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Original Article

Safety and compliance of long-term low-dose ondansetron in alcohol use disorder treatment.

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ABSTRACT

Background: The increasing prevalence of alcohol use disorder (AUD) and the parallel surge in alcohol-associated liver disease (ALD) emphasize the urgent need for comprehensive alcohol management strategies. Low-dose ondansetron (AD04, a 5-HT3 antagonist) was shown recently to be a promising treatment for AUD with a specific genotypic profile (5-marker). The liver safety of AD04 has never been evaluated in subjects with AUD. The aim of the present study was to assess the liver safety profile of AD04 compared with placebo in subjects with AUD.

Methods: Liver biochemical parameters were assessed in subjects with AUD with a 5-marker genetic profile who participated in a Phase 3 randomized controlled trial and received either twice-daily, low-dose AD04 (ondansetron 0.33 mg twice daily) or matching placebo, combined with brief psychosocial counseling. ALT, AST, GGT, Serum Bilirubin, MCV, and Prothrombin were evaluated at weeks 0, 12, and 24. Adverse cardiac events, general well-being, and study completion were also assessed.

Results: Low-dose AD04 did not significantly change biochemical markers of liver injury, such as ALT, AST, and Serum Bilirubin. While patients with AUD displayed elevated GGT levels, typically associated with increased alcohol consumption, this parameter remained unaffected by low-dose AD04. Notably, no significant adverse effects were observed due to oral low-dose AD04 treatment.

Conclusions: Low-dose AD04 has the potential to be a safe treatment option for subjects with AUD and ALD, indicating the need for an RCT for this specific cohort. Such a trial would pave the way for the design of a precision treatment for combined AUD with ALD.

1. Introduction

1.1. Alcohol use disorder

There are geographical differences regarding alcohol-associated morbidity and mortality, the WHO European Region being the most affected area [1]. Europe leads globally in *per capita* alcohol consumption, followed by the Americas [1]. Alcohol use disorder (AUD) refers to impaired control over alcohol use, leading to physiological dependence and tolerance and detrimental psychological, social, and physical

Abbreviations: AUD, Alcohol use disorder; ALD, Alcohol-associated liver disease; 5HT3, Serotonin-3 receptor; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; GGT, Gamma Glutamyl Transferase; MCV, Mean Corpuscular Volume; INR, International Normalized Ratio; BBCET, Brief Behavioral Compliance Enhancement Treatment.

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consequences. These disorders are highly disabling and associated with many physical and psychiatric comorbidities [2]. AUD is a complex, heterogeneous, and chronic relapsing disorder that encompasses both acute binge alcohol consumption episodes as well as prolonged bouts of heavy alcohol consumption. AUD is a major and leading risk factor for death and disability worldwide. Data analysis from the most comprehensive estimate of the Global Burden of Disease gathered for AUD across 195 countries and territories between 1990 - 2016 showed that AUD was the seventh leading risk factor for deaths and disability-adjusted life-years (DALYs), accounting for 6.8 % of male deaths and 2.2 % of female deaths [3]. Strikingly, for those between the ages of 15 – 49, AUD was the leading cause of DALYs, accounting for 12.2 % and 3.8 % of male and female deaths, respectively [3].

Despite evidence supporting the effectiveness of medications, Alcohol Use Disorder (AUD) is frequently underdiagnosed and undertreated [4,5]. Pharmacological interventions are crucial for addressing AUD; however, fewer than 4 % of individuals diagnosed with AUD receive FDA-approved medications [4]. Additionally, less than one-third of subjects with AUD receive any treatment, and only about 10 % are prescribed medication to encourage abstinence or reduce heavy drinking [5].

1.2. AUD current medications

Individuals who have AUD often fail to take medication for the disorder because of general concerns over efficacy and possible adverse events (AEs). Acamprosate, naltrexone, nalmefene, and disulfiram are all approved in the EU and USA for the treatment of AUD [6]. Safety considerations for using psychopharmacological treatments in this patient group include the impact of concurrent alcohol consumption at high levels; multiple physical comorbidities that may interfere with pharmacological effects, distribution, and metabolism; and concomitant medication for the treatment of comorbid physical and psychiatric conditions. The approved drugs exhibit distinct safety profiles that must be weighed against individual treatment objectives, patient preferences, and concurrent conditions [7]. Optimal treatment decisions should be guided by the specific risk-benefit profile in each case [8].

