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Advancing Clinical Trials for Alcohol Use Disorder: Informing Trial Design and Integrating Biological Markers

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Advancing Clinical Trials for Alcohol Use Disorder: Informing Trial Design and Integrating Biological Markers

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Neuroscience

by

Malia Alexandra Belnap

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ABSTRACT OF THE DISSERTATION

Advancing Clinical Trials for Alcohol Use Disorder:

Informing Trial Design and Integrating Biological Markers

by

Malia Alexandra Belnap

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2026

Professor Lara A. Ray, Chair

Alcohol use disorder (AUD) is a prevalent and debilitating condition that has substantial social, psychological, and medical consequences. Despite its significant public health impact, only three medications are approved by the U.S. Food and Drug Administration (FDA) for the treatment of AUD, and no new medications have been approved in over two decades. Thus, there is a critical need to improve the processes for developing and evaluating novel pharmacotherapies. The current dissertation comprises two complementary lines of research. The first line of research examines methodological considerations in AUD pharmacotherapy clinical trials, including the selection of efficacy endpoints (Chapter 1), the influence of trial length on observed medication effects (Chapter 2), and the use of cigarettes and cannabis during an AUD clinical trial (Chapter 3). Together, these studies inform key design considerations for future AUD pharmacotherapy trials. The second line of research examines peripheral biological markers associated with AUD and treatment response, using data from a randomized clinical trial

of the neuroimmune modulator ibudilast. Specifically, it investigates peripheral inflammatory and intestinal permeability markers as moderators of ibudilast treatment response and their associations with clinical characteristics (Chapter 4) and models the relationship between changes in alcohol consumption and peripheral inflammatory markers during treatment (Chapter 5). Collectively, this dissertation contributes to the development and evaluation of pharmacotherapies for AUD by informing key aspects of clinical trial design and advancing understanding of the biological processes relevant to AUD and its pharmacological treatment.

The dissertation of Malia Alexandra Belnap is approved.

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2026

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The dissertation author was the primary researcher and author of the work presented in Chapters 1–5. The coauthors listed in the citations above contributed to the research and preparation of the corresponding chapters. Additional financial support for this dissertation was provided by the UCLA Graduate Division Dissertation Year Award.

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INTRODUCTION

Alcohol Use Disorder

Alcohol use disorder (AUD) is a highly pervasive disorder, and it is estimated that about 27.9 million adults over the age of 12 meet criteria for current AUD (SAMHSA, CBHSQ, 2025). AUD is characterized by the continued use of alcohol despite significant social, psychological, and medical consequences (Ray et al., 2019). It is estimated that more than 178,000 people in the United States die from alcohol-related causes annually (Centers for Disease Control and Prevention, 2024), making excessive alcohol use a leading cause of preventable death. Despite these negative consequences, it is estimated that only 7.6% of adults with past-year AUD received treatment for alcohol use in 2024, and only about 2.4% received medication-assisted treatment (SAMHSA, CBHSQ, 2025).

Part I: Pharmacological Treatment of Alcohol Use Disorder and Challenges in Medication Development

Current Pharmacological Treatment Options

To date, only three medications have been approved by the US Food and Drug Administration (FDA) for AUD, with no new medications approved in two decades (Burnette et al., 2022). The approved medications are disulfiram, acamprosate, and naltrexone. Disulfiram, the first FDA-approved medication for AUD in 1951, is an aldehyde dehydrogenase inhibitor that inhibits the metabolism of alcohol, which leads to unpleasant physiological symptoms and is intended to create an aversive reaction to alcohol (De Sousa, 2019). A meta-analysis of 22 clinical trials found that disulfiram was more effective than placebo only in open-label trials, with no advantage over placebo in blinded randomized controlled trials (Skinner et al., 2014).

Acamprosate was approved by the FDA in 2004 and is thought to normalize dysregulated NMDA-mediated glutamatergic activity caused by chronic alcohol use (Mason & Heyser, 2010).

Results from a Cochrane meta-analysis of 24 acamprosate randomized controlled trials (RCTs), of which 23 required participants to achieve abstinence before randomization, found that acamprosate reduced the risk of returning to any drinking and increased cumulative abstinence duration compared with placebo, but did not reduce the risk of returning to heavy drinking (Rösner et al., 2010). However, findings from individual trials, including the COMBINE study, the largest multisite pharmacotherapy trial for AUD, did not find an advantage of acamprosate over placebo on abstinence- or drinking-based outcomes (Anton et al., 2006; Berger et al., 2013). These mixed efficacy findings may reflect variation in study characteristics, including inpatient versus outpatient treatment settings and the duration of pre-treatment abstinence required before randomization (Anton et al., 2006).

Naltrexone is available in both an oral formulation (approved for AUD in 1994) and an extended-release injectable formulation (approved for AUD in 2006) (Burnette et al., 2022). Naltrexone is a μ-opioid receptor antagonist that is thought to reduce the rewarding effects of alcohol (Burnette et al., 2022). There is evidence that naltrexone is more effective than placebo in increasing abstinence rates, reducing drinking frequency, and decreasing the risk of heavy drinking (Garbutt et al., 2005; Jonas et al., 2014; O'Malley et al., 1992). However, the use of naltrexone may be limited in some patients because it can precipitate opioid withdrawal in those who are physically dependent on opioids and is contraindicated in individuals with acute hepatitis or liver failure (Burnette et al., 2022).

Several medications are also prescribed off-label for AUD, including topiramate, baclofen, gabapentin, and varenicline (Fischler et al., 2022; Litten et al., 2016). Although some of these medications have demonstrated efficacy in clinical trials (Burnette et al., 2022), their off-label status means that prescribing decisions rely largely on clinician judgment and the

available evidence, rather than FDA-approved prescribing information (FDA, 2018). Thus, expanding the number of FDA-approved pharmacotherapies would provide clinicians with additional evidence-based treatment options. Given the substantial clinical heterogeneity of AUD, expanding the number of approved medications may also improve treatment outcomes by increasing the likelihood that individuals will respond to available medications (McManus & Ray, 2026).

Randomized Controlled Trials for Alcohol Use Disorder and Design Considerations

RCTs of medications for AUD are costly and resource-intensive efforts that may ultimately lead to important healthcare advances through the development and approval of novel pharmacotherapies (Hariton and Locascio 2018). Although there has been an effort to develop and test repurposed and novel medications for AUD, these efforts have not resulted in a new FDA-approved medication in more than two decades (Burnette et al. 2022). The wide variability in AUD clinical trial methodology, including the characteristics of study participants, outcome measures used, and analytic techniques, may hinder the development and approval of novel medications (Anton et al. 2012; Donato et al. 2024). Thus, a host of design considerations have been raised in an attempt to refine, and potentially standardize, AUD pharmacotherapy RCT methodology (Anton et al. 2012; Witkiewitz et al. 2015).

The selection of efficacy endpoints is a critical consideration in the design of AUD pharmacotherapy trials (Anton et al., 2012). The FDA currently accepts abstinence, no heavy drinking days, and, as of 2025, reductions in World Health Organization (WHO) risk drinking levels as primary outcomes for phase 3 trials of AUD pharmacotherapy (Witkiewitz et al., 2025). Although these outcomes remain important benchmarks, they may not fully capture the range of clinically meaningful improvements that individuals may experience during treatment for AUD.

Thus, broadening the scope of metrics and approved endpoints accepted by regulatory bodies could support a more comprehensive evaluation of medication efficacy and ultimately enhance the medication approval process.

Trial length, defined as the duration of the active medication period, is another important design consideration in AUD pharmacotherapy trials (Anton et al., 2012). Longer RCTs place greater demands on participants, researchers, and sponsors, resulting in increased burden and cost (Naidoo et al., 2020; Zweben et al., 2009). Trial length also has regulatory implications. The U.S. Food and Drug Administration (FDA) currently recommends a minimum six-month active treatment duration for AUD medication RCTs, as cited in its draft guidance document (U.S. Department of Health and Human Services et al., 2015). Despite regulatory guidance for late-phase AUD pharmacotherapy trials, trial length varies substantially across the broader AUD RCT literature, and there is limited evidence on whether trial length influences the detection of medication effects (Maisel et al., 2013). Therefore, investigating the relationship between trial length and medication effects is critical to improving consistency and optimizing the ability to detect efficacious medications.

The concurrent use of tobacco and cannabis during AUD clinical trials is another important consideration. Alcohol, tobacco, and cannabis are the most frequently used psychoactive substances in the United States, and co-use is highly prevalent among individuals with AUD (Falk et al., 2008; SAMHSA, CBHSQ, 2025; Weinberger et al., 2019). Tobacco and cannabis use are associated with an increased likelihood of developing AUD (Grucza & Bierut, 2006; Weinberger et al., 2016), and individuals commonly report using these substances concurrently (Roche et al., 2019). Moreover, the use of one of these substances is associated with a greater likelihood of engaging in multiple substance use (Roche et al., 2019). Prior research

also indicates that tobacco and cannabis use during AUD treatment may negatively influence drinking-related outcomes. A systematic review by Van Amsterdam and Van Den Brink (2022) found that of the 31 AUD treatment studies reviewed, 16 reported that cigarette smoking during treatment was associated with poorer alcohol-related outcomes (Van Amsterdam & Van Den Brink, 2022). Research also suggests that cannabis use during treatment for AUD is associated with lower rates of alcohol abstinence after treatment and worsened alcohol-related consequences one-year post-treatment (Subbaraman et al., 2017). However, few studies have evaluated the patterns of tobacco and cannabis use during AUD treatment and how these changes relate to drinking. Given the complex and potentially reinforcing relationships between tobacco, cannabis, and alcohol use, it is crucial to assess patterns of tobacco and cannabis use during AUD treatment to determine how their use may relate to treatment effects and alcohol-use outcomes.

Part II: The Inflammatory System in Alcohol Use Disorder and Its Potential as a Novel Pharmacological Target

Alcohol Use Disorder, Gut Permeability and Inflammation

A growing body of literature suggests that the immune system contributes to the development and maintenance of AUD (Coleman & Crews, 2018; Mayfield & Harris, 2017; Meredith et al., 2021). AUD is associated with dysregulation of inflammatory signaling, and alcohol promotes inflammation through multiple pathways, including increased systemic cytokine production and direct activation of immune signaling in the brain (Coleman & Crews, 2018; Crews & Vetreno, 2016; Mayfield & Harris, 2017). Prior studies in individuals with AUD have found that pro-inflammatory cytokine levels are positively associated with alcohol consumption, as well as increased alcohol craving during acute withdrawal (Heberlein et al., 2014; Leclercq et al., 2014). Several systematic reviews and meta-analyses have reported altered

circulating inflammatory marker concentrations in individuals with AUD compared with healthy controls, although findings have varied across specific inflammatory markers and study populations (Adams et al., 2020; Galvez-Sánchez et al., 2026). Overall, the evidence suggests that individuals with AUD generally exhibit elevated levels of pro-inflammatory markers compared with healthy controls (Adams et al., 2020; Galvez-Sánchez et al., 2026). These differences also appear to vary across phases of alcohol use, with the greatest elevations observed during active drinking and alcohol withdrawal and smaller elevations observed with increasing durations of abstinence (Adams et al., 2020; Galvez-Sánchez et al., 2026; Sokolova et al., 2026). Additionally, elevated peripheral cytokine concentrations have been associated with greater AUD severity, alcohol withdrawal, craving, and psychiatric symptoms, including anxiety, depression, and insomnia (Galvez-Sánchez et al., 2026). Preclinical research has demonstrated that experimentally inducing an inflammatory response in mice leads to long-lasting increases in alcohol consumption, whereas inhibiting enzymes critical to inflammatory signaling reduces ethanol intake and preference (Blednov et al., 2011, 2014). Collectively, these findings suggest that inflammatory processes may contribute to alcohol-related behaviors, highlighting the inflammatory system as a potential biological target for AUD pharmacotherapies (Grodin, 2024; Meredith et al., 2021).

Chronic alcohol use is also associated with increased intestinal permeability, colloquially referred to as “leaky gut,” which allows gut-derived microbial products, including the endotoxin lipopolysaccharide (LPS), to enter circulation and promote systemic inflammation (Bishehsari et al., 2017). Consistent with this process, individuals with AUD exhibit higher levels of markers of intestinal permeability and microbial translocation compared with healthy controls (Donnadieu-Rigole et al., 2018). Among individuals with high baseline intestinal permeability, levels

significantly decreased following a period of abstinence from alcohol, further supporting the association between alcohol use and intestinal permeability (Leclercq et al., 2014). Markers of intestinal permeability have also been positively associated with markers of systemic inflammation, including C-reactive protein (CRP) (Lakatos et al., 2011; Lim et al., 2019), although these relationships have not been examined in an AUD sample. These findings suggest that alterations in intestinal permeability may represent an important pathway contributing to inflammatory processes in AUD.

Ibudilast for the treatment of AUD

Ibudilast (IBUD) is a phosphodiesterase (PDE) inhibitor and macrophage migration inhibitory factor (MIF) with anti-inflammatory properties (Cho et al., 2010; Gibson et al., 2006). Given the growing evidence implicating inflammatory processes in AUD, IBUD has been investigated as a potential pharmacological treatment for the disorder (Meredith et al., 2021). Preclinical studies demonstrated that IBUD reduces alcohol consumption in rodent models (Bell et al., 2015), and early clinical studies in non-treatment-seeking individuals with AUD found that IBUD attenuated alcohol craving and reduced heavy drinking compared to placebo (Grodin et al., 2021; Ray et al., 2017). These findings motivated a subsequent 12-week, double-blind, placebo-controlled randomized clinical trial of IBUD (50 mg twice daily) in 102 treatment-seeking individuals with moderate to severe AUD (Ray et al., 2025). The primary analysis revealed no significant differences between IBUD and placebo in drinking outcomes (Ray et al., 2025). Despite these null primary findings, the trial provides an important opportunity to further examine the factors that may influence treatment response to IBUD, given prior evidence that inflammatory status may moderate IBUD's effectiveness (Grodin et al., 2023). Thus, further investigation of biological markers and clinical characteristics associated with treatment response

may improve our understanding of individual differences in medication response in AUD. Data from this clinical trial were used in the final three chapters of this dissertation.

OVERVIEW OF THE DISSERTATION

PART I

The first part of the dissertation examines key methodological considerations in AUD pharmacotherapy clinical trials. Specifically, it focuses on the selection of clinical trial endpoints (**Chapter 1**), the duration of pharmacotherapy trials (**Chapter 2**), and the co-use of cigarettes and cannabis during a clinical trial for AUD (**Chapter 3**).

Chapter 1 provides a narrative review of the endpoints used to evaluate medication efficacy in AUD clinical trials. It discusses endpoints accepted by regulatory agencies, as well as newer approaches to evaluating efficacy, including reductions in WHO risk drinking levels, biological markers of alcohol use, neuroimaging measures, the NIAAA definition of recovery, and patient-reported outcomes. This review highlights opportunities to broaden and refine endpoint selection to improve the evaluation and development of novel pharmacotherapies for AUD.

Chapter 2 is a meta-regression study examining the relationship between trial duration and observed medication effect sizes across 139 AUD pharmacotherapy RCTs. Because clinical trials are costly and burdensome for participants, understanding how trial duration relates to observed treatment effects has important implications for the design of future AUD trials and for regulatory guidance.

Chapter 3 examines trajectories of cigarette and cannabis use during a randomized clinical trial for AUD and whether their use is associated with alcohol consumption across the

trial. Given the high prevalence of cigarette and cannabis co-use among individuals with AUD, it is important to understand how the use of these substances changes during treatment and whether their use relates to alcohol consumption and treatment response, in order to inform how co-use is interpreted in AUD trials.

PART II

The second part of the dissertation focuses on peripheral inflammation and intestinal permeability in AUD and examines their relevance to understanding AUD and its treatment. These studies are secondary analyses of data from a 12-week randomized controlled trial evaluating the efficacy of ibudilast for the treatment of AUD (Ray et al., 2025).

Chapter 4 examines whether baseline peripheral inflammation, as indexed by CRP, and peripheral markers of intestinal permeability moderate the effects of ibudilast on drinking outcomes over the 12-week RCT. Given the novelty of intestinal markers and AUD, this chapter also examines the relationships between intestinal permeability and AUD clinical characteristics, including alcohol consumption, craving, and symptom severity. This chapter highlights the potential utility of inflammatory and gut-related biomarkers for identifying subgroups of individuals who may differentially respond to treatment and underscores the need for further research examining inflammatory and gut-related processes in AUD.

Chapter 5 examines the relationship between changes in alcohol consumption and peripheral inflammatory marker levels during the first four weeks of the IBUD RCT. Although AUD has consistently been associated with elevated systemic inflammation, prior research has largely relied on cross-sectional group comparisons or human laboratory studies assessing inflammatory changes over several hours following acute alcohol administration. This chapter

addresses this gap by examining individual trajectories of change in drinking and peripheral inflammatory markers over several weeks, as well as the associations between these changes.

Collectively, this dissertation contributes to the development and evaluation of pharmacotherapies for AUD by informing key aspects of clinical trial design and advancing understanding of biological processes relevant to AUD and its pharmacological treatment. Together, these lines of research support the broader goal of expanding the limited pharmacological treatment options available for AUD and improving outcomes for the millions of individuals affected by the disorder.

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PART I: Methodological Considerations in Alcohol Use Disorder Clinical Trials

CHAPTER 1: Endpoints for Clinical Trials for Alcohol Use Disorder

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ABSTRACT

Alcohol use disorder (AUD) is a debilitating disorder, yet only three medications are currently approved by the U.S. Food and Drug Administration (FDA) for its treatment. Thus, there is a critical need to expand and improve the pharmacotherapy options available for AUD. Clinical trials investigating novel pharmacotherapies for AUD traditionally use abstinence or no heavy drinking days as endpoints to determine treatment efficacy, both of which are accepted by the FDA as primary endpoints and are derived from patient self-reported drinking. Beyond these traditional outcomes, there has been growing interest in additional endpoints for AUD pharmacotherapy trials. These include reductions in World Health Organization risk drinking levels, which reflect a harm-reduction approach to evaluating treatment response. In contrast to self-report measures, biological markers of alcohol use may serve as objective endpoints, and neuroimaging paradigms may offer insight into the neural circuitry underlying treatment effects. Additionally, the National Institute on Alcohol Abuse and Alcoholism's operational definition of recovery from AUD and patient-reported outcomes offer new frameworks for capturing clinically meaningful change that extends beyond alcohol consumption itself. These developments offer promising opportunities for future pharmacotherapy development, provided that their validity and reliability can be established. A broader range of endpoints may better capture the complexity and heterogeneity of AUD.

INTRODUCTION

Alcohol use disorder (AUD) is a highly pervasive disorder affecting over 28.6 million adults in the United States (SAMHSA, 2023). Moreover, 93.4 million adults are estimated to suffer from AUD during their lifetime (Grant et al., 2015). AUD is characterized by the continued use of alcohol despite significant social, psychological, and medical consequences (Ray et al., 2019). These consequences include health conditions such as cardiovascular disease, cancer, liver cirrhosis, and injuries (World Health Organization, 2014). It is estimated that more than 140,000 people die from alcohol-related causes annually (Mokdad et al., 2004), making excessive alcohol use a leading cause of preventable death (CDC, 2022). Despite these negative consequences, only an estimated 4.6% of adults with AUD received any treatment for alcohol use in 2021 (SAMHSA, 2023), and only 1.6% of adults with AUD received evidence-based medications for AUD in 2019 (Han et al., 2021).

To date, only three medications have been approved by the US Food and Drug Administration (FDA) for the indication of AUD (Burnette et al., 2022; Kranzler & Soyka, 2018). These medications are disulfiram, acamprosate, and naltrexone (formulated for oral administration or extended-release injection). These medications, along with nalmefene, are recognized by the European Medicines Agency (EMA) as established pharmacotherapies for AUD. Disulfiram, the first FDA-approved medication for AUD, is an aldehyde dehydrogenase inhibitor that inhibits the metabolism of alcohol, which leads to an accumulation of acetaldehyde in the blood following alcohol intake (De Sousa, 2019). The accumulation of acetaldehyde leads to unpleasant physiological reactions, including flushing, nausea, vomiting, headache, and tachycardia (De Sousa, 2019). Thus, disulfiram is intended to create an aversive reaction to alcohol. Disulfiram is administered daily to support individuals as they work towards self-

management, with treatment duration varying from months to years, depending on the individual (National Library of Medicine, 2012). Due largely to problems with medication adherence, disulfiram has shown mixed clinical efficacy (Fuller et al., 1986). Moreover, the psychological expectation of physiological reactions from disulfiram renders disulfiram equally efficacious as placebo in blinded randomized controlled trials, but more efficacious than placebo in open label trials (Skinner et al., 2014). Thus, the risk-benefit of disulfiram in comparison to lack of medication is more favorable when generalized to naturalistic settings where patients know what medication they are consuming.

Acamprosate is thought to interact with the glutamatergic system by normalizing dysregulated NMDA-mediated neurotransmission that results from chronic alcohol exposure (Witkiewitz et al., 2012). Acamprosate may be effective in helping individuals achieve and maintain abstinence, with treatment duration varying from months to years, depending on the individual (National Library of Medicine, 2020; Rösner et al., 2008). Compared to placebo, acamprosate has been shown to significantly reduce the risk of returning to any drinking by 86% (Witkiewitz et al., 2012). Adverse effects from acamprosate are generally minimal, with diarrhea being the most common side effect reported (Rosenthal et al., 2008). Considering acamprosate's significant benefits and minimal adverse events, the risk-benefit of acamprosate is favorable compared to placebo.

Naltrexone is a mu-opioid antagonist and is approved as a treatment for both opioid and alcohol use disorder (Heinzerling, 2013). Oral naltrexone results in lower rates of relapse (O'Malley et al., 1992) and reductions in subjective pleasurable effects of alcohol (Drobes et al., 2004; Ray et al., 2019; Ray & Hutchison, 2007), craving for alcohol (O'Malley et al., 2002; Richardson et al., 2008), and drinks per drinking day (O'Malley et al., 2015). Oral naltrexone is

typically prescribed to be taken once daily for a period of up to 12 weeks (National Library of Medicine, 2024). Similarly, injectable naltrexone reduces the number of heavy drinking days (Garbutt et al., 2005; Johnson, 2007). Injectable naltrexone is administered intramuscularly once every 4 weeks and can be used for six months or longer (National Library of Medicine, 2022). Naltrexone is generally well-tolerated with minimal adverse effects (Avery, 2022; Ray & Hutchison, 2007). Placebo-controlled randomized controlled trials of naltrexone indicate a 50% reduction in relapse rates with naltrexone vs. placebo (Berg et al., 1996). This significant benefit, coupled with the high tolerance and low adverse events of naltrexone, indicates a favorable risk-benefit ratio of naltrexone in comparison to the lack of treatment (Berg et al., 1996).

Given the limited number of current FDA-approved pharmacotherapies for AUD, their moderate treatment effects, limited prescription rates, and poor medication adherence (disulfiram), there is a need to facilitate the development and regulatory approval of novel medications that can effectively treat AUD. While continuous research aimed at advancing novel treatments is crucial to this process, it is also important to examine the application of endpoint metrics in clinical trials for AUD. Presently, as of 2024, the FDA accepts abstinence and no heavy drinking days as primary outcomes for phase 3 trials of AUD pharmacotherapy (U.S. Department of Health and Human Services et al., 2015). While these outcomes remain crucial benchmarks, broadening the scope of metrics and approved endpoints accepted by regulatory bodies could enhance the drug development and approval process. In this article, we discuss current methods for measuring alcohol use in pharmacotherapy clinical trials and how these measures are used to support regulatory approval. We also review alternative and emerging approaches to evaluating alcohol use and treatment efficacy, including grace periods in endpoint analyses, biological measures, definitions of recovery from AUD, and patient-reported outcomes.

The goal of this review is to highlight opportunities to broaden and refine endpoint selection to improve the evaluation and development of novel pharmacotherapies for AUD.

2. Measurements of Alcohol Use

Alcohol consumption is typically assessed using methods such as the Timeline Follow-Back (TLFB) interview (Sobell & Sobell, 1992), Quantity-Frequency (QF) questionnaires (Russell et al., 1991), Form 90 (Miller & Del Boca, 1994), and intensive longitudinal measurements. The TLFB is a widely used and well-validated method for assessing daily alcohol consumption over a specified timeframe, typically covering the preceding 7 to 30 days (Sobell & Sobell, 1992). In clinical pharmacotherapy trials, the TLFB may be administered at baseline and then during follow-up time points spanning the treatment period to assess the daily number of drinks consumed. Though the TLFB has a high reliability (Sobell & Sobell, 1992), it may be influenced by recall bias and a tendency to underreport daily alcohol use due to social desirability effects (Searles et al., 2002). While the TLFB is traditionally administered as a semi-structured interview, it can also be self-administered. In a comparative study, participants reported more total drinks, more heavy drinking days, and fewer abstinent days on a repeated self-administered 7-day TLFB than on an interview-administered 30-day TLFB covering overlapping periods (Hoeppner et al., 2010). Daily-level comparisons showed that discrepancies between the two assessments increased with the length of the recall period, suggesting that the 7-day TLFB may more accurately capture drinking events, particularly those further in the past (Hoeppner et al., 2010). Data from the TLFB can be used to quantify several aspects of drinking behavior, including drinks per drinking day, the percentage of heavy-drinking days, and the percentage of abstinent days. These calculations often require manual conversion of participant-reported drinking into standardized measures of beverage volume and alcohol content (Marini et

al., 2023). Despite these considerations, the TLFB remains a valuable and versatile measure that provides day-level information for evaluating changes in alcohol consumption across a range of drinking outcomes

Quantity and frequency (QF) questionnaires measure the quantity and frequency of typical drinking, the frequency of heavy drinking, and the maximum number of drinks consumed on a single occasion (Stevens et al., 2020). In contrast to the semi-structured interview style of the TLFB, QF questionnaires employ a multiple-choice response format. Questions probed by QF questionnaires include the quantity of drinks on a typical day, frequency of drinking, frequency of binge drinking, and maximum number of drinks (Stevens et al., 2020). While QF questionnaires are more efficient to administer, they provide less detailed information about drinking patterns, such as day-to-day variation in alcohol consumption and the number of drinks consumed on individual drinking days (Stevens et al., 2020). There is also evidence suggesting that QF may underestimate the quantity of alcohol consumed to a greater extent than the TLFB (Alcohol Clinical Trials Initiative Workgroup & National Institute on Alcohol Abuse and Alcoholism, 2018). Thus, QF questionnaires may be best suited to contexts that prioritize efficiency over the granular detail needed to capture nuanced patterns of alcohol consumption.

Form 90 consists of a family of structured assessment interviews used to evaluate alcohol consumption over a specified time frame, with different versions designed for initial intake, in-person or telephone follow-ups, and collateral reporting (Miller & Del Boca, 1994). The assessments are designed to provide a continuous retroactive daily drinking record from a 90-day baseline period through the duration of the assessment period (Miller & Del Boca, 1994). Form 90 quantifies drinking behavior by estimating total standard drinks consumed on each day, steady and episodic patterns of drinking, periods of total abstinence, and projected

blood alcohol concentration, or "intoxication peaks," based on the length of time over which alcohol was consumed (Miller, 1996; Miller & Del Boca, 1994). Thus, while Form 90 allows for comprehensive recording of alcohol use, as well as ease of calculating drinking levels, patterns, and outcomes, the complexity and range of Form 90 instruments require specialized interviewer training to ensure valid administration (Miller & Del Boca, 1994).

Intensive longitudinal measurements, such as a daily diary or ecological momentary assessment measurements, can also be utilized to assess daily drinking (Merrill et al., 2020). Smartphone application daily diary interviews are promising tools for measuring alcohol consumption more accurately over time than the TLFB (Dulin et al., 2017). More broadly, ecological momentary assessment approaches, including mobile electronic diaries, personal data assistants, and smartphones, may be useful in capturing alcohol use and its consequences in real-time (Wray et al., 2014), thereby circumventing the potential biases of retrospective recall as seen with the TLFB, QF, and Form 90. Ecological momentary assessment methods can be used to estimate blood alcohol concentrations and to study subjective responses to alcohol in the natural environment, with and without medications (Miranda et al., 2016; Ray et al., 2010). These self-monitoring approaches, however, could lead to compliance issues and missing data due to challenges with sustained participant engagement, technological disruptions, and participants' commitment to regular data input.

The choice of an assessment method for alcohol consumption depends on specific research objectives and the feasibility of the measurement tools. Information collected with these tools can be used to characterize individual patterns of alcohol use, including abstinence and heavy drinking days, which are the basis for currently approved clinical trial outcomes.

