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Original Article

Low-dose ondansetron: A candidate prospective precision medicine to treat alcohol use disorder endophenotypes

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ABSTRACT

Background: Alcohol use disorder (AUD) is among the leading causes of morbidity and mortality worldwide, and over 95 million people live with alcohol dependence globally. The estimated heritability of AUD is 50–60 %, and multiple genes are thought to contribute to various endophenotypes of the disease. Previous clinical trials support a precision medicine approach using ondansetron (AD04, a 5-HT3 antagonist) by segregating AUD populations by the bio-genetic endophenotype of specific serotonergic genotypes and the bio-psychosocial endophenotype of the severity of drinking or both. By targeting the modulation of biogenetic signaling within the biopsychosocial context of AUD, low-dose AD04 holds promise in reducing alcohol consumption among affected individuals while minimizing adverse effects.

Methods: This was a phase III, 6-month, 25-site, randomized, placebo-controlled clinical trial using AD04 to treat DSM-V–categorized AUD individuals who were pre-stratified into the endophenotypes of heavy or very heavy drinking individuals and possessed a pre-defined profile of genetic variants related to the serotonin transporter and serotonin-3AB receptor. Participants ($N = 303$) presented moderate to severe AUD, >80 % were men, mostly in their fifties, and >95 % were of European descent. Low-dose AD04 (approx. 033 mg twice daily) or a matching placebo was administered twice daily for 6 months. Brief Behavioral Compliance Enhancement Treatment (BBCET [53]) was administered every two weeks to enhance medication compliance and clinic attendance.

Results: There was a significant reduction in the monthly percentage of heavy drinking days, PHDD (-46·7 % (2·7 %), 95 %CI: -52·1 % to -41·2 % vs. -38·1 % (2·9 %), 95 %CI: -43·8 % to -32·5 %, respectively; LS mean difference=-8·5 %; $p = 0.03$) among AD04-treated vs. placebo-receiving heavy drinking individuals at month 6. Heavy drinking individuals were also less likely to be diagnosed with AUD [Month 1: -32·0 % (2·8 %), 95 %CI: -37·5 % to -26·5 % vs. -23·2 % (2·9 %), 95 %CI: -28·9 to -17·5 %; LS mean difference= -8·8 %; $p = 0·026$)], and improved on the WHO quality of life BREF scale with a significant effect for at least a 1-level downward shift (OR = 3.4; 95 % CI: 1·03–11·45, $p = 0·044$). Importantly, heavy drinking individuals, as distinct from very heavy drinking individuals, were the bio-psychosocial endophenotype more predictive of therapeutic response to AD04. AD04 had an exceptional safety and tolerability profile, like the placebo's.

Conclusions: In this Phase 3 clinical trial, AD04 was shown to be a promising treatment for currently drinking heavy drinking individuals with AUD who also possess a specific genotypic profile in the serotonin transporter and serotonin-3AB receptor complex. Using AD04 to reduce the harm of AUD in heavy drinking individuals who are currently drinking, without the necessity of abstinence or detoxification from alcohol use, is an important advance in the field of precision medicine. AD04's adverse events profile, which was like placebo, should enhance accessibility and acceptance of modern medical treatment for AUD by lowering the incorrect but commonly perceived stigma of personal failure.

Abbreviations: AUD, (Alcohol Use disorder); 5HT3, (Serotonin-3 receptor); BBCET, Brief behavioral compliance enhancement treatment.

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1. Introduction

Alcohol is an addictive substance that contributes to millions of deaths and disabilities worldwide [1]. Excessive alcohol use accounts for 5.1 % of the global disease burden and is the primary risk factor for premature mortality and disability in people aged 15 years to 49 years, and accounts for 10 % of all deaths in this age group [1]. Vulnerable societies have higher rates of alcohol-related fatalities and hospitalizations [1]. Despite the serious global disease burden, only a minority of individuals with alcohol use disorder (AUD) receive pharmacotherapy due to concerns over their efficacy and the risk of clinically significant or severe adverse events [2-4].

The US FDA has approved disulfiram, acamprosate, and naltrexone for treating AUD. Evidence from randomized, double-blind, placebo-controlled trials (RDBPCTs) has, however, raised doubts about their efficacy. Disulfiram has proved efficacious in supervised open-label trials but not in blinded trials, and its concomitant use with alcohol can lead to serious potential side effects including death [5]. Although the COMBINE trial found naltrexone favourable compared to placebo [6], a large RDBPCT showed no significant impact on alcohol consumption in AUD subjects [7]. Additionally, large-scale RDBPCTs, including the COMBINE trial, did not demonstrate that acamprosate is efficacious at reducing alcohol use or promoting abstinence [6,8]. Meta-analyses of RDBPCTs on naltrexone and acamprosate have also yielded inconclusive results regarding their efficacy [9,10]. Acamprosate is contraindicated in severe renal impairment, which also impairs naltrexone clearance, resulting in increased side effects [54,55].

1.1. A precision medicine approach to using 5-HT3 antagonists for AUD treatment

The estimated heritability of AUD is 50–60 %, and multiple genes contribute to various endophenotypes of the disease [11]. The significant impact of heritability, including genetic factors on the etiology of AUD, indicates that a precision medicine approach that focusses on specific endophenotypes associated with treatment response could enhance the likelihood of identifying individuals with the greatest potential for benefit [12]. Several attempts to identify genetic factors that might reliably predict the efficacy of naltrexone and acamprosate in defined subpopulations have, however, produced negative or inconclusive results [13,14]. In contrast, the systematic prevailing data suggest that low-dose ondansetron (approx. 033 mg twice daily, AD04), a 5-HT3 receptor antagonist, is a promising precision medicine approach for the treatment of AUD [12].

Basic science studies have shown that 5-HT3 receptors located in the meso-cortical-limbic dopamine pathway (MCLDP) mediate the rewarding effects of alcohol and other drugs of abuse [15]. Alcohol activates directly these 5-HT3 receptors to stimulate dopamine release in the MCLDP, and thereby, express its rewarding effects, which are associated, at least in part, with its addictive effects [16].

The 5-HT3 receptor is an important mediator of the rewarding effects of alcohol associated with its abuse liability [17,18]. Alcohol consumption increases the genetic expression of the 5-HT3 receptor, and selective breeding for alcohol preference results in increased genetic expression of 5-HT3 receptors in the MCLDP, with a corresponding enhancement of sensitivity to the rewarding effects of alcohol and 5-HT3 agonists [17,18]. Antagonism of 5-HT3 receptors in the ventral tegmental area (VTA), a structure within the MCLDP, inhibits the acquisition of alcohol self-administration, reduces ongoing alcohol self-administration, and prevents relapse to increased alcohol consumption following a period of alcohol deprivation. Finally, it has been shown that the activation of serotonergic neurons in the dorsal raphe, which project to the posterior part of the VTA and the nucleus accumbens shell, stimulates 5-HT3 receptors and promotes cue- and context-induced alcohol craving [17,18].

1.2. Progress in the development of a precision medicine approach to using AD04 to treat AUD endophenotypes

Previous clinical trials support a precision medicine approach to using ondansetron by segregating AUD populations by the bio-genetic endophenotype of specific serotonergic genotypes and the bio-psychosocial endophenotype of the severity of drinking, or both. Sellers and colleagues [19] showed in a small RDBPCT ($n = 71$) that in the endophenotype of heavy drinking individuals (i.e., those that drank <10 standard drinks per drinking day (DDD) at enrollment), but not in the endophenotype of very heavy drinking individuals (≥10 DDD at enrollment), that low-dose ondansetron (0·5 mg/day) group compared with placebo had a trend towards less alcohol consumption ($p = 0.06$; estimated study power was less than 0.3). In contrast, in another small RDBPCT ($n = 51$), high-dose ondansetron (16 mg/day) compared with placebo was inefficacious at decreasing alcohol consumption but instead increased it [20]. Combined, these results indicated that ondansetron's therapeutic effects were most manifest at lower doses and in the bio-psychosocial endophenotype of heavy drinking individuals (those who consumed <10 DDD).