Emphasizing the development of tailored medications remains essential, particularly in creating personalized precision pharmacotherapies for AUD. However, enhancing medication adherence stands out as a potentially most impactful approach to improving the therapeutic benefits of pharmacotherapy. Hence, the present paper delves into the analysis of patient adherence, compliance, and safety data from a recent long-term, low-dose ondansetron (AD04) study involving the antagonism of 5HT3 receptors [9].

1.3. Serotonin-3 (5HT3) receptor

The Serotonin-3 (5HT3) receptor mediates the effects of alcohol in the cortico-mesolimbic dopamine reward pathway. Preclinical studies indicate that blocking 5HT3 receptors in the ventral tegmental area hinders the acquisition of alcohol self-administration, diminishes ongoing alcohol self-administration, and prevents heightened alcohol relapse consumption after a period of deprivation. Extensive clinical research translates these findings [10,11]. Ondansetron, by blocking the 5-HT3 receptor, is known to affect dopaminergic signaling in the brain, and the scientific rationale for the use of a 5-HT3 antagonist in the treatment of alcohol dependence is well established [12]. Briefly, studies suggest that the rewarding effects of alcohol involve activation of the 5-HT3 receptors, leading to the release of dopamine within the mesolimbic system of the brain and thereby increasing the risk of alcohol craving and misuse [13]. Thus, by blocking the activation of the 5-HT3 receptor, ondansetron may reduce the ethanol-stimulated release of dopamine, leading to reduced feelings of pleasure or reward and, consequently, reduced craving and consumption [14–25].

1.4. Ondansetron – absorption and clearance

Ondansetron, a 5-HT3 receptor antagonist, may indirectly modulate the 5-HT transporter (5-HTT) function, downregulating dopaminergic neurons and decreasing alcohol reward [26]. The use of ondansetron to treat AUD has progressed over the decades. Following oral administration, ondansetron undergoes complete and rapid absorption from the gastrointestinal tract. Its bioavailability is approximately 40 % lower than intravenous administration due to hepatic first-pass metabolism. Ondansetron moderately binds to plasma proteins (70–76 %), is primarily cleared through hepatic metabolism (95 %), and has an average elimination half-life of approximately 4 h [27,24]. Therefore, no dose adjustments for individuals with mild to moderate hepatic impairment are required for ondansetron. In cases of severe hepatic impairment, there is a reduction in ondansetron clearance, leading to an increased plasma half-life. Consequently, a dosage adjustment may be necessary, and the maximum recommended daily intravenous dose is 8 mg [27,24,28].

1.5. Ondansetron – a prospective precision medicine to treat AUD endophenotypes

Currently, ondansetron represents an exciting approach for AUD personalized treatment as it is effective in specific subtypes of individuals (i.e., early onset AUD - the initial onset of AUD at the age of 25 years or younger – and LL genotype) [29,30]. Multiple clinical studies have reported that low-dose ondansetron reduced alcohol consumption in early-onset AUD subjects [30,31]. Recent developments have indicated that there are genetic markers in the serotonin transporter, $5HT_{3A}$ receptor, and $5HT_{3B}$ receptor that predict the efficacy of ondansetron in reducing alcohol consumption in heavy alcohol-consuming subjects [9,32,33]. Our recent study, a 24-week double-blind, randomized, phase-3 clinical trial of AUD subjects, assessed the efficacy of low-dose AD04 twice daily to reduce alcohol consumption in subjects with AUD and specific genotypes that were further pre-stratified by drinking endophenotype (i.e., the heavy drinkers' group (HD) that consumed $<$10 DDD – drinks per drinking day, and the very heavy drinkers' group (VHD) that drank $\geq$10 DDD) [9]. Low-dose AD04 demonstrated an outstanding safety and tolerability profile compared to placebo, featuring a low occurrence of AEs, high medication compliance, and a minimal dropout rate. To date, there is no existing study in alcohol literature where an effective medication exhibits similar AEs profile to a placebo [9].

