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Immune Treatments for Alcohol Use Disorder: Translational Literature Review and Exploration of Clinical Mechanisms

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Immune Treatments for Alcohol Use Disorder:

Translational Literature Review and Exploration of Clinical Mechanisms

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy in Psychology

by

Lindsay Rae Meredith Broussard

2024

ABSTRACT OF THE DISSERTATION

Immune Treatments for Alcohol Use Disorder:

Translational Literature Review and Exploration of Clinical Mechanisms

by

Lindsay Rae Meredith Broussard

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2024

Professor Lara A. Ray, Chair

Background: Excessive alcohol consumption is a major public health burden. Yet less than 8% of individuals with past-year alcohol use disorder (AUD) received treatment. To support individuals in reducing drinking, treatments must target factors sustaining alcohol use from the molecular to psychosocial level. One emerging feature of AUD is alterations in immune signaling and neuroinflammation. Immune treatments that can restore healthy immune functioning may serve to promote recovery from AUD.

Methods: This dissertation project focused on the application of immune interventions as treatments for AUD. Chapter 1 comprised a qualitative literature review on preclinical and clinical studies testing immune compounds for AUD. The subsequent chapters utilized empirical data collected during two clinical trials on a neuroimmune compound to explore its clinical

mechanisms. In the first trial, 52 participants with AUD were randomized to ibudilast or matched placebo for two weeks and completed daily diary assessments on alcohol, mood, and craving. Chapter 2 tested whether ibudilast modulated acute alcohol-induced changes in craving and mood. In a larger clinical trial, 102 treatment-seeking participants with AUD were randomized to ibudilast or placebo and took study medication for 12 weeks. Chapter 3 tested for differences in monthly change rates among clinical measures of alcohol craving, depression, and anxiety between the medication groups. In Chapter 4, exploratory analyses assessed whether ibudilast improved neurocognition, compared to placebo.

Results: In Chapter 1, we highlighted translational findings with an emphasis on safety and clinical implications from randomized controlled trials testing immune treatments for AUD. Results from naturalistic reports in Chapter 2 showed that ibudilast reduced daily alcohol-induced craving but not mood. Similarly, linear growth models from Chapter 3 showed that the ibudilast group had steeper reductions in tonic alcohol craving than placebo but there were no treatment group differences in rates of change for depression or anxiety symptoms. Lastly, as outlined in Chapter 4, ibudilast did not improve neurocognitive functioning compared to placebo.

Conclusion: This dissertation used a translational framework combining neuroimmunology, pharmacology, and experimental psychology to better characterize the clinical application of immune treatments for AUD. Mitigation of craving may be a central clinical mechanism of ibudilast.

The dissertation of Lindsay Rae Meredith Broussard is approved.

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Dedication

I would like to dedicate this dissertation to my husband Kerry and parents Anna and Brian for their years of steadfast support during my education and training in psychology. I am endlessly grateful for all the encouragement and advice I have received from friends, family, and faculty along this journey.

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Acknowledgements

Chapter 1:

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LAR designed and acquired funding for the primary clinical trial. LRM assisted with data collection, cleaning, and management. LRM and LAR contributed to the conceptualization of the manuscript. LRM conducted data analyses and prepared data visualizations, with the assistance of CKE. LRM drafted the initial manuscript. LAR and CKE provided expert review and edits of the work. All authors agree to be accountable for all aspects of the work, including its accuracy and integrity.

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LAR designed and acquired funding for the primary clinical trial. LRM and WAB assisted with data collection, cleaning, and management. LRM and LAR contributed to the conceptualization of the manuscript. LRM conducted data analyses and prepared data visualizations. LRM drafted the initial manuscript. LAR provided expert review and edits of the work All authors reviewed and approved the initial version of this manuscript. All authors agree to be accountable for all aspects of the work, including its accuracy and integrity.

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3. Green, R., **Meredith, L. R.,** Mewton, L., & Squeglia, L. M. (2023). Adolescent neurodevelopment within the context of impulsivity and substance use. *Current Addiction Reports, 10.* https://doi.org/10.1007/s40429-023-00485-4
4. Grodin, E. N., **Meredith, L. R**., Burnette, E. M., Miotto, K., Irwin, M. R., & Ray, L. A. (2022). Baseline CRP levels are predictive of treatment response to a neuroimmune modulator in alcohol use disorder: A preliminary study. *The American Journal and Drug and Alcohol Abuse, 49*(3). PMID: 36282988
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6. **Meredith, L. R.**, Green, R., Grodin, E. N., Chorpita, M., Miotto, K. & Ray, L. A. (2022). Ibudilast moderates the effect of mood on alcohol craving during stress exposure. *Experimental and Clinical Psychopharmacology.* PMCID: PMC9484034
7. **Meredith, L. R.**, Lim, A. C., & Ray, L. A. (2020). Neurocognitive performance in alcohol use disorder using the NIH Toolbox: Role of severity and sex differences. *Drug and Alcohol Dependence, 216*: 108269. PMCID: PMC7972314
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INTRODUCTION TO THE DISSERTATION

Characterizing Alcohol Use Disorder and Treatment

Among individuals aged 15 to 49 years, harmful use of alcohol is the leading risk factor for premature disability and mortality worldwide (WHO, 2018). Excessive alcohol consumption is a major public health burden that, along with other environmental exposures and biological and psychological factors, can lead to the development of alcohol use disorder (AUD; (Sacks et al., 2015). Diagnostically, the DSM-5 classifies AUD as a mild to severe mental disorder comprised of 11 possible symptoms, which include repeated, often uncontrollable alcohol use and negative consequences, such as mood disturbance, liver disease, relationship distress, and withdrawal symptoms (First et al., 2015). Unfortunately, despite these adverse effects, less than 8% of individuals with past-year AUD received any alcohol treatment and even fewer received evidence-based care, such as FDA-approved pharmacotherapies (i.e., naltrexone/ vivitrol, disulfiram, acamprosate) and behavioral interventions (e.g., contingency management, CBT, motivational enhancement therapy; (SAMHSA, 2019). Moreover, the average latency from time of AUD diagnosis to treatment seeking is estimated to be over eight years (Hasin et al., 2017). Among those more likely to receive any alcohol treatment are individuals in middle adulthood, who identify as male, have mental health comorbidities, and exhibit greater AUD severity (Venegas et al., 2021). Health disparities in AUD treatment exist among certain minority groups, including those identifying as Black, Latina/o/x, and Native American, and particularly women belonging to these groups (Alvanzo et al., 2014; Vaeth et al., 2017). These individuals may face longer latencies from diagnosis to treatment receipt (Lewis et al., 2018) and lower quality of care, such as lower likelihood of receiving treatment from a health professional (Chartier & Caetano, 2011).