The importance of the bio-genetic endophenotype of variation in serotonergic function as a predictor of response to ondansetron for treating AUD has also been demonstrated. For instance, early-onset alcoholics have different underlying bio-genetic contributions to AUD compared with late-onset alcoholics by having an earlier age of disease onset, greater propensity to consume alcohol [21], increased impulsivity [22], and possibly altered modulation of serotonin function [23].

A 2-month and 3-week RDBPCT ($n = 271$) by Johnson and colleagues [24] showed that low-dose ondansetron treatment (4 mcg/kg) was associated with a significant reduction in heavy drinking and an increased abstinence rate. In that same study, less compelling therapeutic effects for very low- or high-dose ondansetron (1 mcg/kg and 16 mcg/kg, respectively) to treat AUD were found. Ondansetron's rather inverted U-shape dose specificity for treating AUD was also supported by the fact that craving reductions were only seen in those with early-onset alcoholism compared with their late-onset alcoholism counterparts for the middle ondansetron dose (i.e., 4 mcg/kg) [25]. Partial support for these findings has come from ondansetron studies in adolescents and adults [26].

In a conceptual paper, Johnson [27] hypothesized that the underlying bio-genetic endophenotype of serotonergic dysfunction segregating ondansetron responders from non-responders may be the genotypes associated with increased pre-synaptic release of serotonin in the promoter region of the gene (LL genotype) and enhanced receptor availability (TT genotype) in the untranslated region of the gene. Interestingly, Stoltenberg [28] built a mathematical model to explain this hypothesis more empirically. Ait-Daoud and colleagues [29] showed also that individuals with AUD and the LL genotype of the 5HTTLPR had significantly greater cue-craving than those with the LS or SS genotype.

Subsequently, Johnson and colleagues [30] provided evidence to support the prediction of this specific serotonergic hypothesis in a large Phase 2 RDBPCT ($n = 321$), where ondansetron 4 mcg/kg treatment was associated with significantly greater reductions in heavy drinking and increased abstinence. Partial corroboration for the modulatory effects of the LL single nucleotide polymorphism (SNP) of the 5HTTLPR to predict ondansetron's effect on reducing alcohol consumption has also been shown [31]. Additionally, Johnson and colleagues have shown that the TT SNP of the 5-HTTLPR when co-expressed with the LL SNP was associated with a significant reduction in alcohol consummatory behaviors following treatment with low-dose ondansetron [32].

1.3. Identification of genotypes responsive to AD04

Whilst the Phase 2 RDBPCT of Johnson and colleagues [30] provided evidence that genotypic variants at the serotonin promoter gene, which

gated serotonergic function but did not bind ondansetron, predicted treatment response to low-dose ondansetron, it was important to extend this hypothesis to search for variants that had a direct effect at the 5-HT3 receptor to which ondansetron actually binds or is modulated. The expectation was that these genotypic variants influencing the 5-HT3 receptor directly would be expected to be even more predictive of ondansetron's therapeutic response. Supportive of that prediction, post-hoc genetic analyses and sequencing studies showed that genotypic variations in the 5-HT3A receptor (rs1150226-AG or rs1176713-GG) and 5-HT3B receptor (rs17614942-AC) and the LL/TT SNP at the 5-HTTLPR mediated the therapeutic effects of ondansetron on alcohol consumption in AUD subjects [30].

The 5-HT3 receptor exerts its function via a ligand-gated ion channel. The rs1150226-AG variant of the 5-HT3A receptor is associated with an increase in the time the receptor's ion channel is opened [32]. Notably, even though it is the 5-HT3A receptor that binds ondansetron, this biogenetic effect would be expected to be amplified up to 16-fold by the further actions of specific variants at the 5-HT3B receptor because the postsynaptic 5-HT3 receptors are formed by 5-HT3A homopentamers or 5-HT3A and 5-HT3B heteropentamer (5-HT3AB) complexes that evince even faster ion transport and conduction [34]. The rs1761942-AC variant is also associated with an increase in the time the receptor ion channel is opened [34]. Combined, the effect of the rs1761942-AC and rs1150226-AG genotypes may further enhance the time the receptor ion channel is opened. Even though the 5-HT3B subunits do not bind with ondansetron, they are important for stabilizing the 5-HT3 receptor complex at the cell surface and increasing the duration of ion channel opening [33,34].

In sum, through prospective genotyping and post hoc algorithmic, systematic analyses, we showed that a complex pattern of five genotypic variants may best predict therapeutic response to low-dose ondansetron in treating AUD. Furthermore, gene by environment ($G \times E$) interactions, such as the duration and level of exposure to alcohol consumption, could alter gene expression and, therefore, explain the findings of previous studies for the potential differential effects of ondansetron in heavy drinking individuals and very heavy drinking individuals.

1.4. Lessons learned towards designing a precision medicine approach to the Phase 3 RDBPCT of AD04 to treat AUD

Previous studies provided three critical lessons for the design of the current Phase 3 RDBPCT. First, selecting and randomizing a priori by the bio-genetic endophenotype of specific selected genotypes at both the promoter and receptor level of the 5-HT3 receptor was important. Second, the study also had to randomize subjects *a priori* by the bio-psychosocial endophenotype of drinking severity (heavy drinking individuals vs. very heavy drinking individuals) to validate the earlier findings. Third, ondansetron's therapeutic dose was an inverted U-shape, with optimal effects at about 4 mcg/kg, thus obviating the need for multi-dose testing.

Our stated hypothesis was that in this multi-national 6-month RDBPCT, ondansetron (0.33 mg twice daily; approximating to about 4 mcg/kg in an 80 kg individual) would reduce significantly the monthly PHDD at Months 5 and 6 and/or at Month 6 among individuals with AUD and the pre-specified bio-genetic endophenotype of a 5-panel genotype pattern at the serotonin transporter and 5-HT3 receptor (see 'Methods' description in the supplement). Importantly, the cohort of AUD individuals was a priori randomized into the bio-psychosocial endophenotypes of heavy drinking individuals (consumed <10 DDD) and very heavy drinking individuals (consumed ≥10 DDD). (See detailed supplementary 'Methods').

2. Synopsis of the methods (see supplement for details)

2.1. Study design

A Phase III, 6-month, RDBPCT of AD04 vs. matching placebo in individuals with AUD, all of whom had a pre-specified bio-genetic endophenotype (i.e., specific variants at the 5-HTTLR and 5-HT3 A/B receptor complex) and were pre-stratified by bio-psychosocial endophenotypes (i.e., heavy vs very heavy drinking individuals) was conducted. The trial spanned 25 sites across seven European countries, with ethics approval and adherence to relevant regulations. This study is registered at www.clinicaltrials.gov (NCT04101227).

2.2. Endophenotypes

Bio-genetic endophenotype: Genotypes at the AC/AG, AC with any other (AC+), AG with any other (AG+) at the 5-HT3 AB receptor complex, or LL/TT with any other (LL/TT+) at the serotonin transporter. Bio-psychosocial endophenotypes: heavy drinking individuals who consumed <10 standard DDD and very heavy drinking individuals who consumed ≥ 10 standard DDD.

2.3. Participants

Enrollees diagnosed with AUD [35] met specific inclusion criteria, including age ≥18, heavy alcohol consumption, willingness for DNA analysis, and specific genotypes. While abstinence was not an outcome, participants expressed a desire to reduce alcohol consumption. Compliance with treatment attendance and medication taking was optimized through participation in standardized, manual-driven, Brief Behavioral Compliance Enhancement Treatment (BBCET), and personnel were trained to use Alcohol Timeline Follow Back (TLFB) and BBCET rating scales. BBCET was supervised with recorded sessions, about 10 % of which were examined for fidelity and adherence to the protocol (see supplemental materials for the list of inclusion and exclusion criteria). Participants were pre-stratified based on gender and alcohol consumption and then randomized using a permuted block design and a 1:1 allocation ratio. Medication was delivered in blinded boxes, ensuring quadruple masking. A total of 303 participants were included in the study ($N = 303$). Full inclusion and Exclusion criteria are described in the Online supplementary material.