3. Endpoints

The next section of this review covers a variety of endpoints derived from the alcohol consumption assessments described above, as well as emerging approaches to evaluating treatment efficacy in AUD. Specifically, a host of endpoints are discussed, including abstinence-based endpoints, non-abstinence endpoints, World Health Organization (WHO) risk-drink level endpoints, evolving definitions of recovery from AUD, patient-reported outcomes, neuroimaging endpoints, and biological endpoints. Together, these endpoints provide a comprehensive set of outcomes that can be used alone or in combination to advance treatment development for AUD. To illustrate how these endpoints have been used in practice, **Table 1-1** summarizes the drinking endpoints used across a selected sample of 118 AUD clinical trials. For details on the selection of this sample, see Ray et al. (2021).

3.1 Abstinence-Based Endpoints

Total abstinence serves as a primary outcome for clinical trials of AUD pharmacotherapy and can indicate a successful response to treatment (U.S. Department of Health and Human Services et al., 2015). Total abstinence from alcohol is characterized by no consumption of any alcohol within a designated timeframe. Within a clinical trial for AUD pharmacotherapy, there are various ways to analyze total abstinence data. For example, researchers can calculate the percentage of participants who achieved complete abstinence during the duration of a clinical trial and compare this proportion between treatment groups (Anton et al., 2020). Researchers could also employ abstinence-based measurements to compare the percentage of days abstinent between treatment groups (Agabio et al., 2023).

Abstinence-based outcomes are reasonable predictors of clinical benefits and can serve as indicators of the efficacy of pharmacotherapy for AUD. The dichotomous categorization of

participants into 'abstinent' or 'non-abstinent' can also be valuable for clinical interpretation and establishing guidelines to define a successful treatment (Falk et al., 2010). Variants of the abstinence-based outcome may be constructs such as time to first drinking day or time to first heavy drinking day. Overall, abstinence-based outcomes remain a well-established and interpretable measure of treatment response in AUD pharmacotherapy trials, but their strict, binary nature may overlook more nuanced patterns of clinical improvement.

3.2 Non-Abstinence-Based Endpoints

3.21 Percentage of No Heavy Drinking Days

Non-abstinence goals for treating AUD may be more attractive to patients (Ambrogne, 2002) and could increase motivation to seek and maintain treatment. While achieving total abstinence is a valid and meaningful goal for some, more lenient outcomes, such as a reduction in drinking, may be more appropriate for others (Falk et al., 2010). The concept of moderate drinking as a viable treatment goal for AUD was introduced in the 1970s (Sobell & Sobell, 1973, 1976). It was proposed that the insistence on total abstinence as the only goal for treatment may be unrealistic and discouraging for individuals seeking treatment (Sobell & Sobell, 1973). Furthermore, it was demonstrated that individuals with AUD were capable of acquiring and maintaining patterns of controlled drinking, also described as low-risk drinking (Sobell & Sobell, 1973).

Aligned with a focus on more controlled drinking rather than complete abstinence, the percentage of subjects with no heavy drinking days (PSNHHDs) is also recognized as a primary efficacy endpoint in clinical trials of pharmacotherapy for AUD (U.S. Department of Health and Human Services et al., 2015). A heavy drinking day is defined as 4 or more drinks for women or 5 or more drinks for males consumed in one day (U.S. Department of Health and Human

Services et al., 2015). The PSNHDD outcome is a dichotomous measure that classifies participants as either having no heavy drinking days or having experienced at least one heavy drinking day during the assessment period (Falk et al., 2010). Researchers can then analyze the percentage of participants with no heavy drinking days throughout the trial and compare these percentages across treatment groups. Thus, although this outcome does not require total abstinence, it is dichotomous, and a single heavy drinking day places a participant in the same category as someone who drank heavily throughout the trial, which limits its ability to capture more nuanced patterns of drinking.

3.22 World Health Organization Risk-Drinking Levels as an Endpoint

Total abstinence and PSNHDDs may not capture the magnitude of drinking reductions, which could be an effective measurement of individualized beneficial treatment outcomes. To address this concern, researchers have explored the potential inclusion of measures that are more finely tuned to capture reductions in drinking. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the Alcohol Clinical Trials Initiative (ACTIVE) workgroup have collaborated to discuss reductions in World Health Organization (WHO) risk drinking levels as a primary efficacy endpoint for AUD pharmacotherapy clinical trials (Witkiewitz et al., 2018). The WHO risk drinking levels include: very high-risk drinking, high-risk drinking, moderate-risk drinking, and low-risk drinking (Shmulewitz et al., 2021) and are based on grams of ethanol consumed per day. Therefore, this measure considers the magnitude of change in the volume of alcohol consumed with treatment (Falk et al., 2019). The success rate of achieving full abstinence or no heavy drinking days is generally lower than the success rate for attaining a 1 or 2 WHO risk level reduction (Falk et al., 2019). In a secondary analysis of a randomized clinical trial of varenicline, a promising pharmacotherapy for the treatment of AUD, 7.6% of participants

who received varenicline achieved abstinence, while 53% achieved a WHO 2-level reduction (Falk et al., 2019).

While the NIAAA does not assert that a reduction in WHO risk drinking levels necessarily implies "recovery" from AUD, since drinking may remain at clinically significant levels, evidence suggests that such reductions can indicate a positive response to medication (Hagman et al., 2022). Studies have demonstrated that a 1- or 2-level reduction in the WHO risk drinking level is associated with significant improvements in various aspects of mental and physical health (Hasin et al., 2017; Knox et al., 2019; Witkiewitz et al., 2017, 2018, 2021). Furthermore, reductions in WHO risk drinking levels have been demonstrated to predict improved long-term outcomes for up to 3 years following the trial (Hasin et al., 2017; Knox et al., 2019; Witkiewitz et al., 2019). These outcomes include a lower risk of alcohol dependence, reduced alcohol consumption, decreased risk of a comorbid substance use disorder, improved physical health, and fewer alcohol-related negative consequences (Hasin et al., 2017; Knox et al., 2019; Witkiewitz et al., 2019). Notably, these outcomes may also better align with a patient's goal of reducing drinking rather than achieving full abstinence (Falk et al., 2019).

3.23 Including Grace-Periods in Analyses of Endpoints

Researchers have explored various approaches to endpoint analysis, including the use of grace periods, to improve the precision of drinking outcome assessments in clinical trials. Grace periods are defined as early time periods in a clinical trial that should be excluded from the analysis to allow the pharmacotherapy to reach an optimal therapeutic level (Falk et al., 2010). Additionally, grace periods could allow the novelty of participating in the trial to diminish, especially for participants in the placebo group (Falk et al., 2010). In a secondary analysis of data from the COMBINE study, a multisite trial evaluating several pharmacotherapies for AUD,

multiple grace period durations were examined (Falk et al., 2010). The naltrexone treatment effect increased monotonically with the increase in duration of the grace period. With no grace period, PSNHDD did not differ significantly between the naltrexone and placebo groups. However, with 1-month, 2-month, and 3-month grace periods, PSNHDD was significantly higher in the naltrexone group compared to the placebo group (Falk et al., 2010). Similar findings were observed for topiramate, in which PSNHDD did not differ significantly between topiramate and placebo groups when no grace periods were implemented. However, with 1-month and 2-month grace periods, PSNHDD was significantly higher in the topiramate group (Falk et al., 2010).

Tailoring the grace period to the unique titration periods and pharmacologic actions of the medications under examination is imperative, ensuring its alignment with both the specific drug being tested and the trial's design. For example, if the titration period of a medication is 6 weeks, it would be reasonable to implement a grace period that extends to the end of the titration period. While recognizing the benefits of utilizing a grace period, it is important to acknowledge that extending the duration of this period may result in a prolonged and more costly trial, potentially leading to increased dropout rates and a higher likelihood that participants will be lost to follow-up. When implemented within reason, however, the incorporation of grace periods stands out as a viable strategy to enhance the evaluation of drinking outcomes in clinical trials.

3.24 Biological Endpoints

Apart from patient reports of drinking behavior outcomes, biomarkers of alcohol use can additionally function as objective treatment outcome measurements of pharmacological responses to AUD medications (Heilig et al., 2016; Ray et al., 2021). The incorporation of biomarkers presents a promising opportunity to enhance the reliability of alcohol consumption

measurements in clinical trials by functioning as unbiased treatment outcome indicators of responses to AUD medications (Heilig et al., 2016; Ray et al., 2021). These biomarkers can be defined as measurable behavioral phenotypes (Kwako et al., 2016), biological substrates (Kwako et al., 2016; Ray et al., 2021), or neural activity (Voon et al., 2020). Biomarkers that measure biological substrates fall into two main categories: direct and indirect (Ingall, 2012). Direct biomarkers involve assessing alcohol levels or byproducts of ethanol metabolism, while indirect biomarkers measure the effects of alcohol consumption on various physiological processes or organs (Harris et al., 2021).

Blood Alcohol Concentration (BAC) and Breath Alcohol Concentration (BrAC) are widely used direct biomarkers that detect alcohol concentration in the blood and breath, respectively (Nanau & Neuman, 2015). These measurements are generally closely correlated and are utilized to assess the immediate level of intoxication (Skaggs et al., 2022). Although valuable in clinical trials for assessing very recent alcohol consumption, the limited time window of sensitivity restricts their ability to offer insight into a participant's drinking patterns over the course of a clinical trial. An alternative method for monitoring alcohol consumption is through the use of transdermal alcohol sensors (TAS). These are devices that were developed to monitor alcohol consumption continuously over extended periods of time by measuring alcohol vapors that are excreted through the skin in sweat (Piasecki, 2019). There is a moderate to strong correlation between TAS data and both breath alcohol content (BrAC) and self-reported drinking (Brobbin et al., 2022; Van Egmond et al., 2020). However, TAS may have decreased accuracy in detecting low-to-moderate alcohol consumption (Van Egmond et al., 2020). Further development and validation are critical to ensure reliability and accuracy across various levels of alcohol consumption in real-world contexts (Fairbairn & Bosch, 2021). Phosphatidylethanol (PEth),

another widely used direct biomarker, is a cellular membrane phospholipid that forms in the presence of ethanol (Hansson et al., 1997) and has been shown to be more sensitive than other direct biomarkers (Neumann et al., 2020). It can be detected in blood for up to 12 days after a single drinking event (Schröck et al., 2017), and the measured PEth concentration correlates with the amount of alcohol consumed (Aradottir et al., 2006). Other direct biomarkers include Fatty Acid Ethyl Ester (FAEE), which is present in blood for at least 24 hours following alcohol consumption (Soderberg et al., 2003). Indirect biomarkers include Mean Corpuscular Volume (MCV) and gamma-glutamyl transpeptidase (GGT), which have detection times ranging from days to weeks, providing a more extended timeframe for assessing alcohol consumption than immediate measures like BAC and BrAC (Harris et al., 2021). Several biomarkers of recent alcohol consumption, such as the ethanol metabolite ethyl glucuronide (EtG), can also be detected in urine (McDonell et al., 2015). The sensitivity of this assay to detect heavy drinking depends on the EtG cutoff level used; higher cutoffs can generally detect EtG for only about a day, while lower cutoffs can detect heavy drinking for up to 5 days (McDonell et al., 2015). However, the validity of urinary EtG tests has shown mixed results, and they are not yet considered reliable enough to be used as an objective biomarker (Grodin et al., 2020). Nevertheless, urinary EtG tests can still be useful in qualitatively assessing recent alcohol consumption, especially when used in conjunction with other measures of alcohol use and when blood sampling is not feasible. Hair analysis for EtG is another method to assess alcohol consumption. While these tests may be capable of indicating alcohol consumption for up to 90 days, these tests have shown low sensitivity and are prone to false positives due to external contamination from alcohol-based cosmetic products (Lees et al., 2012; Morini et al., 2018).

Rather than serving as sole endpoint measurements, biomarkers could be used at multiple time points throughout the trial, providing supplementary evidence of participants' drinking patterns and enhancing data reliability. While biomarkers for alcohol consumption offer valuable insights, several limitations should be considered. A major limitation is the inter-individual variability of alcohol pharmacokinetics, influenced by factors including age, sex, and comorbid conditions (Harris et al., 2021; Kwo et al., 1998; Vatsalya et al., 2023). As such, the reliance on direct biomarkers in clinical trials may lead to challenges in establishing consistent and reliable endpoints, as individual responses to alcohol can significantly vary. Furthermore, these biomarkers would need to be collected at multiple time points throughout the trial to yield temporal information about drinking patterns across the study duration. Biomarker collection would also increase concerns related to cost, logistical complexities of collection, and specialized storage and analysis requirements (Vaught & Henderson, 2011).

When used appropriately, biomarkers can facilitate a more nuanced understanding of alcohol-related behaviors following an intervention. Their integration alongside other clinical outcome measurements can offer a comprehensive and robust evaluation of interventions, mitigating the potential limitations associated with sole reliance on subjective measures of alcohol consumption. While the development of direct biomarkers of alcohol consumption has evolved, there is a strong call for indirect biomarkers that can capture disease processes beyond alcohol use itself (Heilig et al., 2016; Heilig & Leggio, 2016).

3.25 Neuroimaging Endpoints

Neuroimaging paradigms may noninvasively assess the efficacy of treatments for AUD and the functional neurocircuitry underlying these effects (Garrison & Potenza, 2014). Task-based functional magnetic resonance imaging (fMRI) paradigms, for instance, have enabled

researchers to examine the impact of medication on brain activity within tasks specifically designed to recruit neural circuitry implicated in AUD pathology. For example, researchers can employ an alcohol neural cue-reactivity paradigm in the scanner at baseline and following medication administration to understand how the medication influences neural activity in response to alcohol-related cues, designed to induce craving (Grodin & Ray, 2019). Furthermore, researchers can utilize resting-state fMRI, a technique that captures functional brain connectivity in the absence of a stimulus or task. This approach can help to elucidate how a medication influences resting-state functional connectivity within neural networks associated with addiction (Grundinger et al., 2022). Incorporating neuroimaging into pharmacotherapy clinical trials allows researchers to better understand how pharmacotherapies modulate specific brain regions implicated in AUD. It is important to note that utilizing fMRI in a clinical trial setting can incur significant costs. Additionally, participants could be excluded due to the strict MRI-safety scanning criteria. However, when utilized, this integrated approach holds the potential to bridge the gap between observed alterations in neural activity and improvements in clinical outcomes, effectively creating a biomarker for treatment efficacy. Thus, several efforts are underway to standardize fMRI drug cue-reactivity in order to advance it as an acceptable endpoint for clinical trials (Ekhtiari et al., 2022; Sadraee et al., 2021).

3.26 Recovery Definitions

More recently, researchers in the field have continued to discuss harm reduction approaches in AUD treatment (NIAAA, 2023). This interest stems from the recognition that the emphasis on recovery exclusively defined as abstinence from alcohol and the absence of AUD symptoms may inadequately represent the heterogeneous and multifaceted nature of recovery from AUD (Witkiewitz, 2020). Thus, researchers have actively worked towards establishing a

clear definition of recovery from AUD. Presently, stakeholder groups such as researchers, clinicians, policymakers, the FDA, and individuals with AUD define "recovery" in various ways. The ongoing discussions regarding the measurement of non-abstinence treatment outcomes include subjective symptom reduction outcomes to better capture symptom relief and functioning throughout treatment (Ray et al., 2019), improvements in social factors associated with harm reduction (Jordan et al., 2023), and biomarkers to assess biological and brain-based indicators of recovery (Ray et al., 2019).

The NIAAA recently developed an operational definition of recovery from AUD to further facilitate consistency within the field (Hagman et al., 2022). This definition includes three primary components of recovery (Hagman et al., 2022). The first component of this definition is remission from AUD symptoms in accordance with the diagnostic guidelines in the DSM-5 (Hagman et al., 2022). This entails assessing the severity of the disease based on the number of AUD criteria endorsed, as well as paying attention to criteria associated with clinical improvement in psychosocial functioning and well-being, which can serve as markers of recovery (Hagman et al., 2022). The second component of the definition is cessation from heavy drinking, with heavy drinking defined as no more than three (for females) or no more than four (for males) drinks on a single day (Hagman et al., 2022), aligning with the primary endpoint language utilized by the FDA in pharmacotherapy trials for AUD. The third component of the definition is improvements in biopsychosocial functioning and quality-of-life criteria as markers of recovery (Hagman et al., 2022). This approach is consistent with the recognition that changes in drinking may be necessary but not sufficient to re-establish healthy functioning across multiple life domains (Ray et al., 2019).

The NIAAA's definition of recovery recognizes that recovery is an ongoing process and that improvements in biopsychosocial functioning and well-being are an important part of recovery (Hagman et al., 2022). Establishing and adopting a standardized definition of recovery is important for advancing research in the field by allowing professionals more consistent and precise ways to measure recovery, ultimately enhancing our comprehension of the clinical trajectory of AUD. In terms of endpoints for AUD pharmacotherapy trials, researchers can utilize this aspect of the definition to capture clinically meaningful improvements in well-being throughout the recovery process. There is a tremendous opportunity for methods development and standardization in order to reach the overarching goal of a more comprehensive assessment of recovery. To that end, measures of well-being and functioning could potentially be incorporated as accepted endpoints for measuring the efficacy of a medication. Furthermore, the revised definition of recovery could also guide the development of more holistic public health strategies and interventions that extend beyond promoting abstinence.

3.27 Patient-Reported Outcomes

Throughout the treatment development process, it is also important to consider outcome efficacy from the patient's perspective. The Patient-Reported Outcomes Measurement Information System (PROMIS) is a framework launched by the National Institute of Health to guide the development of measurements assessing patient-reported outcomes on several constructs (pain, fatigue, physical functioning, emotional distress, sleep) across a range of diseases (Cella et al., 2007). The PROMIS contains item banks, which led to the creation of specific patient-reported outcome measures (PROMs) for constructs including alcohol use (Pilkonis et al., 2016). Specific PROMs assess a patient's perception of their physical and mental health at specific points in time, such as baseline and follow-up visits to monitor the patient's

subjective progress (Neale & Strang, 2015). These assessments can provide valuable insights into the patient's personal experiences and well-being, which may not be fully captured by abstinence or drinking reduction-based measurements. Five PROMIS item banks are related to alcohol use. These item banks include alcohol use, negative and positive consequences of use, and negative and positive expectancies of drinking (Pilkonis et al., 2013). These items are highly positively correlated with Alcohol Use Disorder Identification Test scores (r=0.79), supporting the validity of using PROMIS alcohol measures (Pilkonis et al., 2016). In addition, PROMIS health status profile measures evaluating emotional distress, sleep disturbance, and pain could be effectively utilized as outcome measures in individuals with substance use disorders. Both patients and providers in addiction medicine settings rated those constructs highly important in assessing their perceived recovery (Johnston et al., 2016). These patient and provider opinions are especially important to consider when developing treatments for AUD, as they will be the primary beneficiaries of new AUD pharmacotherapies.

Beyond PROMIS-based measures, other patient-reported outcome measures have also been developed specifically for individuals recovering from substance use disorders. The Substance User Recovery Evaluator (SURE), for example, was developed to be administered specifically to patients recovering from drug and alcohol use disorder (Neale et al., 2016). This assessment comprises 5 factors: substance use, material resources, outlook on life, self-care, and relationships (Neale et al., 2016). In brief, the positive patient and clinician responses to these measures support the potential use of patient-reported holistic health outcomes in AUD treatment trials.

4. Regulatory Perspectives

Currently, the FDA accepts abstinence and no heavy drinking days as primary endpoints for phase 3 trials of AUD pharmacotherapy (U.S. Department of Health and Human Services et al., 2015). Similarly, the EMA accepts full abstinence as a primary endpoint in AUD pharmacotherapy clinical trials, but it additionally takes into consideration the willingness of patients to achieve full abstinence (European Medicines Agency, 2010). If patients are not yet able to achieve full abstinence, the EMA accepts an intermediate harm reduction primary endpoint with the intention of achieving eventual abstinence maintenance (European Medicines Agency, 2010). Intermediate harm reduction refers to significantly reducing alcohol intake, thereby reducing alcohol-related harms. Efficacy for this endpoint is measured by the change in total consumption of pure alcohol, in grams per day, from baseline, and by the reduction in the number of heavy drinking days (European Medicines Agency, 2010). The EMA additionally evaluates improved patient health as a secondary efficacy endpoint. Improved patient health is measured by changes from baseline in validated liver markers, effects on the participant's social relationships, adherence to medication, changes in alcohol dependence severity measures, and 2-level reductions in WHO drinking risk levels (European Medicines Agency, 2010).

5. Conclusions

AUD is a highly pervasive and debilitating disorder, yet treatment-seeking rates and utilization of available approved pharmacotherapies are low (SAMHSA, 2023). Therefore, the current landscape of pharmacotherapies for AUD suggests opportunities for improvement. While continuous research aimed at developing novel pharmacotherapies is crucial, it is equally as important to examine the practices and accepted endpoints utilized in clinical trials for AUD. Traditionally, the preponderance of clinical trials have used abstinence-based or heavy-drinking

based endpoints as primary outcomes (U.S. Department of Health and Human Services et al., 2015). These outcomes are derived from patient self-reports about their drinking, which are transformed into quantifiable measurements of days abstinent or days without heavy drinking. Apart from these traditional self-report outcomes, there is movement in new directions. One important direction is the use of WHO risk drinking level reductions, which would allow a harm reduction approach that harnesses the health and psychological benefits of non-abstinence endpoints and controlled drinking (Witkiewitz et al., 2018). The WHO risk drinking levels as an endpoint have received ample empirical support and have the potential to move forward in terms of FDA review and approval (Hasin et al., 2017; Knox et al., 2019; Witkiewitz et al., 2017, 2018, 2021). Additionally, there have been developments in biological measurements of alcohol use, such as PEth, which can be utilized with the primary caveat of the consideration of the time periods for which they are implemented (Heilig et al., 2016; Ray et al., 2021). Transdermal alcohol sensors represent a significant advancement in monitoring alcohol consumption continuously over extended periods of time, but require further enhancement and development before they are able to be used as a primary endpoint (Piasecki, 2019). While clinical self-reported abstinence and non-abstinence-based endpoints are not affected by the alcohol pharmacokinetics, biological measurements of alcohol use can be influenced by inter-individual variability of alcohol pharmacokinetics, which is shaped by a variety of factors. Other novel endpoints include the utilization of neuroimaging, in which neuroimaging tasks that assess the incentive salience for alcohol have been developed and employed in clinical trials (Grodin & Ray, 2019).

Conceptually, the newly expanded recovery definition encompasses more than the consumption of alcohol itself, which presents further opportunities to refine clinical endpoints

(Hagman et al., 2022). However, its operational definition for clinical trials has yet to be finalized. Further, patient-oriented outcomes offer a necessary way to engage patients in the treatment development process, allowing them to report subjective benefits and perceived acceptability of treatments (Cella et al., 2007). Trial duration is another important factor to consider, as placebo effects may be more likely to occur during early treatment or in short-term trials. Treatment intervals vary greatly across randomized clinical trials, typically ranging from 4 weeks to 6 months (Jonas et al., 2014; Srisurapanont & Jarusuraisin, 2005). Placebo-controlled trials that utilize treatment intervals greater than 12 weeks may be more reliable (Jonas et al., 2014). However, there is an ethical obligation to limit the duration in which participants might receive a placebo when effective treatments exist. Therefore, utilizing active-controlled trials or placebo-controlled trials with a sufficient yet reasonable duration of care, in addition to implementing grace periods, may help balance these ethical considerations while still detecting the benefits of active pharmacotherapies over placebo (Falk et al., 2010).

The breadth of significant attention paid to clinical endpoints for AUD trials within the past decade speaks largely to the lack of success in the development of novel compounds for this indication. New pharmacotherapies meeting industry standards are necessary in order to address the complexity and heterogeneity of AUD symptom presentations. Moreover, given the complexity of AUD, a holistic approach to endpoint assessment is ideal. This holistic approach is feasible given the major developments in clinical endpoints of AUD pharmacotherapy trials including recognizing WHO risk drinking levels, utilizing biomarkers, and advancing the recovery definition. However, in practical terms, it is imperative that these endpoints be measurable in valid and reliable fashion in order to ultimately improve treatment development. There is a promising opportunity to leverage the attention paid to clinical endpoints to continue

their innovation, validation, and implementation, thereby ensuring the expansion of treatment development to capture the complexities of AUD.

Table 1-1. Frequency of endpoints utilized in 118 pharmacotherapy randomized clinical trials for alcohol use disorder (AUD)

	Randomized clinical trials (1985–2018)	
Characteristic	**No.**	**%**
AUD Required for Trial Inclusion	110	93%
Abstinence Required for Trial Inclusion	34	29%
Outcome Domain		
Abstinence	27	22.9%
Heavy Drinking	17	14.4%
Both	74	62.7%
Selected Outcomes		
Return to Any Drinking	66	55.9%
Return to Heavy Drinking	51	43.2%
Percent Heavy Drinking Days	48	40.7%
Percent Days Abstinent	78	66.1%
Drinks per Day	17	14.4%
Drinks per Drinking Day	35	29.7%

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CHAPTER 2: Examining the Impact of Trial Length on Detecting Medication Effects for Alcohol Use Disorder: A Meta-Regression Study

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ABSTRACT

Background

The U.S. Food and Drug Administration (FDA) recommends a minimum duration of 6 months for randomized controlled trials (RCTs) evaluating pharmacotherapies for alcohol use disorder (AUD). The relationship between trial length and treatment effects is not well understood. The current study conducted meta-regression analyses of RCTs for AUD to examine the association between trial length and observed medication effect sizes in clinical trials.

Methods

This secondary data analysis utilized data from a systematic literature review that identified 139 pharmacotherapy RCTs for AUD conducted between 1985 and 2023. Meta-regressions were conducted using the metafor package in R to examine whether shorter (< 12 weeks) or longer (> 12 weeks) trials differed in observed medication effect sizes for abstinence- and drinking-based endpoints, relative to 12-week trials, which represented the median trial duration. Publication bias was addressed using a weight-function model.

Results

For abstinence-based endpoints, neither shorter (< 12 weeks; $\beta = 0.13$, SE = 0.21, $p = 0.54$) nor longer (> 12 weeks; $\beta = 0.11$, SE = 0.11, $p = 0.30$) trials differed significantly from the 12-week reference group in observed medication effect sizes. Similarly, for drinking-based endpoints, neither shorter (< 12 weeks; $\beta = -0.07$, SE = 0.09, $p = 0.39$) nor longer (> 12 weeks; $\beta = 0.06$, SE = 0.05, $p = 0.26$) trials differed significantly from 12-week trials in observed medication effect sizes. All results remained nonsignificant after adjusting for publication bias. In sensitivity analyses restricted to trials with statistically significant effect sizes, trials > 12 weeks showed smaller estimated treatment effects compared to trials ≤ 12 weeks.

Conclusions

Observed medication effect sizes did not differ significantly across trial lengths for abstinence and drinking-based endpoints, except for analyses restricted to trials with significant effect sizes, where there was an advantage for trials that were 12 weeks or shorter. These findings inform efforts to optimize AUD RCT design.

INTRODUCTION

Randomized controlled trials (RCTs) of medications for alcohol use disorder (AUD) are costly and resource-intensive efforts that may ultimately lead to important healthcare advances through the development of novel pharmacotherapies (Hariton and Locascio 2018). Although there has been an effort to develop and test repurposed and novel medications for AUD, only three are currently U.S. Food and Drug Administration (FDA)-approved, and they demonstrate modest efficacy (Burnette et al. 2022). The wide variability in AUD clinical trial methodology, including the characteristics of study participants, outcome measures used, and analytic techniques, may hinder the development and approval of novel medications (Anton et al. 2012; Donato et al. 2024). Thus, a host of design considerations have been raised in an attempt to refine, and potentially standardize, AUD pharmacotherapy RCT methodology (Anton et al. 2012; Witkiewitz et al. 2015).

The Alcohol Clinical Trials Initiative (ACTIVE) workgroup has long recognized trial length as an important design consideration (Anton et al., 2012), where the trial length is defined as the period of medication treatment duration (i.e., active medication period). Longer RCTs place greater demands on participants, researchers, and sponsors, resulting in increased burden and cost (Naidoo et al., 2020; Zweben et al., 2009). Trial length also has regulatory implications. The U.S. Food and Drug Administration (FDA) currently recommends a minimum six-month active treatment duration for AUD medication RCTs, as cited in its draft guidance document (U.S. Department of Health and Human Services et al., 2015). In this document, the FDA cites findings from Zweben and Cisler (2003) (U.S. Department of Health and Human Services et al., 2015) which reanalyzed data from a large, behavioral intervention multisite treatment trial (Project Match Research Group, 1998), and showed that fewer individuals maintained abstinence

status (the most conservative outcome criteria) over 12 months than at 3 months (Zweben and Cisler, 2003). The FDA used these data to justify the need for longer duration trials; however, these limited findings also need to be contextualized in light of the chronic nature of AUD and the need for ongoing treatment and chronic disease management. Indeed, research conducted by the ACTIVE group has shown that drinking outcomes are stable during treatment episodes of 4 months and that outcomes achieved at the end of treatment tend to be stable over 1- to 3-years following a treatment episode (Witkiewitz et al., 2021, 2017). The FDA also based its recommendation on clinical observational findings from Schuckit et al. (1997), which found that individuals with alcohol dependence recruited from community settings commonly report abstinence periods lasting at least three months, which may confound the interpretation of results in shorter trials by augmenting a placebo response conferred by natural recovery (Schuckit et al., 1997).