1.6. Alcohol-associated liver disease

ALD encompasses various alcohol-induced liver conditions, progressing from steatosis and steatohepatitis to fibrosis, cirrhosis, and hepatocellular carcinoma [27]. The course of ALD differs significantly among individuals, with 80 % to 90 % of heavy drinkers developing steatosis, 10–35 % of whom progress to alcoholic hepatitis, and 10 % advancing to cirrhosis [9,34]. Liver transplantation is the treatment of choice for alcohol-associated cirrhosis, being nowadays the leading cause of liver transplantation in Europe and North America [35]. At present, most randomized clinical trials (RCTs) that have evaluated the effectiveness of pharmacological treatments for AUD have excluded patients with ALD due to concerns that effective medications for AUD may worsen liver disease [36]. No approved therapies target ALD pathogenesis or stop its progression, and alcohol abstinence is the most effective and primary treatment for all stages of alcohol disease [35]. It is worth mentioning that both European [37] and American [38,39] guidelines acknowledge the possible utilization of baclofen [40] and acamprosate [41] in these individuals, although the supporting evidence is constrained. This underscores the imperative to explore novel treatments for patients with AUD impacted by ALD. ALD rates are increasing among young individuals and women across diverse demographics.

With no apparent decline in alcohol use, it is anticipated that ALD rates will persist in their upward trajectory. Without proactive measures, the mortality rate for ALD is projected to double by 2040 [42].

1.7. Ondansetron – safety profile

Ondansetron is generally considered to have a very safe profile [9,33]. In clinical oncology settings, ondansetron is well-tolerated with no observed end-organ toxicity. Previous assessments of its safety have primarily focused on its use in preventing chemotherapy-induced nausea and vomiting, revealing a broad therapeutic index [43]. In the context of cardiology, administering ondansetron orally rather than intravenously prevented QT interval corrected for heart rate by Fridericia's formula elongation (QTcF) [9,44,45]. Despite its overall safety profile, a formal evaluation of its impact on liver safety in individuals with Alcohol Use Disorder (AUD) has not been conducted [28]. Therefore, the current study aims to compare the liver safety profile of ondansetron to placebo in subjects with AUD.

2. Methods

2.1. Study design

The data originated from clinical analyses conducted during our recent precision medicine trial [9]. It consisted of 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 HD and VHD and also had a prospectively determined 5-Marker serotonin-related SNP panel (AC/AG, AC with any other (AC+), AG with any other (AG+), or LL/TT with any other LL/TT+). A total of 303 participants were included in the study ($N = 303$).

The main goal of this precision medicine trial was to assess the efficacy of low-dose AD04 (0.33 mg, orally (p.o.) twice a day for 24 weeks) combined with brief psychosocial counseling (BBCET [42]) to reduce alcohol consumption among subjects with AUD who possessed specific genotypes at serotonin transporter and 5-HT3 receptor genes. The low dose of AD04 was selected both on the basis of previous evidence of its efficacy to reduce alcohol intake in AUD patients and on the basis of its safety [30]. The study aimed to test the hypothesis that low-dose AD04 compared with placebo would decrease heavy drinking significantly in individuals with AUD and a specific 5-Marker serotonin-related SNP panel [9]. The primary endpoint for the analysis of efficacy was the change from baseline in the monthly number of heavy drinking days (HDD) during the last eight weeks (weeks 16–24) of the 24-week treatment period [9], where the World Health Organization defined heavy drinking as the consumption of ≥60 g of pure alcohol on at least one single occasion at least monthly [46,47].

The primary outcome of the present study was to assess the liver safety profile of low-dose AD04 compared with placebo in subjects in AUD patients. The secondary outcome was to evaluate AD04′s general safety compared to the placebo in AUD patients. General well-being was assessed by the 9-item Patient Health Questionnaire (PHQ-9) for general well-being, Suicide risk was assessed by the Columbia Suicide Severity Rating Scale (C-SSRS).

Safety and data integrity in the study were maintained rigorously through various independent and blinded processes. An independent Data Monitoring Committee monitored the study's progress and participant safety, while data quality and cleaning were managed by Optimapharm. Strict measures, including third-party oversight, sealed envelope randomization, and double-blinding techniques, were used to minimize bias. The trial was registered and transparently reported, with the study team fully disclosing all potential conflicts of interest.