Medications Development for AUD

Guidelines recommend that physicians and other prescribers offer approved medications to patients with moderate to severe AUD (Kranzler & Soyka, 2018), yet they are largely under-prescribed due to various provider and patient factors. One study using a retail pharmacy database suggested that fewer than 10% of patients who needed AUD treatment received a prescription for FDA-approved pharmacotherapies (Mark et al., 2009). Further, only around 3% of Veterans diagnosed with an AUD received approved medications for alcohol use within the Veterans Health Administration (Harris et al., 2012). Even among those receiving front-line treatment, relapse and continued heavy drinking is common, as existing pharmacotherapies are only moderately effective (Heilig et al., 2019). For example, naltrexone significantly reduces the risk of binge drinking by 10% and any drinking by 5%, but these effects are modest (Kranzler & Soyka, 2018). Acamprosate is approved to sustain abstinence, as it associated with a reduction in drinking risk among abstinent individuals but it is not predictive of reductions in binge drinking (Jonas et al., 2014). Disulfiram, the earliest FDA-approved medication for AUD, lacks evidence of efficacy among blinded trials, although has shown benefits when its ingestion is supervised (Skinner et al., 2014). The modest efficacy rates of established treatments are a result of the heterogeneity of AUD profiles, individuals' differential response to treatment, psychosocial factors, and multisystem symptomatology (Litten et al., 2020). As such, the development of novel and more efficacious treatments for AUD is a high research priority in the alcohol field and one aspect of a complex system that may help improve recovery rates and the implementation and utilization of evidence-based care (Litten et al., 2012). Over the past several decades, repurposed and novel agents with a wide range of treatment targets (e.g., gabapentin, topiramate, prazosin, GET73) have been tested in animal models and in clinical samples with

AUD (Burnette et al., 2022), but no new pharmacotherapies have received FDA-approval for the treatment of AUD since 2006 (i.e., extended-release naltrexone injections).

To support individuals in reducing drinking or achieving abstinence, pharmacotherapies must target factors sustaining alcohol use from the molecular to psychosocial level. A dominant neurobiological theory of addiction, the allostatic model, posits that the development of addiction is characterized by an allostasis, or an aberrant homeostatic process, which involves changes in reward and stress circuits following regular exposure to alcohol or other substances (Koob, 2015; Koob & Le Moal, 2008; Koob & Volkow, 2016). Through this lens, individuals with AUD are thought to experience three stages of the addiction cycle: (1) binge and intoxication, (2) withdrawal/ negative affect, and (3) preoccupation/ anticipation, which map onto progressive neuroadaptive changes in brain regions and circuits relevant to addiction (Koob & Volkow, 2016). This model emphasizes the change from initial heavy alcohol use for its salient, rewarding effects, followed by chronic and often uncontrollable alcohol intake that is driven by negative reinforcement and craving associated with dysphoria and withdrawal states. Clinically, this cycle places individuals at high risk for relapse or resurgence of heavy drinking after a period of reduced alcohol intake. A variety of psychological maintenance factors are connected to these three stages of addiction and represent meaningful treatment targets. Thus, research initiatives have sought to more accurately capture the clinical neuroscience of addiction using transdiagnostic, neuroscience-based frameworks with the hopes of understanding how treatments might successfully alter factors sustaining addiction among individuals with diverse symptom profiles and backgrounds (Kwako et al., 2016; Nieto et al., 2021).

One such initiative that is clinically relevant is the Addictions Neuroclinical Assessment (ANA) framework (Kwako et al., 2016). The ANA framework consists of three domains: (1)

executive function, (2) incentive salience, and (3) negative emotionality, which complement the three phases of the addiction cycle. Deficits in a wide range of neurocognitive functions, including response inhibition, working memory, attention, and episodic memory are well-documented among inpatient samples with AUD (Bernardin et al., 2014; Stavro et al., 2013). Greater neurocognitive dysfunction among individuals with AUD is associated with worse treatment outcomes, as these important processes promote one's engagement in goal-directed actions, self-regulation, planning, etc. (Bates et al., 2006). Continuing, incentive salience is the perceptual transformation of certain stimuli, such that they become more attractive and strongly influence motivation and behavioral responses (Cofresi et al., 2019). Regarding incentive salience in AUD, these stimuli take the form of cues that have become associated with alcohol and its consumption. Enhanced reactivity to alcohol-related cues is shown to increase risk for relapse through craving induction and habit formation (Valyear et al., 2017). Negative emotionality plays several roles in addiction. Anti-reward and stress systems drive motivation to drink via negative reinforcement, such as to diminish withdrawal-induced dysphoria or via self-medication of psychiatric symptoms (Koob, 2013). Not surprisingly, negative affectivity is positively associated with alcohol problem severity and intake (Cano et al., 2017; Pavkovic et al., 2018).