The European Medicines Agency (EMA) guidance document recommends active treatment of 3 to 6 months to establish efficacy of a medication in double blind placebo-controlled trials for alcohol dependence, and also notes that a withdrawal phase of 12 to 15 months following randomization should be conducted to assess potential symptom rebound and drug withdrawal effects following discontinuation of medications (European Medicines Agency, 2010). Despite regulatory guidance for late-phase AUD pharmacotherapy trials, there is substantial variability in trial length across the broader AUD RCT literature. Meta-analytic work examining RCTs of naltrexone and acamprosate highlights notable variability in trial length, with acamprosate trials averaging about 25 weeks and naltrexone trials averaging around 14 weeks (Maisel et al., 2013). These differences may reflect variation in trial sponsorship and study location, as acamprosate trials were more often industry-sponsored and conducted in

Europe, whereas naltrexone trials were largely non-industry-sponsored and conducted in the United States (Maisel et al., 2013). Therefore, determining an optimal active treatment length is critical to improve consistency and optimize the ability to detect efficacious medications. In clinical practice, the optimal treatment duration for pharmacotherapy treatment for AUD is also unclear, and thus, a better understanding of how treatment length is associated with clinical outcomes could inform broader clinical decision-making (Substance Abuse and Mental Health Services Administration, 2015).

Prior research has examined the impact of trial length on placebo responses in AUD pharmacotherapy RCTs with abstinence-based endpoints. A previous meta-regression of 19 AUD pharmacotherapy RCTs conducted by Scherrer et al. (2021) found that, after controlling for baseline AUD severity measures, trial length was not significantly associated with the rate of abstinence in the placebo group (Scherrer et al., 2021). Similarly, in a meta-regression of 51 acamprosate and naltrexone RCTs for alcohol dependence, trial length was not significantly associated with placebo response or effect size in percent days abstinent (Litten et al., 2013). Notably, both meta-regressions only included studies that required abstinence from alcohol prior to starting study medication, which may not be representative of the majority of AUD pharmacotherapy RCTs (Donato et al., 2024). Importantly, neither analysis evaluated the effect of trial length on non-abstinence-based endpoints. With the FDA's recent expansion of approved endpoints beyond abstinence and no heavy drinking days to include reductions in drinking (Anton et al., 2025; Witkiewitz et al., 2025), it is critical to determine whether trial length influences the ability to detect efficacy across a wide range of drinking endpoints.

We previously conducted a systematic review of AUD pharmacotherapy RCTs that identified 139 trials testing 19 medications that were published between 1985 and 2023 (Donato

et al., 2024; Nieto et al., 2024; Ray et al., 2021). Donato et al. (2024) reviewed these identified trials to summarize common methodological practices in order to inform standardization efforts (Donato et al., 2024). The median trial length was found to be 12 weeks, and most of the trials had a trial length shorter than 6 months (Donato et al., 2024). Importantly, most of the trials included in this review were not testing medications via an FDA or EMA regulatory process, and thus did not follow FDA or EMA regulatory guidance for medication development with respect to trial length. Nonetheless, this comprehensive dataset affords the opportunity to test design features that may impact the likelihood of detecting treatment effects. By quantitatively examining the effects of AUD RCT design features on treatment outcomes, we can inform the refinement of trial designs. The present study leverages data from our meta-analytic work to examine the relationship between trial length and observed medication effect sizes in AUD pharmacotherapy RCTs. Specifically, we conducted meta-regressions on both abstinence- and drinking-based endpoints to compare observed effect sizes from RCTs with the median trial length of 12 weeks relative to those with shorter (<12 weeks) and longer (>12 weeks) trial lengths.

METHODS

Systematic Literature Search

The current secondary analysis utilizes data from two previously conducted literature searches of AUD pharmacotherapy RCTs published between 1985 and 2023 (Donato et al., 2024; Ray et al., 2021). The first literature search was conducted in 2018, which identified RCT pharmacotherapy studies published between 1985 and 2018 (Ray et al., 2021). The literature search was conducted algorithmically in collaboration with UCLA librarians and included searches of both Cochrane reviews and PubMed. Based on established guidelines by the

Cochrane Collaboration, study inclusion criteria were (a) a randomized controlled trial, (b) double or single-blinded, (c) placebo or active control condition, (d) alcohol use was the primary endpoint, (e) ≥4 weeks of treatment, (f) ≥12 weeks of follow-up. A total of 118 studies testing 17 medications were identified in the first literature search (Ray et al., 2021). A subsequent follow-up systematic literature search was conducted in 2023 using the same procedure and inclusion criteria, which identified 21 additional studies published between 2018 and 2023 (Donato et al., 2024). In total, 139 studies testing 19 medications, including both FDA-approved and non-FDA-approved drugs for AUD treatment, were included in the dataset. Complete literature search procedures and PRISMA flowcharts are provided in the primary papers (Donato et al., 2024; Ray et al., 2021). The current study was not formally preregistered. The protocol for the larger meta-analytic project underwent scientific review at the National Institutes of Health (Project R21AA029771), and the study codebook and analytic code can be accessed upon reasonable request.

Effect sizes of RCT endpoints

Cohen's *d* was calculated as the target effect size for six clinical trial endpoints: percent days abstinent, percent heavy drinking days, return to any drinking (yes/no), return to heavy drinking (yes/no), drinks per day, and drinks per drinking day. It was defined as the mean difference between the treatment and placebo/control group divided by the pooled standard deviation and multiplied by a correction factor to account for small sample size bias (Hedges, 1981). Negative effect sizes indicate a more favorable medication effect (e.g., more abstinence, less heavy drinking) over the control condition. Multiple effect sizes were contributed to the dataset only when they corresponded to distinct endpoints (e.g., a study reported both abstinence- and drinking-based outcomes) or when the same outcome was reported for multiple dosing arms

of the same medication. No multiple effect sizes were included for subgroup analyses based on gender, age, or other demographic characteristics.

Data Analysis

For analysis, the RCT endpoints were categorized into two groups that included both continuous and categorical endpoint measures: (1) abstinence-based endpoints (percent days abstinent and return to any drinking) and (2) drinking-based endpoints (return to heavy drinking, percent heavy drinking days, drinks per day, and drinks per drinking day). With respect to comparisons to FDA-approved endpoints, the drinks per day (continuous metric) endpoint is related to the recently approved World Health Organization risk drinking levels (categorical metric) that are calculated based on grams consumed per day, and the percent heavy drinking days (continuous metric) is related to the heavy drinking day endpoint (categorical metric). Separate meta-regressions were conducted for abstinence-based endpoints and drinking-based endpoints. This approach is consistent with previous meta-regressions that have pooled effect sizes across multiple substance use endpoints (Grodin et al., 2022; Magill et al., 2019; Nieto et al., 2024; Ray et al., 2021).

Studies were categorized by the length of active treatment into three groups: <12 weeks, 12 weeks, and >12 weeks. These bins were chosen based on representativeness of trials in the dataset, as reported below, with most trials being 12 weeks and fewer trials being less than or more than 12 weeks. Random-effects meta-regressions were conducted in R using the *metafor* package (Hedges and Vevea, 1998; Viechtbauer, 2010). Trial length was entered as a three-level categorical moderator (<12, 12, >12 weeks), specifying 12 weeks as the reference group. Twelve-week trials were chosen as the reference group because this duration represented the modal and median treatment length in this dataset. Effect sizes and their sampling variances

were modeled so that the coefficients estimate the differences for <12 vs 12 and >12 vs 12. We corrected for publication bias using the Vevea and Hedges weight-function model (Vevea and Hedges, 1995), which was conducted using the WEIGHTR R package (Coburn and Vevea, 2019). Results were visualized using bar graphs with standard errors generated in R.

To evaluate the robustness of our findings, we conducted several sensitivity analyses. We first analyzed RCT endpoints individually to determine whether trial length effects varied across outcomes. Additionally, because there were relatively few effect sizes from trials shorter than 12 weeks, we combined effect sizes from <12-week trials with those from 12-week trials to increase statistical power and then compared the observed effect sizes for ≤12 vs >12. We also conducted analyses that included only FDA-approved AUD medications (naltrexone and acamprosate) with established clinical efficacy, given the variability in efficacy across the tested medications. Finally, we conducted analyses limited to statistically significant effect sizes ($p < .05$) to evaluate trial length effects among studies that demonstrated efficacy. Statistical significance was assessed by calculating 95% confidence intervals for each effect size. Given the limited number of significant effect sizes from trials <12 weeks, they were combined with 12-week trials to create a ≤12-week group for this analysis. Results from sensitivity analyses are reported in the Supplementary Material.

RESULTS

This study included 139 RCTs for AUD conducted between 1985 and 2023, representing 19 medications and 390 effect sizes (Table 1). Naltrexone (k=42; 30.22%), acamprosate (k=24; 17.27%), and baclofen (k=11; 7.91%) were the most frequently investigated medications in this dataset. The complete list of medications included in this dataset is shown in Supplementary Table S1. The median trial length was 12 weeks (IQR = 12–16; min = 4, max = 52). Overall,

52% (k=68) of the RCTs investigated FDA-approved medications, 46% (k=60) investigated off-label medications, and 2% (k=3) investigated medications without FDA indications. There were 113 effect sizes (28.97%) that were statistically significant and indicated a favorable medication effect. The median trial length was 12 weeks (IQR = 12–16; min = 4, max = 52). The majority of RCTs (k=87; 62.59%) reported outcomes from both abstinence-based and drinking-based domains. Accordingly, most studies contributed more than one effect size (79.14%). One hundred sixty-nine (43.33%) effect sizes were from abstinence-based endpoints, and 221 (56.67%) effect sizes were from drinking-based endpoints (**Table 2-1**).

Thirty-eight (9.74%) effect sizes were from trials shorter than 12 weeks (k = 12), 227 (58.21%) effect sizes were from trials that were 12 weeks (k = 81), and 125 (32.05%) effect sizes were from trials that were greater than 12 weeks (k = 46; **Table 2-1).** Full distributions of trial lengths across the included studies are shown in **Figure 2-1**. In the shorter-than 12-weeks-trial group, the mean age was 42.67 (SD = 3.20) years, 77.74% (SD = 16.10) of participants were male, and 83.33% of the trials required a pre-trial AUD diagnosis (**Table 2-2**). In the 12-week trial group, the mean age was 44.89 (SD = 4.52) years, 74.31% (SD = 18.31) of participants were male, and 92.50% of the trials required a pre-trial AUD diagnosis. In the longer-than-12-weeks-trial group, the mean age was 44.73 (SD = 3.61) years, 73.60% (SD = 14.27) of participants were male, and 93.48% of the trials required a pre-trial AUD diagnosis. The proportion of trials sponsored by pharmaceutical companies was 33.33% for shorter trials, 35.53% for 12-week trials, and 55.00% for longer trials. Median publication year was 2008 for shorter trials, 2007 for 12-week trials, and 2007 for longer trials. Full details of study characteristics are reported in Donato et al., 2024.

Abstinence-Based Endpoints

A total of 169 effect sizes were included for abstinence-based endpoints, which pooled data from percent days abstinent and return to any drinking endpoints. The analysis of the 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.36, SE = 0.07, P < .0001, 95% CI [-0.49, -0.22]). Neither shorter (<12 weeks; β = 0.13, SE = 0.21, P = .54, 95% CI [-0.29, 0.55]) nor longer (>12 weeks; β = 0.11, SE = 0.11, P = .30, 95% CI [-0.10, 0.33]) trials differed significantly from the 12-week reference **(Figure 2-2).** After applying the weight-function model to correct for publication bias, the contrasts remained non-significant (shorter vs. 12 weeks: β = 0.19, SE = 0.44, *p* = .67, 95% CI [-0.68, 1.05]; longer vs. 12 weeks: β = 0.31, SE = 0.24, *p* = .18, 95% CI [-0.15, 0.78]). A sensitivity analysis limited to significant effect sizes (n = 51) indicated that trials >12 weeks showed a smaller magnitude of treatment effect compared to trials ≤ 12 weeks (β = 0.62, SE = 0.28, *p* = .03, 95% CI [0.06, 1.17]). Across all other abstinence-based sensitivity analyses (examining individual endpoints, restricting analyses to FDA-approved medications, and comparing ≤12-week with >12-week trials), observed effect sizes did not differ significantly by trial length. Detailed results are provided in the Supplementary Material.

Drinking-Based Endpoints

Drinking-based endpoints included 221 effect sizes, which pooled data from return to heavy drinking, percent heavy drinking days, drinks per day, and drinks per drinking day endpoints. The analysis of the 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.25, SE = 0.03, P < .0001, 95% CI [-0.31, -0.19]). Neither shorter (<12 weeks; β = -0.07, SE = 0.09, P = .39, 95% CI [-0.24, 0.10]) nor longer (>12 weeks; β = 0.06, SE = 0.05, P = .26, 95% CI [-0.04, 0.17]) trials differed significantly from the 12-week reference **(Figure 2-3).**

After applying the weight-function model to correct for publication bias, the contrasts remained non-significant (shorter vs. 12 weeks: β = -0.10, SE = 0.10, p = .36, 95% CI [-0.30, 0.11]; longer vs. 12 weeks: β = 0.07, SE = 0.07, p = .27, 95% CI [-0.06, 0.21]). A sensitivity analysis limited to significant effect sizes (n = 62) indicated that trials >12 weeks showed a smaller magnitude of treatment effect compared to trials ≤ 12 weeks (β = 0.34, SE = 0.07, p < .0001, 95% CI [0.20, 0.48]). Across all other drinking-based sensitivity analyses (examining individual endpoints, restricting analyses to FDA-approved medications, and comparing ≤12-week with >12-week trials), observed effect sizes did not differ significantly by trial length. Detailed results are provided in the Supplementary Material.

DISCUSSION

RCTs are essential for advancing medication development for AUD. Refine trial design features, including trial length, is critical for informing future trial design and regulatory guidelines. In this secondary analysis of a representative sample of 139 AUD pharmacotherapy RCTs conducted between 1985 and 2023, we tested whether trial length influenced the observed estimated effect sizes of medications. Findings indicated that observed effect sizes from both shorter (<12 weeks) and longer (>12 weeks) trials did not differ significantly from the median 12-week duration. However, the small number of trials in the <12-week group resulted in wider confidence intervals, and these results should be interpreted with caution. Nevertheless, observed effect sizes were generally consistent across trial length groups, with no clear evidence that medication effects differed by trial length. Findings reported in the Supplementary Materials indicated that observed effect sizes were also similar across trial-length groups when RCT endpoints were analyzed individually. This is critical in demonstrating consilience across endpoints, such that clinical trial outcomes are not differentially affected by trial length. When

we limited the analyses to the subset of effect sizes that were statistically significant and indicated a favorable treatment response, longer trials (>12 weeks) showed observed treatment effects that were smaller in magnitude than those from trials that were ≤12 weeks. The smaller observed effect sizes in longer trials may reflect factors that become more pronounced as study duration increases, including higher attrition rates, lower medication adherence, and increased missing data, which may lead to biased treatment effect estimates (Brorson et al., 2013; Hallgren and Witkiewitz, 2013).

One of the longstanding issues in AUD clinical trials is placebo response (Litten et al., 2013). Specifically, treatment-seeking individuals who enroll in a clinical trial for AUD routinely demonstrate improvements in their alcohol use, including those receiving placebo (Litten et al., 2012). Although preliminary analyses of abstinence endpoints in RCTs did not detect an effect of trial length on placebo response, the generalizability of these findings to other trial designs, outcome measures, and a broader set of medications has not been established (Litten et al., 2013; Scherrer et al., 2021). Prior work has suggested that placebo responses may be more pronounced in shorter trials, potentially reflecting the novelty of early trial participation (Litten et al., 2013). However, this association was not observed in the present analysis. At the same time, maintaining individuals who are non-responders on an experimental medication (or placebo) for a longer duration of time (i.e., 6-month trials or longer) raises ethical issues, given that extant FDA-approved medications for AUD are efficacious. Additionally, as previously noted, attrition rates increase as study duration is extended, leading to increased missing data, which poses a significant challenge for analyzing AUD clinical trials due to the need for various missing drinking data imputation strategies (Enos, 2025; Hallgren and Witkiewitz, 2013). Notably, only 20 studies in this analysis met or exceeded 6 months, and thus, a fine-grained analysis of longer-

length AUD studies is warranted. Future research aimed at clarifying how trial length influences factors such as retention rates, medication adherence, and missing data can provide further insight into determining the optimal trial length for AUD pharmacotherapy trials. Although our findings indicate that trial length was not associated with differences in observed medication effect sizes, the findings from the current study do not directly address other considerations that purportedly informed the FDA guidance for a minimum six-month trial length, including references to evidence that drinking outcomes assessed over shorter durations may not reflect stable drinking patterns or reliably predict future drinking behavior. Importantly, other work has shown that trials of shorter duration do reflect stable drinking patterns and reliably predict future drinking (Witkiewitz et al., 2021, 2017); however, these outcomes were not the focus of the current study. Rather, the findings from the current study provide additional context for evaluating the potential advantages and limitations of different trial lengths and demonstrate that all trial lengths evaluated had equivalent medication effect sizes.

These findings should be considered in the context of their strengths and limitations. A significant strength is the inclusion of a comprehensive sample of medication RCTs spanning four decades of research. This robust sample of studies includes effect sizes for several FDA-approved and non-approved trial endpoints, which represent a range of alcohol-related endpoints relevant to the evaluation of treatment efficacy. Regarding generalizability, these findings should be interpreted in the context of treatment-seeking adults with AUD enrolled in medication RCTs. Additional features of this dataset and analytic approach should also be taken into account when interpreting these findings. For example, the majority of the studies contributed multiple effect sizes to the analyses because they reported several endpoints within the same trial. Additionally, multiple effect sizes for a study may reflect outcomes from different arms of the trial (e.g.,

varying dosing regimens). The inclusion of multiple effect sizes from the same trial may have given greater weight to studies that reported multiple endpoints, which could affect estimates. Another consideration is the heterogeneity of the included trials. This dataset includes studies that tested both FDA-approved and experimental/off-label medications that may differ in efficacy. To address this consideration, sensitivity analyses limited to FDA-approved AUD medications and to statistically significant outcomes (i.e., positive trials) were conducted. The dataset also includes trials with varying study designs and participant populations, which may contribute to variability in the estimates (Donato et al., 2024). For instance, length of abstinence prior to randomization has previously been shown to affect placebo response rates (Scherrer et al., 2021). In addition, baseline drinking level or severity of trial populations may also affect observed outcomes. Finally, the number of studies in the <12-week length group is limited relative to the other trial length groups, contributing to wider confidence intervals for this group.

In summary, observed medication effect sizes did not differ significantly across trial lengths for abstinence and drinking-based endpoints. These initial findings are important given ongoing efforts to optimize AUD clinical trial design. The goals of reducing trial costs, participant burden, and risks are not trivial; therefore, more attention to this important issue is critical for future development of AUD medications and to inform regulatory guidance.

Table 2-1. Distribution of effect sizes by endpoint, trial length, and medication representation.

Domain	Endpoint	Total Effect Sizes	< 12 Weeks	12 Weeks	> 12 Weeks
Abstinence-based	Percent days abstinent	91	10	54	27
	Return to any drinking	78	3	40	35
	Total	**169**	**13**	**94**	**62**
Drinking-based	Return to heavy drinking	63	5	41	17
	Percent heavy drinking days	68	8	41	19
	Drinks per day	33	4	16	13
	Drinks per drinking day	54	8	32	13
	Total	**221**	**25**	**133**	**63**
Top-three represented medications	Naltrexone (k=42)	166	11	119	36
	Acamprosate (k=24)	121	10	39	72
	Baclofen (k=11)	36	0	22	14

Table 2-2. Sample and study characteristics by trial length group.

	< 12 Weeks (k = 12)	**12 Weeks** (k = 81)	**> 12 Weeks** (k = 46)	***P* value**
Sample Characteristics				
Age, mean (SD)	42.67 (3.20)	44.89 (4.52)	44.73 (3.61)	.25
Male, % (SD)	77.74 (16.10)	74.31 (18.31)	73.60 (14.27)	.77
Trial Characteristics				
Trials requiring baseline AUD diagnosis, %	83.33	92.41	93.48	.50
Industry-sponsored trials, %	33.33	35.53	55.00	.11
Publication year, median (IQR)	2008 (2002–2011)	2007 (2002–2014)	2008 (2000-2015)	.93

Trial-length group comparisons were conducted using one-way ANOVA for continuous variables, chi-square tests for categorical variables, and Kruskal–Wallis tests for publication year.

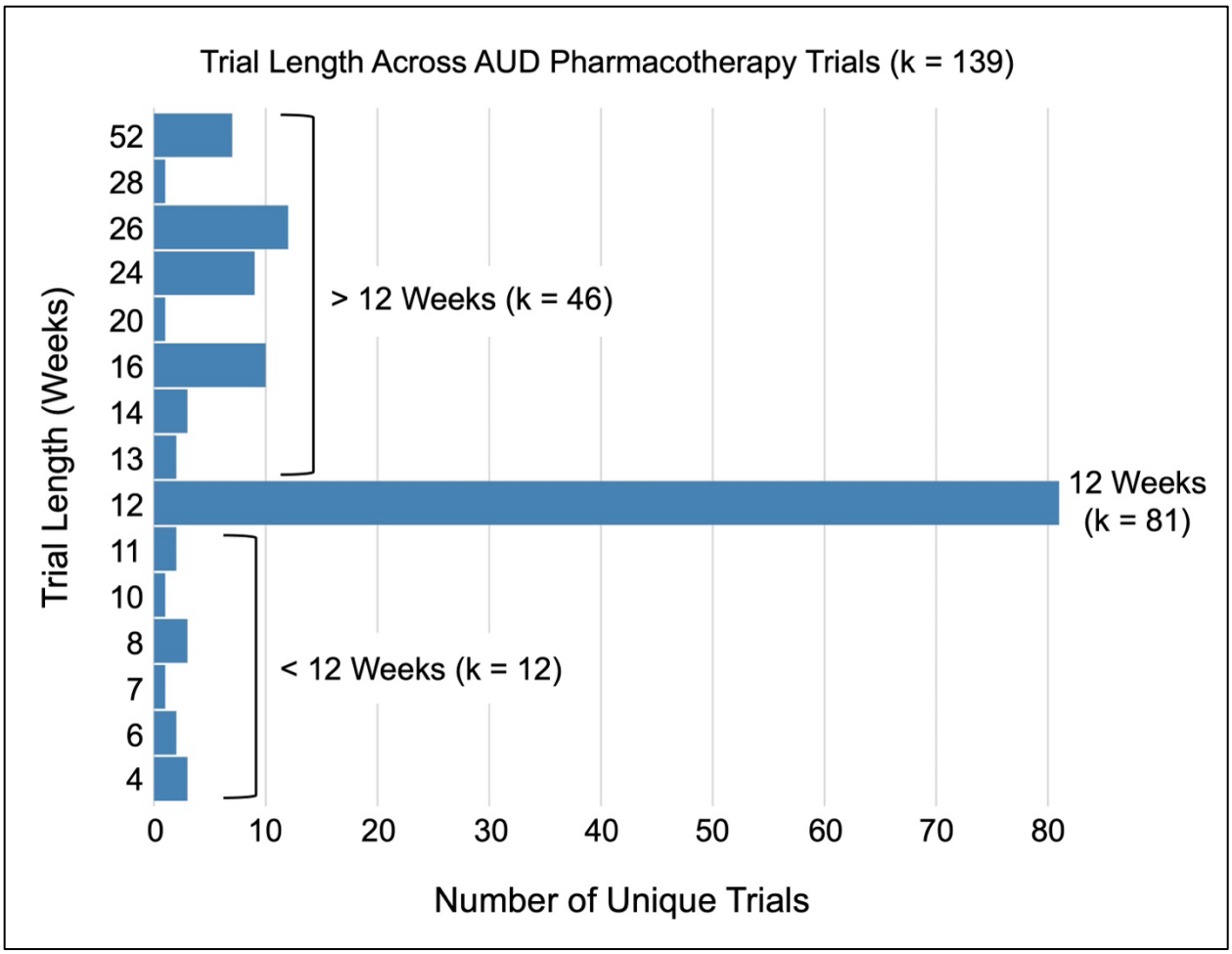


Figure 2-1. Distribution of trial length across alcohol use disorder pharmacotherapy randomized controlled trials ($k = 139$). Twelve trials were shorter than 12 weeks, 81 trials were 12 weeks, and 46 trials were longer than 12 weeks.

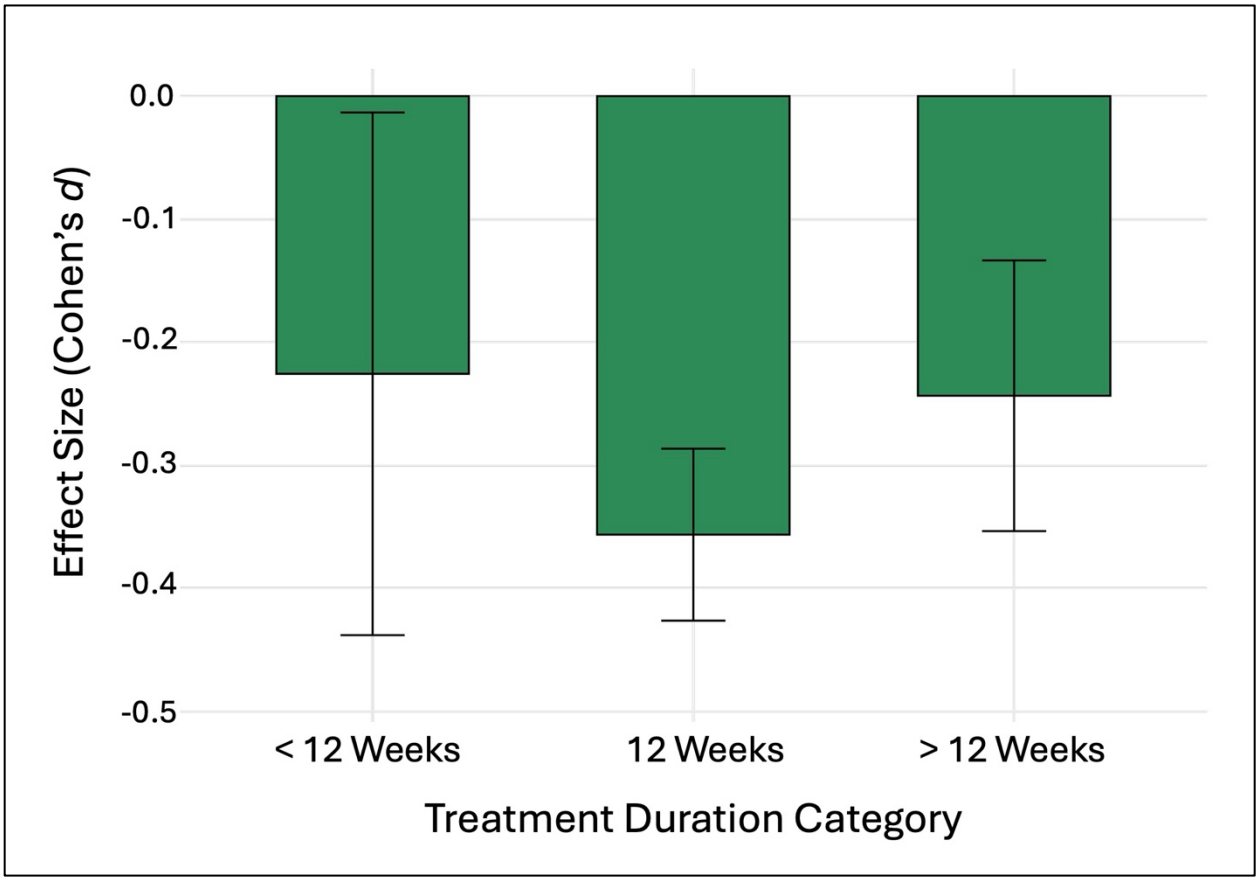


Figure 2-2. Effect sizes (Cohen's *d*) for abstinence-based endpoints by trial length category. There were no significant differences between shorter (< 12 weeks) or longer (> 12 weeks) trials compared to the 12-week reference. Negative effect sizes indicate a favorable medication effect over placebo. Error bars represent standard error. See Figure S2.1 for corresponding violin plots.