2.4. Procedures

Enrollment occurred between February 2020 and August 2021 and included comprehensive assessments at Screening and baseline (Visit 2). Participants received double-blind treatment for 6 months, with safety assessments at scheduled intervals. BBCET, administered twice monthly, aimed to enhance medication compliance and clinic attendance. Training, certification, and performance monitoring ensured consistent administration. Safety assessments occurred at 2-month intervals, including ECG recordings. At months 3 and 6, physical examinations and other tests were conducted. The study concluded with a safety follow-up at month 7. AD04 dosing was determined based on our previous Phase 2 study. Both active medication and matching placebo were identical, supplied by Catalent, with pill-taking frequency calculated from the amount returned subtracted from the dispensed amounts. In summary, this precision medicine clinical trial used rigorous randomization, blinding, and compliance measures. Safety assessments and follow-ups were done throughout the study (see Online supplementary material for the schedule of assessments for the measures).

2.5. Safety assessment and adverse events

Safety assessments occurred at 2-month intervals, including ECG recordings. Adverse events were collected at every study visit and as

reported.

Synopsis of Statistical Analysis (See Online supplementary material for details)

Optimapharm managed data quality supervision, cleaning, and dataset delivery. PharPoint Research Inc. handled safety and efficacy determination. Professor Lei Liu and Dr. Joe Hirman provided additional data and statistical verification.

Out of enrolled subjects, 303 received at least one dose, forming the safety population (AD04: 156; placebo: 147). Intent-to-treat analysis was employed for efficacy evaluation based on randomized treatment. An independent Data Monitoring Committee (DMC) ensured safety, reviewing unblinded data every three months. The DMC monitored adherence to study procedures and recommended study continuation without modification.

Demographic and baseline characteristics were described using summary statistics. Primary and secondary outcomes, except the modified WHO Quality BREF scale (DRL), were analyzed using a Mixed Model for Repeated Measures (MMRM) with an unstructured covariance structure for repeated measures. Restricted maximum likelihood estimation and the Kenward-Roger approximation for degrees of freedom were employed. For the drinking data, covariates in the model included the stratification factors of gender and baseline DDD category (i.e., heavy drinking individuals vs very heavy drinking individuals), together with baseline PHDD, study month (as a categorical variable), and a study month by treatment interaction. For the DRL, logistic models were used to describe the proportion of subjects with a significant categorical shift from baseline compared with study Month 6. Missing data were addressed using the MMRM analysis, assuming random missingness.

Incomplete dates were systematically imputed, minimizing bias. Reasonable imputation methods were applied for analyses requiring complete dates. Planned subgroup analyses were conducted to examine treatment effects.

2.6. Ethical considerations

The committee provided ethics approval for protecting human subjects at each participating site. The trial complied with the protocol, the International Conference of the Harmonization of Good Clinical Practice, the applicable regulatory requirement(s), and the Declaration of Helsinki.

3. Results

Fig. 1. Trial Flow Diagram.

The baseline drinking levels for heavy drinking individuals and very heavy drinking individuals averaged 70.8 g ethanol/day and 118.5 g ethanol/day, respectively (Table 1). The heavy drinking individuals' alcohol consumption was like that of recruits reported for previous large-scale multicenter registration trials [36,37]. Participants at "very high risk" for the consequences of AUD were 32.5 % and 51.5 % for heavy drinking individuals vs. very heavy drinking individuals, respectively, and 99 % of subjects in each category consumed ≥6 heavy drinking days (HDD)/month. Supplementary Table 7 lists the disposition and genotype testing by subject.

Three hundred and three subjects (AD04: 156; placebo: 147) received at least one dose of the study drug and had at least one valid post-baseline assessment of the TLFB. A power calculation prior to study initiation estimated the power would be 0.95 to detect significant differences in the PHDD at Months 5 and 6 between the treatment groups. Post-study power analyses on the PHDD measure indicated that the overall power was 0.533 (Field bootstrapping) [38].

For the total cohort, the MMRM model yielded no significant main effect ($p = 0.55$) or interaction effect of treatment x visit ($p = 0.23$). For Months 5 and 6, the least-squares (LS) mean difference in the change in PHDD from baseline was 2.1 % between the AD04 and the placebo groups ($p = 0.53$) in favor of the AD04 group. For Month 6 only, the LS mean difference in the change in PHDD from baseline was 2.9 % between the AD04 and the placebo groups ($p = 0.41$) in favor of the AD04 group. Examination of the treatment effect in the pre-specified endophenotype of the drinking characteristic of the heavy drinking individuals showed no main effect ($p = 0.11$) or interaction effect of treatment x visit ($p = 0.28$) in PHDD change during Months 5 and 6 from baseline. For very heavy drinking individuals, the corresponding p-values were 0.38 and 0.59, respectively.

Planned analyses PHDD showed the average PHDD for Months 5 and 6 and each study month (Months 1–6; Fig. 2). For Months 5 and 6 in the heavy drinking individuals' group, the difference in the LS mean change in PHDD from baseline was greater, but non-significant (LS mean difference of −7.0 %; $p = 0.07$), for the AD04 group (LS mean (SD): −45.0 % (2.7 %), 95 % confidence interval (CI): −50.3 % to −39.7 %) compared with the placebo group (LS mean (SD): −38.0 % (2.8 %), 95 % CI: −43.6 % to −32.5 %). The planned contrasts that examined PHDD for each month showed a significant reduction in PHDD for the AD04-treated heavy drinking individuals group compared with placebo controls during Month 1 and Month 6 of the study (Fig. 2). During Month 1, the LS mean (SD) change in PHDD was significantly greater in the AD04 group compared with placebo (−32.0 % (2.8 %), 95 %CI: −37.5 % to −26.5 % vs. −23.2 % (2.9 %), 95 %CI: −28.9 to −17.5 %; LS mean difference=−8.8 %; $p = 0.026$). Similarly, during Month 6, the LS mean (SD) change in PHDD from baseline was greater in the AD04 group compared with placebo (−46.7 % (2.7 %), 95 %CI: −52.1 % to −41.2 % vs. −38.1 % (2.9 %), 95 %CI: −43.8 % to −32.5 %, respectively; LS mean difference= −8.5 %; $p = 0.03$). For the very heavy drinking individuals group, no significant effects were observed.

In response to the significant AD04-induced reduction in PHDD during Months 1 and 6, we performed an additional analysis (not listed in the SAP) to examine AD04 treatment efficacy throughout the entire treatment period. On PHDD, a Mixed Factor Repeated Measures Analysis of Variance (MFRM ANOVA) was done. Significant effects were found for Study Months alone (WL = 0.6251; F[5216] = 25.91; $p < 0{\cdot}001$) and the Study Months* DDD Stratum (WL = 0.9246; F[5216] = 3.52; $p = 0.004$). For the heavy drinking individuals' group, the Study Month*-Drug Treatment interaction effect was not found to be significant (WL = 0.9576, F[5133] = 1.18, $p = 0.323$); however, the between-subjects effect of Drug Treatment was significant (F[1137] = 4.57, $p = 0.034$). This indicated that for the 6-month study period, individuals in the heavy drinking individuals' group who received AD04 had consistently reduced PHDD compared with placebo.

Given that AD04 reduced PHDD in the heavy drinking individuals' group during Months 1 and 6, we performed a correlational analysis on PHDD values between Month 1 and Month 6. In the heavy drinking individuals' group, those who received AD04 had a significant negative correlation ($r = -0.33$; $p = 0.005$) on PHDD value during Month 1 and Month 6. Additionally, we did a correlational analysis on the change in PHDD from baseline between Months 1 and 6. Fig. 3 shows that the change in PHDD during Month 1 was correlated with the value during Month 6 of AD04 treatment ($r = 0.76$; $p < 0.001$). Additional sensitivity models examining the effects of treatment on the PHDD outcome using different statistical analytic plans and a lower DDD cut-off for women are presented as supplementary information (Supplementary Tables 1–6 and 8–22).

Fig. 4 shows the effects of AD04 and placebo in the heavy drinking individualss group on the mean (standard error (SE)) total alcohol consumed (TAC) during the 6-month clinical trial. For the AD04 group compared with the placebo group, there was no significant effect on TAC in the very heavy drinking individuals' group ($p > 0.05$) for any time duration; however, there was a significant, predictable directional effect ($p = 0.05$; one-tailed) to reduce TAC at Month 6, but not for any other duration.