To further the treatment of AUD, including personalized medicine, researchers must assess whether potential pharmacotherapies sufficiently alter alcohol intake, along with salient maintenance factors to promote long-term recovery and improved quality of life. Establishing treatment effectiveness and identifying mechanisms of action through human laboratory paradigms, randomized controlled trials (RCTs), and collection of real-world data represent vital steps in medications development for AUD (Carpenter et al., 2020; Litten et al., 2020; Ray et al.,

2018). The empirical chapters of this dissertation explored the effects of a novel compound and potential pharmacotherapy for AUD, ibudilast, on each of the ANA domains, which represent meaningful treatment targets. We leveraged data collected from RCTs of ibudilast enrolling individuals diagnosed with AUD.

The Immune System as a Treatment Target

Over the past two decades, the body of literature implicating the critical role of the immune system in the development and maintenance of addiction has grown dramatically (Crews, Lawrimore, et al., 2017; Crews, Walter, et al., 2017; Erickson, Grantham, et al., 2019; Gao et al., 2019; Mayfield et al., 2013; Mayfield & Harris, 2017; Ozburn et al., 2020). While the immune system is essential for survival, an excessive inflammatory response, such as from sustained heavy alcohol use, can negatively impact the individual and contribute to compulsive drinking and other consequences of AUD (Slavich & Irwin, 2014). Alcohol intake is thought to alter immune signaling and increase neuroinflammation through two primary mechanisms: (a) indirectly by initiating production of proinflammatory cytokines systemically (e.g., leaky gut, binding at vagal afferent sites), and (b) directly via actions in the brain, wherein alcohol and potentially alcohol-induced neural damage stimulate the release of inflammatory molecules (Barak et al., 2015; Blednov et al., 2011; Mayfield et al., 2013). In human samples, peripheral proinflammatory markers are consistently elevated in AUD and correlate with alcohol use, craving, and severity (Adams et al., 2020; Crews, Walter, et al., 2017). Further, studies on postmortem brains of heavy drinkers shows a differential expression of proinflammatory neuroimmune genes compared to controls (Mayhugh et al., 2018). As a result of these intriguing findings, the field has started to identify and test compounds targeting the peripheral immune

system and neuroimmune system with the hopes of reducing alcohol use and mitigating factors sustaining symptoms of AUD. An overview of immune signaling and its role in the maintenance of AUD will be reviewed in further detail in Chapter 1-- a qualitative review paper.

Continuing, toll-like receptors (TLRs), which are essential contributors to immune signaling, are considered key components of alcohol-stimulated neuroimmune activation (Erickson, Grantham, et al., 2019). Activation of TLR4 results in the nuclear translocation of NF-κB, a regulator of proinflammatory cytokine expression (Erickson, Grantham, et al., 2019) that has widespread effects on physiological and behavioral responses (Dinarello, 2000). In contrast, inhibition of NF-κB signaling results in specific reductions in ethanol intake (Truitt et al., 2016). Thus, compounds targeting TLR and NF-κB signaling pathways are now being tested as potential pharmacological treatments. For example, several phosphodiesterase (PDE) inhibitors, including rolipram, apremilast, and, germane to this proposal-- ibudilast, have been tested in animal or human models of AUD and demonstrated initial efficacy (Bell et al., 2015; Blednov et al., 2014; Blednov, Da Costa, Harris, et al., 2018; Blednov, Da Costa, Tarbox, et al., 2018; Hirose et al., 2007; Li et al., 2020; Ray et al., 2017; Schafer et al., 2010; Wen et al., 2018a). PDE inhibitors uniquely regulate cyclic adenosine monophosphate (cAMP) signaling, which plays a role in neural functioning and the downregulation of NF-kB and proinflammatory cytokine release (Wen et al., 2018a). This pathway is implicated in several features of compulsive alcohol use and represents a promising treatment target (Parry & Mackman, 1997).

Despite progress in understanding the connection between the neuroimmune system and AUD, few clinical trials testing immune treatments for AUD have been conducted to date. As such, little is known about which compounds or interventions might be most effective in human samples and how these immune therapies influence various maintenance factors of AUD, such as

reward, executive function, and negative emotionality. This dissertation pursues an examination of neuroimmune treatment for AUD with a focus on testing ibudilast's mechanisms of action.

Overview of Dissertation Chapters

Overview for Chapter 1.

Chapter 1 of this dissertation serves as a qualitative literature review that covers both preclinical and clinical studies of pharmacological and behavioral interventions targeting immune mechanisms, which have been tested in animal models of ethanol dependence and humans with AUD. Several literature reviews on immune signaling in AUD and addiction have been published to date. However, these reviews focused primarily on the contribution of molecular immune mechanisms, inflammation, and peripheral immune and neuroimmune pathways to the development and maintenance of addiction. In contrast, the present review sought to integrate preclinical and human research on immunological intervention to provide a translational perspective supporting safe and effective clinical application of immune treatments for AUD.

We first provided a brief theoretical rationale for immune therapies in the management of AUD and then discussed progress in medications development for AUD with the immune system as a treatment target. Through this approach, the present review more comprehensively covered clinically relevant factors, such as safety profiles and approval status of the tested immune compounds and outlines applicable completed and ongoing RCTs and their published findings. Importantly, we further discussed these compounds' effectiveness in the context of clinically significant drinking outcomes and other maintenance factors of AUD, such as negative affectivity, withdrawal, craving, stress, neurocognitive function, and subjective response to

alcohol. In doing so, we hope this qualitative review provides researchers with up-to-date knowledge on promising immune interventions for the treatment of AUD tested in both animal models and human samples with clinical safety and applicability at the forefront. We concluded by providing recommendations for future research in this area.

Overview for Chapter 2, 3 and 4.