2.2. Participants

A total of 303 patients received at least one dose of study medication and were included in the safety population (AD04: 156; placebo: 147). Participants were included if they were of European descent, presented a diagnosis of moderate to severe AUD [48], and met defined inclusion criteria, such as being aged ≥18 years, engaging in heavy alcohol consumption, demonstrating willingness for DNA analysis, and possessing a bio-genetic endophenotype of a 5-Marker genotype panel. Exclusion criteria included other substance use disorders except nicotine use disorder, alcohol withdrawal symptoms, and several health assessments. In particular, subjects with AUD who had advanced liver disease were excluded because there was insufficient information available on the liver safety of the drug at the time of the study. Although abstinence was not a primary outcome, participants expressed an intent to decrease alcohol consumption. Compliance with the intent to decrease alcohol consumption was strengthened by participation in BBCET [9]. For compliance assessment, we employed direct (pill counting at each visit as a subtraction of the amount of medication returned from the amount dispensed) and indirect (patient self-reporting) methods. Inclusion and Exclusion criteria are described in the Online supplementary material.

2.3. Liver safety clinical assessment

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamyl Transferase (GGT), Serum Bilirubin, Mean Corpuscular Volume (MCV), and International Normalized Ratio (INR) were evaluated [48] at Eurofins laboratory, Brussels. The outcome for each variable was determined by the difference from week 0 to week 12 and week 24.

2.4. Ethical considerations

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

2.5. Statistical analysis

For the clinical laboratory assessments for safety, outcome variables of interest were ALT, AST, GGT, serum bilirubin, MCV, and INR. We first summarized these outcome variables by their mean and standard deviations at each time point. A mixed effects model with an unstructured covariance structure was employed for each variable to accommodate repeated measures at week 12 and week 24. This approach allowed us to assess each variable's change from week 0 to week 12 and week 24. Treatment, visit, and their interaction (i.e., treatment * visit) were included in each model. Stratification of analyses was done based on the DDD level at baseline (<10 vs. ≥10).

3. Results

No elevated liver biochemical parameters were observed among subjects administered low-dose AD04 compared with the corresponding placebo, demonstrating good tolerance to the drug. Additionally, there was no notable discrepancy in liver enzyme levels between subjects receiving low-dose AD04 and those given a matching placebo at each treatment interval. The combined results are shown in Table 1.

3.1. Alanine Aminotransferase (ALT)

A standard reference range for an ALT blood test is 7 to 56 U/L (units per liter) [49,50]. ALT mean values for DDD < 10 and DDD ≥ 10 in the low-dose AD04 and placebo groups at weeks 0, 12, and 24 were not significantly altered (p values for the main effect of treatment and its temporal changes were not statistically significant, $p \geq 0.05$). Combining the DDD<10 and the DDD≥10 groups, there was no

Table 1
Liver Parameters and Enzymes.

Liver Parameters & Enzymes						
	week 0	**week 12**	**week 24**	**week 0**	**week 12**	**week 24**
	DDD < 10 + ondansetron (AD04)			DDD < 10 Placebo		
ALT	31.2	31.6	28.2	28.4	26.5	25.7
AST	28.6	34.7	27.6	28.3	28.5	26.5
GGT	65.0	64.9	119.8	74.8	57.8	65.1
BR	0.399	0.475	0.424	0.474	0.497	0.474
MCV	100.3	99.4	98.6	99.5	99.7	98.4
P/INR	1.00	1.01	1.00	1.02	1.05	1.01
	DDD ≥ 10 + ondansetron (AD04)			DDD ≥ 10 Placebo		
ALT	33.7	32.7	33.7	29.6	31.3	40.7
AST	30.5	31.3	29.1	29.2	33.9	39.3
GGT	84.1	62.9	70.4	69.0	97.7	89.5
BR	0.518	0.581	0.507	0.470	0.552	0.470
MCV	100.2	99.3	98.9	101.0	100.3	99.2
P/INR	1.01	1.00	0.98	1.01	0.99	0.98

ALT: Alanine Aminotransferase.
AST: Aspartate Aminotransferase.
GGT: Gamma Glutamyl Transferase.
BR: Serum Bilirubin (total: conjugated + unconjugated).
MCV: Mean Corpuscular Volume.
P/INR Prothrombin Int. Normalized Ratio.
DDD: Drinks per Drinking Day, standard US drink = 10 g of absolute ethanol.

statistically significant change in ALT levels from baseline to week 12 between the low-dose AD04 group and the placebo group (p-value = 0.91), while the p-value was 0.17 for the change from baseline to week 24. All values were within the expected standard reference range.