Fig. 5 shows the effects of AD04 vs. placebo on mean (SE) DDD during the clinical trial in the heavy drinking individuals' group. An MFRM ANOVA was done for the DDD variable, comprising a within-

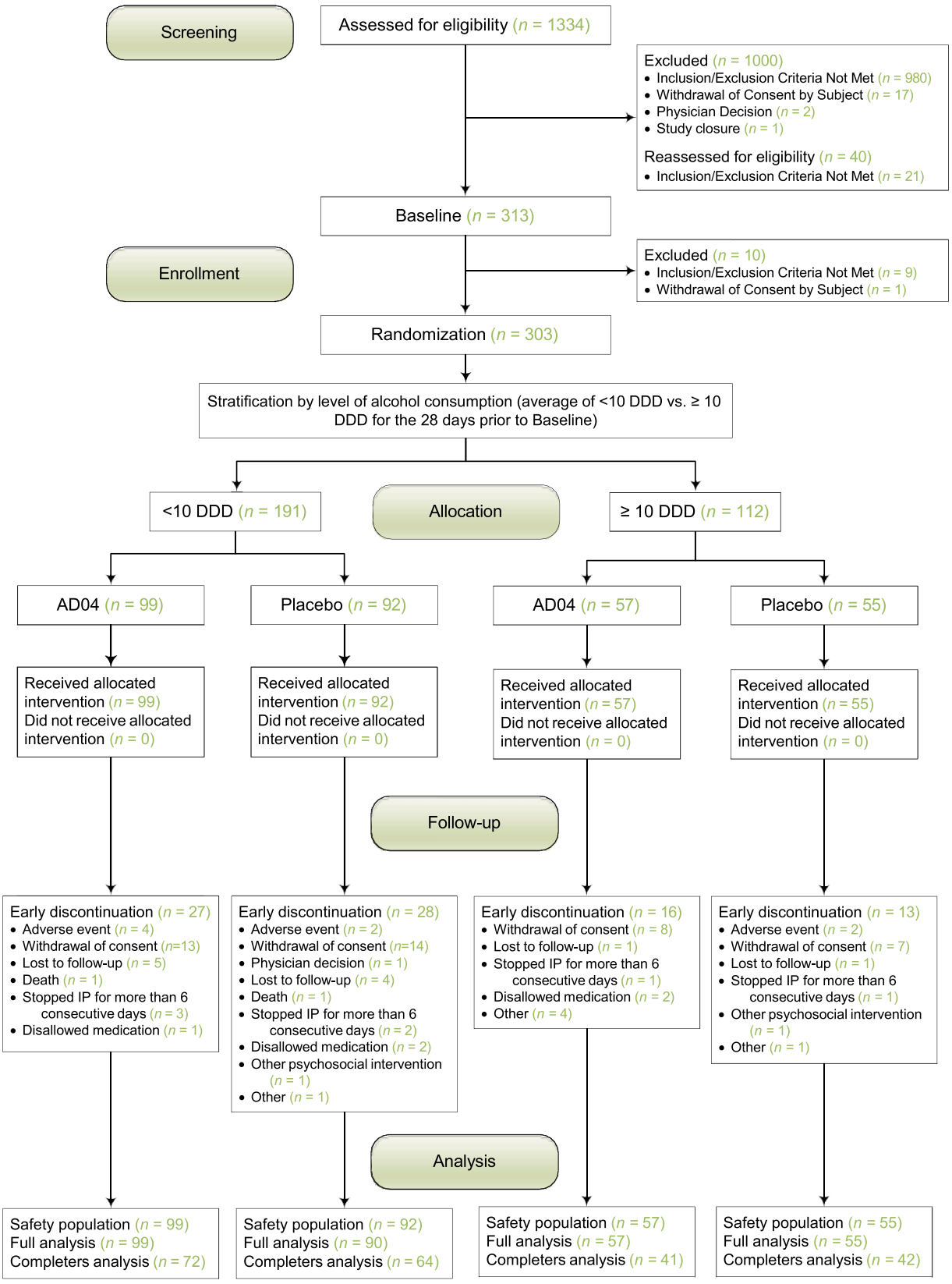


Fig. 1. Trial Flow Diagram.
DDD: standard drinks/drinking day; IP: investigational product.

Table 1
Demographic And Baseline Characteristics of The Study Subjects.

	Heavy (n = 191)			Very heavy (n = 112)		
	AD04 (n = 99)	Placebo (n = 92)	P-value	AD04(n = 57)	Placebo (n = 55)	P-value
Demographic Characteristics						
Age	48 (23 – 78)	50 (23 – 76)	0.31	50 (27 – 67)	47 (23 – 73)	0.21
Gender, No. (%)			0.88			0.42
Men	63 (64 %)	57 (62 %)		47 (82 %)	49 (89 %)	
Women	36 (36 %)	35 (38 %)		10 (18 %)	6 (11 %)	
Height, cm	173.5 (147.0 – 196.0)	172.3 (157.0 – 190.0)	0.32	175.5 (156 – 190)	175·8 (156 – 192)	0.86
Weight, kg	81.6 (42.0 – 138.0)	79.1 (48·6 – 127·3)	0·30	84·9 (55·0 – 121·5)	86·6 (50·0 – 170·0)	0·64
BMI, kg/m^2	27·0 (15·1 – 46·1)	25·8 (17·6 – 44·8)	0·58	27·5 (18·8 – 38·6)	27·9 (17·7 – 51·3)	0·70
Genotype*, No. (%)						
LL/TT	39 (39 %)	41 (45 %)	0·56	28 (49 %)	25 (45 %)	0·71
AG	43 (43 %)	37 (41 %)	0·66	22 (39 %)	26 (47 %)	0·45
GG	17 (17 %)	20 (22 %)	0·47	10 (18 %)	6 (11 %)	0·42
AC	30 (30 %)	31 (34 %)	0·64	21 (37 %)	15 (27 %)	0·32
Measures of Alcohol Drinking						
Baseline Diagnosis of AUD per DSM-5			0·38			>0·99
Moderate	44 (44 %)	35 (39 %)		12 (21 %)	12 (22 %)	
Severe	55 (56 %)	55 (61 %)		45 (79 %)	43 (78 %)	
Percent of heavy drinking days	65·6 % (14·3 % – 100 %)	62.5 % (21·4 % – 100 %)	0·95	76·3 % (7·1 % – 100 %)	75·8 % (25·0 % – 100 %)	0·93
Total alcohol consumed, g/day	70·8 (20·6 – 162·9)	70·5 (27·0 – 291·0)	0·95	118·2 (16·7 – 387·0)	118·8 (37·0 – 322·5)	0·96
Mean drinks per drinking day	8·9 (3·8 – 22·1)	9·2 (3·4 – 29·1)	0·67	14·8 (4·7 – 67·7)	14·1 (4·7 – 32·3)	0·59
Risk drinking level at baseline			0·55			0·68
Abstinence	0	0		0	0	
Very low risk	0	0		1/57 (2 %)	0	
Low risk	2/99 (2 %)	1/92 (1 %)		1/57 (1 %)	0	
Medium risk	32/99 (32 %)	29/92 (32 %)		5/57(9 %)	8/55 (15 %)	
High risk	34/99 (34 %)	40/92 (43 %)		17/57 (30 %)	17/55 (31 %)	
Very high risk	31/99 (31 %)	22/92 (24 %)		33/57 (58 %)	30/55 (55 %)	
Heavy alcohol consumption patterns			>0·99			>0·99
0 HDD	0	0		0	0	
1 HDD	0	0		0	0	
2 HDD	0	0		1/57 (2 %)	0	
3 HDD	0	0		0	0	
4 HDD	1/99 (1 %)	0		0	0	
5 HDD	0	0		0	0	
≥6 HDD	98/99 (99 %)	92/92 (100 %)		56/57 (98 %)	55/55 (100 %)	

subject factor of 'Time' and between-subject factors of 'Treatment' and 'drinking characteristic endophenotype'. The overall analysis showed a significant three-way interaction ($F_{6,212} = 13.64$; $p < 0.0001$). The significant three-way interaction term was decomposed by examination of the effects of 'Time' and 'Treatment' in heavy drinking individuals and very heavy drinking individuals. In the heavy drinking individuals' group, there was a significant effect of 'Time' ($F_{6,129} = 30.46$; $p < 0.001$).