Chapters 2, 3, and 4 of this dissertation present empirical data from our laboratory collected during two double-blind randomized controlled trials of ibudilast for AUD. In the first trial, 52 participants with AUD were randomized to ibudilast or matched placebo for two weeks and completed daily diary assessments on alcohol, mood, and craving. Chapter 2 pulled data from these naturalistic reports to test whether ibudilast modulated acute alcohol-induced changes in craving and mood during a reported drinking episode. In a larger, full-scale clinical trial, 102 treatment-seeking participants with AUD were randomized to ibudilast or placebo and took study medication for 12 weeks. Chapter 3 tested for differences in monthly change rates among clinical measures of alcohol craving, depression, and anxiety between the medication groups. In Chapter 4, exploratory analyses assessed whether ibudilast improved neurocognition, compared to placebo. Ibudilast is a promising and novel neuroimmune treatment for AUD. These chapters involve secondary data analyses exploring ibudilast's potential clinical mechanisms of action, including alcohol craving/ reward, negative affectivity, and neurocognitive functioning. The background, rationale, and methods for these empirical chapters on ibudilast are provided in detail following Chapter 1.

CHAPTER 1:

Immune Treatments for Alcohol Use Disorder:

A Translational Framework

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ABSTRACT

While the immune system is essential for survival, an excessive or prolonged inflammatory response, such as that resulting from sustained heavy alcohol use, can damage the host and contribute to psychiatric disorders. A growing body of literature indicates that the immune system plays a critical role in the development and maintenance of alcohol use disorder (AUD). As such, there is enthusiasm for treatments that can restore healthy levels of inflammation as a mechanism to reduce drinking and promote recovery. In this qualitative literature review, we provide a conceptual rationale for immune therapies and discuss progress in medications development for AUD focused on the immune system as a treatment target. This review is organized into sections based on primary signaling pathways targeted by the candidate therapies, namely: (a) toll-like receptors, (b) phosphodiesterase inhibitors, (c) peroxisome proliferator-activated receptors, (d) microglia and astrocytes, (e) other immune pharmacotherapies, and (f) behavioral therapies. As relevant within each section, we examine the basic biological mechanisms of each class of therapy and evaluate preclinical research testing the role of the therapy on mitigating alcohol-related behaviors in animal models. To the extent available, translational findings are reviewed with discussion of completed and ongoing randomized clinical trials and their findings to date. An applied and clinically focused approach is taken to identify the potential clinical applications of the various treatments reviewed. We conclude by delineating the most promising candidate treatments and discussing future directions by considering opportunities for immune treatment development and personalized medicine for AUD.

INTRODUCTION

A growing body of literature indicates that the immune system plays a critical role in the development and maintenance of alcohol use disorder (AUD) (Mayfield & Harris, 2017). Hence, there is increasing interest in the development of medications and therapies that target the immune system in an effort to treat AUD. This review addresses the conceptual rationale for immune treatments and highlights the potential for treatment approaches modulating the immune system to mitigate mechanisms contributing to AUD. The link between the immune system and AUD is supported by both basic and clinical findings.

Briefly, the immune system, which is comprised of both innate and adaptive immune mechanisms, serves as the body's primary defense against pathogens and is critical for human well-being and health (Slavich & Irwin, 2014). Although the brain is protected by the blood-brain barrier (BBB), it has resident immune defenses, including innate immune cells, to help protect against threats (Coleman & Crews, 2018). Microglia are considered resident macrophages of the brain and, along with astrocytes and neurons, contain receptors capable of immune signaling (Coleman & Crews, 2018). Innate immune signaling in the periphery can cross the BBB through several mechanisms, including immune-mediated active transport and disruptions in the BBB (Banks, 2015; Erickson & Banks, 2018; Quan & Banks, 2007). The innate immune branch responds rapidly and includes immune cells like monocytes and dendritic cells that circulate throughout the body. It is the first line of defense against bacterial infection or tissue injury and can initiate inflammatory cascades and activate adaptive immune processes (Medzhitov, 2008). Adaptive immunity takes over when the innate immune response is insufficient; it is slower but more specific (Bonilla & Oettgen, 2010). Adaptive immune

mechanisms like T and B lymphocytes target antigens through an immunological memory of the pathogen (Slavich & Irwin, 2014).

During initial innate immune activation, inflammatory responses are triggered by detection of conserved features of microbes, termed pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS) (Bonilla & Oettgen, 2010). LPS is an endotoxin component of the outer membrane of Gram-negative bacteria (Raetz & Whitfield, 2002). LPS levels are shown to be elevated in individuals with AUD (Qin et al., 2008); however, these levels normalize after 3 weeks of abstinence (Leclercq, Cani, Neyrinck, Stärkel, et al., 2012). Toll-like receptors (TLRs) are a common family of receptors found on immune cells and are known to recognize PAMPs and subsequently activate transcription factors, including nuclear factor-κB (NF-κB), interferon (IFN) regulatory factors, and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) (Aurelian et al., 2016; Balan, Warnock, Puche, Gondre-Lewis, & Aurelian, 2018; Medzhitov, 2008). These activated factors then drive the expression of proinflammatory immune protein molecules, termed cytokines, which are released from immune cells, coordinate inflammatory cell functions, and have wide-ranging effects on physiological and behavioral responses (Dinarello, 2000). Types of cytokines have specific mechanisms and proinflammatory cytokine types include interleukin (IL)-1, IL-2, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) (Erickson, Grantham, et al., 2019).

Alcohol is thought to alter immune signaling and increase neuroinflammation via two primary mechanisms: (a) indirectly by initiating systemic production of proinflammatory cytokines; and (b) directly through actions in the brain, whereby alcohol and potentially alcohol-induced neural damage (de la Monte & Kril, 2014) stimulate the release of inflammatory molecules (Crews & Vetreno, 2016). Individuals with AUD display peripheral immune