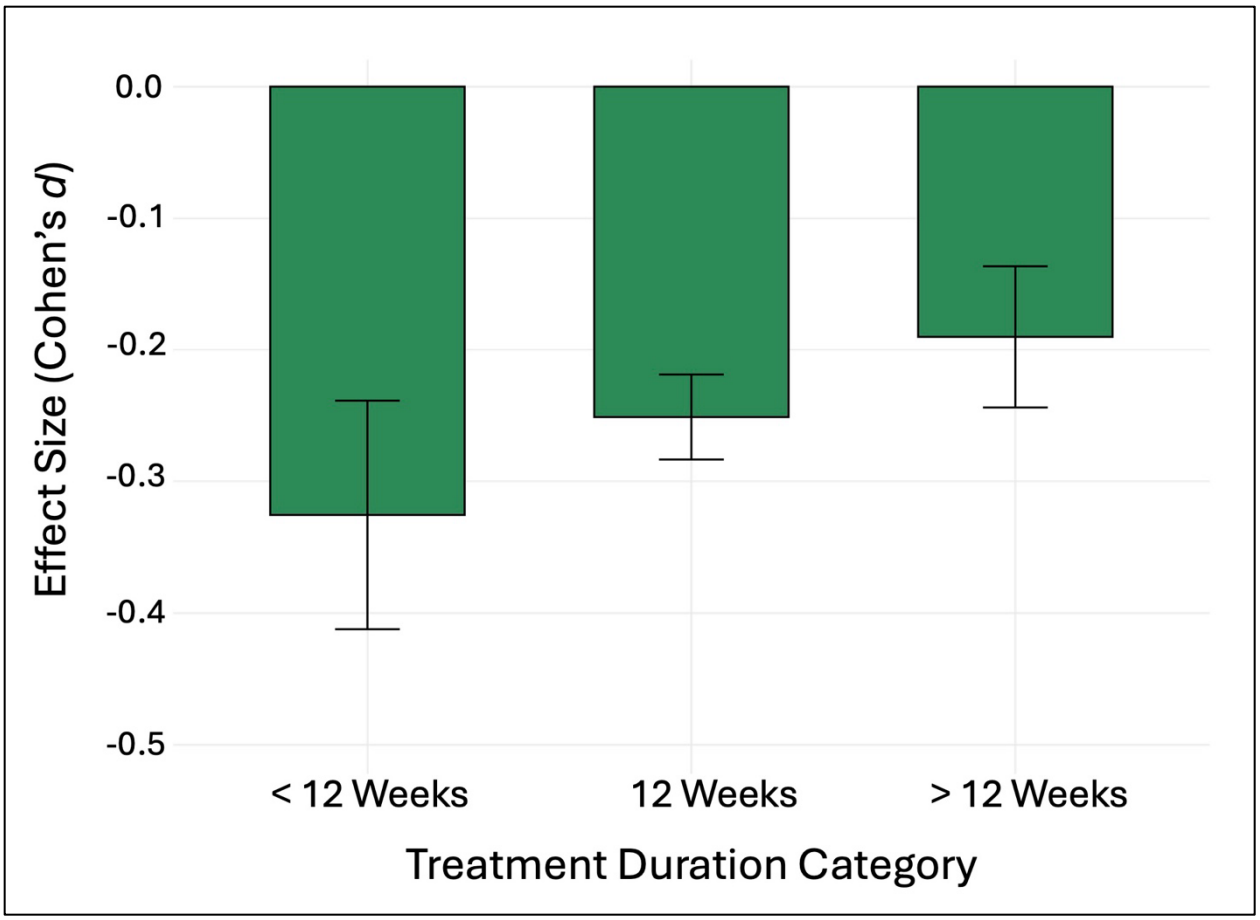


Figure 2-3. Effect sizes (Cohen's *d*) for drinking-based endpoints by trial length category. There were no significant differences between shorter (< 12 weeks) or longer (> 12 weeks) trials compared to the 12-week reference. Negative effect sizes indicate a favorable medication effect over placebo. Error bars represent standard error. See Figure S2.2 for corresponding violin plots.

CHAPTER 2 SUPPLEMENTAL MATERIAL

SENSITIVITY ANALYSES

Examining Endpoints Individually

To assess the robustness of the primary analyses, we evaluated trial length effects for each endpoint individually.

Return to any drinking. This outcome included 78 effect sizes. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.18, SE = 0.05, P < .001, 95% CI [-0.28, -0.08]). Neither shorter (<12 weeks; β = -0.23, SE = 0.27, P = .40, 95% CI [-0.76, 0.31]) nor longer (>12 weeks; β = -0.06, SE = 0.07, P = .37, 95% CI [-0.21, 0.08]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Percent days abstinent. This outcome included 91 effect sizes. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.47, SE = 0.12, P < .001, 95% CI [-0.71, -0.23]). Neither shorter (<12 weeks; β = 0.30, SE = 0.32, P = .36, 95% CI [-0.33, 0.92]) nor longer (>12 weeks; β = 0.25, SE = 0.21, P = .24, 95% CI [-0.17, 0.66]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Return to heavy drinking. This outcome included 63 effect sizes. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.23, SE = 0.04, P < .001, 95% CI [-0.32, -0.15]). Neither shorter (<12 weeks; β = -0.02, SE = 0.16, P = .89, 95% CI [-0.33, 0.28]) nor longer (>12 weeks; β = 0.08, SE = 0.08, P = .29, 95% CI [-0.07, 0.23]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Percent heavy drinking days. This outcome included 68 effect sizes. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.33, SE = 0.08, p < .001, 95% CI [-0.49, -0.17]). Neither shorter (<12 weeks; β = -0.12, SE = 0.22, p = .60, 95% CI [-0.55, 0.31]) nor longer (>12 weeks; β = 0.06, SE = 0.14, p = .66, 95% CI [-0.21, 0.33]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Drinks per day. This outcome included 33 effect sizes. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.24, SE = 0.09, p = .008, 95% CI [-0.42, -0.07]). Neither shorter (<12 weeks; β = 0.04, SE = 0.19, p = .82, 95% CI [-0.33, 0.41]) nor longer (>12 weeks; β = 0.02, SE = 0.12, p = .85, 95% CI [-0.21, 0.26]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Drinks per drinking day. This outcome included 54 effect sizes. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.18, SE = 0.06, p = .002, 95% CI [-0.29, -0.07]). Neither shorter (<12 weeks; β = -0.15, SE = 0.14, p = .27, 95% CI [-0.42, 0.12]) nor longer (>12 weeks; β = 0.10, SE = 0.10, p = .29, 95% CI [-0.09, 0.29]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

FDA-Approved Medications

Given the variability in efficacy across the tested medications, we conducted analyses that included only effect sizes from trials of FDA-approved AUD medications (naltrexone and acamprosate) with established clinical efficacy.

Abstinence-based endpoints. A total of 100 effect sizes were included from naltrexone and acamprosate trials with abstinence-based endpoints. The analysis of 12-week trials indicated a

significant benefit of medication compared to placebo (β = -0.45, SE = 0.11, P < .0001, 95% CI [-0.68, -0.23]). Neither shorter (<12 weeks; β = 0.26, SE = 0.34, P = .77, 95% CI [-0.41, 0.93]) nor longer (>12 weeks; β = 0.16, SE = 0.17, P = .36, 95% CI [-0.18, 0.49]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Drinking-based endpoints. A total of 80 effect sizes were included from naltrexone and acamprosate trials with drinking-based endpoints. The analysis of 12-week trials indicated a significant benefit of medication compared to placebo (β = -0.23, SE = 0.04, P < .0001, 95% CI [-0.32, -0.15]). Neither shorter (<12 weeks; β = 0.11, SE = 0.12, P = .36, 95% CI [-0.12, 0.34]) nor longer (>12 weeks; β = 0.04, SE = 0.08, P = .60, 95% CI [-0.11, 0.20]) trials differed significantly from the 12-week reference. Results were consistent after adjusting for potential publication bias.

Comparing ≤12-Week And >12-Week Trials

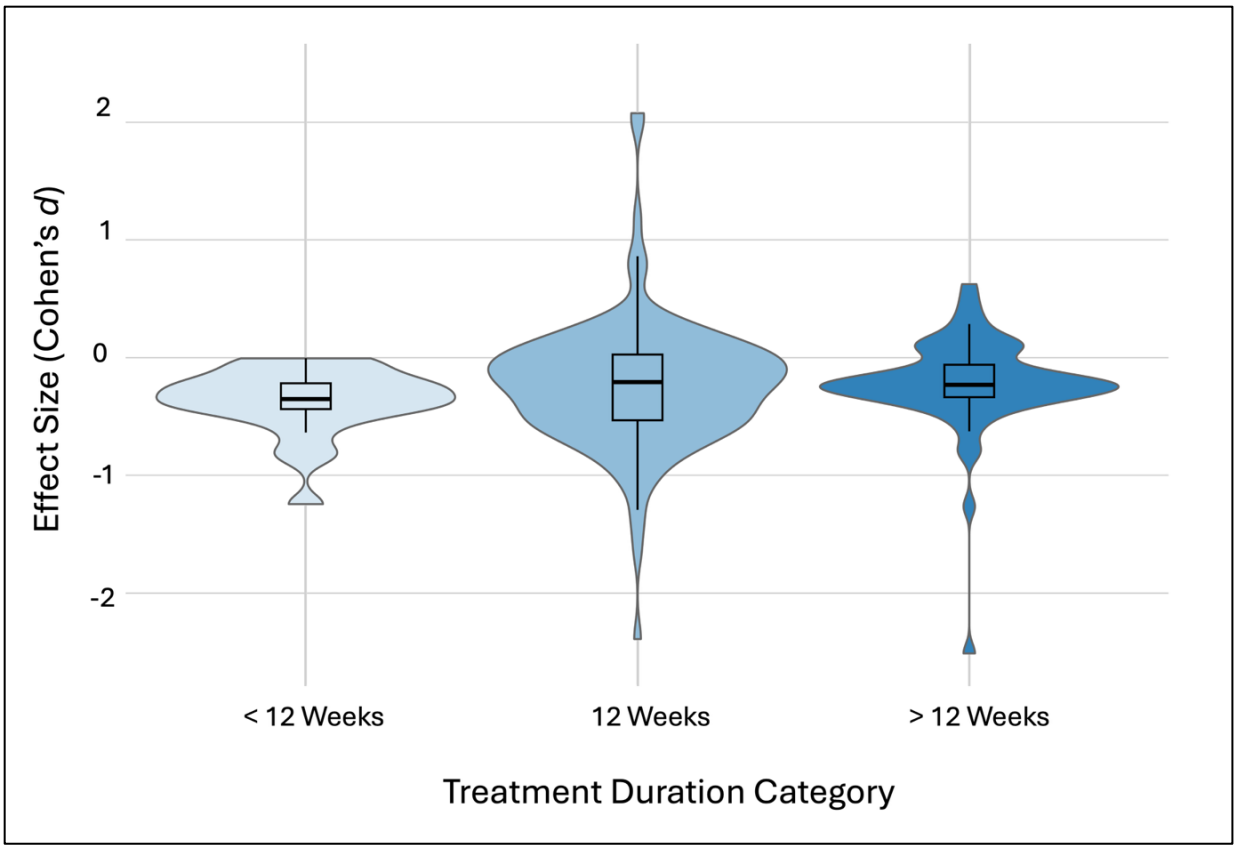
Given the relatively small number of effect sizes from trials shorter than 12 weeks, effect sizes from <12-week trials were combined with those from 12-week trials to increase statistical power. We then compared observed effect sizes from ≤ 12-week trials with those from >12-week trials.

Abstinence-based endpoints. For abstinence-based endpoints, a total of 169 effect sizes were included, with 107 effect sizes from ≤12-week trials and 62 effect sizes from >12-week trials. The analysis of ≤12-week trials indicated a significant benefit of medication compared to placebo (β = -0.34, SE = 0.07, *p* = < .001, 95% CI [-0.47, -0.21]). There were no significant differences in observed effect sizes between the ≤12-week trials and the >12-week trials (β = 0.10, SE = 0.11, P = .36, 95% CI [-0.11, 0.31]). Results were consistent after adjusting for potential publication bias.

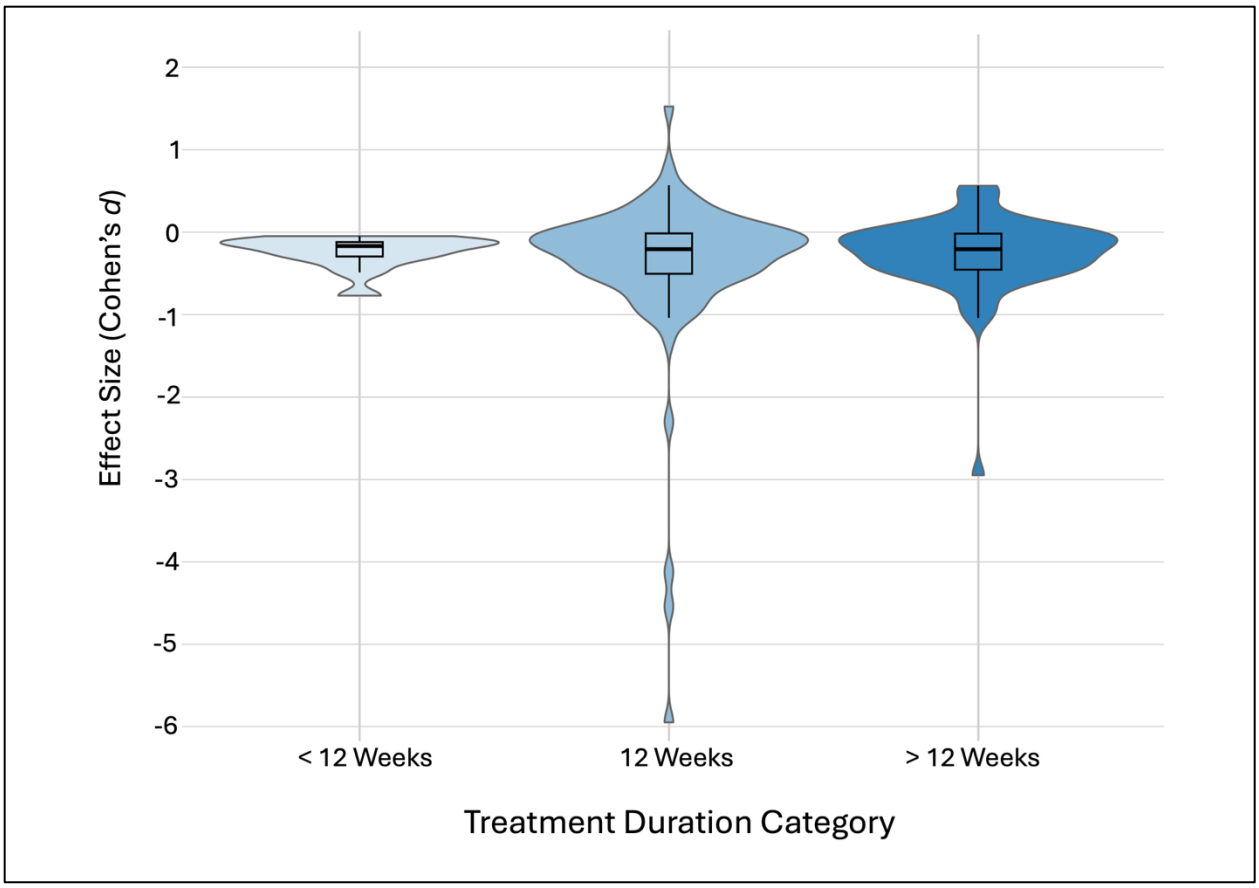
Drinking-based endpoints. For drinking-based endpoints, a total of 221 effect sizes were included, with 158 effect sizes from ≤12-week trials and 63 effect sizes from >12-week trials. The analysis of ≤12-week trials indicated a significant benefit of medication compared to placebo (β = -0.26, SE = 0.03, *p* = < .001, 95% CI [-0.32, -0.20]). There were no significant differences in observed effect sizes between the ≤12-week trials and the >12-week trials (β = 0.07, SE = 0.05, P = .17, 95% CI [-0.03, 0.17]). Results were consistent after adjusting for potential publication bias.

Supplementary Table 2-1. Medications examined across the included studies.

Medication	Number of Studies
Naltrexone	44
Acamprosate	24
Baclofen	11
Topiramate	10
Nalmefene	8
Multiple/combination	8
Gabapentin	7
Quetiapine	5
Varenicline	5
Levetiracetam	2
Olanzapine	2
Ondansetron	2
Prazosin	2
Ritanserin	2
Valproate	2
Aripiprazole	1
Carbamazepine	1
Memantine	1
Rimonabant	1
Zonisamide	1



Supplementary Figure 2-1. Violin plots of effect sizes (Cohen's *d*) for drinking-based endpoints by trial length category. There were no significant differences between shorter (<12 weeks) or longer (>12 weeks) trials compared to the 12-week reference. Negative effect sizes indicate a favorable medication effect over placebo. Internal boxplots indicate the median, IQR and whiskers extending to 1.5 x IQR.



Supplementary Figure 2-2. Violin plots of effect sizes (Cohen's *d*) for drinking-based endpoints by trial length category. There were no significant differences between shorter (<12 weeks) or longer (>12 weeks) trials compared to the 12-week reference. Negative effect sizes indicate a favorable medication effect over placebo. Internal boxplots indicate the median, IQR and whiskers extending to 1.5 x IQR.

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CHAPTER 3: Reductions in cigarette and cannabis use during a randomized clinical trial for alcohol use disorder

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ABSTRACT

Objectives: Alcohol, tobacco, and cannabis are the most frequently used psychoactive substances in the United States and are commonly used concurrently. This study analyzed patterns of cigarette and cannabis use during a randomized controlled trial (RCT) for alcohol use disorder (AUD).

Methods: This secondary analysis of a 12-week randomized controlled trial of ibudilast for alcohol use disorder (N = 102; 61M/41F) used multilevel models to examine trajectories of cigarette and cannabis use over the trial and whether their use was associated with alcohol consumption.

Results: Individuals significantly reduced their cigarette use over the trial ($P = .002$). Cannabis use significantly decreased during the early phase of the trial ($P = .006$) and subsequently increased during the remainder of the trial ($P = .03$). The primary drinking outcome, percent heavy drinking days, was not significantly associated with cigarette or cannabis use ($Ps \geq .22$). However, the secondary drinking outcome, drinks per drinking day, was positively associated with cigarette use across the trial and negatively associated with cannabis use during the early phase of the trial ($Ps < .05$).

Conclusions: Individuals enrolled in an RCT for AUD changed their cigarette and cannabis use without being prompted to, and their use was significantly associated with drinks per drinking day. These findings emphasize the importance of evaluating co-occurring substance use in AUD clinical trials, given that significant changes in cigarette and cannabis use behaviors may occur even when not directly targeted.

INTRODUCTION

Alcohol, tobacco, and cannabis are the most frequently used psychoactive substances in the United States (Substance Abuse and Mental Health Services Administration, 2025). Tobacco and cannabis use are associated with an increased likelihood of developing alcohol use disorder (AUD; Grucza and Bierut, 2006; Weinberger, Platt, and Goodwin, 2016), and individuals commonly report using these substances concurrently (Roche et al., 2019). Moreover, the use of one of these substances is associated with a greater likelihood of engaging in multiple substance use (Roche et al., 2019). Human-laboratory studies have demonstrated that alcohol and nicotine may bidirectionally increase craving and self-administration of each other (Verplaetse and McKee, 2017). Research on the relationship between cannabis and alcohol use is mixed. Several studies suggest that cannabis may enhance alcohol's effects, leading to increased drinking, while other studies support a substitution effect, where cannabis is used in place of alcohol and associated with reduced drinking (Risso et al., 2020; Gunn, Aston, and Metrik, 2022).

Prior research indicates that tobacco and cannabis use during AUD treatment may negatively influence drinking-related outcomes. A systematic review by Van Amsterdam and Van Den Brink (2022) found that of the 31 AUD treatment studies reviewed, 16 reported that cigarette smoking during treatment was associated with poorer alcohol-related outcomes (Van Amsterdam and Van Den Brink, 2022). Research also suggests that cannabis use during treatment for AUD is associated with lower rates of alcohol abstinence after treatment and worsened alcohol-related consequences one year post-treatment (Subbaraman et al., 2017, 2018). However, most existing research has conceptualized the impact of tobacco and cannabis use on treatment outcomes in terms of baseline tobacco/cannabis use status or severity. Few studies

have evaluated the patterns of tobacco and cannabis use during AUD treatment and how these changes relate to drinking.

Given the complex and potentially reinforcing relationships between tobacco, cannabis, and alcohol use, it is crucial to assess patterns of tobacco and cannabis use during AUD treatment. Further, it is important to elucidate how they are related to changes in alcohol use. AUD medication-development clinical trials provide an opportunity to evaluate these patterns and identify potential incidental effects of medication on tobacco and cannabis use. Several AUD randomized controlled trials (RCTs) have reported reductions in cigarette use among individuals enrolled in pharmacotherapy trials for AUD. In a recent RCT evaluating semaglutide for AUD among non-treatment-seeking individuals, participants who smoked cigarettes and received semaglutide showed greater reductions in cigarette use compared to those receiving the placebo (Hendershot et al., 2025). Similarly, in an RCT evaluating mecamylamine for alcohol dependence in treatment-seeking individuals, participants showed reductions in cigarette smoking throughout treatment, across both mecamylamine and placebo groups (Roberts et al., 2018). Moreover, among individuals with more severe nicotine dependence, reductions in drinking were associated with reductions in smoking across the trial (Roberts et al., 2018). Together, these findings provide initial evidence that cigarette use can change over the course of AUD treatment without targeted smoking cessation interventions. Patterns of cannabis use during AUD treatment, to our knowledge, have not yet been examined. Thus, further research is needed to examine how tobacco and cannabis use patterns evolve during AUD treatment and to determine how these changes may relate to alcohol outcomes. This research can inform clinical practice, given that the co-use of tobacco and cannabis in AUD samples is ubiquitous in clinical populations.

To advance our understanding of tobacco and cannabis co-use during AUD treatment, we conducted a secondary analysis of a 12-week phase-2 RCT of ibudilast (IBUD) for the treatment of AUD, to examine patterns of cigarette and cannabis use during the trial (Ray et al., 2025). AUD is associated with elevated systemic inflammation, which is believed to promote continued alcohol consumption and contribute to the development of AUD (Mayfield and Harris, 2017). IBUD, a neuroimmune modulator with anti-inflammatory properties, has been investigated for its potential therapeutic effects in reducing alcohol consumption (Johnson, Matsuda, and Iwaki, 2014). This approach is supported by preclinical findings demonstrating that attenuation of inflammatory signaling in mice leads to reductions in alcohol intake and preference (Blednov et al., 2014). IBUD has shown efficacy in reducing alcohol use in both preclinical and early clinical studies (Bell et al., 2015; Grodin et al., 2021). However, the primary findings from the current trial revealed no significant differences between IBUD and placebo on drinking outcomes, with both groups showing significant reductions in drinking relative to baseline (Ray et al., 2025).

In the current study, we examined (a) whether cigarette and cannabis use changed over the 12-week trial and (b) whether their use was associated with alcohol consumption across the trial. We hypothesized that individuals would show decreases in cigarette and cannabis use and that their use would be positively associated with alcohol consumption. Although the primary trial showed no overall medication effects on drinking outcomes, we conducted exploratory analyses to evaluate the potential impact of IBUD on cigarette and cannabis use outcomes. Given the established pro-inflammatory effects of cigarette use and preliminary yet mixed evidence suggesting cannabis may also engage inflammatory pathways, it is plausible that IBUD may have influenced use trajectories through its anti-inflammatory properties (Lee, Taneja, and Vassallo, 2012; Chandy, Jimenez-Tellez, and Wu, 2025). Finally, we conducted exploratory

analyses to evaluate whether baseline cigarette or cannabis use status moderated alcohol use outcomes and the effect of medication on alcohol use outcomes.

METHODS

Trial Design

The current study is a secondary analysis of a Phase 2, 12-week, double-blind, placebo-controlled, randomized clinical trial of IBUD (50 mg twice daily) for the treatment of AUD (Ray et al., 2025; ClinicalTrials.gov identifier: NCT03594435). 102 treatment-seeking individuals with moderate or severe AUD (61M/41F) were randomized into the study to receive either IBUD (50 mg twice daily) or matched placebo. Participants completed a telephone screening, an in-person eligibility assessment, and a medical examination prior to being randomized. Participants completed in-person follow-up visits at 4, 8, and 12 weeks and telephone assessments of substance use at weeks 2, 6, and 10. This trial was approved by the UCLA Institutional Review Board and monitored by a Data and Safety Monitoring Board. All study participants provided written informed consent. A full description of the trial design can be found in the primary publication of the study (Ray et al., 2025).

Setting and Participants

The trial was conducted at an outpatient research facility at UCLA. Participants were recruited through print, social media, and mass transit advertisements. Inclusion criteria were: (1) aged between 18 and 65; (2) met the DSM-5 diagnostic criteria for moderate or severe alcohol use disorder in the past 12 months; (3) sought treatment for AUD; and (4) reported drinking at least 14 drinks per week for males (7 drinks per week for females) in the 28 days prior to consent. A DSM-5 diagnosis of any substance use disorder other than alcohol or nicotine in the

past 12 months was exclusionary, except for mild cannabis use disorder. Full exclusionary criteria can be found in the primary publication (Ray et al., 2025).

During the initial screening visit, a trained clinician assessed participants for a current AUD diagnosis and exclusionary diagnoses using the Structured Clinical Interview for DSM-5 (SCID-5; First et al., 2015). Eligible participants then met with a physician to review medical history, vital signs, electrocardiogram, and laboratory tests to ensure medical safety for IBUD treatment.

Interventions

Randomization was conducted in a 1:1 ratio, with participants assigned to either IBUD or placebo. This allocation was carried out using a stratified block randomization procedure with gender and heavy drinking status (heavy drinking defined as ≥ 28 drinks/week for men and ≥ 21 drinks/week for women). The allocation sequence was computer-generated by the study statistician, who was not involved in participant enrollment. The research pharmacist managed the blind and implemented the stratified randomization sequence. MediciNova supplied the medication and matched placebo. The target dose was 50 mg/twice daily (5 × 10 mg capsules twice daily). The medication came in blister packaging, and compliance was monitored by the study staff using pill count at each visit. Participants completed the computer-delivered "Take Control" program (Devine et al., 2016), developed for use in AUD pharmacotherapy trials to provide behavioral support for modifying drinking behaviors. Take Control is derived from bibliotherapy and from the interactive web-based tools employed in Rethinking Drinking, an NIAAA-supported online resource for individuals seeking help to quit or cut down on alcohol use (Devine et al., 2016).

Substance Use Assessments and Outcomes

Study staff administered the Timeline Follow-Back (TLFB; Sobell and Sobell, 1992) interview biweekly to participants throughout the 12-week trial. Participants reported on their daily alcohol consumption, number of combustible cigarettes smoked, and cannabis use days (yes/no). Use of alternative nicotine products, including e-cigarettes or oral pouches, were not assessed. Baseline substance use reflected the 30 days before randomization, with follow-up substance use outcomes calculated in 2-week intervals through week 12.

Cigarette Use. Participants were considered to have smoked cigarettes if they reported any use during the 30-day baseline period. Among identified smokers, those who reported smoking on 29–30 days were considered to have engaged in daily smoking. The Fagerström Test for Nicotine Dependence (FTND) was administered at baseline to assess nicotine dependence severity (Heatherton *et al.*, 1991). The cigarette use outcome was the average number of cigarettes smoked per day.

Cannabis Use. Individuals were considered to have used cannabis if they reported any use during the 30-day baseline period. Among identified cannabis users, those who reported use on 10 or more days were considered to have engaged in regular cannabis use (McManus et al., 2025). The Cannabis Use Identification Test – Revised (CUDIT-R; Adamson et al., 2010) and substance use module on the SCID-5 (First et al., 2015) were administered at baseline to assess the severity of problematic cannabis use and cannabis use disorder (CUD) symptom criteria. Individuals with moderate or severe CUD were excluded from the trial. The cannabis use outcome was the percentage of cannabis use days.

Alcohol Use. To maintain consistency with the preregistered protocol of the parent RCT, percent heavy drinking days (PHDD) was the primary drinking outcome. Heavy drinking was

defined as 4+ drinks for women and 5+ for men (NIAAA, 2026). Average drinks per drinking day (DPDD), average drinks per day (DPD), and percent days abstinent (PDA) were analyzed as secondary drinking outcomes and are reported in the supplementary material.

Statistical Analyses

Assessing Cigarette and Cannabis Use Patterns

Multilevel models were conducted in SAS 9.4 to evaluate changes in the average number of cigarettes smoked per day and the percentage of cannabis use days across the trial, assessed at 2-week intervals. Cigarette and cannabis outcomes were analyzed in separate models, and analyses were restricted to participants who reported use of the relevant substance at baseline. For each substance, we estimated models for participants who reported any use at baseline and for those who reported more frequent use at baseline (i.e., daily cigarette smoking or regular cannabis use). Results for the frequent-use models are reported in the supplementary material.