Individual ANOVAs were performed at each time point on data derived from the heavy drinking individuals' group. The pre-specified endpoint (i.e., the average value for Months 5 and 6) showed a non-significant effect of AD04 ($F_{1,131} = 1.67$; $p = 0.097$). Examination of the effects of AD04 vs. placebo treatment in the heavy drinking individuals' group during Month 6 also showed a non-significant finding ($F_{1,131} = 1.83$; $p = 0.07$). In the very heavy drinking individuals'group, there was no significant effect on DDD in the AD04 group compared with the placebo for the average value at Months 5 and 6 ($p = 0.072$) or Month 6 ($p = 0.14$).

The USA FDA has indicated that a valid clinical endpoint in RDBPCTs investigating treatments for AUD is the establishment of alcohol abstinence [51] (i.e., no drinking days (NDD)). In the present study, abstinence was not a stated goal. NDD, a qualitative variable, was analyzed using the non-parametric Wald's Chi-Square. The overall results showed no significant effect of AD04 compared with placebo on NDD ($p = 0.098$; data presented in Table 2). In the heavy drinking individuals' group, there was also no significant effect of AD04 compared with placebo on NDD during the average of Months 5 and 6 ($p = 0.21$) or Month 6 ($p = 0.11$).

There was a significant overall improvement in AUD severity from baseline to study end for both heavy drinking individuals' and very heavy drinking individuals ($p = 0.04$). At month 6, for the heavy drinking individuals, the AUD severity pattern in the AD04 group was significantly different from the placebo group: (mild symptoms: AD04 group 33% vs. placebo group 39 %; severe symptoms: AD04 group 10 % vs. placebo group 24 %) ($p = 0.05$, Fisher's exact test). At month 6, for the very heavy drinking individuals'group, the AD04 group compared with placebo showed a non-significant pattern (mild symptoms: AD04 group 33 % vs. placebo group 39 %; severe symptoms: AD04 group 21 % vs. placebo group 26 %) ($p = 0.10$, Fishers' exact test).

For the change in WHO Quality of Life BREF, the overall treatment effect in the combined heavy drinking individuals and very heavy drinking individuals was non-significant for at least 1-level change (odds ratio (OR) = 1.55, $p = 0.31$), and at least 2-level change (OR = 1.01, $p = 0.96$), or an at least 3-level change (OR = 1.08, $p = 0.79$). Notably, however, in the heavy drinking individuals' group, the treatment effect was significant for an at least 1-level change, with an increase in the OR of an at least 1-level downwards shift of 3.4 (95 % CI: 1.03–11.45, $p = 0.044$). The treatment effect was, however, not significant for an at least 2-level shift (OR = 1.60, $p = 0.20$) or an at least 3-level shift (OR = 1.74, $p = 0.12$). For the very heavy drinking individuals' group, the treatment effect was not significant for at least a 1-, 2-, or 3-level shift.

Previous post hoc analyses have shown that five genotypes (LL, TT, AC, AG, and GG) were associated with AD04's efficacy in treating AUD [29]. We performed similar post hoc exploratory analyses on genotypes for the clinical endpoints of the present study (Table 2). For PHDD, in the heavy drinking individuals'group, data analysis showed a significant difference between the AD04 group and the placebo group among subjects with an AC/AG, AC with any other (AC+), AG with any other

(AG+), or LL/TT with any other (LL/TT+) for the average PHDD at Months 5 and 6 and for Month 6. There was no significant effect of AD04 vs. placebo treatment among the heavy drinking individuals with only a single SNP (AC alone, AG alone, or GG alone). There was a similar genotype association with the TAC analysis. In the heavy drinking individuals' group, AD04 compared with placebo reduced TAC significantly among individuals with the AC/AG, AC/AG, and any other, and GG with any other genotype profile (Month 6 only).

For the Alcohol Consumption Risk Responder analysis, AD04 compared with placebo non-significantly increased the rate of NDD for the average of Months 5 and 6 in AC/AG, GG, and all others, and LL/TT (Table 2). In the heavy drinking individuals' group, there was a significant increase in the AD04 group compared with the placebo group on NDD for all subjects except those with only the AC genotype (point estimate –2.025; $p = 0.05$). For DDD, in the heavy drinking individuals' group, there was a significant reduction for the AD04 group compared with the placebo group on the average of Months 5 and 6 and at Month 6 in subjects with the AG or AC/AG genotypes (Table 2). Fig. 6 shows the effect sizes for the individual genotype analyses displayed as mean change (95 % CI) in PHDD.

4. Discussion

AUD is a heterogeneous disorder characterized by specific endophenotypes and diagnoses with the identification of non-overlapping psychosocial and behavioral symptoms [39], with a low likelihood of developing a pharmacological agent that will, robustly, treat all AUD subjects. A precision medicine approach for specific

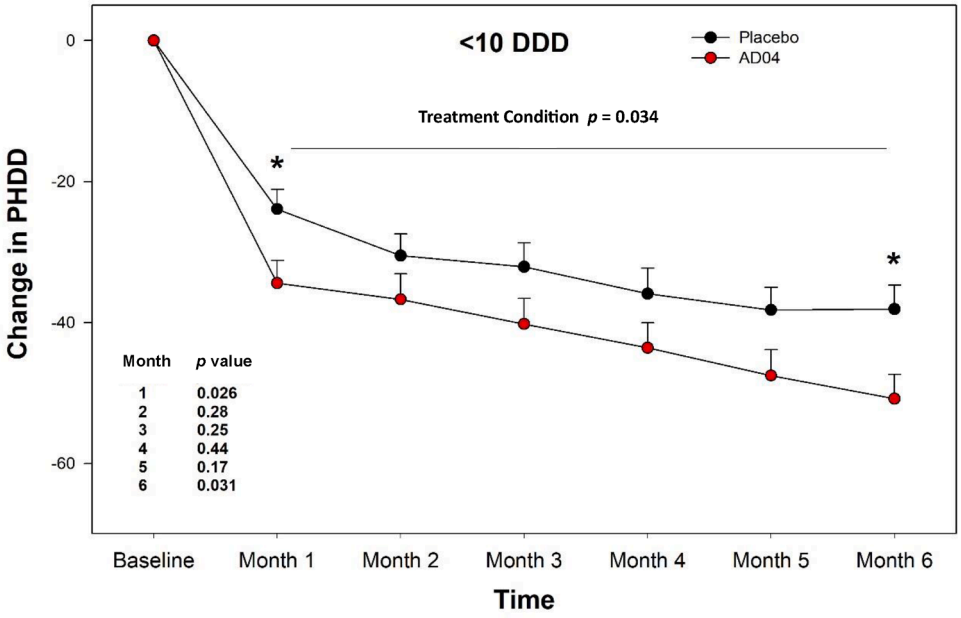


Fig. 2. Trajectory of change (LS Mean [SD]) for heavy drinking individuals in Percentage of Heavy Drinking Days during treatment, AD04 and placebo groups. *Indicates significant differences between AD04 and placebo. Individual analyses by month are inserted in the graph. Overall, statistical analysis showed that in the heavy drinking individuals (<10 DDD), AD04 reduced PHDD for the duration of the 6-month clinical trial.

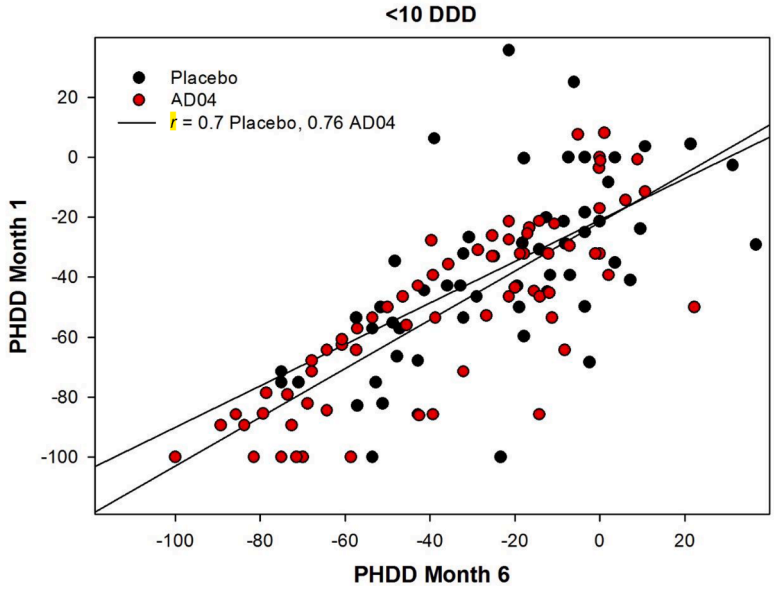


Fig. 3. Correlational analysis for change in PHDD between months 1 and 6 in the heavy drinking individuals (<10 DDD).