dysregulation and are vulnerable to viral or bacterial infections (Keshavarzian et al., 2009). Systemic inflammation appears to be induced by alcohol when it acts on peripheral immune receptors in the gut (Erickson, Grantham, et al., 2019) and also by breaking down lymphatic duct lining and endothelial cell junctions, allowing inflammatory molecules to leak into the bloodstream, termed "leaky gut" (Gorky & Schwaber, 2016). The resultant proinflammatory molecules in the periphery then provoke neuroinflammation, i.e., an inflammatory response within the central nervous system (CNS) as opposed to in the periphery. This provocation occurs through several mechanisms, such as inflammatory molecules crossing the BBB via immune-mediated active transport or by entering the brain through disruptions in the BBB (Banks, 2015; Quan & Banks, 2007). Additionally, receptor binding of inflammatory cytokines at vagal afferent sites (e.g., in stomach and liver) rapidly results in the transduction of inflammatory signaling in the CNS (Quan & Banks, 2007). Proinflammatory molecules in the brain impact neural circuit functioning and neuronal plasticity (Erickson, Grantham, et al., 2019). Notably, neuroinflammation can be both adaptive, such as in response to brain injury to promote repair, or maladaptive, such as in response chronic social stressors (DiSabato et al., 2016). Thus, while the immune system is essential for survival, an excessive or prolonged inflammatory response, such as that resulting from sustained heavy alcohol use, can damage the host and contribute to psychiatric and physical disorders (Slavich & Irwin, 2014).

Initial support for the relationship between alcohol use and neuroinflammation came from gene expression studies of post-mortem brain tissue (McBride et al., 2014; Osterndorff-Kahanek et al., 2015). These studies demonstrated consistent upregulation in the expression of genes involved in inflammatory responses in the brains of individuals with AUD (Liu et al., 2004; Liu, Lewohl, et al., 2006; Robinson et al., 2014). Similar findings were obtained in a

reverse-translation (i.e., leveraging insights from human studies to inform mechanistic and preclinical work) to rodents exposed to chronic ethanol. Voluntary ethanol consumption increased cytokines and chemokines in the CNS and periphery for mice (Pascual et al., 2015) and monkeys (Beattie et al., 2018). In rats, 24-48 hours of ethanol withdrawal, a critical window of reinstatement, resulted in the upregulation of mRNA proinflammatory expression of innate immune markers (e.g., TNF-α, IL-1β) in cortical tissue (Freeman et al., 2012; Whitman et al., 2013). These findings indicate immune signaling upregulated during alcohol withdrawal may contribute to the maintenance of AUD. Importantly, immune factors mediate not only neuroinflammation but a broad set of neural functions, including neurotransmitter systems and synaptic function, neurogenesis and neurodevelopment, and endocrine function (Cui et al., 2014). For instance, research suggests that alcohol disrupts the ability of astrocytes to properly regulate glutamate homeostasis, which contributes to the development of sustained drinking (Bachtell et al., 2017). Emerging work also supports the involvement of neuroimmune signaling in adolescent binge drinking and subsequent changes in brain physiology (Crews et al., 2019; Crews, Walter, et al., 2017; Montesinos et al., 2016; Pascual et al., 2018). Further, preclinical work suggests that neuroinflammation and modulation of immune signaling induced by chronic alcohol use heighten motivation for intake, enhances alcohol-related reward, and contributes to substance-related cognitive impairments and depression-like behavior (Alfonso-Loeches et al., 2010; Blednov, Da Costa, Harris, et al., 2018; Breese et al., 2008; Briones & Woods, 2013; Frank et al., 2011).

Evidence for heightened CNS activation of inflammatory signaling in human samples with AUD is very limited and further research is necessary to establish the neuroimmune hypothesis of AUD. Studies evaluating this hypothesis have largely used positron emission

tomography (PET) to image the translator protein (TSPO), a mitochondrial protein that is upregulated during neuroinflammation. Three studies have reported reduced binding of PET TSPO ligands in the brains of individuals with AUD relative to controls (Feldman et al., 2020); however, these findings are discrepant with in vitro animal studies and complicated by genotype specific responding to TSPO ligands (Kreisl et al., 2013). Elevations in TSPO mRNA in postmortem brains from individuals with an AUD provide initial evidence for the neuroinflammation hypothesis (De Carvalho et al., 2021). Several studies demonstrate elevated peripheral inflammation in clinical AUD samples. Whereas this work has been largely correlational, it generally supports the hypothesized link between inflammation and AUD. In treatment-seeking individuals, elevated levels of circulating LPS were found at treatment onset but decreased after 3-weeks of detoxification, reaching levels comparable to controls (Leclercq, Cani, Neyrinck, Stärkel, et al., 2012). Proinflammatory proteins, including TNF-α, IL-6, and C-reactive protein (CRP), were positively correlated with craving at treatment entry among individuals with AUD (Leclercq, Cani, Neyrinck, Stärkel, et al., 2012). However, not all studies have found elevations in LPS proinflammatory protein levels, indicating that this peripheral inflammatory response may be present in only a subset of individuals with AUD (Adams et al., 2020). To what extent alcohol induction of peripheral inflammation increases neuroimmune signaling in clinical samples is not known, although this link is established in basic studies. Translational work is beginning to guide these efforts. For example, when fecal microbiota were transplanted from patients with AUD to germ-free mice (Leclercq et al., 2020), CNS alterations in myelination, neurotransmission, and inflammation occurred, with evidence of an increased expression of proinflammatory cytokines and chemokines and elevated markers of microglial activation. To that end, novel treatment targets, such as peripheral and neural immune pathways,

represent an important direction in the development of novel and more effective treatment options for AUD (Litten et al., 2016; Ray et al., 2014) and psychiatric diseases more broadly. While the current review focuses on the application of immune interventions for AUD, literature in this area is broad and recent reviews have addressed other topics relevant to immunity and AUD in detail (Coleman & Crews, 2018; Crews, Lawrimore, et al., 2017; Cui et al., 2014; Erickson, Grantham, et al., 2019; Gao et al., 2019; Jimenez-Gonzalez et al., 2021).