3.2. Aspartate Aminotransferase (AST)

The upper standard limit of serum AST is 40 IU/l on average, ranging from 30 to 50 IU/l [51]. AST mean values for DDD < 10 and DDD ≥ 10 in the low-dose AD04 and placebo groups at weeks 0, 12, and 24 were not significantly altered ($p \geq 0.05$ for the main effect of treatment and its temporal changes), and all values were below 40 IU/l. Combining the DDD<10 and the DDD≥10 groups, there was no statistically significant change in AST levels from baseline to week 12 between the AD04 group and the placebo group (p-value = 0.42), while the p-value was 0.32 for the change from baseline to week 24. AST/ALT ratio (De Ritis Ratio) was calculated for DDD < 10 and DDD ≥ 10 in the low-dose AD04 and placebo groups at weeks 0, 12, and 24 (Table 2). No ratio value was higher than 1.1.

3.3. Gamma glutamyl transferase (GGT)

The reference serum of GGT is 9 to 85 U/L for males and 5 to 55 U/L for females [52]. GGT mean values for DDD < 10 and DDD ≥ 10 in the low-dose AD04 and placebo groups at weeks 0, 12, and 24 were not significantly altered ($p \geq 0.05$ for the main effect of treatment and its temporal changes). All values were below 85 U/L, with the exception of 119.8 (SD 443.4) for DDD < 10 in the low-dose AD04 group (week 24) and 97.7 (SD 153.8) and 89.5 (SD 207.7) for DDD ≥10 in the placebo group (weeks 12 and 24, respectively). Combining the DDD<10 and the DDD≥10 groups, there was no statistically significant difference in GGT levels from baseline to week 12 between the low-dose AD04 group and the placebo group (p-value = 0.35), whilst the p-value is 0.77 for the change from baseline to week 24.

Table 2
AST/ALT ratio (De Ritis Ratio).

	week 0	**week 12**	**week 24**	**week 0**	**week 12**	**week 24**
	DDD < 10 + ondansetron (AD04)			DDD < 10 Placebo		
ALT	31,2	31,6	28,2	28,4	26,5	25,7
AST	28,6	34,7	27,6	28,3	28,5	26,5
AST/ALT	**0,9**	**1,1**	**1,0**	**1,0**	**1,1**	**1,0**
	DDD ≥ 10 + ondansetron (AD04)			DDD ≥ 10 Placebo		
ALT	33,7	32,7	33,7	29,6	31,3	40,7
AST	30,5	31,3	29,1	29,2	33,9	39,3
AST/ALT	**0,9**	**1,0**	**0,9**	**1,0**	**1,1**	**1,0**

3.4. Serum bilirubin

The reference total serum bilirubin levels vary between 0 and 1.2 mg/dL [53]. The mean values of serum bilirubin for DDD < 10 and DDD ≥ 10 in the low-dose AD04 group and in the placebo group at weeks 0, 12, and 24 were not significantly altered ($p \geq 0.05$ for the main effect of treatment and its temporal changes). At first, in the DDD<10 group, the p values for the main effect of treatment and its interaction with time were 0.04 and 0.80, respectively, suggesting some significant difference in the average values but no significant difference in their change over time due to the treatment ($p \geq 0.05$). However, after adjustment for multiple comparisons, the difference in average values was no longer statistically significant ($p \geq 0.05$). Combining the DDD<10 and the DDD≥10 groups, there was no statistically significant difference observed in the change of serum bilirubin levels from baseline to week 12 between the AD04 group and the placebo group (p-value = 0.50), while the p-value was 0.52 for the change from baseline to week 24. No serum bilirubin value was higher than 1.2 mg/dL.

3.5. Mean corpuscular volume (MCV)

The reference mean value of MCV varies between 80 and 99 fL [54, 55]. MCV mean values for DDD< 10 and DDD ≥ 10 in the low-dose AD04 and placebo groups at weeks 0, 12, and 24 were not significantly altered ($p \geq 0.05$ for the main effect of treatment and its temporal changes). Combining the DDD<10 and the DDD≥10 groups, there was no statistically significant difference observed in the change of MCV levels from baseline to week 12 between the AD04 group and the placebo group (p-value = 0.58), whilst the p-value was 0.58 for the change from baseline to week 24. As observed in Table 1, most of the MCV values are above 99 fL.