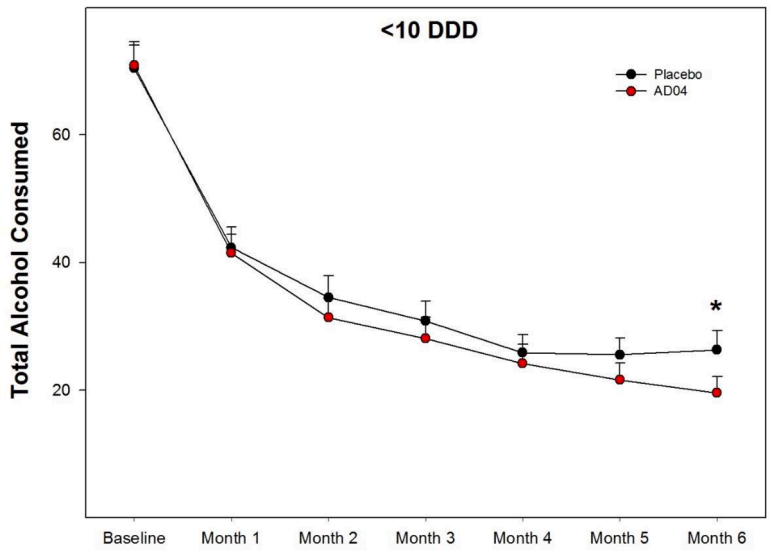


Fig. 4. Trajectory of change for the heavy drinking individuals' group in MEAN (*SE*) Total Alcohol Consumed (TAC, grams ethanol/day) during THE SIX-MONTH treatment WITH AD04 OR placebo.
*Denotes a *p* value=0.05 (one tailed).

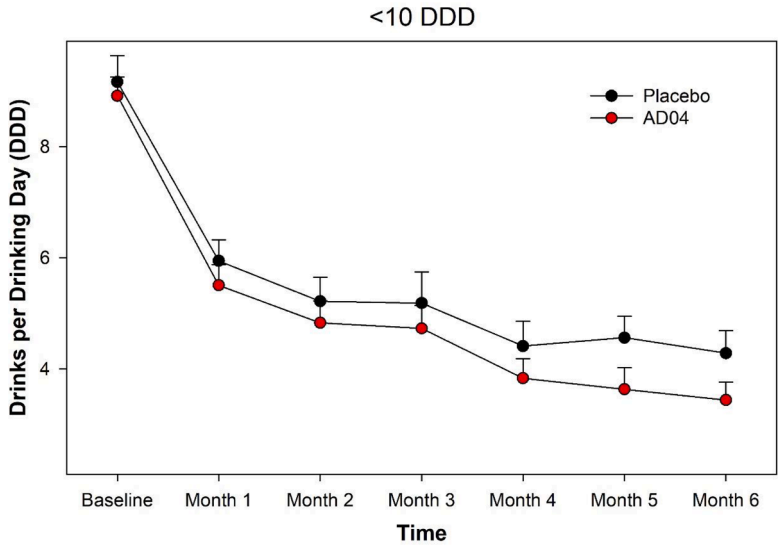


Fig. 5. Trajectory of the mean (se) in the heavy drinking individuals for Drinks per Drinking Day (DDD) during treatment with AD04 or placebo.

treatment-responsive endophenotypes of individuals with AUD should, however, produce a more powerful response to treat the disorder.

In the present RDBPCT, in the heavy drinking individuals' group (<10 DDD), during Months 1 and 6, the mean change in the PHDD from baseline was 8.5 % greater in the AD04 vs placebo group. However, the contrast between the AD04 and placebo groups on PHDD for the primary clinical endpoint (average PHDD for Months 5 and 6) was not statistically significant ($p = 0.07$). The marginal primary clinical endpoint finding could be predicated on the study being statistically underpowered on post-study analysis (0.53) and increased variability in the primary clinical endpoint (i.e., the variability of combined values increases unless the correlation between the two variables is 1, 0, or −1). Possibly, the COVID-19 pandemic, during which time the present study was done, was associated with reduced availability of alcohol products and, consequently, lower than expected alcohol consumption for all groups, thereby magnifying the placebo response and creating a "floor" effect in our ability to detect differences between the AD04 and placebo groups. Despite the statistical insignificance of the primary clinical endpoint, there were additional indications of the beneficial effects AD04 of treatment (Figs. 2 and 4-6).

We propose that the present RDBPCT supports AD04 as a viable precision medicine for reducing alcohol consumption in AUD subjects with specific bio-genetic and bio-psychosocial endophenotype markers of genotype and severity of drinking characteristics. Specifically, the heavy drinking individuals AD04 group compared with the placebo group had a significant reduction in PHDD during Months 1 and 6 (Fig. 2), decreased TAC during Month 6 (one-tailed statistical test due to the predicted direction of effect) (Fig. 4), improved quality of life measures, reduced likelihood of a DSM-5 diagnosis for AUD at Month 6, and showed promising trends to reduce DDD (Fig. 5) and increase NDD (Table 2).

These positive observations replicated our previous findings (Phase 2a clinical study) wherein low-dose ondansetron was shown to be efficacious at reducing various markers of alcohol consumption in early-onset (but not late-onset) AUD [24] and among the same specific genotypes [30]. Other studies have found similar directional results [19,

Table 2
Effects of AD04 on measures of alcohol consumption in the heavy drinking individuals (Percent Heavy Drinking Days - PHDD; No Drinking Days - NDD; Change in Total Alcohol Consumed - ΔTAC; Change in Drinks per Drinking Day - ΔDDD).

Genotype	N	PHDD (M5&6)	PHDD (M6)	p (M5&6)	p (M6)	NDD (M5&6)	NDD (M6)	p (M5&6)	p (M6)	DTAC (M5&6)	DTAC (M6)	p (M5&6)	p (M6)	DDDD (M5&6)	DDDD (M6)	p (M5&6)	p (M6)
AC	7	7.11	2.86	0.75	0.91	NA	NA	NA	NA	25.39	18	0.19	0.38	3.2678	1.6063	0.75	0.88
AG	17	4.29	0.03	0.77	0.99	NA	NA	NA	NA	8.5	7.89	0.47	0.5	−3.9799	−3.3817	0.03	0.067
GG	22	−5.88	−10.91	0.64	0.42	2.222	3.814	0.54	0.287	1.9	−0.98	0.9	0.95	−2.1263	−1.9583	0.159	0.195
AC/AG	26	−30.01	−31.22	0.0045	0.0062	5.705	2.495	0.055	0.26	−23.28	−24.45	0.038	0.048	−3.2925	−2.7577	0.038	0.116
LL/TT	58	−4.4	−5.9	0.18	0.14	0.591	0.603	0.389	0.377	5.1	5.3	0.46	0.49	0.2436	0.1458	0.75	0.86
AC+	61	−12.51	−14.37	0.03	0.018	1.623	2.133	0.365	0.157	−8.25	−10.67	0.15	0.07	−0.7715	−1.1061	0.44	0.27
AG+	80	−12.07	−13.46	0.02	0.01	3.158	3.439	0.022	0.015	−6.27	−7.6	0.24	0.16	−2.0947	−1.9635	0.008	0.016
GG+	37	−11.27	−11.92	0.25	0.25	4.018	3.094	0.139	0.16	−16.67	−17.64	0.052	0.039	−1.8572	−1.6402	0.11	0.17
AC/AG+	48	−19.96	−20.95	0.6	0.55	3.042	3.314	0.071	0.051	−15.1	−16.38	0.0306	0.0261	−2.0624	−1.9053	0.048	0.076
LL/TT+	77	−3.32	−3.99	0.003	0.004	0.744	0.762	0.55	0.582	1.25	1.44	0.83	0.81	0.7018	0.5346	0.371	0.504
All	191	−7	−8.8	0.07	0.031	1.495	1.629	0.21	0.11	−3.07	−4.3	0.39	0.23	−0.8378	−0.9335	0.1	0.071

M: Month; p: p-value.