In this qualitative literature review, we discuss recent advances in medications development for AUD focusing on the immune system as a treatment target. Based on the implication of immune mechanisms to the phenomenology of AUD, briefly reviewed above, there is enthusiasm for treatments that can restore healthy levels of inflammation and immune signaling as a mechanism to reduce drinking and promote recovery (see **Figure 1-1**). This review is organized into sections based on the primary signaling pathway targeted by the candidate therapies, namely: (a) TLRs, (b) phosphodiesterase (PDE) inhibitors, (c) peroxisome proliferator-activated receptors (PPARs), (d) microglia and astrocytes, (e) other immune pharmacotherapies, and (f) behavioral therapies. We use these categories for organizational purposes, as we recognize that these distinctions are inherently arbitrary and that there is complex interplay across signaling pathways and molecules. After reviewing the relevant literature, we consider future directions and opportunities for treatment development and personalized medicine for AUD. As the field continues to evolve and more clinical studies are added to the robust preclinical literature on alcohol and inflammation, a refined understanding of immune targets for AUD will continue to emerge. Consistent with the ongoing challenge of translational science in AUD (L.A. Ray et al., 2021), we emphasize avenues for applying these findings to clinical populations. To that end, we take an applied and clinically focused approach

to identifying potential clinical applications of the various treatments reviewed herein. We contend that biological and clinical plausibility are both necessary to optimize treatment development.

Targets for Candidate Immune Therapies for AUD

The following sections describes various candidate immune therapies for AUD. Within each section, we examine the basic biological mechanisms of each class of therapy and evaluate the basic and preclinical research testing the role of the therapy in mitigating alcohol behaviors in animal models. To the extent available, translations findings are reviewed with discussion of completed and ongoing interventional trials and findings to date.

Toll-Like Receptors

TLRs are members of the IL-1 receptor/ TLR superfamily. As reviewed above, TLRs, along with proinflammatory cytokines and their associated receptors, share signaling pathways that converge on NF-κB, an innate immune transcription factor that regulates inflammatory cytokine expression (Crews, Lawrimore, et al., 2017). These pathways are widely implicated in alcohol-induced neuroinflammation (Bajo et al., 2016; Crews, Lawrimore, et al., 2017; Montesinos et al., 2017), with human brain tissue from individuals with AUD showing an upregulation of several TLRs.

Ten TLRs have been identified in humans and the most widely studied subtype within this family is TLR4, which is thought to contribute significantly to alcohol-related neuroimmune activation. TLR4 activation plays an important role in regulating neuroimmune signals that influence alcohol intake. The TLR4 signal is innately activated in neurons from alcohol-

preferring rats and TLR4-MyD88 proinflammatory cytokines are inhibited after acute exposure (Muralidharan et al., 2018). The TLR4 signal is activated through the non-canonical TLR4 binding of the $GABA_AR$ α2 subunit (Balan, Warnock, Puche, Gondre-Lewis, June, et al., 2018; Liu et al., 2011). During alcohol self-administration, this signal is sustained through increased expression of the stress hormone corticotropin-releasing factor (CRF) and its feedback regulation of TLR4 signaling (Balan, Warnock, Puche, Gondre-Lewis, & Aurelian, 2018; June et al., 2015). The balance of the resulting pro- and anti-inflammatory chemokines likely contributes to the transition to alcohol dependence. Further, in neurons from the central nucleus of the amygdala (CeA) and ventral tegmental area (VTA), TLR4 signals through the chemokine CCL2 (Aurelian & Balan, 2019; Zhou et al., 2011) localizing in dopaminergic neurons, and inducing the expression of tyrosine hydroxylase through CREB signal (Aurelian et al., 2016; Banisadr et al., 2005). In CeA neurons, CCL2 is localized to the synapse and transported via axons to downstream brain regions, such as the bed nucleus of the stria terminalis, and is thought to affect behaviors such as anxiety. Neurons are thus an important target for chemokine function independent of inflammation but related to relevant behavior, including impulsivity and alcohol intake (Harper et al., 2020).

Results from studies of TLR-affecting medications in the context of AUD implicate TLRs as promising targets for the development of AUD therapeutics (see **Table 1-1**). TLR4 blockade by opioid antagonists, including naltrexone and naloxone, has been extensively tested in animal models. Both the (+) and (-) isomers of naltrexone and naloxone are considered TLR4 antagonists (Skolnick et al., 2014; Wang et al., 2016). The fact that the opioid-inactive (+) isomer acts similarly on TLR4 as the opioid-active (-) isomer, denotes an immune mechanism likely independent of the opioid effects of these pharmacotherapies (Hutchinson et al., 2008;

Wang et al., 2016). In regards to alcohol effects, TLR4 blockade by (+)-naltrexone reduces binge drinking in adolescent mice (Jacobsen et al., 2018b) and decreases ethanol preference (Jacobsen et al., 2018a). In healthy mice using opioid-inactive naloxone to block TLR4 reduced acute alcohol-induced motor impairment and sedation (Wu et al., 2012). In contrast, another study found that (+)-naloxone produced very modest inhibition of intake among rodents and only at the highest dose (Harris et al., 2017). Moreover, nalmefene, another opioid receptor antagonist that inhibits TLR4 signaling, reduced ethanol-induced inflammation and binge-like drinking behaviors in adolescent female mice by preventing TLR4 activation (Montesinos et al., 2017).

While TLR4 is the most studied member of the TLR family in the context of AUD, other TLR pathways modulating NF-κB signaling, including TLR2 and TLR3 (Erickson, Grantham, et al., 2019) are similarly implicated in the neuroimmune effects of alcohol in several preclinical studies (Blednov et al., 2017; Fernandez-Lizarbe et al., 2013; McCarthy et al., 2018; Pascual et al., 2015). Given that NF-κB is a target of multiple TLRs, it is likely that multiple receptors work in concert and convergently act on NF-κB. Therefore, exploring pharmacological antagonists of TLR subclasses beyond TLR4 may be worthwhile. Neuroimmune therapies that bypass TLR binding to act directly on NF-κB have also shown promise in preclinical work for the treatment of AUD. Immunotherapies such as sulfasalazine and TPCA-1 act on NF-κB through IKKβ, an inhibitor of the NF-κB kinase subunit beta. Both of these pharmacological inhibitors of IKKβ have been shown to decrease ethanol consumption and preference in mice (Truitt et al., 2016). Amlexanox, another NF-κB inhibitor and anti-inflammatory, anti-allergic pharmacological immunomodulator involved in several TLR pathways (Reilly et al., 2013) reduced ethanol consumption and preference in mice completing a two-bottle-choice paradigm (McCarthy et al., 2018).