Based on model fit and observed cigarette use patterns, linear mixed models were used to examine the cigarette use outcome. Observed patterns of cannabis use showed an initial decrease from Baseline to Week 2, followed by a slight increase over the remainder of the trial. Based on these observed patterns and model fit indices, piecewise linear mixed models with two linear phases were used for the cannabis use outcome. Phase 1 modeled change from baseline to the two-week follow-up, and Phase 2 captured change from Week 2 to Week 12. All models included random intercepts and slopes to account for individual variability in baseline levels and rates of change in cigarette or cannabis use. Sex was tested as a covariate in each model but was removed because it did not improve model fit or impact results.

To evaluate whether cigarette and cannabis use were associated with drinking over the trial, PHDD (primary drinking outcome) was included as a time-varying predictor in the model.

For cannabis use models, this relationship was estimated separately for Phase 1 and Phase 2. Additional drinking outcomes (DPDD, DPD, and PDA) were examined in parallel models and are reported in the supplementary material.

Exploratory Medication-Focused Analyses

To evaluate whether patterns of cigarette and cannabis use differed by medication condition (IBUD vs. Placebo), medication condition and its interaction with Time (for cigarettes) or Phase (for cannabis) were included as predictors in the model. Baseline CUDIT-R score was included as a covariate in the cannabis model due to significant baseline differences between the IBUD and Placebo groups.

Exploratory Moderation Analyses

To evaluate whether baseline cigarette or cannabis use status moderated the primary drinking outcome (PHDD) and secondary drinking outcomes (DPDD, DPD, and PDA), we extended the primary model described in Ray et al. (2025) to include each use status variable (0 = no use, 1 = use) as a moderator. We also evaluated whether baseline cigarette and cannabis use status moderated the medication effect on drinking outcomes by testing the interaction between medication condition and Phase. Consistent with Ray et al. (2025), longitudinal analyses employed piecewise linear mixed models with two linear phases to model drinking outcomes (Ray *et al.*, 2025). Phase 1 modeled changes in drinking from baseline to the two-week follow-up, and Phase 2 modeled changes in drinking from Week 2 to Week 12.

RESULTS

Participant Characteristics

A total of 102 individuals were randomized to receive either IBUD or placebo (Ray *et al.*, 2025). The average age was 44.26 years (SD = 10.81), and 59.80% were male (**Table 3-1**).

During the 30-day baseline period, participants reported an average of 66.60% heavy drinking days (SD = 34.55), 22.56 drinking days (SD = 7.40), and 7.54 drinks per drinking day (SD = 5.36).

Baseline Cigarette Use Characteristics

Thirty-four (33.33%) individuals reported smoking at least one cigarette during the 30-day baseline period (**Table 3-1**), with an average of 22.53 days (SD = 11.53) of use. Among these individuals, cigarette use was reported on 79.40% (SD = 36.44) of drinking days at baseline. Twenty-three individuals reported daily smoking (29–30 days), with an average of 29.96 days (SD = 0.21). Among individuals who reported any baseline cigarette use, the average number of cigarettes smoked per day was 6.22 (SD = 6.41). Among the individuals who reported daily cigarette use at baseline, the average number of cigarettes smoked per day was 8.76 (SD = 6.33). 50.00% of individuals who reported any smoking were male, and 69.57% of individuals who reported smoking daily were male.

Baseline Cannabis Use Characteristics

Thirty-eight (37.25%) individuals reported consuming cannabis at least once during the 30-day baseline period (**Table 3-1**), with an average of 15.84 (SD = 11.47) use days. Among these individuals, cannabis use was reported on 52.81% (SD = 39.03) of drinking days at baseline. Twenty-four individuals reported regular cannabis use (≥10 days during the 30-day baseline period) with an average of 22.88 (SD = 8.21) use days. Among individuals who reported any cannabis use at baseline, 12 (31.58%) met criteria for mild CUD, and 10 (41.67%) of those who reported regular cannabis use met criteria for mild CUD. 50.00% of individuals who consumed any cannabis were male, and 58.33% of individuals who reported regular cannabis use were male.

Cigarette Smoking Outcomes

Among individuals who reported any cigarette use at baseline (n = 34), average cigarettes per day decreased significantly throughout the trial (β = –0.46, SE = 0.14, 95% CI [–0.74, –0.18], P = .002; **Figure 3-1**). The primary drinking outcome, PHDD, was not associated with cigarette use (β = 0.64, SE = 0.52, 95% CI [–0.39, 1.67], P = .22). However, the secondary drinking outcome, DPDD, showed a significant positive association with cigarette use (β = 0.12, SE = 0.06, 95% CI [0.01, 0.23], P = .04), suggesting that heavier alcohol use, as measured by this outcome, corresponded to heavier cigarette use across the trial. There were no significant associations between cigarette use and the other secondary drinking outcomes (DPD, PDA; *P* > .05). There were no significant differences in cigarette-use trajectories by medication condition (β = 0.34, SE = 0.27, 95% CI [–0.20, 0.88], P = .22). Parallel models conducted in the subgroup of individuals who reported daily cigarette use at baseline produced similar results and are reported in Supplementary Material.

Cannabis Use Outcomes

Among participants who reported any cannabis use at baseline (n = 38), percent cannabis use days decreased significantly during Phase 1 (β = –0.13, SE = 0.05, 95% CI [–0.23, –0.04], P = .006) and increased significantly during Phase 2 (β = 0.02, SE = 0.01, 95% CI [0.003, 0.04], P = .03; **Figure 3-2**). The primary drinking outcome, PHDD, was not associated with cannabis use during Phase 1 (β = -0.02, SE = 0.07, 95% CI [-0.15, 0.11], P = .77) or Phase 2 (β = 0.009, SE = 0.02, 95% CI [-0.03, 0.05], P = .68). However, the secondary drinking outcome, DPDD, showed a significant negative association with cannabis use during Phase 1 (β = −0.01, SE = 0.01, 95% CI [−0.03, −0.00], P = .049), and no association during Phase 2 (β = 0.00, SE = 0.00, 95% CI [−0.00, 0.01], P = .74). This negative association indicates an inverse relationship between

DPDD and cannabis use during the early phase of the trial, such that lighter alcohol use corresponded to heavier cannabis use. There were no significant associations between cannabis use and the other secondary drinking outcomes (DPD, PDA; $P > .05$). There were no significant differences in cannabis-use trajectories by medication condition during Phase 1 (β = -0.10, SE = 0.09, 95% CI [–0.29, 0.08], P = .27) or Phase 2 (β = -0.01, SE = 0.02, 95% CI [–0.05, 0.03], P = .57). Parallel models conducted in the subgroup of individuals who reported regular cannabis use at baseline produced similar results and are reported in Supplementary Material.

Exploratory Moderation Analyses

Lastly, we utilized piecewise linear mixed models to test whether baseline cigarette and cannabis use status influenced changes in alcohol use (PHDD) throughout the trial. Consistent with analyses in the primary publication, PHDD was modeled in two phases: Phase 1 captured changes in PHDD from Baseline to Week 2, and Phase 2 captured changes in PHDD from Week 2 to Week 12 (Ray *et al.*, 2025).

Cigarette smoking status did not significantly moderate PHDD trajectories during Phase 1 (β = -0.11, SE = 0.08, 95% CI [-0.26, 0.04], P = .14) or Phase 2 (β = 0.10, SE = 0.08, 95% CI [-0.07, 0.26], P = .24) of the trial. Additionally, smoking status did not moderate the effect of medication on PHDD trajectories during Phase 1 (β = 0.02, SE = 0.15, 95% CI [-0.29, 0.32], P = .92) or Phase 2 (β = -0.06, SE = 0.17, 95% CI [-0.40, 0.27], P = .70). Additional drinking outcomes were evaluated in these moderation analyses and showed similar null effects (see Supplementary Material).

Cannabis use status did not significantly moderate PHDD trajectories during Phase 1 (β = 0.01, SE = 0.07, 95% CI [-0.14, 0.16], P = .91) or Phase 2 (β = -0.05, SE = 0.08, 95% CI [-0.22, 0.11], P = .51) of the trial. Additionally, cannabis use status did not moderate the effect of

medication on PHDD trajectories during Phase 1 (β = 0.02, SE = 0.15, 95% CI [-0.29, 0.32], *P* = .92) or Phase 2 (β = -0.06, SE = 0.17, 95% CI [-0.40, 0.27], *P* = .70). Additional drinking outcomes were evaluated in these moderation analyses and showed similar null effects (see Supplementary Material).

DISCUSSION

In this secondary analysis of a 12-week randomized controlled medication trial for AUD, cigarette use significantly decreased across the trial, while cannabis use decreased early in the trial and subsequently increased. Among the individuals who smoked cigarettes at baseline, average daily cigarette use decreased from 6.22 to 3.40 cigarettes per day, and from 8.43 to 4.09 cigarettes per day among the subset of individuals who smoked daily at baseline. This roughly 50% reduction in cigarette use from baseline to the end of the treatment period represents a clinically significant decrease (Chang et al., 2021). Changes in cigarette smoking and cannabis use were not significantly associated with changes in the primary drinking outcome of percent heavy drinking days. However, analyses of the secondary drinking outcomes showed that changes in cigarette use were positively associated with changes in drinks per drinking day, while changes in cannabis use were negatively associated with changes in drinks per drinking day during the early phase of the trial. Exploratory analyses revealed no medication effects on cigarette and cannabis use, indicating that both the ibudilast and placebo groups experienced similar decreases in use. Baseline cigarette or cannabis use status did not influence changes in drinking outcomes or the impact of medication on drinking outcomes, indicating that co-use at baseline did not influence treatment response.

These findings indicated that participants were able to change their cigarette and cannabis use without being prompted to, and that these changes were not influenced by ibudilast. Several factors may explain the observed reductions in cigarette use and early-phase cannabis use. One possibility is assessment reactivity, where repeatedly reporting substance use increases its salience, making participants more aware of their behaviors and potentially prompting a reduction in use (Schrimsher and Filtz, 2011; Meier et al., 2017). Participants had regular visits with study staff throughout the trial, which may have provided participants with a sense of support and accountability. Additionally, all participants completed "Take Control" behavioral modules**,** which, although designed to support alcohol reduction, included strategies (e.g., coping with urges, managing triggers) that may have applied to cigarette and cannabis use (Devine et al., 2016). Another possibility is broad motivation for behavior change. Although the trial focused on reducing alcohol consumption, participants may have entered the study with a general intention to improve their health, which may have led to the observed reductions in other substance use (Grosso et al., 2013).

Interestingly, while cigarette use decreased in a linear pattern throughout the trial, cannabis use showed an initial decline followed by an increase in use after Week 2. While the initial decrease may be attributable to factors previously noted, such as assessment reactivity, one possible explanation for the subsequent increase is that participants may perceive cannabis as less harmful than cigarettes or alcohol and therefore may not view it as a health behavior in need of change (Chambers et al., 2023). This trial was conducted in the state of California, where recreational cannabis use was legalized in 2016 (Hill et al., 2025). The legalization and commercialization of recreational cannabis have been associated with lower perceived risk and more positive attitudes toward cannabis use (Schuermeyer et al., 2014; Hill et al., 2025). Thus,

the observed increase in cannabis use following an initial period of reduction may be specific to the perception of cannabis use in California and should be explored in additional AUD medication settings.

The current findings demonstrated that cigarette and cannabis use were significantly associated with drinks per drinking day during the trial, though in opposite directions. The association between cigarette use and drinks per drinking day was positive, suggesting that lower alcohol use corresponded to lower cigarette use across the trial. These findings are consistent with prior research in both human laboratory and clinical trial settings showing that cigarette and alcohol use are positively associated (Verplaetse and McKee, 2017; Roberts et al., 2018; Roche et al., 2019). In contrast, the association between cannabis use and drinks per drinking day was negative during the first phase of the trial, when alcohol use showed the steepest reductions, indicating that lower alcohol use corresponded to higher cannabis use during this period. This negative association may align with the substitution theory, in which reductions in alcohol use coincide with increased cannabis use as a form of compensation (Gunn et al., 2022). Notably, we did not see these significant associations with the other drinking measures, suggesting that alcohol use outcomes may vary in how they relate to the examined cigarette and cannabis use measures.

In this trial, baseline cigarette or cannabis use status did not significantly influence drinking outcomes or medication effects on drinking outcomes. These findings suggest that cigarette and cannabis use may not compromise treatment response in AUD trials, at least when medication effects are null, and there are strong placebo effects. They also challenge traditional beliefs that addressing other substance use compromises alcohol treatment, and support integrating care for co-occurring substances, especially tobacco, into AUD treatment (Chiauzzi

and Liljegren, 1993; Substance Abuse and Mental Health Services Administration, 2021; American Society of Addiction Medicine, 2022).

The current findings should be interpreted in light of the study's strengths and limitations. Study strengths include a focus on tobacco and cannabis use in individuals with AUD, which is associated with worsened drinking and health-related outcomes (Durazzo, Gazdzinski, and Meyerhoff, 2007; Venegas et al., 2022). Another study strength was the longitudinal data on tobacco, cannabis, and alcohol use across the 12-week medication RCT. Collecting data on all three substances allowed for a comprehensive analysis of substance use trajectories during AUD treatment. The study is limited by the relatively small number of participants who reported cigarette or cannabis use at baseline in this trial, which reduced statistical power, especially for subgroup analyses. The small sample size also prohibited examining potential sex differences in cigarette smoking and cannabis use throughout the trial. Additionally, data were only collected on combustible cigarette use, with no information on alternative forms of nicotine consumption, which are increasing in popularity and may influence overall nicotine exposure (Osterman et al., 2025; Felicione et al., 2025). Similarly, cannabis use was recorded in terms of use days, but did not capture quantity, potency, or mode of administration. Furthermore, biochemical verification of cigarette and cannabis use was not conducted. Future research should more thoroughly characterize nicotine and cannabis use during AUD clinical trials. Lastly, individuals with moderate or severe CUD were excluded from the trial, and the sample exhibited relatively low nicotine dependence. Consequently, these findings may not generalize to individuals with more severe cannabis and cigarette use.

In summary, this secondary analysis found that reductions in cigarette smoking and early-stage reductions in cannabis use occurred during an AUD randomized controlled treatment trial,

despite these substances not being direct targets of intervention. These findings highlight the value of evaluating concurrent substance use in clinical trials, given that significant changes in cigarette and cannabis use behaviors can occur beyond the primary focus of intervention. Clinically, these results support a more flexible approach to treating AUD that also considers cigarette and cannabis use, rather than focusing solely on alcohol use.

Table 3-1. Participant demographics and baseline substance use characteristics.

All Randomized Participants				
Variable (SD)	**Total (n = 102)**	**IBUD (n = 53)**	**Placebo (n = 49)**	***p* value**
Sex (M/F)	61/41	32/21	29/20	.90
Age (Years)	44.26 (10.81)	42.74 (10.01)	45.92 (11.49)	.14
Drinking Characteristics				
% Heavy Drinking Days	66.60% (34.55)	67.5% (34.55)	65.7% (34.89)	.79
No. Drinking Days	22.56 (7.40)	22.42 (7.46)	22.71 (7.41)	.84
Drinks per Drinking Day	7.54 (5.36)	7.57 (4.30)	7.52 (3.36)	.95
PACS Alcohol Craving	13.78 (6.17)	13.83 (5.85)	13.73 (6.56)	.94
AUDIT Score	20.24 (7.35)	21.23 (8.24)	19.16 (6.14)	.15
Participants Who Smoke Cigarettes				
Variable (SD)	**Total (n = 34)**	**IBUD (n = 17)**	**Placebo (n = 17)**	***p* value**
Sex (M/F)	17/17	10/7	7/10	.30
Age (Years)	42.88 (11.62)	41.41 (9.86)	44.35 (12.30)	.45
No. Cigarette Use Days	22.53 (11.58)	25.88 (9.61)	19.18 (12.66)	.09
Average Cigarettes per Day	6.22 (6.41)	8.10 (7.44)	4.34 (4.68)	.09
Cigarettes per Smoking Day	6.91 (6.01)	8.50 (7.12)	5.33 (4.30)	.13
FTND Score	2.91 (2.77)	3.47 (2.60)	2.35 (2.89)	.24
Drinking Characteristics				
% Heavy Drinking Days	64.73% (34.68)	68.70 (35.49)	60.76% (34.45)	.51
No. Drinking Days	21.24 (7.46)	21.24 (8.46)	21.24 (6.56)	1.00
Drinks per Drinking Day	7.00 (4.01)	8.23 (4.76)	5.77 (2.68)	.08
PACS Alcohol Craving	16.20 (5.41)	16.76 (4.94)	15.65 (5.95)	.56
AUDIT Score	22.71 (7.01)	24.35 (8.05)	21.06 (5.56)	.18
Participants Who Use Cannabis				
Variable (SD)	**Total (n = 38)**	**IBUD (n=20)**	**Placebo (n=18)**	***p* value**
Sex (M/F)	19/19	9/11	10/8	.52
Age (Years)	40.47 (11.65)	38.25 (10.85)	42.94 (12.31)	.22
% Cannabis Use Days	52.81% (38.23)	44.17% (37.48)	62.41% (37.76)	.14
Mild CUD, No. (%)	12 (31.58%)	6 (30.00%)	6 (33.33%)	.83
CUDIT-R Score	8.61 (5.43)	9.30 (5.81)	7.83 (5.01)	**.03**

Drinking Characteristics				
% Heavy Drinking Days	64.06% (33.36)	68.25% (34.77)	59.40% (6.97)	.28
No. Drinking Days	21.66 (7.46)	22.15 (8.27)	21.11 (6.64)	.67
Drinks per Drinking Day	6.93 (4.19)	6.91 (3.74)	6.95 (4.75)	.98
PACS Alcohol Craving	14.34 (6.41)	14.7 (6.05)	13.94 (6.94)	.72
AUDIT Score	19.97 (6.87)	21.10 (7.06)	18.72 (6.61)	.29

Substance use measures were derived from the Timeline Follow Back and reflected substance use during the 30 days before the baseline visit. Medication group comparisons were conducted using chi-square tests for categorical variables and Welch's two-tailed T-tests for continuous variables. Bolded p-values indicate statistically significant differences between groups. Abbreviations: PACS, Penn Alcohol Craving Scale; AUDIT, Alcohol Use Disorders Identification Test; FTND, Fagerström Test for Nicotine Dependence; CUDIT-R, Cannabis Use Identification Test - Revised.

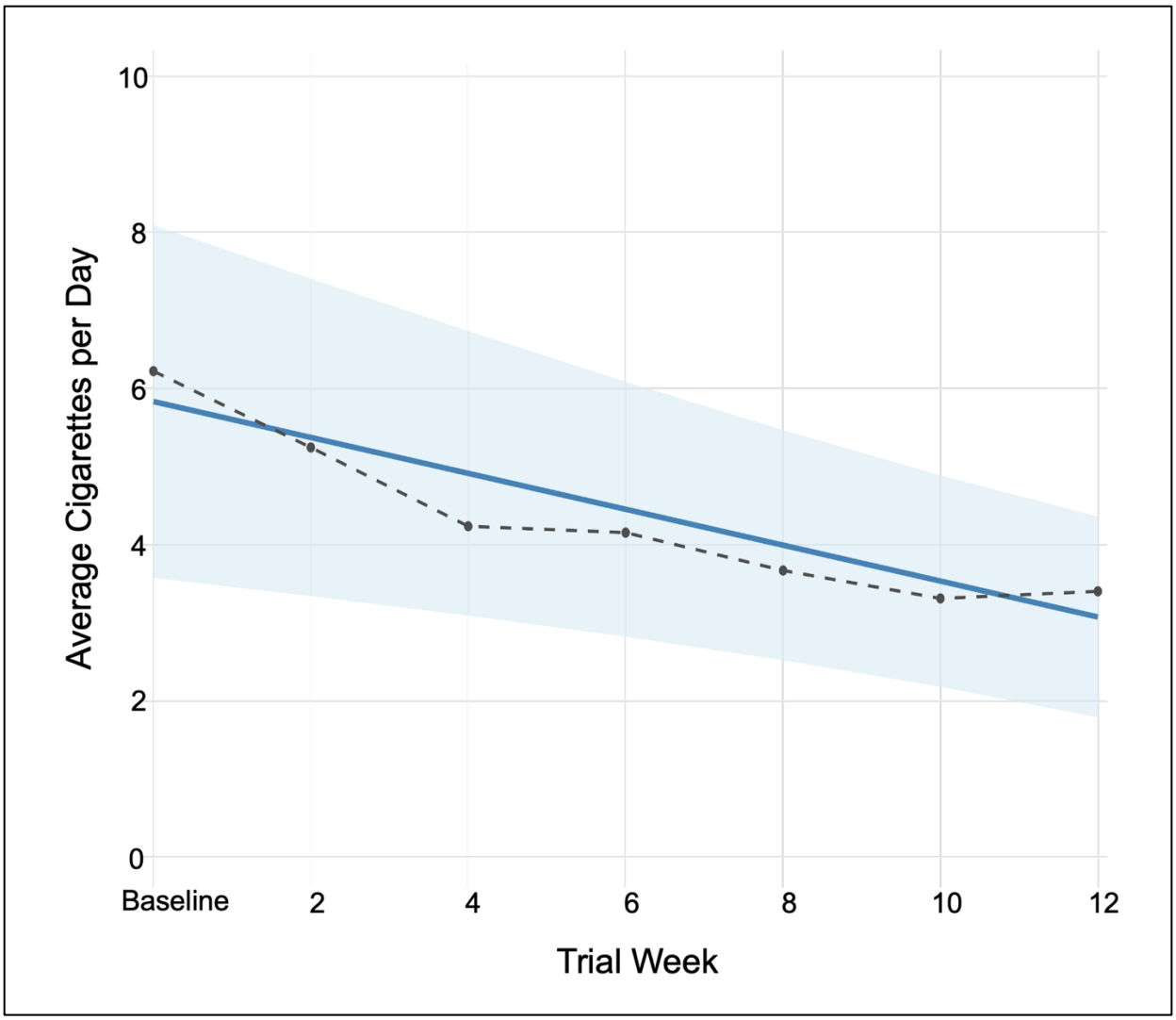

Figure 3-1. Change in average number of cigarettes smoked per day over the 12-week trial among participants who reported any cigarette use at baseline. Solid lines represent model-estimated means with 95% CIs, and dashed lines indicate observed means.

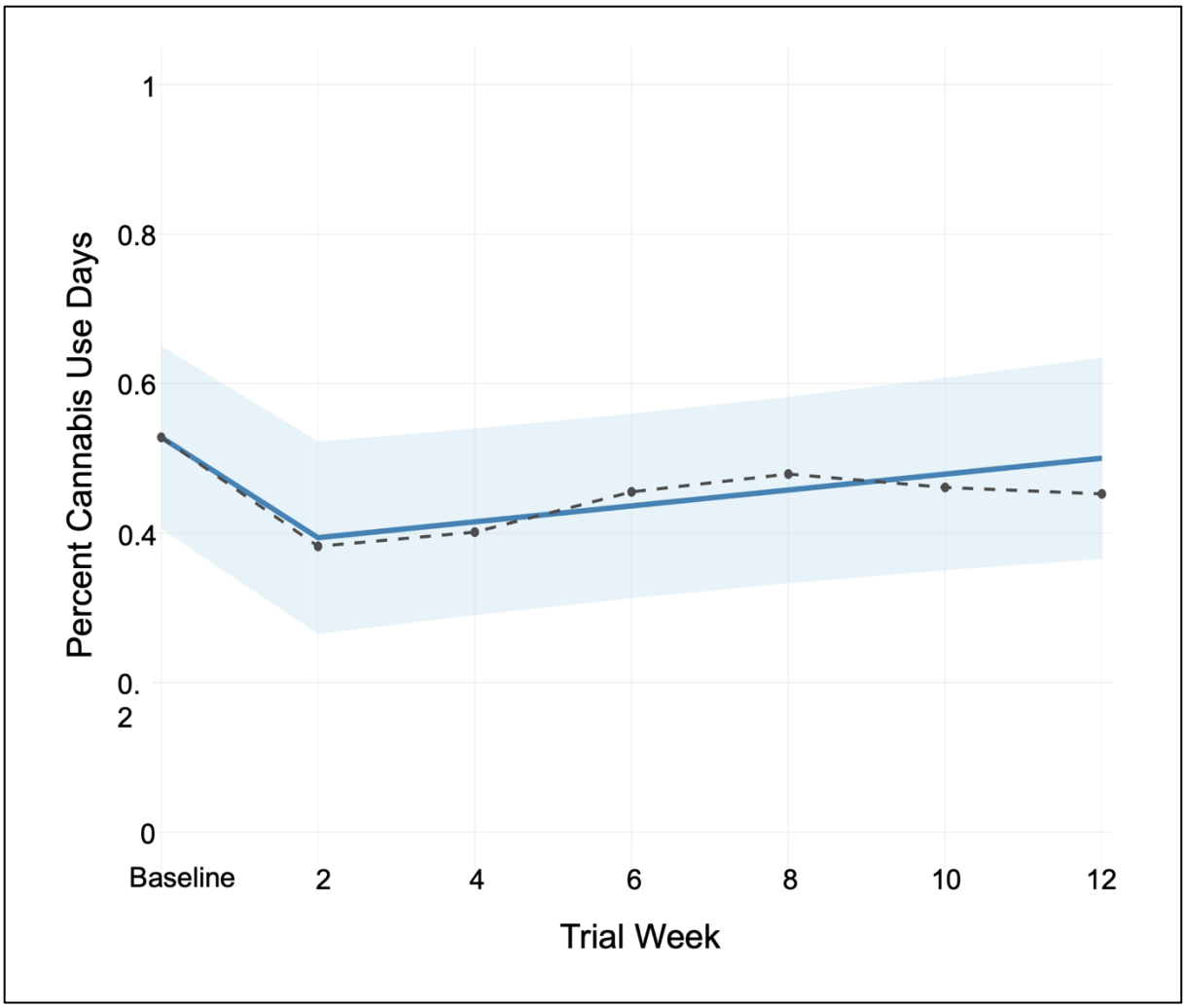

Figure 3-2. Change in percent cannabis use days per day over the 12-week trial among participants who reported any cannabis use at baseline. Solid lines represent model-estimated means with 95% CIs, and dashed lines indicate observed means.

CHAPTER 3 SUPPLEMENTAL MATERIAL

Frequent Use Subgroup Analyses

Cigarette Smoking Outcomes. Among individuals who reported daily cigarette use at baseline (n = 23), cigarette use decreased significantly throughout the trial (β = –0.67, SE = 0.17, 95% CI [–1.03, –0.31], P = .001).

Cannabis Use Outcomes. Among individuals who reported regular cannabis use at baseline (n = 24), percent cannabis use days decreased significantly during Phase 1 (β = –0.19, SE = 0.06, 95% CI [−0.32, −0.07], P = .003), but did not change significantly during Phase 2 (β = 0.01, SE = 0.01, 95% CI [−0.01, 0.04], P = .27).

Associations Between Cigarette Use and Secondary Drinking Outcomes

Percent heavy drinking days (daily use subgroup). Among individuals who reported daily cigarette use at baseline, cigarette use was not significantly associated with PHDD (β = 0.39, SE = 0.64, 95% CI [−0.88, 1.66], P = .54).

Drinks per drinking day. Among individuals who reported any cigarette use at baseline, cigarette use was significantly associated with DPDD (β = 0.12, SE = 0.06, 95% CI [0.01, 0.23], P = .04), but not among the subgroup of individuals who reported daily cigarette use at baseline (β = 0.09, SE = 0.07, 95% CI [−0.04, 0.22], P = .16).

Drinks per day. Among individuals who reported any cigarette use at baseline, and among the subgroup of individuals who reported daily cigarette use, cigarette use was not significantly associated with DPD (any use: β = 0.18, SE = 0.09, 95% CI [−0.00, 0.36], P = .06; daily use: β = 0.18, SE = 0.11, 95% CI [−0.04, 0.40], P = .11).

Percent days abstinent. Among individuals who reported any cigarette use at baseline, and among the subgroup of individuals who reported daily cigarette use, cigarette use was not significantly associated with PDA (Any use: β = −1.16, SE = 0.78, 95% CI [−2.72, 0.39], P = .14; daily use: β = −1.84, SE = 1.01, 95% CI [−3.84, 0.17], P = .07).

Associations Between Cannabis Use and Secondary Drinking Outcomes

Percent heavy drinking day (regular use subgroup). Among individuals who reported regular cannabis use at baseline, cannabis use was not significantly associated with PHDD during Phase 1 (β = −0.06, SE = 0.08, 95% CI [−0.22, 0.10], P = .49) or Phase 2 (β = 0.03, SE = 0.02, 95% CI [−0.02, 0.08], P = .31).