24,25,26,30] except for a recent study [40], which may have failed because of insufficient statistical power, failure to choose the correct drinking characteristic endophenotype, and the non-standardized use of the psychosocial intervention (BBCET), with possible related inflation of the placebo response [41].

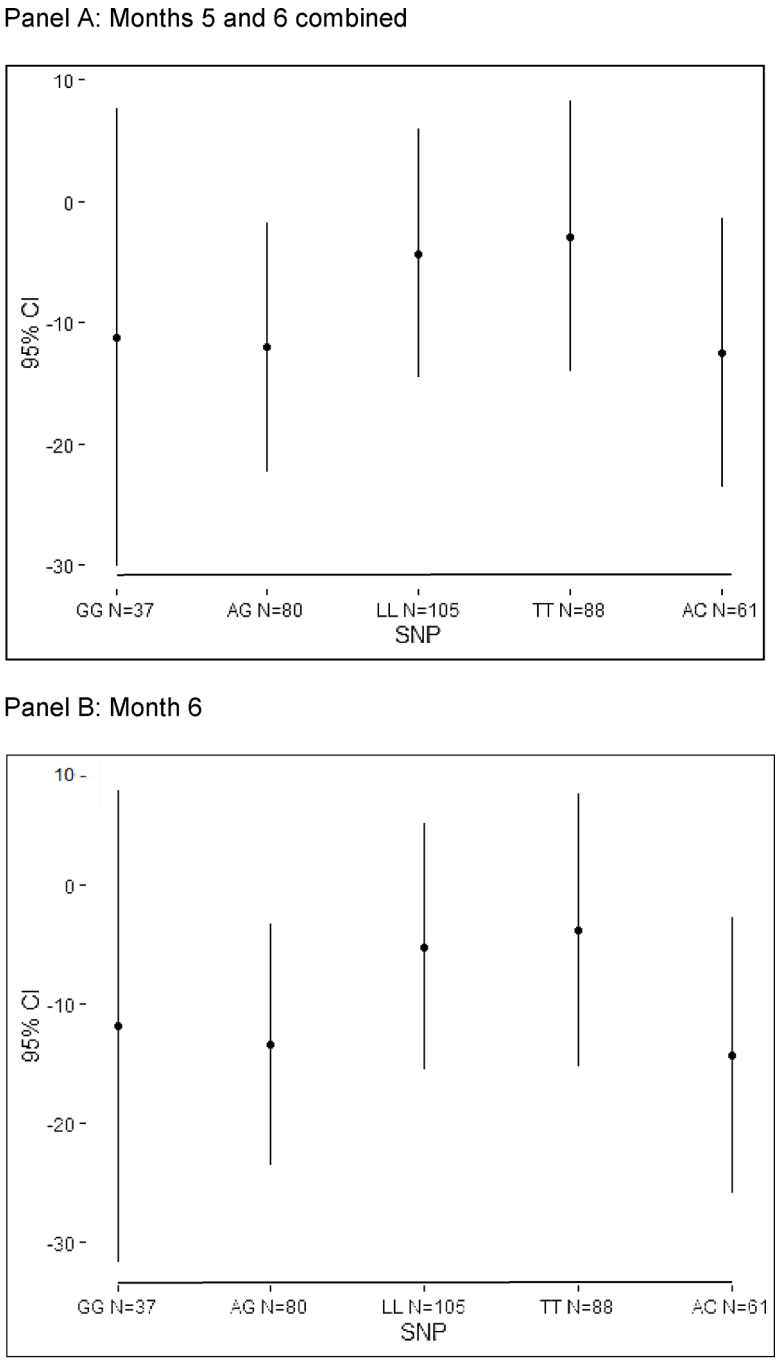
Substantial placebo effects are commonly observed in clinical trials for AUD, and their complete mechanisms remain elusive. Possible factors include patients' anticipations of overcoming their AUD, as well as the non-specific psychosocial support derived from extensive interactions with staff during visits. Moreover, consistent exposure to educational materials and encouragement to alter drinking behavior, coupled with the commitment to attend sessions regularly, contribute to fostering motivation. In the COMBINE trial [6], factors such as pill-taking, interactions with healthcare professionals, and optimism about medication effectiveness contributed significantly to the placebo response. In the present study, we speculated that the global COVID-19 pandemic may have increased the placebo response due to movement restrictions in Europe, thereby limiting the opportunity to go out and purchase alcohol or even have it delivered. Placebo response varied widely across 51 naltrexone and acamprosate trials, showing a negative correlation with the treatment effect size [42]. In the present study, we also found important effects of placebo on treatment outcomes that appeared to reduce the observed size of the therapeutic effect. Nevertheless, these placebo effects appeared more moderated, presumably by implementing BBCET treatment rather than formal psychotherapy for psychosocial support.

It was important in the present study to segregate our cohort of individuals with AUD by bio-genetic and bio-psychosocial endophenotypes. This segregation allowed us to identify clinically distinct endophenotypes that may be amenable to a precision medicine approach. This is consistent with the hypothesis that AD04′s potential therapeutic effect was based on a complex Gene × Environment interaction, whereby "Environment" was the type of heavy drinking. Indeed, we have shown that the level of alcohol consumption affects the expression levels of different serotonin genotypes and clinical response to ondansetron, such that mRNA may in future be used as a biomarker to measure clinical response to AD04 [30,40]; however, further validation studies are needed to establish this finding.

Advances in precision medicine have led to significant discoveries and FDA-approved treatments tailored to individuals' characteristics [57]. Research into precision medicine for AUD, aiming for better treatment responses and fewer side effects, remains a priority. Promising pharmacotherapies and potential genetic moderators for AUD are progressing, but limited pharmacogenetic data must be interpreted cautiously, necessitating translational approaches to effectively translate novel medications and precision strategies from basic and human research into clinical practice [56,58,59].

Binge drinking is an important endophenotype of AUD. This broad category of endophenotype can be subdivided into heavy drinking individuals and very heavy drinking individuals, as there appears to be a distinction in binge drinking severity based on the amount of alcohol consumed within a specific time period. While the very heavy drinker group compared with the heavy drinker group did differ by segregation criterion on DDD, their actual TAC overlapped significantly, as seen in the present study (Fig. 7). Indeed, the differentiation between these endophenotypes seems to go beyond the simple frequency of heavy drinking but may include variations in behavioral and neurophysiological characteristics. These variations may be associated with the recruitment of different or supplemental brain pathways associated with neuroplasticity and may respond differentially to various medications for AUD. Indeed, preclinical research has shown that repeated binge alcohol consumption results in distinct behavioral changes and alters pharmacological responses to potential therapeutics for the treatment of AUD compared with organisms that have consistent heavy drinking for the same duration [43].

Furthermore, there is a positive correlation between alcohol



Note: Because genotypes overlap, total *N* exceeds the study cohort.

SNP: Single nucleotide polymorphism.

Fig. 6. Comparison of effect sizes for change in PHDD in the heavy drinking individuals' group for AD04 vs. placebo shown as the mean (95 % CI) for the five different genotypes.

consumption and use/dependency of other drugs of abuse, leading to more alcohol-related and other drug use-related problems than just alcohol consumption. In young adults, very heavy drinking is associated with polydrug use. The present RDBPCT allowed for the use of other drugs of abuse (e.g., nicotine and marijuana). Extended excessive consumption of alcohol and nicotine results in unique alterations in neurochemistry (i.e., a 4-fold increase in basal glutamate levels in the medial prefrontal cortex), enhanced drug-seeking behaviors, and alterations in neurotrophic factors (BDNF) within the nucleus accumbens shell compared with equivalent alcohol and nicotine alone consumption. Therefore, preclinical and clinical data indicate that very heavy drinking compared with heavy drinking, combined with other drugs of abuse, may result in individuals that are more resistant to pharmacotherapeutics for AUD, or may require other distinct treatment options to observe efficacious effects.