While the majority of work exploring neuroimmune modulators of TLR and NF-κB are preclinical in nature, numerous human studies have evaluated established opioid antagonists, such as naltrexone and nalmefene. Naltrexone is FDA-approved for treatment of AUD and nalmefene is approved in Europe for harm-reduction (Swift & Aston, 2015). Naltrexone was associated with drinking reductions, including longer latency to return to any drinking and reduction in heavy drinking days (Jonas et al., 2014). Nalmefene was also associated with moderate reductions in heavy drinking days and drinks per drinking day (Karhuvaara et al., 2007). Importantly, existing human studies have not specifically examined their neuroimmune mechanisms and instead focus on opioid receptor mechanisms. Therefore, it remains uncertain whether drinking outcomes relate to neuroimmune properties.

In sum, it is likely that several subclasses of TLRs work together along with other neuroimmune factors to influence drinking-related behaviors. While naltrexone and nalmefene are well-established pharmacotherapies for addiction, questions remain about the biological mechanisms (e.g., opioid and/or immune system) through which they affect AUD-related behavioral outcomes in humans. Experimental trials seeking to test their specific anti-inflammatory actions would help address this knowledge gap, particularly by comparing the opioid-inactive vs. -active isomers. NF-κB, IKKε, and direct TLR inhibitors, such as sulfasalazine and amlexanox, have not yet progressed to use in human clinical trials for AUD, although preclinical results demonstrate beneficial effects on ethanol consumption and preference, which support their potential for further medications development. Several of these compounds demonstrate safety and tolerability in other clinical samples for the treatment of inflammatory medical conditions like rheumatoid arthritis and ulcerative colitis (Liu, Zeng X Fau - Chen, et al., 2006; Plosker & Croom, 2005). However, while sulfasalazine shows a

relatively safe side effect profile, serious adverse events like low white blood cell count (i.e., leukopenia) are known to occur in rare cases (Plosker & Croom, 2005). Future work that tests novel TLR inhibitors for their safety, particularly medication × alcohol interactions, and early efficacy markers would be critical in facilitating the progression of medications development for this class of drugs from preclinical to clinical studies of AUD.

Phosphodiesterase Inhibitors

Cyclic nucleotide PDEs are a family of phosphohydrolases. PDEs are the only known enzymes to regulate the intracellular levels of cAMP and cyclic guanosine monophosphate (cGMP) (Wen et al., 2018a). Thus, PDEs play a critical role in regulating the intracellular levels of cAMP and cGMP as well as their downstream signal transductions. There are 11 PDE subtypes widely distributed in the central nervous system (CNS) (Menniti et al., 2006), which can be divided into three categories based on their substrate specificity: (1) cAMP specific; (2) cGMP specific; and (3) dual-substrate PDEs. These subtypes are differentially distributed in the brain and have unique roles regulating neuronal function, indicating that targeted inhibition of specific isoforms may provide the best therapeutic benefits.

cAMP and cGMP signaling pathways play a key role in neural functions and synaptic transmission in the CNS as well as the downregulation of NF-κB and proinflammatory cytokine release (Parry & Mackman, 1997; Wen et al., 2018a). PDEs play a crucial role in maintaining cyclic nucleotide levels, and therefore, regulate intracellular signaling cascades that use cAMP and cGMP as second messengers. Of particular importance, PDEs modulate the cAMP protein kinase (PKA) pathway, which has been implicated in the regulation of response to acute and chronic alcohol exposure (Logrip, 2015). Acute alcohol exposure leads to activation of cAMP

signal transduction; conversely, chronic alcohol exposure attenuates this signaling pathway in a brain-region specific manner (Wen et al., 2018a). Alcohol withdrawal, thought to be an important driver of severe AUD, also decreases cAMP signal transduction in the cortex and amygdala in the rat (Pandey et al., 2003). cGMP signaling may also be involved in alcohol-drinking behavior; however, it has been less studied than cAMP. Rats exposed to chronic ethanol have increased cGMP levels in various brain regions including the striatum, hippocampus, and cortex; abstinence from ethanol lowers the cGMP levels back to normal (Uzbay et al., 2004). Given the critical role of these signaling pathways in alcohol drinking behaviors, normalization of their signaling is of interest for treating AUD. Specifically, PDE sub-family inhibitors have been proposed as promising therapeutics for AUD (see **Table 1-2**).

PDE inhibitors have been widely studied using preclinical animal models of AUD with particular focus on PDE4 inhibition, as PDE4 is expressed in several brain regions that underly the reinforcing effects of alcohol (e.g., nucleus accumbens, amygdala, and VTA) (Perez-Torres et al., 2000). Rolipram, a selective PDE4 inhibitor, has shown promising preclinical efficacy. Rolipram reduced alcohol intake and preference in several strains of mice (Blednov et al., 2014; Hu et al., 2011; Ozburn et al., 2020), decreased alcohol seeking in alcohol-preferring drinking rats (Franklin et al., 2015; Wen et al., 2012), and attenuated abstinence-like anxious and depressive behavior in mice (M. F. Gong et al., 2017). Despite these promising findings, rolipram does not have a desirable side effect profile in humans as it commonly induces significant nausea and emesis thought to be caused by its high affinity for the PDE4 subtype D. Other selective PDE4 inhibitors have been evaluated in preclinical mouse models, including mesopram, piclamilast, and CDP840 (Blednov et al., 2014). In a 24-hour two-bottle choice test, all three compounds showed efficacy at reducing ethanol intake and preference but only