3.6. International normalized ratio (INR)

The reference value INR for a healthy individual is 1.1 or below [56]. INR mean values for DDD< 10 and DDD ≥ 10 in the low-dose AD04 and placebo groups at weeks 0, 12, and 24 were not significantly altered ($p \geq 0.05$ for the main effect of treatment and its temporal changes). Combining the DDD<10 and the DDD≥10 groups, there was no statistically significant difference observed in the change of INR levels from baseline to week 12 between the ondansetron group and the placebo group (p-value = 0.82), whilst the p-value was 0.25 for the change from baseline to week 24.

In summary, of all the previous clinical tests evaluated, serum bilirubin in the DDD < 10 group was the only one that had a p-value <0.05 ($p = 0.04$) related to the main effect of the treatment (but not related to its interaction with time), which became nonsignificant after multiple comparison adjustments. These findings indicate that the liver exhibited good tolerance to AD04.

Low-dose AD04 treatment was well tolerated. No treatment-emergent serious adverse events (SAEs) were attributed to low-dose AD04. SAEs were reported for three subjects with nine occurrences in the low-dose AD04 group and for three subjects with seven occurrences in the placebo group. One subject in the low-dose AD04 group had a fatal outcome due to COVID-19 infection. One subject in the placebo group died suddenly of an undetermined cause. One subject in each treatment group had an SAE due to infection with the COVID-19 virus. One subject in each treatment group had an SAE due to alcohol poisoning.

3.7. Adverse events

Adverse Events (AEs) were similar in frequency in the low-dose AD04 and placebo groups, with no meaningful clinical differences (data not shown). No AE was significantly more prevalent in the low-dose AD04 group. The most common AEs by system organ classes in the low-dose AD04 group were nervous system disorders (34 subjects, 22 %) and gastrointestinal disorders (30 subjects,19 %). The most common AEs by system organ classes in the placebo group were nervous system disorders (26 subjects, 18 %) and infections and infestations (22 subjects, 15 %). In the low-dose AD04 group and placebo group, 12 (7 %) and 18 (12 %) subjects, respectively, had gastrointestinal disorders. For both treatment groups, AEs were generally rated as mild (about two-thirds) or moderate (about one-third). One individual in the low-dose AD04 group reported a severe AE in the nervous system disorders category. For the AD04 group and placebo group, respectively, at the symptom level within the nervous system, headaches were reported in 19 (12 %) and 16 (11 %) subjects, and dizziness in six (4 %) and seven (5 %) subjects. No AE required any medical intervention, and all resolved spontaneously. In either treatment group, no other AE occurred with a frequency more significant than 5 %.

3.8. Cardiac events

QT time is the time taken for ventricular depolarization and repolarization. QTcF, as a marker for QT time, is intricately tied to the overall heart rate, making it more responsive to medication side effects than QT alone. QTcF is the foremost prognostic electrocardiogram (ECG) parameter for evaluating potential drug-related cardiac events. Despite the reported uncommon incidence of intravenous ondansetron elongating the QTcF interval on the electrocardiogram (ECG) [44,45], we did not observe any significant effect for oral ondansetron at our study dose (0.33 mg twice daily) [9]. Specifically, there was no significant difference in QTcF time between the low-dose AD04 and the placebo groups, regardless of their risk profile (HD or VHD) [9]. No participant exhibited clinically significant QTcF prolongation, and none were withdrawn from the study due to such prolongation. Nevertheless, clinicians must be vigilant regarding the possibility of QT interval prolongation with intravenous AD04 administration, especially in high doses and among high-risk patients [57].

3.9. General well-being, suicide events and pregnancy

On the Columbia Suicide Severity Rating Scale (C-SSRS), no subject met the criterion for clinically significant suicidality during the study. On the 9-item Patient Health Questionnaire (PHQ-9) for general well-being, there was no worsening in any group during the study. No woman tested positive for pregnancy at any time during the study.

3.10. Study completion and compliance

Compliance rate (i.e., pill-taking frequency) was unexpectedly high for a 24-week study in AUD, with no significant difference between the low-dose AD04 and placebo groups (low-dose AD04: 95.60 %, placebo: 98.90 %) nor between the HD or VHD groups (98.80% vs. 274 99.40 %, respectively). The study completion rate was also high for a 24-week study in AUD, with no significant group difference between the treatment groups (low-dose AD04: 72.7 %, placebo: 69.6 %) and between the HD and VHD groups (71.9% vs. 76.4 %, respectively).