Drinks per drinking day. Among individuals who reported any cannabis use at baseline, cannabis use was significantly associated with DPDD during Phase 1 (β = −0.01, SE = 0.01, 95% CI [−0.03, −0.00], P = .049) but not Phase 2 (β = 0.00, SE = 0.00, 95% CI [−0.00, 0.01], P = .74). Among the subgroup of individuals who reported regular cannabis use at baseline, cannabis use was significantly associated with DPDD during Phase 1 (β = −0.02, SE = 0.01, 95% CI [−0.03, −0.00], P = .02) but not Phase 2 (β = 0.00, SE = 0.00, 95% CI [−0.00, 0.01], P = .39).

Drinks per day. Among individuals who reported any cannabis use at baseline, cannabis use was not significantly associated with DPD during Phase 1 (β = −0.01, SE = 0.01, 95% CI [−0.04, 0.01], P = .23) or Phase 2 (β = 0.00, SE = 0.00, 95% CI [−0.01, 0.01], P = .57). Among the subgroup of individuals who reported regular cannabis use at baseline, cannabis use was not significantly associated with DPD during Phase 1 (β = −0.01, SE = 0.01, 95% CI [−0.03, 0.02], *P* = .56) or Phase 2 (β = –0.00, SE = 0.00, 95% CI [−0.01, 0.01], P = .81).

Percent days abstinent. Among individuals who reported any cannabis use at baseline, cannabis use was not significantly associated with PDA during Phase 1 (β = 0.09, SE = 0.09, 95% CI [−0.09, 0.27], P = .35) or Phase 2 (β = −0.01, SE = 0.03, 95% CI [−0.06, 0.04], P = .66). Among the subgroup of individuals who reported regular cannabis use at baseline, cannabis use was not significantly associated with PDA during Phase 1 (β = −0.13, SE = 0.11, 95% CI [−0.35, 0.10], P = .26) or Phase 2 (β = 0.04, SE = 0.03, 95% CI [−0.02, 0.10], P = .17).

Cigarette Use Status as a Moderator of Secondary Drinking Outcomes and Medication Effects

Drinks per drinking day. Cigarette smoking status did not significantly moderate DPDD trajectories during Phase 1 (β = −0.68, SE = 0.95, 95% CI [−2.54, 1.19], P = .48) or Phase 2 (β = 0.34, SE = 0.96, 95% CI [−1.55, 2.23], P = 0.72). The effect of medication on DPDD was not moderated by cigarette smoking status (Phase 1: β = -3.27, SE = 1.88, 95% CI [−6.97, 0.43], P = 0.08; Phase 2: β = 3.28, SE = 1.91, 95% CI [−0.48, 7.04], P = 0.09).

Drinks per day. Cigarette smoking status did not significantly moderate DPD trajectories during Phase 1 (β = −0.45, SE = 0.68, 95% CI [−1.80, 0.89], P = .51) or Phase 2 (β = 0.27, SE = 0.69, 95% CI [−1.09, 1.63], P = .69). The effect of medication on DPD was not moderated by cigarette smoking status (Phase 1: β = −1.72, SE = 1.37, 95% CI [−4.42, 0.98], P = .21; Phase 2: β = 1.51, SE = 1.39, 95% CI [−1.23, 4.25], P = .28).

Percent days abstinent. Cigarette smoking status did not significantly moderate PDA trajectories during Phase 1 (β = −0.00, SE = 0.06, 95% CI [−0.12, 0.12], P = .99) or Phase 2 (β = 0.01, SE = 0.07, 95% CI [−0.12, 0.15], P = 0.83). The effect of medication on PDA was not moderated by cigarette smoking status (Phase 1: β = −0.07, SE = 0.12, 95% CI [−0.32, 0.17], P = .55; Phase 2: β = 0.11, SE = 0.14, 95% CI [−0.16, 0.37], P = .44).

Cannabis Use Status as a Moderator of Secondary Drinking Outcomes and Medication Effects

Drinks per drinking day. Cannabis use status did not significantly moderate DPDD trajectories during Phase 1 (β = −0.54, SE = 0.93, 95% CI [−2.36, 1.29], P = .56) or Phase 2 (β = 0.36, SE = 0.94, 95% CI [−1.49, 2.20], P = .70). Additionally, the effect of medication on DPDD was not moderated by cannabis use status (Phase 1: β = −0.95, SE = 1.87, 95% CI [−4.63, 2.72], P = .61; Phase 2: β = 1.37, SE = 1.89, 95% CI [−2.34, 5.08], P = .72).

Drinks per day. Cannabis use status did not significantly moderate DPD trajectories during Phase 1 (β = −0.41, SE = 0.67, 95% CI [−1.73, 0.91], P = .54) or Phase 2 (β = 0.29, SE = 0.68, 95% CI [−1.05, 1.62], P = .67). The effect of medication on DPD was not moderated by cannabis use status (Phase 1: β = 0.13, SE = 1.36, 95% CI [−2.54, 2.80], P = .92; Phase 2: β = 0.01, SE = 1.37, 95% CI [−2.68, 2.70], P = .99).

Percent days abstinent. Cannabis use status did not significantly moderate PDA trajectories during Phase 1 (β = 0.01, SE = 0.06, 95% CI [−0.11, 0.12], P = .91) or Phase 2 (β = 0.01, SE = 0.07, 95% CI [−0.12, 0.14], P = .90). The effect of medication on PDA was not moderated by cannabis use status (Phase 1: β = −0.02, SE = 0.12, 95% CI [−0.26, 0.22], P = .87; Phase 2: β = 0.03, SE = 0.13, 95% CI [−0.23, 0.29], P = .84).

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PART II: Integrating Biological Markers into Alcohol Use Disorder Clinical Trials

CHAPTER 4: C-reactive Protein and Intestinal Permeability as Moderators of Ibudilast Treatment Response and Correlates of Clinical Characteristics in Alcohol Use Disorder

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ABSTRACT

Alcohol use disorder (AUD) is associated with heightened systemic inflammation, and increased intestinal permeability has been proposed as a potential contributor to this elevated inflammatory state. This secondary analysis examined whether baseline peripheral inflammatory and gut permeability markers moderated the efficacy of ibudilast (IBUD), a phosphodiesterase-4 inhibitor with anti-inflammatory properties, in a 12-week randomized clinical trial for the treatment of AUD. Participants with moderate-to-severe AUD (N = 99) were enrolled in a double-blind, placebo-controlled trial of IBUD (50 mg twice daily). Baseline plasma concentrations of C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), soluble cluster of differentiation 14 (sCD14), and fatty acid-binding protein 2 (FABP2) were assessed. Linear mixed-effects models examined whether these biomarkers moderated treatment effects on drinking outcomes across the treatment period. Correlations among the markers and associations between markers and baseline clinical characteristics were evaluated. Baseline CRP levels significantly moderated the effects of IBUD on percent heavy drinking days and drinks per day, with treatment effects differing across time. Similarly, FABP2 significantly moderated treatment effects on drinks per day, with effects varying across time. Baseline levels of CRP, sCD14, and LBP showed associations with alcohol-related clinical characteristics, supporting their potential clinical relevance in AUD. These findings highlight the potential utility of inflammatory and gut-related biomarkers for identifying subgroups of individuals who may differentially respond to treatment and underscore the need for further research examining inflammatory and gut-related processes in AUD.

INTRODUCTION

A growing body of literature suggests that the immune system contributes to the development and maintenance of alcohol use disorder (AUD) (Coleman & Crews, 2018; Mayfield & Harris, 2017; Meredith et al., 2021). Alcohol promotes inflammation through multiple pathways, including increased systemic cytokine production and direct activation of immune signaling in the brain (Coleman & Crews, 2018; Crews & Vetreno, 2016). Additionally, chronic alcohol use is associated with increased intestinal permeability, colloquially referred to as "leaky gut," which allows gut-derived microbial products, including the endotoxin lipopolysaccharide (LPS), to enter circulation and promote systemic inflammation (Bishehsari et al., 2017). Consistent with this process, individuals with AUD exhibit higher levels of markers of intestinal permeability and microbial translocation compared with healthy controls (Donnadieu-Rigole et al., 2018). Among individuals with high baseline intestinal permeability, levels significantly decreased following a period of abstinence from alcohol, further supporting the association between alcohol use and intestinal permeability (Leclercq et al., 2014). Markers of intestinal permeability have also been positively associated with markers of systemic inflammation, including C-reactive protein (CRP) (Lakatos et al., 2011; Lim et al., 2019), although these relationships have not yet been examined in an AUD sample. These findings suggest that alterations in intestinal permeability may represent an important pathway contributing to inflammatory processes in AUD.

Prolonged inflammatory responses associated with chronic alcohol use, arising in part through increased microbial translocation from greater intestinal permeability, are believed to contribute to the maintenance of AUD (Coleman & Crews, 2018). Prior studies in individuals with AUD have found that pro-inflammatory cytokine levels are positively associated with

alcohol consumption, as well as increased alcohol craving during acute withdrawal (Heberlein et al., 2014; Leclercq et al., 2014). Preclinical research has demonstrated that experimentally inducing an inflammatory response in mice leads to long-lasting increases in alcohol consumption, whereas inhibiting enzymes critical to inflammatory signaling reduces ethanol intake and preference (Blednov et al., 2011, 2014). Collectively, these findings suggest that inflammatory processes may contribute to alcohol-related behaviors, highlighting the inflammatory system as a potential biological target for AUD pharmacotherapies (Grodin, 2024; Meredith et al., 2021).

Ibudilast (IBUD) is a small compound molecule that preferentially inhibits phosphodiesterase (PDE)3A, PDE4, PDE10A, and PDE11A and inhibits macrophage migration inhibitory factor (MIF) (Cho et al., 2010; Gibson et al., 2006). PDE4 and MMIF are critically involved in proinflammatory signaling (Fan et al., 2024; Mitchell et al., 2002), and thus, inhibition of these targets is theorized to reduce inflammation (Johnson et al., 2014). There is also evidence suggesting that PDE4 inhibition may reduce intestinal inflammation and support intestinal barrier functioning (Chen et al., 2025; Spadaccini et al., 2017). IBUD has been investigated as a potential pharmacological treatment for AUD, given its putative anti-inflammatory properties. Preclinically, IBUD has been shown to reduce ethanol intake by approximately 50% in selectively bred ‘alcohol-preferring’ and ‘high alcohol drinking’ rats, both under conditions of maintenance and relapse testing (Bell et al., 2015). Prior clinical work from our group has demonstrated the initial efficacy of IBUD in non-treatment-seeking individuals. Specifically, a 7-day human laboratory study and a 14-day double-blind micro-longitudinal trial demonstrated that IBUD, compared to placebo, attenuated tonic alcohol craving and reduced rates of heavy drinking (Grodin et al., 2021; Ray et al., 2017). A secondary analysis of the micro-

longitudinal trial found that baseline levels of peripheral inflammation, as indexed by circulating C-reactive protein (CRP) levels, moderated the response to IBUD, such that individuals in the IBUD group with higher baseline CRP levels reported fewer drinks per drinking day than those with lower baseline CRP levels (Grodin et al., 2023). To build on these promising early efficacy findings, our laboratory conducted a 12-week, double-blind, randomized controlled trial of IBUD (50 mg twice daily) versus placebo in 102 treatment-seeking individuals with moderate to severe AUD (Ray et al., 2025). The primary analysis of 102 randomized participants showed no significant differences in drinking outcomes between IBUD and placebo (Ray et al., 2025). However, over the course of the 12-week trial, both groups showed significant decreases in drinking from baseline. Interestingly, there were no differences in peripheral inflammatory markers and cytokines between IBUD and placebo over the 12-week trial, nor was there an overall change in these inflammatory markers across the trial (Ray et al., 2025).

Notably, baseline inflammation in this sample varied substantially across participants. For example, CRP, a widely used marker of systemic inflammation (Amezcua-Castillo et al., 2023), showed high variability, indicating significant heterogeneity in baseline inflammatory status. This variability aligns with the broader characterization of AUD as a highly heterogeneous disorder (Litten et al., 2015) and with prior research showing that individuals with AUD do not uniformly exhibit elevated inflammation (Adams et al., 2020). Given IBUD's proposed anti-inflammatory mechanism, its efficacy as an AUD treatment may depend on the presence of heightened baseline inflammation. Given that increased intestinal permeability is thought to contribute to inflammatory signaling in AUD, and that PDE4 inhibition may influence intestinal inflammation and barrier function, intestinal permeability-related processes may also be relevant to understanding variability in response to IBUD.

The current study extends Grodin et al.'s (2023) findings by examining whether baseline CRP, as well as markers of intestinal permeability, moderate the effects of IBUD on drinking outcomes in the 12-week, double-blind, randomized controlled trial of IBUD versus placebo in treatment-seeking individuals with moderate to severe AUD. Peripheral inflammation was indexed by CRP, given its well-established clinical utility as a marker of systemic inflammation (Amezcua-Castillo et al., 2023) and prior findings implicating baseline CRP in clinical response to IBUD (Grodin et al., 2023). Intestinal permeability markers were examined as exploratory measures reflecting alcohol-related gut barrier disruption, which may serve as a more proximal index of inflammation relevant to alcohol use (Bishehsari et al., 2017). Specifically, we examined plasma levels of lipopolysaccharide-binding protein (LBP) and soluble cluster of differentiation 14 (sCD14), which reflect LPS exposure and immune activation, as well as fatty acid-binding protein 2 (FABP2), which reflects intestinal epithelial integrity (Bibolar et al., 2025). These markers have been used as indices of intestinal permeability (Bibolar et al., 2025; Stevens et al., 2018; Tabung et al., 2017). We hypothesized that individuals with higher baseline levels of CRP and intestinal permeability markers would exhibit a greater reduction in drinking, as measured by the percentage of heavy drinking days and average drinks per day, following IBUD treatment compared to placebo, relative to individuals with lower baseline levels. Given the novelty of the intestinal permeability markers in AUD, we examined the relationships among these markers, their associations with CRP, and their associations with AUD clinical characteristics, including alcohol consumption, craving, and symptom severity. We hypothesized that higher levels of intestinal permeability markers would be positively associated with one another and with CRP, and that higher levels of intestinal permeability and inflammation would be associated with greater alcohol consumption, craving, and AUD symptom severity.

METHODS

Trial design

This secondary analysis utilized data from a Phase 2, 12-week, double-blind, randomized placebo-controlled trial of IBUD for the treatment of AUD (Ray et al., 2025) (Clinical trials identifier: NCT03594435). 102 individuals with moderate or severe AUD (61 M/41F) who were seeking treatment for AUD were randomized to receive either IBUD (50 mg twice a day) or placebo. Participants completed eligibility assessments and a medical examination prior to being randomized into the study. After randomization, participants completed in-person follow-up visits at weeks 4, 8, and 12. Additionally, follow-up telephone assessments of substance use were conducted at weeks 2, 6, and 10. This trial was approved by the UCLA Institutional Review Board and monitored by a Data and Safety Monitoring Board. All study participants provided written informed consent before participation in study procedures. Additional information regarding the trial design can be found in the trial protocol and primary publication of the study (Burnette et al., 2020; Ray et al., 2025).

Setting and Participants

The trial was conducted at an outpatient research facility at UCLA. Participants were recruited through print, social media, and mass transit advertisements. The inclusion criteria for participants included: (1) being between 18 and 65 years of age; (2) meeting DSM-5 diagnostic criteria for moderate or severe AUD within the past 12 months; (3) seeking treatment for AUD; and (4) reporting consumption of at least 14 drinks per week for males or 7 drinks per week for females in the 28 days before consent. A DSM-5 diagnosis of any substance use disorder other than alcohol or nicotine in the past 12 months was exclusionary, except for mild cannabis use disorder (CUD). Additionally, participants were excluded if they had a medical condition that

may have interfered with their participation in the study, including unstable cardiac, renal, or liver disease, or uncontrolled hypertension or diabetes. Full exclusionary criteria are reported in the trial protocol and primary publication (Burnette et al., 2020; Ray et al., 2025).

During the initial screening visit, a trained clinician administered the Structured Clinical Interview for DSM-5 (SCID−5) (First et al., 2015) to assess participants for a current AUD diagnosis and exclusionary diagnoses. A physician then met with eligible participants to review their medical history and conduct vital sign assessments, electrocardiograms, and laboratory tests to ensure medical safety for IBUD treatment.

Interventions

Participants were randomized to receive IBUD or the matched placebo in a 1:1 ratio. The allocation was carried out using a stratified block randomization procedure based on sex and heavy drinking status, with heavy drinking defined as ≥28 drinks/week for men and ≥21 drinks/week for women. A research pharmacist managed the study blind and implemented the stratified randomization sequence. MediciNova supplied IBUD and the matched placebo. The target dose was 50 mg/twice daily. Medication compliance was monitored by the study staff using pill count at each visit. Throughout the trial, participants completed the computer-delivered "Take Control" program, which provided behavioral support for modifying drinking behaviors and was developed for use in AUD pharmacotherapy trials (Devine et al., 2016).

Alcohol Use Assessments and Outcomes

To assess daily alcohol consumption, study staff administered the Timeline Follow-Back (TLFB) (Sobell & Sobell, 1992) interview to participants at baseline and every two weeks throughout the 12-week trial. Consistent with the primary analysis, alcohol use was calculated

for a 30-day baseline period, and follow-up drinking was assessed in 2-week intervals across the 12-week treatment period.

To maintain consistency with the preregistered RCT protocol, percent heavy drinking days (PHDD) was analyzed as the primary drinking outcome and defined as the percentage of drinking days on which participants engaged in heavy drinking. Heavy drinking was classified as 4 or more drinks for women and 5 or more drinks for men (National Institute on Alcohol Abuse and Alcoholism, n.d.). Average drinks per day (DPD) was analyzed as a secondary drinking outcome to capture overall alcohol consumption beyond heavy drinking episodes.

For analyses examining the associations between CRP and intestinal permeability markers with baseline drinking and alcohol-related clinical characteristics, alcohol use disorder severity was measured using the Alcohol Use Disorders Identification Test (AUDIT) (Barbor et al., 2021), and tonic alcohol craving was measured using the Penn Alcohol Craving Scale (PACS) (Flannery et al., 1999).

Inflammatory and Intestinal Permeability Markers

Blood samples were collected in EDTA tubes from participants at randomization, before the first dose of medication. Whole blood was centrifuged at 4°C to obtain plasma, which was aliquoted and stored at −80°C. Plasma CRP levels and intestinal permeability markers were measured using ELISA kits from R&D Systems. Specifically, CRP was measured using the Human CRP Quantikine ELISA kit. LBP, sCD14, and FABP2 were measured using the Human LBP DuoSet®, Human sCD14 DuoSet®, and Human FABP2/I-FABP DuoSet® ELISA kits, respectively. Assays were performed according to the manufacturer's protocol and in duplicate. The average intra-assay CV% for CRP, LBP, sCD14, and FABP2 was 3.3%, 8.6%, 14.6%, and

8.3%, respectively. Due to limited sample volume, one sample in the LBP assay and eight samples in the FABP2 assay were run in singlicate.

Statistical Analysis

Statistical analyses were performed using SAS Statistical Software Version 9.4. Consistent with the primary trial analyses (Ray et al., 2025), drinking outcomes were modeled using piecewise linear mixed-effects models with two linear trajectories. The first linear phase captured initial changes in drinking from baseline to the two-week follow-up, and the second, or maintenance, phase captured drinking from week 2 to week 12.

To test baseline CRP and intestinal permeability markers as moderators of medication effects on drinking outcomes, we fit separate models for each marker. Each model extended the primary model by including the marker, all corresponding lower-order terms, and two three-way interaction terms: Marker × Medication × Time 1 and Marker × Medication × Time 2, which were the primary effects of interest. In line with Grodin et al. (2023), age, sex, cigarette use, and body mass index were examined as covariates and included in the final models if significant. Medication compliance was also examined as a covariate. Participants were deemed compliant if they did not miss any doses over the 12-week treatment period and non-compliant if they missed one or more doses.

Consistent with Grodin et al. (2023), CRP levels were categorized as low (≤3.0 mg/L; n = 69) or high (>3.0 mg/L; n = 30) and modeled as a binary variable, based on the clinically established American Heart Association cutoff for cardiovascular risk (Ridker, 2003a). LBP indicated a non-unimodal distribution (Hartigan's dip test, $p < .001$), with visual evidence of bimodality. Participants were classified into low (n = 36) and high (n = 63) LBP groups using a two-component Gaussian mixture model. LBP was then included in the model as a binary

variable (0 = low, 1 = high). Levels of sCD14 and FABP2 were right-skewed (skewness: sCD14 = 1.75, FABP2 = 2.06) and were therefore log-transformed and modeled as continuous variables. All models included random intercepts and slopes.

To examine the associations between CRP and intestinal permeability markers with baseline drinking and alcohol-related clinical characteristics, drinking variables were calculated for the 7 days before the baseline blood draw to align with the timing of biomarker collection. AUD severity was assessed using the AUDIT, and tonic alcohol craving was measured using the PACS. Total scores were used for both measures. Multiple linear regression models were used to examine whether baseline inflammatory markers were associated with these outcomes. In contrast to the clinically defined subgroup approach used in the moderation analyses, CRP, which was right-skewed (skewness = 4.81), was log-transformed and modeled as a continuous variable to preserve variability for association analyses. Consistent with the moderation analyses, LBP was modeled as a binary variable based on its bimodal distribution, and levels of sCD14 and FABP2 were modeled as log-transformed continuous variables. Sex, age, baseline cigarette use status, and baseline BMI were included as covariates in each model.

RESULTS

Of the 102 randomized participants, 99 (IBUD: n = 50; placebo: n = 49) provided baseline blood samples and were included in the analyses (**Table 4-1**). The sample was 60.61% male, with a mean age of 44.30 years (SD = 10.95). During the 30-day baseline period, participants reported drinking on 22.50 days on average, with 65.59% heavy drinking days. Mean drinks per day and drinks per drinking day were 5.24 (SD = 3.53) and 7.47 (SD = 5.39), respectively. The average AUDIT score was 19.99 (SD = 7.29), consistent with hazardous and harmful alcohol use (Barbor et al., 2021). There were no significant differences between the

IBUD and placebo groups on these demographic or clinical characteristics. Full demographic information for all randomized participants is reported in Ray et al. (2025).

There were no significant differences in baseline levels of circulating CRP or intestinal permeability markers between the IBUD and placebo groups ($p > .22$; **Table 4-1**). Notably, average CRP levels (3.90 mg/L, SD = 8.64) exceeded the American Heart Association cutoff for elevated cardiovascular risk (3.0 mg/L)(Ridker, 2003b). This suggests that, on average, the sample exhibited clinically elevated CRP levels, although there was substantial variability across participants. To examine the relationships between levels of CRP and the intestinal permeability markers, Pearson correlation coefficients were calculated for relationships between continuous variables, and point-biserial correlations were used for LBP, which was dichotomized due to the bimodal distribution described in the Methods (**Table 4-2**). There were no strong correlations between markers ($|r| \leq 0.08$), indicating that these markers were largely independent.

Moderation Analyses

Outcome: Percentage of Heavy Drinking Days

Moderation by Baseline CRP

There was a significant CRP × Medication × Time 1 interaction ($\beta = -0.45$, SE = 0.17, 95% CI [-0.79, -0.12], $p = .01$) and a significant CRP × Medication × Time 2 interaction ($\beta = 0.48$, SE = 0.19, 95% CI [0.10, 0.85], $p = .01$). During the first phase of the trial (baseline–week 2), there was a faster rate of decrease in PHDD for the placebo versus IBUD group among participants with high CRP, compared to participants with low CRP (**Figure 4-1**). Conversely, during the second phase of the trial (week 2–week 12), there was a faster rate of decrease in

PHDD for the IBUD versus placebo group among participants with high CRP, compared to participants with low CRP (**Figure 4-1)**.

Moderation by LBP Group: There were no significant LBP Group × Medication × Time interactions on PHDD during either phase of the trial (Time 1: β = −0.14, SE = 0.16, 95% CI [−0.45, 0.17], *p* = .37; Time 2: β = 0.18, SE = 0.17, 95% CI [−0.16, 0.52], *p* = .30).

Moderation by Baseline sCD14: There were no significant sCD14 × Medication × Time interactions on PHDD during either phase of the trial (Time 1: β = −0.49, SE = 0.38, 95% CI [−1.23, 0.26], *p* =.20; Time 2: β = 0.54, SE = 0.42, 95% CI [−0.29, 1.36], *p* = .20).

Moderation by Baseline FABP2: There were no significant FABP2 × Medication × Time interactions on PHDD during either phase of the trial (Time 1: β = −0.41, SE = 0.37, 95% CI [−1.13, 0.32], *p* = .27; Time 2: β = 0.45, SE = 0.40, 95% CI [−0.35, 1.24], *p* = .27).

Outcome: Average Drinks per Day

Moderation by Baseline CRP: There was a significant CRP Group× Medication × Time 1 interaction (β = -2.99, SE = 1.40, 95% CI [-5.76, -0.23], p = .03) and a significant CRP Group × Medication × Time 2 interaction (β = 3.24, SE = 1.40, 95% CI [0.49, 5.98], p = .02). During the first phase of the trial (baseline–week 2), there was a faster rate of decrease in DPD for the placebo versus IBUD group among participants with high baseline CRP, compared to participants with low CRP (**Figure 4-2**). Conversely, during the second phase of the trial (week 2–week 12), there was a faster rate of decrease in PHDD for the IBUD versus placebo group among participants with high CRP, compared to participants with low CRP (**Figure 4-2)**.

Moderation by LBP Group: There were no significant LBP Group × Medication × Time interactions on DPD during either phase of the trial (Time 1: β = 1.60, SE = 1.37, 95% CI [−1.08, 4.29], *p* = .24; Time 2: β = −1.55, SE = 1.39, 95% CI [−4.28, 1.19], *p* = .27)

Moderation by Baseline sCD14: There were no significant sCD14 × Medication × Time interactions on DPD during either phase of the trial (Time 1: β = 0.41, SE = 3.50, 95% CI [−6.49, 7.30], *p* = .91; Time 2: β = -0.06, SE = 3.51, 95% CI [-6.97, 6.85], *p* = .99)

Moderation by Baseline FABP2: There were significant FABP2 × Medication × Time interactions on DPD during both the early phase (β = -6.75, SE = 2.82, 95% CI [−12.31, −1.19], p = .02) and the later phase of the trial (β = 7.36, SE = 2.82, 95% CI [1.82, 12.91], *p* = .01). During the first phase of the trial (baseline–week 2), there was a faster rate of decrease in DPD for the placebo versus IBUD group among participants with higher FABP2, compared to participants with lower FABP2 (**Figure 4-3**). Conversely, during the second phase of the trial (week 2–week 12), there was a faster rate of decrease in DPD for the IBUD versus placebo group among participants with higher FABP2, compared to participants with lower FABP2 (**Figure 4-3**).

Associations Between Biological Markers and AUD-Related Clinical Characteristics

Baseline log CRP and log sCD14 were significantly positively associated with PHDD (log CRP: β = 0.24, SE = 0.08, p = .002; log sCD14: β = 0.62, SE = 0.20, p = .002), such that higher levels of these markers were associated with greater PHDD. Other markers were not significantly associated with PHDD (*p*'s > .70). Baseline log sCD14 was significantly positively associated with DPD (β = 3.33, SE = 1.53, p = .03), and log CRP was positively associated with DPD at a trend level (β = 1.12, SE = 0.59, p = .06), such that higher levels of these markers were associated with greater DPD. LBP group (high vs low) was significantly negatively associated

with DPDD (β = −1.76, SE = 0.84, p = .04) and DPD at a trend level (β = −1.15, SE = 0.62, p = .06), such that individuals in the low LBP group had higher DPDD and DPD compared to those in the high LBP group. Log FABP2 was not significantly associated with DPD (β = 1.67, SE = 1.45, p = .25).

Baseline log CRP and log sCD14 were significantly positively associated with AUDIT scores (log CRP: β = 3.10, SE = 1.40, p = .03; log sCD14: β = 9.36, SE = 3.60, p = .01), such that higher levels of these markers were associated with greater AUD severity. The other markers were not significantly associated with AUDIT scores (*p*'s >.57). Log sCD14 was significantly positively associated with PACS scores (β = 7.77, SE = 3.07, p = .01), such that higher baseline sCD14 was associated with greater tonic alcohol craving. The other markers were not significantly associated with PACS score (*p's*> 25). Full results are presented in **Table 4-3**.