The FDA and EMA have indicated that potential pharmacological treatments for AUD should improve patients' quality of life. The relevance of this goal is supported by research showing a positive association between AUD symptoms and greater negative consequences on health and quality of life, even if the individual is not consuming alcohol

excessively at that time. Hence, the burden to overall health in subjects with AUD and to society is magnified if there is a high number of AUD symptoms as well as excessive alcohol consumption. The current study indicates that AD04 treatment appears to reduce AUD symptoms and improve the quality of life in heavy drinking individuals, which is relevant to the targeted goals of the FDA and EMA.

In the present study, multiple serotonin-related SNPs were necessary for AD04 compared with placebo to be efficacious at reducing multiple measures of alcohol consumption. Previous research has also shown that the LL and TT SNPs needed to be co-expressed in heavy drinking individuals for AD04 to be efficacious in reducing alcohol consumption [30]. Indeed, it is likely that individuals with AUD are highly polygenic, but the etiological pathways and genetic variations involved may vary among different populations [45]. Recent Genome-wide association studies (GWAS) on AUD confirm its polygenic nature and the emergence of polygenic risk scores (PRS) for AUD risk assessment [46]. Nevertheless, small GWAS samples reduce PRS accuracy, and studying large and diverse sample populations is crucial [47-50]. Despite current limitations, GWAS and PRS are powerful tools for predicting disease risk and identifying potential candidates for clinical applications. Further refinements in the selection of SNP panels for future studies may be needed.

Our findings also showed an exceptional safety and tolerability profile of AD04 compared with placebo, with a low incidence of AEs, high medication compliance, and a low dropout rate. In addition, low-dose AD04 showed no significant changes in key liver biochemical parameters in individuals with AUD, suggesting it may be a safe treatment candidate for both AUD and Alcohol-associated Liver Disease (ALD) [52].

There is currently no published study in alcohol literature whereby an efficacious medicine had an AE profile like placebo. For example, naltrexone is associated with high intolerability ($\leq$15 % of patients); acamprosate has been associated with high rates of study withdrawal and non-compliance due to severe gastrointestinal discomfort, with $<$30 % of patients tolerating it long-term; and disulfiram can lead to potentially fatal AEs with concomitant alcohol consumption. AD04 is, therefore, a unique medicine that could significantly gain favor among non-specialist clinical practitioners, as both 6-month pill-taking and study completion exceeded 95 % and 70 %, respectively. A potential/likely reason for the high compliance to AD04 treatment in heavy drinking AUD subjects is that there was a significant effect as early as Month 1. The efficacy at Month 1 was associated with the efficacy of AD04 during Month 6, and for the 6-month study duration, AD04 reduced alcohol consumption (Figs. 2, 4, and 5). Furthermore, the psychosocial intervention platform used in this study, BBCET, has been associated with high medication and study compliance rates in several studies [43,44].

A significant barrier to the widespread treatment of patients with AUD is stigma. Stigma has resulted in only about 10 % of individuals with an AUD seeking treatment, and perhaps only about 1 % receiving medication [44]. Furthermore, there is great reluctance in the youth to admit the need for help with an AUD, let alone to agree to become abstinent for life. Thus, a treatment that focuses on harm reduction in currently heavily drinking individuals, perhaps especially the youth, may gain wide acceptance. Also, in our view, a medicine that is based on the genetic profile of the patient may be more socially accepted as conceptualizing AUD as a "medical problem." Coupling an efficacious medicine with a standardized, brief psychosocial intervention that takes about the same time as an average medical visit to deliver, and perhaps monitoring response to medication through mRNA expression levels, could change favorably how AUD disease is perceived and could increase the demand for treatment to many who would not have otherwise considered it.

The present study has five caveats. First, because this is the first prospective study to examine the effect of our selected genotypes on the treatment of AUD, a replication study confined to the heavy drinking population would need to validate the results. Second, a more prolonged study duration, perhaps 9–12 months, would be essential as AD04′s therapeutic effects on PHDD and quality of life were still improving at Month 6. Third, a follow-up post-treatment period could determine if, and for how long, AD04′s therapeutic effects are sustained. Fourth, the ethnic constitution of enrolled subjects was mostly Caucasians. Further studies will, therefore, need to expand the ethnic diversity of the cohort. Fifth, we did not include traditional biomarkers of alcohol consumption (e.g., ethyl glucuronide) and alcohol consequences (e.g., carbohydrate-

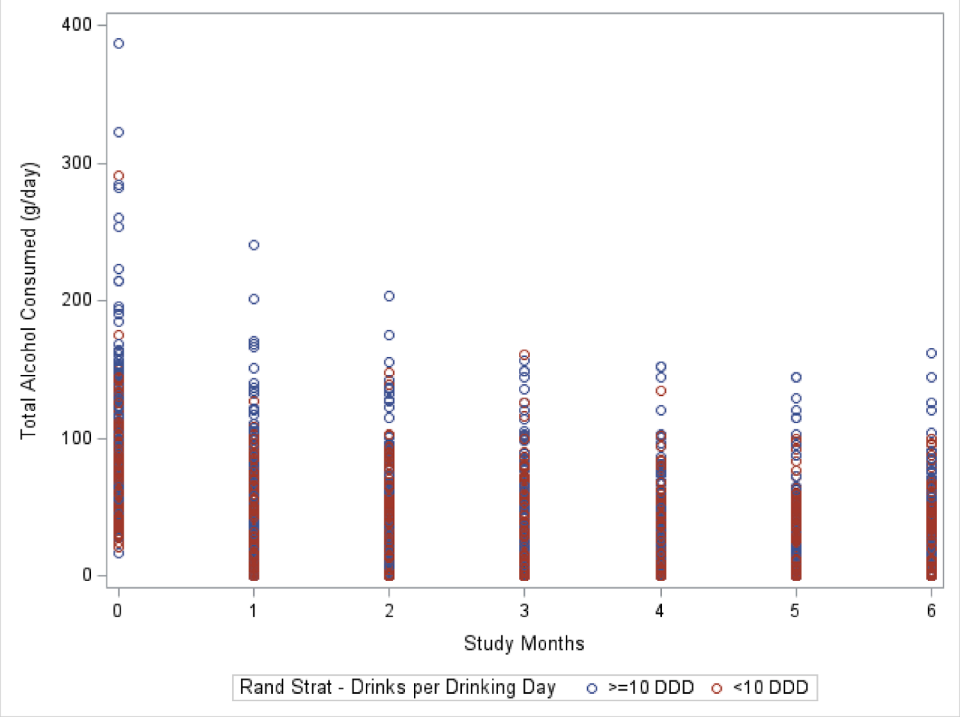


Fig. 7. Scatterplot showing overlap on total alcohol consumed for the heavy drinking individuals and the very heavy drinking individuals.

deficient transferrin) to add to the self-reported effect indicators because self-report has been shown to be more sensitive [42].

5. Conclusions

In conclusion, the present study's results provide support for AD04 for the treatment of currently drinking heavy drinking individuals who possess a genetic profile targeted to selected genotypes in the serotonin transporter and serotonin-3AB receptor complex. Because our genetically defined cohort targets about 35 % of the AUD population, further research is needed to define other endophenotypes that could respond to other specific medicines. Also, lengthier studies with a follow-up period among heavy drinking individuals with diverse ethnic backgrounds would be needed to determine the longer-term effects of AD04 on alcohol consumption and to determine whether such effects are sustained post-treatment.

CRediT authorship contribution statement

Bankole Johnson: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Validation, Funding acquisition, Writing – original draft, Writing – review & editing. **Hannu Alho:** Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Giovanni Addolorato:** Formal analysis, Writing – original draft, Writing – review & editing. **Otto Michael Lesch:** Supervision, Validation, Writing – original draft. **Jonathan Chick:** Supervision, Writing – original draft, Writing – review & editing. **Lei Liu:** Data curation, Formal analysis. **Vinzant Schuyler:** Project administration.

Declaration of competing interest

All authors are paid consultants of Adial Pharmaceuticals Inc. Vinzant Schuyler and Bankole Johnson are officers of Adial Pharmaceuticals Inc.

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Writing assistance

Paula Bresciani M. de Andrade, PhD.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2024.06.001.

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