4. Discussion

Hepatotoxicity caused by drugs is a prevalent adverse reaction and a primary factor contributing to attrition rate in drug development, issuance of black box warnings, and post-marketing withdrawals [58]. AST, ALT, and GGT are key liver test markers, showing elevated levels in individuals with ALD [52].

The present study showed no significant difference in liver enzyme levels between subjects treated with low-dose AD04 and those receiving a placebo at each treatment interval. This finding confirms the liver safety profile of low-dose AD04 in AUD patients. Notably, no significant difference in AST and ALT levels during the study was found between low-dose AD04 and placebo-treated subjects. AST levels typically surpass ALT levels in ALD patients, with an AST: ALT ratio over 2.0 as a crucial indicator of ALD [59]. In a study differentiating ALD from metabolic dysfunction-associated steatotic liver disease (MASLD) and nonalcoholic liver disease, ALD would be indicated with an AST: ALT ratio greater than 2 [60]. None of the cases were seen in the four groups of the present study, as the AST/ALT ratios were all below 1.1.

The GGT levels were elevated in the four groups under investigation. Although an elevated level of GGT can be an indicator of liver disease, it is not always specific. It may only be present in 30–50 % of excessive drinkers in the general population [53,61]. Notably, an increase in GGT levels can also result from enzyme induction caused by alcohol or certain medications, even in the absence of liver disease [59]. While consuming three or more alcoholic drinks per day (45 g of ethanol or more) can cause an increase in GGT levels, it is not a definitive marker of chronic heavy alcohol consumption [59]. Additionally, individuals with digestive ailments like pancreatitis or prostate disease may also experience elevated GGT levels [61].

Serum Bilirubin levels in all groups were below 1.2 mg/dL and unaffected by AD04. Slightly higher MCV values than reference values found in this study, not altered by AD04, might be due to MCV Macrocytosis or enlarged red blood cells, often caused by alcohol toxicity rather than a folate deficiency.

Safety and medication adherence are key factors for successful pharmacotherapy. In a controlled research study setting, adherence is usually better than in general or specialist practice for AUD and AUD clinical trials; often, half of patients adhere to their long-term therapy [62]. Thus, our finding on the shallow dropout rate and high pill count in 24-week-long research is even more critical in real-life settings. These findings may be due to low adverse events and the fact that the participant's pill-taking compliance was supported on each visit with a simple, short BBCET session.

Participant beliefs about their condition (e.g., consequences of nonadherence and the perceived impact of illness on daily life) are an essential determinant of medication adherence, taking account of which may require a more personalized treatment approach and support. Additionally, risk-taking behaviors such as actively drinking in individuals with AUD may add to medication nonadherence, further complicating the care delivery.

Treatment of AUD plays a pivotal role in ALD, as alcohol abstinence and/or reduction of alcohol intake represent the only effective goal for these patients. The persistence of high alcohol consumption significantly increases mortality in patients with ALD [57]. The cornerstone of treatment for AUD is the combination of psychosocial and pharmacological intervention coupled with medical management. However, at present, the effectiveness of anti-craving medications in patients with ALD is still poorly investigated, even because AUD patients with ALD are usually excluded from pharmacological trials due to concerns about the liver safety of these medications.

The safety profile of ondansetron shown in the present study led to the hypothesis of possible use of this medication in individuals with AUD and concomitant ALD, which suggests the utility of planning an RCT in this cohort. To date, although ondansetron has not been tested in AUD patients with ALD, this medication seems to be one of the most promising emerging safe pharmacotherapies for AUD.

The research has some limitations. First, the study's participants were all European with a specified bio-genetic endophenotype, which may limit the generalizability of the findings. Additionally, while standard biochemical markers were used to evaluate possible hepatotoxicity, incorporating imaging evaluations into future research could

enhance liver safety assessments. Finally, it cannot be established whether hepatic safety in patients without liver disease is identical to that in patients with liver disease. However, the present findings allow us to hypothesize an RCT in AUD with ALD with reasonable confidence.

5. Conclusions

In conclusion, our results suggest that ondansetron, because of its liver safety, could have an essential role in the treatment of AUD patients with ALD. However, RCTs are needed before drafting definitive conclusions.

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Declaration of competing interest

All authors are paid consultants of Adial Pharmaceuticals Inc. Bankole Johnson is an officer of Adial Pharmaceuticals Inc.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2024.03.017.

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