DISCUSSION

This study examined whether baseline peripheral inflammation, as indexed by CRP levels, and intestinal permeability markers moderated the effects of IBUD on drinking outcomes in a 12-week, double-blind, randomized controlled trial of treatment-seeking individuals with moderate to severe AUD. Results were nuanced and suggested that, among participants with higher baseline CRP, the rate of decrease in drinking (percent heavy drinking days and average drinks per day) was superior in the IBUD condition, versus placebo, during the maintenance phase of the trial (week 2 – week 12), while placebo was superior to IBUD during the initial change period (baseline – week 2). These time-specific effects for CRP suggest that individuals with high baseline inflammation may show a delayed response to IBUD that is more pronounced later in the treatment process. Considering that psychiatric medications, notably selective

serotonin−reuptake inhibitors (SSRIs), may show a time lag in efficacy (Machado-Vieira et al., 2010), it is plausible that the effects of IBUD on alcohol use may have a lagged effect in individuals with high levels of baseline inflammation. To that end, longer-duration clinical trials for anti-inflammatory medications may be warranted to capture clinical efficacy beyond 12 weeks in treatment-seeking individuals with AUD. This may be particularly relevant given the high placebo response rates observed in this trial, which are consistent with high placebo effects reported in other RCTs for AUD (Litten et al., 2013). While our findings are consistent with those of Grodin et al. (2023) in demonstrating that baseline CRP moderates drinking outcomes in response to IBUD, the pattern of effects differed across studies. Grodin et al. (2023) found that individuals with elevated CRP who received IBUD reported fewer drinks per drinking day on average. In contrast, the present findings suggest that the moderating effect of CRP varied across the two phases of treatment. These differences may reflect variation in sample characteristics (treatment-seeking versus non-treatment-seeking participants), trial duration, and the more complex drinking trajectories captured in the present study as a function of the clinical trial design.

Baseline intestinal permeability markers did not significantly moderate the effects of IBUD on the primary drinking outcome of percent heavy drinking days. However, baseline FABP2 significantly moderated the effects of IBUD on the secondary drinking outcome of average drinks per day. During the first time period (baseline–week 2), participants with higher baseline FABP2 showed a faster rate of decrease in drinks per day in the placebo group relative to the IBUD group, compared to participants with lower FABP2. During the maintenance phase (week 2–week 12), participants with higher baseline FABP2 showed a greater rate of decrease in drinks per day in the IBUD group relative to the placebo group, compared to participants with

lower FABP2. Although these results showed a similar pattern to the CRP findings, visual examination of the plotted trajectories suggests that the interaction may also reflect differences in drinks per day trajectories among participants with lower baseline FABP2 levels, where the IBUD group showed a less steep decrease during the maintenance phase relative to placebo. Thus, the observed interaction may not solely reflect greater IBUD-related reductions in drinks per day compared to placebo among participants with higher FABP2 levels.

Towards understanding the clinical relevance of these markers, preliminary findings suggest that higher levels of sCD14, a proxy for intestinal permeability and exposure to gut-derived LPS (Bibolar et al., 2025), were positively associated with higher drinking levels, greater AUD severity, and higher tonic alcohol craving. CRP levels were similarly associated with alcohol-related clinical characteristics despite CRP and sCD14 levels not being significantly correlated, suggesting that gut-related and systemic inflammatory processes may represent distinct, but clinically relevant features of AUD phenomenology. These findings are consistent with the general notion that individuals with heavier alcohol use and alcohol-related problems display higher levels of inflammation (Lékó et al., 2023). In contrast, baseline LBP was significantly negatively associated with drinks per drinking day and, at the trend level, with drinks per day, indicating that the low-LBP group reported higher levels of drinking than those in the high-LBP group. These contrasting associations suggest that these markers may reflect more complex biological processes than intestinal permeability alone. Although both LBP and sCD14 are commonly used as indirect markers of microbial translocation, CD14 is produced primarily by monocytes and macrophages and reflects innate immune activation in response to LPS (Na et al., 2023), whereas LBP is an acute-phase protein synthesized primarily by the liver and is upregulated during an acute inflammatory response (Schumann et al., 1996). Therefore, LBP

concentrations may reflect both gut-derived endotoxin exposure and hepatic function, which may complicate the interpretation of LBP as a marker of intestinal permeability. These preliminary findings highlight the need for additional research to better understand the clinical significance of these markers in AUD. Future studies including healthy controls or lower-severity comparison groups may help further clarify these relationships.

Although prior clinical and mechanistic research suggests that increased intestinal permeability can contribute to systemic inflammation (Bishehsari et al., 2017), we did not find evidence that the intestinal permeability markers measured in the current study were significantly associated with one another or with CRP levels. While higher levels of these markers are generally thought to reflect greater intestinal permeability and related immune activation, the current findings suggest they may reflect distinct components of gut barrier and immune functioning rather than a single underlying biological process (Ioannou et al., 2025). These findings differ from prior work showing significant associations between CRP and intestinal permeability markers, although these studies were conducted in clinical populations with conditions characterized by elevated inflammation, including Crohn's disease and kidney disease requiring hemodialysis (Lakatos et al., 2011; Lim et al., 2019). Intestinal permeability markers have not been widely studied in AUD, despite growing evidence implicating the gut in alcohol-related inflammation. Thus, additional work is needed to better characterize these markers in individuals with AUD, including how they relate to broader inflammatory processes and alcohol-related clinical characteristics.

This study should be interpreted in the context of its strengths and limitations. A major strength was the use of data from a 12-week randomized, double-blind, placebo-controlled clinical trial in treatment-seeking individuals with AUD. This allowed for the assessment of

inflammatory and intestinal permeability markers in relation to longitudinal drinking outcomes during pharmacological treatment. Additional strengths include repeated assessments of drinking outcomes across the 12-week treatment period and the inclusion of multiple markers reflecting intestinal permeability-related processes. One important consideration is that individuals with significant medical conditions, including alcohol-related liver disease and cirrhosis, were excluded from this trial during the medical screening visit. Thus, the current findings may not generalize to individuals with more severe alcohol-related medical complications, particularly liver disease, who may respond differently to anti-inflammatory medications such as IBUD. Additionally, the current study did not account for the potential influence of commonly comorbid psychiatric conditions, such as anxiety and mood disorders, that have also been linked to disruptions in inflammatory processes and intestinal permeability (Bauer & Teixeira, 2019; Wasiak & Gawlik-Kotelnicka, 2023). Furthermore, although sex differences have been observed in inflammatory processes and response to IBUD (Ray et al., 2025), the current sample size limited our ability to adequately examine sex-specific effects. Future studies are needed to determine whether inflammatory and intestinal permeability markers differentially moderate treatment response in males and females. Finally, intestinal permeability was assessed using peripheral plasma biomarkers available from the parent clinical trial. Although these markers provide an indirect index of gut barrier dysfunction and endotoxin-related immune activation, future work would benefit from incorporating more direct assessments of gut health, including fecal-based measures, to better characterize gut-related mechanisms in AUD.

In summary, the current findings suggest that baseline CRP levels may influence treatment-related changes in drinking outcomes during IBUD treatment for AUD, with these effects varying across treatment phases, from initial change in alcohol use to maintenance of

drinking reductions. FABP2, but not the other gut permeability markers, moderated treatment effects on the secondary drinking outcome of drinks per day. Additionally, CRP, sCD14, and LBP showed associations with alcohol-related clinical characteristics, supporting their potential clinical relevance in AUD. These findings highlight the potential utility of inflammatory and gut-related biomarkers for identifying subgroups of individuals who may differentially respond to treatment and underscore the need for further research examining inflammatory and gut-related processes in AUD.

Table 4-1. Participant demographics, baseline drinking characteristics, and biological marker levels.

Variable (SD)	Total (n = 99)	IBUD (n = 50)	Placebo (n = 49)	p value
Sex (M/F)	60/39	31/19	29/20	.77
Age (Years)	44.30 (10.95)	42.72 (10.26)	45.92 (11.49)	.15
Baseline Drinking Characteristics				
% Heavy Drinking Days	65.59% (34.57)	65.52% (34.61)	65.70% (34.89)	.98
No. Drinking Days	22.50 (7.43)	22.28 (7.51)	22.71 (7.41)	.78
Drinks per Day	5.24 (3.53)	5.39 (3.78)	5.09 (3.28)	.67
Drinks per Drinking Day	7.47 (5.39)	7.42 (4.29)	7.52 (3.36)	.90
AUDIT Score	19.99 (7.29)	20.80 (8.24)	19.16 (6.14)	.26
Baseline Circulating CRP and Intestinal Permeability Markers				
CRP (mg/L)	3.90 (8.64)	4.01 (8.56)	3.75 (8.81)	.87
CRP Category (high/low)	30/69	18/32	12/37	.21
LBP (ng/mL)	10.52 (4.61)	10.15 (4.10)	10.90 (5.09)	.42
LBP Category (high/low)	63/36	30/20	33/16	.45
sCD14 (μg/mL)	1.38 (0.75)	1.36 (0.77)	1.41 (0.74)	.76
FABP2 (pg/mL)	722.47 (432.90)	775.21 (422.82)	668.65 (440.75)	.22

Note. Substance use measures were derived from the TLFB and reflected substance use during the 30-day baseline period. Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; CRP, C-reactive protein; LBP, lipopolysaccharide-binding protein; sCD14, cluster of differentiation 14; FABP2, fatty acid-binding protein 2. Group differences between IBUD and placebo were tested using Welch's two-sample t-tests for continuous variables and χ^2 tests for categorical variables. No significant baseline differences were observed between the IBUD and placebo groups for the reported characteristics. Biological marker values are presented as the mean and standard deviation of raw (non-log-transformed) values. LBP category reflects classification into high versus low groups based on a two-component Gaussian mixture model.

Table 4-2. Baseline correlations among CRP and intestinal permeability markers.

Biomarker	CRP	LBP	sCD14	FABP2
CRP	—	—	—	—
LBP	0.03	—	—	—
sCD14	0.08	-0.06	—	—
FABP2	0.08	-0.06	-0.07	—

Note. Pearson correlation coefficients among log-transformed marker levels. LBP was dichotomized into low and high groups due to its bimodal distribution; correlations with LBP represent point-biserial correlations. Abbreviations: CRP, C-reactive protein; LBP, lipopolysaccharide-binding protein; sCD14, soluble cluster of differentiation 14; FABP2, fatty acid-binding protein 2.

Table 4-3. Associations of baseline CRP and intestinal permeability markers with clinical characteristics.

Predictor	β	SE	p
Percent Heavy Drinking Days			
CRP	**0.24**	**0.08**	**.002**
sCD14	**0.62**	**0.20**	**.002**
LBP Group	0.03	0.08	.72
FABP2	0.07	0.19	.70
Drinks per Day			
CRP	1.12	0.59	.06
sCD14	**3.33**	**1.53**	**.03**
LBP Group	-1.15	0.62	.06
FABP2	1.67	1.45	.25
Drinks per Drinking Day			
CRP	**2.09**	**0.80**	**.01**
sCD14	2.98	2.14	.17
LBP Group	**-1.76**	**0.84**	**.04**
FABP2	1.71	2.00	.39
AUDIT Score			
CRP	**3.10**	**1.40**	**.03**
sCD14	**9.36**	**3.60**	**.01**
LBP Group	-0.75	1.49	.61
FABP2	1.99	3.46	.57
PACS Score			
CRP	1.41	1.21	.25
sCD14	**7.77**	**3.07**	**.01**
LBP Group	-0.17	1.27	.90
FABP2	1.00	2.95	.74

Note. Linear regression analyses examining associations between baseline inflammatory and intestinal permeability markers with alcohol-related clinical characteristics. CRP, sCD14, and FABP2 were modeled as log-transformed continuous variables. LBP was modeled as a dichotomous variable (low vs. high) based on a two-component Gaussian mixture model. Sex, age, and baseline BMI were tested in each model as covariates. Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; PACS, Penn Alcohol Craving Scale; CRP, C-reactive protein; LBP, lipopolysaccharide-binding protein; sCD14, cluster of differentiation 14; FABP2, fatty acid-binding protein 2.

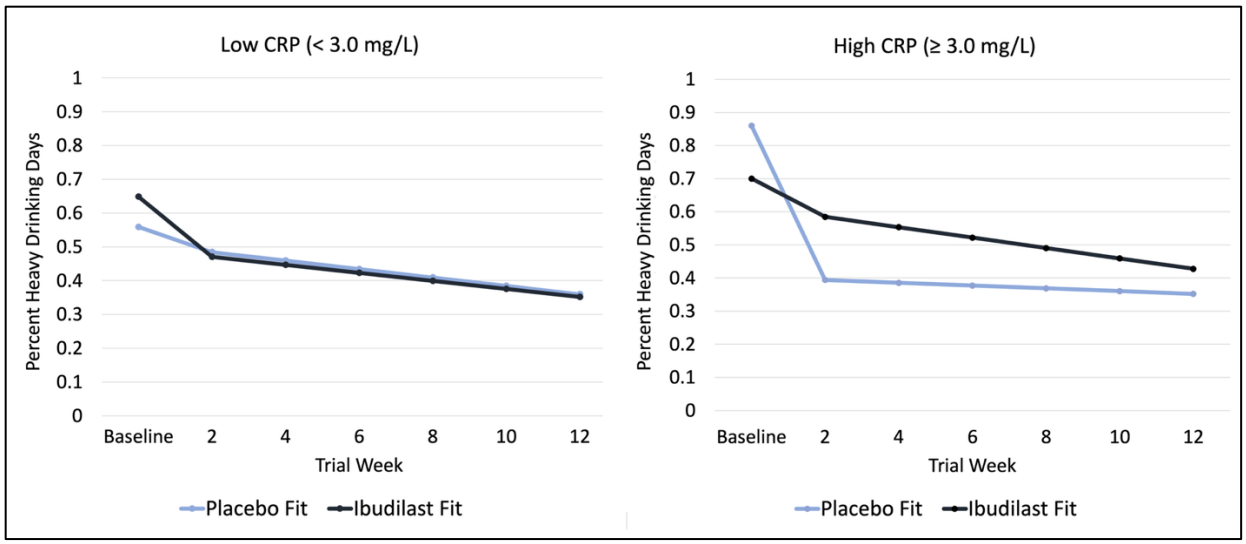

Figure 4-1. Predicted percent heavy drinking days trajectories by baseline CRP group. CRP groups were defined using established clinical risk guidelines, with low CRP representing ≤3.0 mg/L and high CRP representing >3.0 mg/L (Ridker, 2003a).

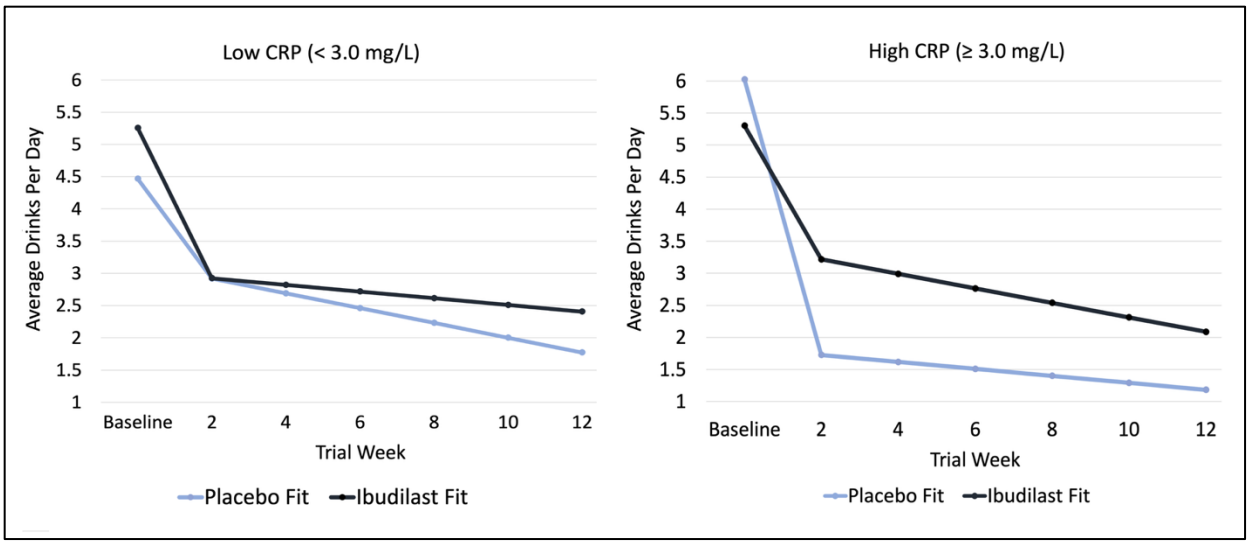

Figure 4-2. Predicted average drinks per day trajectories by baseline CRP group. CRP groups were defined using established clinical risk guidelines, with low CRP representing ≤3.0 mg/L and high CRP representing >3.0 mg/L (Ridker, 2003a).

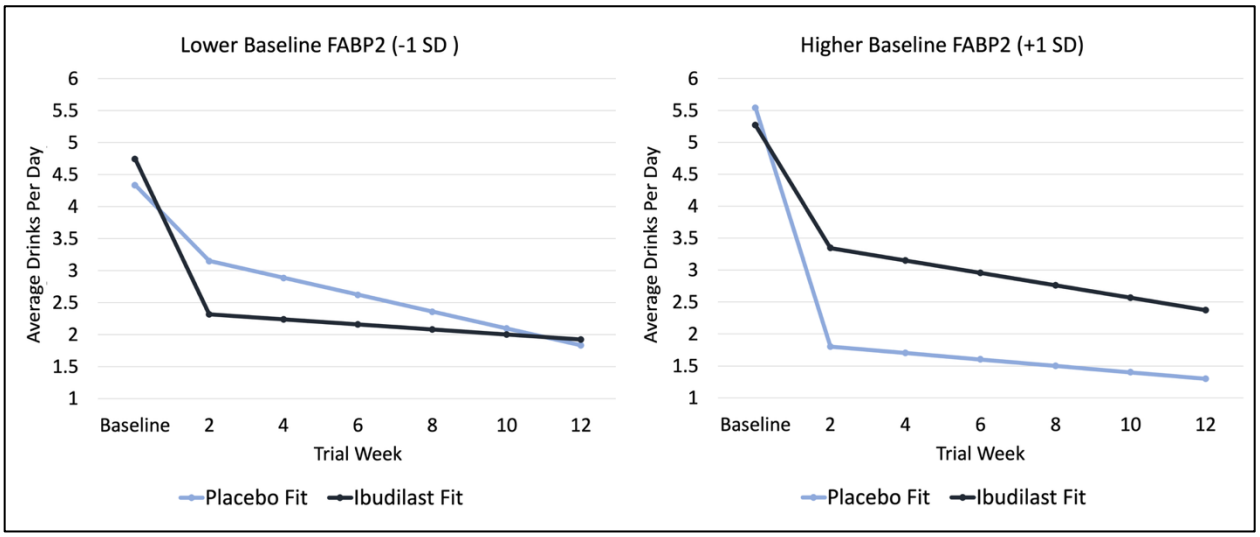

Figure 4-3. Predicted average drinks per day trajectories by baseline FABP2 level. For plotting purposes, lower FABP2 represents one standard deviation below the mean baseline FABP2 level, and higher FABP2 represents one standard deviation above the mean baseline FABP2 level.

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CHAPTER 5: Reductions in Drinking Are Associated with Reductions in C-Reactive Protein During Treatment for Alcohol Use Disorder

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ABSTRACT

Alcohol use disorder (AUD) is associated with elevated systemic inflammation. However, few studies have examined the relationship between changes in alcohol consumption and changes in peripheral inflammation over time. To address this gap, the current study examined the association between changes in alcohol consumption and peripheral inflammatory markers using data from a 12-week, double-blind, randomized controlled trial of ibudilast, a neuroimmune modulator, for AUD. Analyses focused on the first four weeks of treatment, when participants showed steep average reductions in drinking. Bivariate latent growth models were used to estimate individual trajectories of change in drinking (drinks per day, drinks per drinking day, and percent heavy drinking days) and peripheral inflammatory marker levels (CRP, IL-6, IL-8, TNF-α, and IFN-γ). Statistical models tested associations between these latent trajectories and whether these associations differed by medication condition. Results revealed that greater reductions in drinking, across all three drinking measures, were associated with greater reductions in CRP. Reductions in drinks per drinking day were also associated with decreases in IL-6 and IFN-γ. Medication condition did not moderate the association between changes in drinking and changes in inflammatory marker levels. Overall, these findings suggest that CRP may be a particularly robust marker of treatment-related reductions in drinking over several weeks, while cytokines such as IL-6 and IFN-γ may be uniquely sensitive to specific drinking outcomes. These results highlight the importance of examining individual trajectories of change to better understand the relationship between drinking and inflammation during AUD treatment.

INTRODUCTION

Alcohol use disorder (AUD) is associated with dysregulation of inflammatory signaling, and there is growing interest in developing medications that target the inflammatory system for the treatment of AUD (Mayfield & Harris, 2017; Meredith et al., 2021). Several systematic reviews and meta-analyses have reported altered circulating inflammatory marker concentrations in individuals with AUD compared with healthy controls, although findings have varied across specific inflammatory markers and study populations (Adams et al., 2020; Galvez-Sánchez et al., 2026). Overall, the evidence suggests that individuals with AUD generally exhibit elevated levels of pro-inflammatory markers compared with healthy controls (Adams et al., 2020; Galvez-Sánchez et al., 2026). These differences also appear to vary across phases of alcohol use, with the greatest elevations observed during periods of active drinking and alcohol withdrawal, with smaller elevations observed with increasing durations of abstinence (Adams et al., 2020; Galvez-Sánchez et al., 2026; Sokolova et al., 2026). Additionally, elevated peripheral cytokine concentrations, which are signaling proteins that regulate immune and inflammatory responses, have been associated with greater AUD severity, alcohol withdrawal, craving, and psychiatric symptoms, including anxiety, depression, and insomnia (Galvez-Sánchez et al., 2026). While these findings provide insights into inflammatory alterations associated with AUD, much of the existing literature has relied on cross-sectional comparisons between groups representing different phases of alcohol use (e.g., healthy controls and individuals in early alcohol withdrawal). Thus, there is limited understanding of how inflammatory marker levels change within individuals with AUD over time and how these changes relate to changes in drinking behavior.

Although longitudinal studies are limited, experimental human laboratory studies have demonstrated that inflammatory marker levels can change within individuals over periods of hours following acute alcohol administration. In a human laboratory study of moderate drinkers without AUD who abstained from alcohol for several days before testing, acute alcohol administration to a target blood alcohol concentration (BAC) of 0.12% was associated with transient increases in interleukin (IL)-8 and decreases in tumor necrosis factor (TNF)-α six hours after alcohol consumption (Hillmer et al., 2020). Both markers returned to baseline levels by 24 hours (Hillmer et al., 2020). In contrast, levels of IL-6, IL-12, and interferon (IFN)-γ did not change, highlighting the marker-specific nature of alcohol-induced immune responses. Similarly, in a placebo-controlled, alcohol administration study of light and heavy drinkers, IL-6, IL-8, TNF-α, and monocyte chemoattractant protein (MCP-1) exhibited distinct acute responses that varied according to alcohol dose, time following alcohol administration, and drinking history (Monnig et al., 2025). Together, these studies demonstrate that acute alcohol administration can alter inflammatory marker levels, although responses differ across biomarkers. However, because these studies examine changes on a timescale of hours, they do not address whether longer-term changes in alcohol consumption are associated with corresponding changes in inflammatory marker levels, nor has prior work examined these relationships in a clinical sample of individuals with moderate-to-severe AUD.

The current study addresses this gap in the literature using data from a 12-week, double-blind, randomized controlled trial (RCT) of ibudilast (IBUD) for AUD. IBUD is a phosphodiesterase-3, -4, -10, and -11 inhibitor with anti-inflammatory properties that has been investigated as a potential treatment for AUD (Burnette et al., 2020; Grodin et al., 2021; Ray et al., 2017, 2025). Interest in IBUD as a potential novel medication was driven by the growing

evidence implicating inflammatory processes in AUD, positive findings from early-phase clinical studies (Grodin et al., 2021; Ray et al., 2017), and preclinical evidence that modulation of neuroimmune signaling in rodents can reduce ethanol consumption and preference (Blednov et al., 2014; Meredith et al., 2021). The primary analysis of the 12-week RCT found no overall effect of IBUD, relative to placebo, on drinking outcomes or peripheral inflammatory marker levels (Ray et al., 2025). However, participants in both groups showed, on average, steep reductions in alcohol consumption over the course of treatment. The primary analyses focused on medication effects and did not examine longitudinal changes in inflammatory markers, individual variability in these changes, or their relationship to changes in drinking.

The present study examined the association between changes in alcohol consumption and changes in peripheral inflammatory marker levels during the first four weeks of the RCT. This period was selected because it captured the greatest reductions in drinking and included the largest number of participants with available blood samples to assess inflammatory markers. We hypothesized a positive association between changes in drinking and changes in peripheral inflammatory marker levels, such that individuals showing greater reductions in drinking would also show greater reductions in inflammatory marker levels. Additionally, although the primary analyses found no overall effect of IBUD on peripheral inflammatory markers, it is possible that, given its putative anti-inflammatory mechanism, IBUD may have influenced the relationship between changes in drinking and changes in these markers. Therefore, medication condition (IBUD vs. placebo) was evaluated as an exploratory moderator. We hypothesized that the association between changes in drinking and changes in peripheral inflammatory marker levels would be stronger in the IBUD group, as IBUD's anti-inflammatory properties may act

synergistically with reductions in drinking to produce more pronounced reductions in peripheral inflammation.

METHODS

Trial design

The current secondary analysis utilized data from a Phase 2, 12-week, double-blind, randomized placebo-controlled trial of IBUD for the treatment of AUD (Ray et al., 2025) (Clinical trials identifier: NCT03594435). Analyses examined changes from baseline to week 4 because COVID-19-related disruptions limited blood sample collection at subsequent follow-up visits (baseline: N = 99; week 4: N = 72; week 8: N = 28; week 12: N = 60). A total of 102 treatment-seeking individuals with moderate-to-severe AUD (61 male, 41 female) were randomized to receive either IBUD (50 mg twice daily) or placebo. The present analyses focused on the 99 participants who provided baseline blood samples.

Participants completed eligibility assessments and a medical examination prior to randomization. Blood samples were collected at the baseline/randomization visit and again during the week 4 in-person follow-up visit. Alcohol use data were collected at baseline, week 2 via telephone assessment, and week 4. This trial was approved by the UCLA Institutional Review Board and monitored by a Data and Safety Monitoring Board. All study participants provided written informed consent prior to participation in study procedures. Additional information regarding the trial design can be found in the trial protocol and primary publication of the study (Burnette et al., 2020; Ray et al., 2025).

Setting and participants

The trial was conducted at UCLA in an outpatient research facility. Social media, mass transit, and printed flyer advertisements were used to recruit participants. Participants were

required to (1) be between 18 and 65 years old; (2) meet DSM-5 diagnostic criteria for moderate or severe AUD within the past 12 months; (3) be seeking treatment for AUD; and (4) report drinking at least 14 drinks per week for males or 7 drinks per week for females in the 28 days before consent. Participants were excluded if they met criteria for a DSM-5 diagnosis of any substance use disorder other than alcohol or nicotine in the past 12 months, except for mild cannabis use disorder. Participants were also excluded if they had a medical condition that would interfere with their safety or study participation, such as unstable cardiac, renal, or liver disease, or uncontrolled hypertension or diabetes. The full inclusion and exclusion criteria are reported in the trial protocol and primary publication (Burnette et al., 2020; Ray et al., 2025).

During the screening visit, a trained clinician administered the Structured Clinical Interview for DSM-5 (SCID−5) (First et al., 2015) to assess participants for a current AUD diagnosis and exclusionary psychiatric diagnoses. Eligible participants then met with a physician to review their medical history and complete a physical exam, electrocardiograms, and laboratory tests to ensure medical safety for IBUD treatment.

Interventions

Participants were randomized in a 1:1 ratio to receive either IBUD or matched placebo using a stratified block randomization procedure based on sex and heavy drinking status, with heavy drinking defined as ≥28 drinks/week for men and ≥21 drinks/week for women. A research pharmacist who was not involved in participant recruitment or study activities maintained the study blind and implemented the randomization sequence. IBUD and matched placebo were supplied by MediciNova. The target dose was 50 mg twice daily.

Alcohol Use Assessments and Outcomes

To assess retrospective daily alcohol consumption, study staff administered the Timeline Follow-Back (TLFB) (Sobell & Sobell, 1992) interview to participants at baseline, week 2, and week 4. Drinking outcomes were assessed at baseline (day of randomization), week 2, and week 4. Each assessment reflected drinking during the preceding 2-week interval (baseline: 14 days before baseline; week 2: baseline to week 2; week 4: week 2 to week 4), resulting in three drinking observations per participant. Average drinks per day (DPD), drinks per drinking day (DPDD), and percent heavy drinking days (PHDD) were analyzed as the drinking outcome variables. PHDD was defined as the percentage of drinking days on which participants engaged in heavy drinking. Heavy drinking was classified as 4 or more drinks for women and 5 or more drinks for men (National Institute on Alcohol Abuse and Alcoholism, n.d.).

Peripheral Inflammatory Markers

Peripheral inflammatory markers were evaluated from blood samples collected at baseline (day of randomization) and at week 4 of treatment. Due to scheduling variability, the timing of the Week 4 assessment varied across participants. The mean interval between the baseline and Week 4 visits was 29.92 days, and 80.56% of participants completed the Week 4 assessment within ±5 days of the target 28-day visit. Blood samples were assayed for C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukins 6 and 8 (IL-6, IL-8). Inflammatory marker levels were log-transformed to normalize the distributions due to right skew. CRP levels were measured using the high-sensitivity Human CRP R&D Systems Quantikine ELISA assay in duplicate, according to the manufacturer's protocol, with a lower limit of detection of 0.2 mg/L (Grodin et al., 2023). Fifteen of 171 CRP values (8.8%) were below the limit of detection of 0.2 mg/L and were assigned a value of 0.2 mg/L. The mean inter- and intra-assay coefficients of variation for CRP were 5.7% and 3.3%, respectively. IL-6, IL8, IFN-γ, and TNF-α

were assessed using the Meso Scale Discovery MULTI-SPOT Proinflammatory Panel 1 Human Kit Assay System (Rockville, MD) in duplicate according to the manufacturer's protocol. Mean inter- and intra-assay CVs were 2.7% and 2.1% for IL-6, 2.0% and 1.1% for IL-8, 4.5% and 1.5% for IFN-γ, and 12.0% and 7.6% for TNF-α, respectively. TNF-α, IL-8, and IFN-γ levels were detectable in all subjects. For IL-6, 13 of 171 values (7.6%) were below the limit of detection (0.2 pg/mL) and were assigned a value of 0.2 pg/mL.

Statistical Analysis

A series of bivariate latent growth models were estimated in R using Markov Chain Monte Carlo (MCMC) estimation in the *rblimp* package (Keller & Enders, 2026) to examine whether individual differences in rate of change in alcohol consumption were associated with individual differences in rate of change in inflammatory marker levels over the first four weeks of the RCT. Separate models were estimated for each inflammatory marker and drinking variable. Models were estimated using MCMC procedures, with 50,000 burn-in iterations followed by 50,000 retained iterations for posterior inference.

For each model, person-level latent intercepts and linear slope factors were specified for both drinking and inflammation. For drinking, growth models were estimated separately for each drinking variable using assessments at baseline, week 2, and week 4, with time scores fixed at 0, 2, and 4**,** so the intercept represents each participant's baseline drinking and the slope represents linear change in drinking per week. For inflammation, growth models were estimated from log-transformed marker values at baseline and week 4. The visits were scaled so that the intercept represented each participant's week 4 inflammatory marker value. This allowed the correlations between drinking and inflammatory markers to reflect the intended temporal ordering in which drinking preceded inflammation. The slope loading was fixed to each participant's actual time

between assessments to account for variability in follow-up timing. Residual variances for the inflammation indicators were fixed to near-zero values because only two assessment occasions were available for each marker, which allowed each participant's linear trajectory to fully account for the observed data, leaving no residual variance to estimate.

The inflammation slope was regressed on the drinking slope to test whether change in drinking over the first four weeks was associated with change in inflammatory marker levels. The inflammation intercept was regressed on the drinking intercept to account for the association between baseline drinking and endpoint inflammation. To test whether medication condition moderated the association between changes in drinking and changes in inflammation, a second set of models added medication condition and a medication condition × drinking slope interaction term as predictors of the inflammation slope. The drinking intercept and slope were allowed to correlate, as were the inflammation intercept and slope, to account for residual covariance not explained by the regressions. Consistent with the MCMC estimation framework, inference was based on the posterior distribution of each parameter, representing the range of plausible parameter values given the observed data. The posterior mean served as the point estimate, and 95% credible intervals were used to quantify uncertainty. Parameters were considered statistically significant if their 95% credible interval did not include zero. Throughout the Results section, standard deviation (SD) refers to the standard deviation of the posterior distribution for each parameter estimate (i.e., “Bayesian standard errors”). For variance parameters, the reported SD reflects the uncertainty in the estimated variance parameter.

Covariates were specified as predictors of the latent intercepts, to account for associations between each covariate and the outcome at the modeled intercept, and of the latent slopes, as moderators of trajectories of change. Age and sex were included as predictors of drinking and

inflammation latent intercepts and slopes. Medication condition (ibudilast vs. placebo) was included as a predictor of the drinking slope, but not the drinking intercept (representing baseline, prior to medication initiation), and was included as a predictor of the inflammation intercept (representing Week 4) and slope. Body mass index (BMI), depressive symptoms (BDI-II total score (Beck et al., 1996)), and cigarette smoking status (smoker vs. non-smoker) were included as predictors of the inflammation intercept and slope, based on evidence of their associations with levels of peripheral inflammatory markers (Howren et al., 2009; Levitzky et al., 2008; Su et al., 2024).

RESULTS

Participant Characteristics

The sample consisted of 99 adults with moderate-to-severe AUD (IBUD: N = 50; placebo: N = 49). The sample was 60.61% male, with a mean age of 44.30 years (SD = 10.95) and a mean AUDIT score of 19.99 (SD = 7.29), consistent with harmful alcohol use (Barbor et al., 2021), on average, participants reported mild depressive symptoms (BDI-II: M = 11.14, SD = 7.83) and were in the overweight BMI range (BMI: M = 27.93, SD = 5.92); 32.32% reported current cigarette smoking. The IBUD and placebo groups did not differ on any demographic or clinical characteristic (**Table 5-1**). Baseline drinking levels and rates of change for each drinking measure are reported in the following section. Full demographic information for the parent trial is reported in Ray et al. (2025).

Analyses

The results are first presented for the unconditional growth models of each drinking and inflammatory marker variable, followed by regression analyses examining the associations

between changes in drinking and changes in inflammation, and whether these associations differ by medication condition (IBUD vs. placebo).

Unconditional Growth Models of Drinking and Inflammation

The growth model estimates presented below are from unconditional latent growth models, which were estimated prior to fitting the regression models. These results are provided to characterize the overall levels and rates of change in drinking and inflammatory marker variables.

Drinking Measures

On average, participants reduced their drinking across all three measures from baseline to week 4. Each week, participants decreased drinks per day (DPD) by 0.26, drinks per drinking day (DPDD) by 0.28, and percent heavy drinking days (PHDD) by 3 percentage points, with 95% credible intervals for all three slopes excluding zero. Slope variances indicated meaningful differences in rates of change for all three drinking measures. For example, based on the DPDD slope variance, the majority of participants (±1 SD) had weekly slopes ranging from −0.77 to 0.21. This indicates that participants at the lower end of the change distribution reduced their drinking at nearly three times the average rate, whereas those at the higher end showed a slight increase in drinking. Parameter estimates are presented in **Table 5-2.**

Peripheral Inflammatory Markers

Across the examined peripheral inflammatory markers, average slopes were near zero, with credible intervals that contained zero, suggesting little average change in inflammation from baseline to week 4. Slope variances indicated meaningful differences in the rates of change for all inflammatory markers. For example, the average weekly rate of change for log CRP was −0.01, but based on the slope variance, the majority of participants (±1 SD) had weekly slopes

between −0.27 and 0.25, indicating that some individuals decreased in log CRP over time, whereas others increased. Full parameter estimates are presented in **Table 5-3.**

Regression Models

Separate models tested whether changes in each drinking outcome were associated with changes in each inflammatory marker and whether these associations differed by medication condition (IBUD vs. placebo). All models included the covariates described in the Methods. Full parameter estimates are presented in **Table 5-4.**

Changes in drinking were positively associated with changes in log CRP across all three drinking measures: drinks per day (DPD, $b = 0.60$, $SD = 0.30$, 95% CrI [0.21, 1.31]), drinks per drinking day (DPDD, $b = 0.42$, $SD = 0.19$, 95% CrI [0.16, 0.91]), and percent heavy drinking days (PHDD, $b = 2.24$, $SD = 1.88$, 95% CrI [0.07, 6.76]). Changes in DPDD were also positively associated with changes in log IL-6 ($b = 0.14$, $SD = 0.16$, 95% CrI [0.02, 0.42]) and log IFN-γ ($b = 0.43$, $SD = 0.32$, 95% CrI [0.11, 1.24]). These positive associations indicate that participants with greater reductions in drinking also showed greater reductions in the corresponding inflammatory markers. There was no evidence of associations between changes in drinking and changes in log IL-8 or log TNF-α, or between changes in DPD or PHDD and changes in log IL-6 or log IFN-γ. Medication condition did not moderate any associations between changes in drinking and inflammation.

DISCUSSION

The current study examined the relationship between changes in alcohol consumption and changes in peripheral inflammatory marker levels during a randomized controlled trial of the neuroimmune modulator ibudilast (IBUD) for the treatment of AUD. These analyses focused on the first four weeks of treatment, when participants experienced a reduction in drinking, as

measured by drinks per day (DPD), drinks per drinking day (DPDD), and percent heavy drinking days (PHDD). While there were no significant average changes in CRP or the pro-inflammatory markers assayed during this period, participants varied significantly in their trajectories of both drinking and inflammatory marker levels. In line with our hypothesis, changes in drinking were positively associated with changes in several peripheral inflammatory markers. Greater reductions in drinking, across all three drinking measures, were associated with greater reductions in CRP. Reductions in drinks per drinking day were also associated with greater reductions in IL-6 and IFN-γ. Changes in drinking were not associated with changes in IL-8 or TNF-α. These findings indicate that inflammatory markers differed in the extent to which they were associated with changes in drinking over the first four weeks of treatment in the trial.

Among the inflammatory markers examined, changes in CRP showed the most consistent relationship with changes in drinking, suggesting that CRP may be sensitive to multiple dimensions of drinking behavior, including overall amount (DPD), quantity per drinking occasion (DPDD), and frequency of heavy drinking (PHDD). In contrast, IL-6 and IFN-γ were associated only with changes in drinks per drinking day, suggesting sensitivity to a more specific aspect of drinking, namely quantity per drinking occasion. Notably, IL-8 and TNF-α were not associated with changes in any drinking measure over this period. These findings are consistent with meta-analytic and review evidence showing that the relationship between alcohol-related characteristics and inflammation is not uniform across markers, even among those characterized as pro-inflammatory (Adams et al., 2020; Galvez-Sánchez et al., 2026). Findings also vary across studies, and several factors have been proposed to account for this variability, including differences in the recency and patterns of alcohol use being examined, sample characteristics such as AUD severity and comorbid conditions, study setting, and other methodological features

(Galvez-Sánchez et al., 2026). The present findings suggest that the timescale over which drinking and inflammation are assessed may additionally contribute to variability across studies. While cross-sectional studies compare inflammatory marker levels between groups at a single time point, and human laboratory studies capture within-person inflammatory responses over hours following acute alcohol administration (Hillmer et al., 2020; Monnig et al., 2025), the present study examined relationships between trajectories of change in drinking and inflammation over four weeks. Notably, the aforementioned human laboratory studies did not assess CRP and instead focused on pro-inflammatory cytokines and chemokines (Hillmer et al., 2020; Monnig et al., 2025). This is consistent with the shorter timescale of these studies, as CRP, which is a hepatic acute-phase protein, responds more slowly than cytokines and chemokines, with its levels peaking approximately 48 hours after an inflammatory stimulus (Pepys & Hirschfield, 2003). CRP may therefore be better suited to detecting the more gradual, cumulative changes in inflammation that accompany sustained reductions in drinking over several weeks.

Although the primary analysis of this trial found no overall effect of IBUD on drinking or peripheral inflammatory markers relative to placebo (Ray et al., 2025), we examined whether medication condition moderated the association between changes in drinking and changes in inflammation. Given IBUD's anti-inflammatory properties, we hypothesized that it might amplify the reductions in peripheral inflammation associated with reduced drinking. However, we did not find evidence that medication condition moderated this association, which may reflect several factors. Although participants varied in their trajectories of peripheral inflammation, with some showing reductions in marker levels, these decreases may not have been attributable to IBUD. Consistent with the primary analysis, IBUD appeared to have little effect on peripheral inflammation in this sample (Ray et al., 2025). Alternatively, even if IBUD reduced peripheral

inflammation in some participants, these effects may have occurred independently of the reductions associated with decreased drinking and therefore would not have strengthened the relationship between changes in drinking and peripheral inflammation. Additionally, IBUD crosses the blood-brain barrier and acts on glial signaling within the central nervous system, and some of its anti-inflammatory effects may therefore not be reflected in peripheral markers (Angelopoulou et al., 2022). Thus, future work should also examine the relationship between IBUD and central markers of inflammation (Grodin et al., 2022).

The present findings should be interpreted in light of its strengths and several important considerations. Strengths include the use of bivariate latent growth modeling, which allowed us to simultaneously estimate individual trajectories of change in alcohol consumption and peripheral markers of inflammation, and to examine the association between latent changes in these variables. Drinking was characterized using multiple endpoints reflecting different aspects of consumption, and peripheral inflammation was assessed using multiple markers, enabling us to examine whether associations were consistent across inflammatory markers and dimensions of drinking. An important consideration when interpreting these findings is that the sample was limited to treatment-seeking individuals with moderate-to-severe AUD who reported heavy drinking. Additionally, nearly one-third of the sample had elevated CRP at baseline, with 30 of 99 participants exceeding the American Heart Association cutoff of 3.0 mg/L for high cardiovascular risk (Ridker, 2003). These sample characteristics may have influenced the associations observed in this study, as elevated baseline inflammation and higher initial levels of alcohol consumption allow greater room for change. The associations between changes in drinking and inflammation may therefore differ among individuals with lower baseline inflammation or smaller reductions in drinking. Future work should therefore examine these

relationships in more diverse samples, including individuals with varying levels of baseline inflammation, alcohol consumption, and AUD severity. Another important consideration is the directionality of the relationship between drinking and inflammation. Although change in drinking was modeled as predicting change in inflammation, both slopes represented change occurring over the same four-week period, so the temporal ordering of these changes cannot be determined. Because the relationship between alcohol consumption and inflammation is thought to be bidirectional (Mayfield & Harris, 2017), and studies that examine whether change in one precedes subsequent change in the other are needed to establish directionality. Finally, because the study was conducted, in part, during the COVID-19 pandemic, follow-up blood collection was disrupted over the remainder of the 12-week trial. Future studies would benefit from assessing inflammatory markers at more than two time points to characterize trajectories of change over a longer period of treatment.

In summary, the current findings indicate that reductions in alcohol consumption over the first four weeks of treatment were associated with reductions in CRP across all three drinking measures examined, whereas associations with the examined pro-inflammatory cytokines were limited to drinks per drinking day outcome. These findings suggest that CRP may be a more sensitive marker of the inflammatory change accompanying reductions in alcohol use over the course of treatment, consistent with its broad use as an index of systemic inflammation in clinical and research settings (Amezcua-Castillo et al., 2023). Notably, these associations were observed despite no significant average change in peripheral inflammatory marker levels, highlighting the value of examining individual trajectories to better understand relationships between changes in drinking and inflammation that may be obscured by group-level analyses.

Table 5-1. Participant Demographic and Clinical Characteristics

Variable	Total (n = 99)	IBUD (n = 50)	Placebo (n = 49)	p
Sex (male/female)	60/39	31/19	29/20	.77
Age (years)	44.30 (10.95)	42.72 (10.26)	45.92 (11.49)	.15
AUDIT score	19.99 (7.29)	20.80 (8.24)	19.16 (6.14)	.26
BDI-II score	11.14 (7.83)	11.54 (8.51)	10.73 (7.13)	.61
BMI (kg/m^2)	27.93 (5.92)	27.34 (6.62)	28.54 (5.11)	.27
Current smoker (yes/no)	32/67	15/35	17/32	.62

Note. BDI-II = Beck Depression Inventory–II; BMI = body mass index; AUDIT = Alcohol Use Disorders Identification Test. Values are mean (SD) unless otherwise indicated. Group differences were tested using Welch's two-sample t tests for continuous variables and χ^2 tests for categorical variables.

Table 5-2. Unconditional Latent Growth Curve Model Estimates for Drinking Measures

	Intercept		Slope		Slope variance	
Measure	*b* (*SD*)	95% CrI	*b* (*SD*)	95% CrI	σ^2 (*SD*)	95% CrI
DPD	3.61 (0.29)	[3.04, 4.19]	−0.26 (0.07)	[−0.39, −0.12]	0.14 (0.06)	[0.04, 0.29]
DPDD	6.00 (0.45)	[5.12, 6.87]	−0.28 (0.10)	[−0.48, −0.09]	0.24 (0.12)	[0.08, 0.54]
PHDD	0.56 (0.04)	[0.49, 0.63]	−0.03 (0.01)	[−0.05, −0.01]	0.004 (0.002)	[0.001, 0.01]

Note. DPD = drinks per day; DPDD = drinks per drinking day; PHDD = percent heavy drinking days; CrI = credible interval. Intercepts represent estimated baseline drinking levels. Slopes represent weekly change from baseline to week 4.

Table 5-3. Unconditional Latent Growth Curve Model Estimates for Log-Transformed Peripheral Inflammatory Markers

	Intercept		Slope		Slope variance	
Marker	*b* (*SD*)	95% CrI	*b* (*SD*)	95% CrI	σ^2 (*SD*)	95% CrI
log CRP	0.37 (0.15)	[0.07, 0.66]	−0.01 (0.03)	[−0.07, 0.05]	0.07 (0.01)	[0.05, 0.10]
log IL-6	−0.42 (0.09)	[−0.60, −0.25]	0.02 (0.01)	[−0.002, 0.05]	0.01 (0.002)	[0.009, 0.02]
log IL-8	1.37 (0.06)	[1.25, 1.48]	−0.01 (0.01)	[−0.03, 0.02]	0.02 (0.003)	[0.01, 0.02]
log IFN-γ	1.83 (0.10)	[1.63, 2.02]	−0.02 (0.03)	[−0.07, 0.03]	0.06 (0.01)	[0.04, 0.09]
log TNF-α	−0.26 (0.03)	[−0.33, −0.20]	−0.002 (0.005)	[−0.01, 0.01]	0.002 (<0.001)	[0.001, 0.002]

Note. CRP = C-reactive protein; IL-6 = interleukin-6; IL-8 = interleukin-8; IFN-γ = interferon-gamma; TNF-α = tumor necrosis factor-alpha; CrI = credible interval. All markers were log–transformed prior to analysis. Intercepts represent marker levels at week 4. Slopes represent weekly change from baseline to week 4.

Table 5-4. Associations Between Change in Drinking and Change in Inflammatory Markers, and Moderation by Medication Condition

		Drinking–Inflammation Association		Medication Moderation	
Marker	Drinking	*b* (*SD*)	95% CrI	*b* (*SD*)	95% CrI
Log CRP	DPD	**0.60 (0.30)**	**[0.21, 1.31]**	−0.03 (0.22)	[−0.47, 0.38]
	DPDD	**0.42 (0.19)**	**[0.16, 0.91]**	0.004 (0.20)	[−0.29, 0.49]
	PHDD	**2.24 (1.88)**	**[0.07, 6.76]**	−0.39 (2.08)	[−5.27, 2.75]
Log IL-6	DPD	0.15 (0.16)	[−0.01, 0.52]	−0.07 (0.20)	[−0.48, 0.32]
	DPDD	**0.14 (0.16)**	**[0.02, 0.42]**	0.09 (0.16)	[−0.12, 0.51]
	PHDD	0.65 (0.75)	[−0.22, 2.32]	0.16 (1.11)	[−2.17, 2.22]
Log IL-8	DPD	−0.04 (0.31)	[−0.76, 0.37]	−0.34 (0.70)	[−1.60, 1.03]
	DPDD	0.05 (0.32)	[−0.25, 0.67]	0.09 (0.29)	[−0.61, 0.62]
	PHDD	−0.28 (0.95)	[−2.19, 0.92]	0.07 (0.44)	[−0.70, 0.97]
Log IFN-γ	DPD	0.35 (0.51)	[−0.08, 1.59]	0.24 (0.34)	[−0.40, 0.96]
	DPDD	**0.43 (0.32)**	**[0.11, 1.24]**	0.24 (0.25)	[−0.13, 0.86]
	PHDD	0.92 (2.32)	[−2.55, 5.42]	2.42 (2.27)	[−0.88, 7.65]
Log TNF-α	DPD	0.03 (0.06)	[−0.06, 0.17]	0.08 (0.13)	[−0.12, 0.37]
	DPDD	0.04 (0.04)	[−0.01, 0.16]	0.06 (0.05)	[−0.02, 0.19]
	PHDD	0.10 (0.65)	[−0.45, 0.67]	0.56 (0.49)	[−0.10, 1.79]

Note. CRP = C-reactive protein; IL-6 = interleukin-6; IL-8 = interleukin-8; IFN-γ = interferon-gamma; TNF-α = tumor necrosis factor-alpha; DPD = drinks per day; DPDD = drinks per drinking day; PHDD = percent heavy drinking days; CrI = credible interval. All markers were log–transformed prior to analysis. Positive association coefficients indicate that greater reductions in drinking were associated with greater reductions in inflammation. Moderation coefficients represent the difference in association between ibudilast and placebo.

CHAPTER 5 REFERENCES

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OVERALL DISCUSSION

To date, only three medications have been approved by the FDA for the treatment of alcohol use disorder (AUD), despite its high prevalence, debilitating health consequences, and significant public health burden (Burnette et al., 2022). Given the highly heterogeneous nature of AUD in terms of its etiology, biology, and consequences, medications are not one-size-fits-all, and it is therefore important to expand the menu of approved pharmacotherapy options to increase the likelihood that an individual will find a medication that works for them (Koob et al., 2009; McManus & Ray, 2026). Despite ongoing efforts toward drug development for AUD, there has not been a new medication approved by the FDA for AUD in 20 years. This difficulty may be partially attributable to a lack of clear methodological guidelines for AUD randomized controlled trials (RCTs) (Anton et al., 2012). Thus, improving how AUD pharmacotherapy trials are designed and evaluated has become a high priority in the field, as a critical step toward developing novel and effective treatments for AUD. Additionally, investigating biological markers of AUD and their relationships with treatment outcomes may advance AUD pharmacotherapy research and guide the evaluation of novel therapeutic targets, including the inflammatory system.

The current dissertation addressed these priorities through two lines of research. The first part of the dissertation focused on key clinical trial design considerations: trial endpoints (**Chapter 1**), trial length (**Chapter 2**), and the co-use of cigarettes and cannabis during AUD clinical trials (**Chapter 3**). The second part of the dissertation focused on peripheral inflammation and intestinal permeability in AUD, given the inflammatory system's emergence as a novel treatment target for AUD. This work examined these markers as moderators of medication response and correlates of alcohol-related clinical characteristics (**Chapter 4),** and

the relationship between drinking and peripheral inflammation over the course of treatment (**Chapter 5**).

Chapter 1 provided a narrative review of the endpoints used to evaluate medication efficacy in AUD clinical trials. Traditionally, the FDA has accepted abstinence and no heavy drinking days as primary endpoints in phase 3 trials, both of which are derived from self-reported drinking (U.S. Department of Health and Human Services et al., 2015). While these outcomes serve as important benchmarks, they may not fully capture clinically meaningful changes, including overall reductions in drinking and improvements in psychosocial functioning and quality of life. This review, therefore, covered commonly used methods of assessing alcohol consumption and the endpoints derived from them, as well as alternative endpoints that had gained attention in the field, including reductions in WHO risk drinking levels, biological markers of alcohol use, neuroimaging measures, the NIAAA definition of recovery, and patient-reported outcomes. Since the publication of this dissertation chapter, the FDA has accepted a reduction of two WHO risk drinking levels as a primary endpoint, reflecting growing recognition of clinically meaningful change beyond abstinence and greater acceptance of harm-reduction approaches in AUD trials (Witkiewitz et al., 2025). Broadening the range of validated endpoints may better reflect the heterogeneity of AUD and support the development and regulatory approval of novel pharmacotherapies.

Chapter 2 is a meta-regression study that examined the relationship between trial duration and observed medication effect sizes in AUD pharmacotherapy RCTs. The FDA currently recommends an active treatment duration of at least six months for AUD pharmacotherapy RCTs, yet the relationship between trial length and treatment effects remains poorly understood (U.S. Department of Health and Human Services et al., 2015). In this

secondary analysis of a representative sample of 139 AUD pharmacotherapy RCTs conducted between 1985 and 2023, we tested whether trial length influenced the observed estimated effect sizes of medications. We found that observed effect sizes were generally consistent across trial length groups, with no clear evidence that medication effects differed by trial length. These findings contribute to ongoing efforts to improve AUD clinical trial design and provide additional context for considering trial length in future medication development and regulatory guidance decisions, particularly when weighing the detection of medication effects against trial costs and participant burden.

Chapter 3 examined cigarette and cannabis use over the course of a 12-week trial of ibudilast for AUD, in which drinking significantly decreased, but there was no significant effect of medication. Participants reduced their cigarette use over the trial, while cannabis use decreased during the early phase of treatment and then increased over the remainder of the trial. These findings show that cigarette and cannabis use may also change during AUD treatment, even when they are not directly targeted by the intervention. Drinks per drinking day was positively associated with cigarette use across the trial and negatively associated with cannabis use during the early phase of the trial. These findings contribute to growing evidence that cigarette and cannabis use may relate to drinking in distinct ways, with cigarette use potentially complementing alcohol use and cannabis use potentially substituting for it during AUD treatment. This study also found that baseline cigarette or cannabis use status did not significantly influence drinking outcomes or medication effects. These findings suggest that cigarette and cannabis use may not necessarily compromise treatment response in AUD trials and challenge concerns that addressing co-occurring substance use could interfere with alcohol use outcomes during treatment.

Chapter 4 examined whether baseline peripheral inflammation, indexed by C-reactive protein (CRP), and peripheral markers of intestinal permeability, including lipopolysaccharide-binding protein (LBP), soluble cluster of differentiation 14 (sCD14), and fatty acid-binding protein 2 (FABP2), moderated the effects of ibudilast on drinking outcomes over the 12-week RCT. Given the novelty of intestinal markers and AUD, this chapter also examined the relationships between intestinal permeability and AUD clinical characteristics, including alcohol consumption, craving, and symptom severity. Among participants with higher baseline CRP, ibudilast was associated with steeper reductions in percent heavy drinking days and average drinks per day than placebo during the maintenance phase (weeks 2–12), whereas placebo was associated with steeper reductions during the early phase (baseline–week 2). FABP2 similarly moderated medication effects on average drinks per day, with effects varying across trial phases. Baseline levels of CRP, sCD14, and LBP showed associations with alcohol-related clinical characteristics, supporting their potential clinical relevance in AUD. Overall, Chapter 4 highlights the potential utility of inflammatory and gut-related biomarkers for identifying subgroups of individuals who may differentially respond to treatment and underscores the need for further research examining inflammatory and gut-related processes in AUD.

Finally, **Chapter 5** examined the relationship between changes in alcohol consumption and changes in peripheral inflammatory marker levels during the first four weeks of the ibudilast RCT. Although AUD has consistently been associated with elevated systemic inflammation, prior research has largely relied on cross-sectional group comparisons or human laboratory studies assessing inflammatory changes over several hours following acute alcohol administration. This chapter addressed this gap by examining individual trajectories of change in both drinking and peripheral inflammatory markers, and the associations between these changes.

Reductions in alcohol consumption over the first four weeks of the trial were associated with reductions in CRP across all examined drinking measures, whereas associations with interleukin-6 and interferon-gamma were limited to the drinks per drinking day outcome. Changes in drinking were not associated with changes in interleukin-8 or tumor necrosis factor-alpha. CRP may therefore be better suited to detecting more gradual, cumulative changes in inflammation that accompany sustained reductions in drinking over several weeks. These findings highlight the value of examining individual trajectories of inflammation and drinking, which may be obscured in group-level analyses.

In summary, this dissertation makes several important contributions to the field of pharmacotherapy development for AUD. The first line of research highlights important methodological considerations for RCTs, which remain a high priority given the difficulty of obtaining FDA approval for novel AUD medications. The findings from Chapters 1–3 can inform decisions about efficacy endpoints and the length of active treatment periods, both of which may have regulatory implications, as well as how cigarette and cannabis co-use is considered in AUD trials. The second line of research explores how biological markers can improve our understanding of AUD and its treatment. Findings from Chapters 4 and 5 provide novel evidence that peripheral markers of intestinal permeability are relevant to AUD and that baseline peripheral inflammation may help identify subgroups of individuals who respond differently to anti-inflammatory medications. This work also demonstrates the value of modeling individual trajectories of change in drinking and inflammation to detect relationships that traditional group-level analyses may miss. The ultimate goal of this work, is to maximize our ability to develop and approve effective medications that will expand treatment options and improve outcomes for the millions of individuals affected by AUD.

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