mesopram produced long-lasting reductions. Yet, these compounds are not without their own side effect concerns, as there is a correlation between high affinity binding of these PDE4 inhibitors and emetic activity (M. F. Gong et al., 2017). Roflumilast is a second generation PDE4 inhibitor with FDA approval for chronic obstructive pulmonary disease. In mice, roflumilast decreased ethanol intake and preference in two drinking paradigms and did not impact sucrose or quinine drinking (Liu et al., 2017). However, it was much less potent for reducing drinking than rolipram, which may be attributable to its poor ability to penetrate the BBB. Finally, apremilast, a partial competitive PDE4 inhibitor, is FDA-approved for the treatment of psoriasis. Apremilast has a better side effect profile than the PDE4 inhibitors reviewed above, possibly because it does not demonstrate PDE4 subfamily (A to D) selectivity (Schafer et al., 2010). Favorably, apremilast reduced ethanol intake and preference in mice but did not modify sucrose preference, indicating its effects may be alcohol-specific (Blednov, Da Costa, Harris, et al., 2018). This compound may impact ethanol consumption and preference by increasing the aversive properties of ethanol, including decreasing functional tolerance and increasing sedative effects (Blednov, Da Costa, Harris, et al., 2018).

Other preclinical work has investigated the inhibition of other PDE-subtypes. PDE10 inhibition has been evaluated in preclinical rat models through TP-10, a specific PDE10A inhibitor (Logrip et al., 2014). TP-10 reduced alcohol self-administration in alcohol-preferring and dependent- and non-dependent rats. However, TP-10 also reduced saccharin self-administration, indicating that it may have a broader effect on reinforcing substances, which could limit translation to humans. Of note, inhibitors of PDE1 (vinpocetine), PDE3 (olprinone, milrinone), PDE5 (zaprinast), and a non-selective PDE inhibitor (propentofylline) have all been tested in animal models with null results (Blednov et al., 2014). Finally, ibudilast, which is a

selective PDE inhibitor, with preferential inhibition of PDE3A, PDE4, PDE10A, and PDE11A, has been tested in preclinical research (Gibson et al., 2006). Ibudilast reduced drinking and relapse in multiple animal models of AUD, and critically, has been shown to preferentially reduce drinking in dependent, compared to non-dependent mice (Bell et al., 2015). Specifically, ibudilast reduced drinking by ~50% in alcohol-preferring and high-alcohol drinking rats, during both maintenance and relapse tests. It is suspected that ibudilast's effects on alcohol drinking are primarily driven by the inhibition of PDE4 and PDE10A.

At present two completed randomized controlled trial investigating the effect of PDE inhibition in humans with AUD have been published. A human laboratory trial of ibudilast with a crossover design was conducted in a non-treatment seeking sample with AUD. Ibudilast decreased tonic craving for alcohol and improved mood following alcohol cue and stress exposure (Ray et al., 2017). A two-week experimental medicine trial of ibudilast conducted by the same laboratory similarly enrolled a non-treatment seeking sample with AUD and results demonstrated that ibudilast reduced rates of heavy drinking and neural alcohol cue-reactivity compared with placebo (Grodin et al., 2021).

Taken together, PDE inhibitors represent promising novel compounds to treat AUD and may be particularly effective at reducing alcohol preference, relapse, and negative mood associated with withdrawal. Ibudilast and apremilast have the best translational potential, particularly due to their tolerability, and are under investigation in large scale clinical trials. Specifically, ibudilast (50mg, bis in die (b.i.d. or twice a day) is being evaluated in a 12-week randomized clinical trial in treatment-seeking individuals with AUD (NCT03594435) with a primary outcome of percent heavy drinking days and an additional aim to examine peripheral markers of inflammation, and depressive symptomology. Apremilast (50mg, b.i.d.) is being

investigated in a two-week clinical trial in non-treatment-seeking individuals with AUD (NCT03175549) to assess alcohol cue-induced craving and drinking. At this time, first generation PDE4 inhibitors do not show translational potential due to their unfavorable side effect profile. However, next-generation PDE4 inhibitors, particularly those targeting the PDE4B subtype, hold promise as they are designed with human translation at the forefront. While PDE10A inhibitors may have translational potential, more preclinical work must be done to evaluate if PDE10A inhibition causes unfavorable, wide-ranging reductions in reward seeking behaviors. Future research should validate the immunomodulatory actions of PDE inhibitors by measuring medication-induced changes in markers of inflammation in samples of AUD and further connect these changes to meaningful clinical outcomes.

Peroxisome Proliferator-Activated Receptors

PPARs are transcription factors and members of the nuclear hormone receptor superfamily that have been tested for their potential role in addiction processes (Cippitelli et al., 2017; Ray et al., 2014). PPARs form a ligand-activated heterodimer partnership with retinoid X receptors; this dimer binds to a particular DNA sequence element, referred to as the peroxisome proliferator response element. PPAR actions can attenuate proinflammatory innate immune signaling (Michalik et al., 2006) and regulate other cellular and physiological processes, such as glucose metabolism, cellular differentiation and proliferation, and lipid-homeostasis. PPARs are thought to modulate pathways involved in NF-κB and nitric oxide (NO) production and inhibit expression of TNF-α (Berger & Moller, 2002; Scirpo et al., 2015). The three known isoforms, PPARα, PPARβ/δ, and PPARγ, are each transcribed from different genes (Berger & Moller, 2002) and are located in peripheral tissues and neural regions implicated in AUD (Moreno et al.,

2004). These isoforms are activated by eicosanoids and fatty acids and display broad albeit tissue-specific expression patterns (Michalik et al., 2006). PPARα is highly expressed in organs carrying out catabolism of fatty acids, PPARβ/δ shows the broadest expression patterns, and PPARγ is expressed in adipose tissue and more widely in the brain, gut, and immune cells. Generally, PPARs are distributed throughout the brain in neuronal and glial cell types and are suggested to be involved in neuromodulation through the regulation of genes encoding for neurotransmitter receptors, metabolism, and release (Moreno et al., 2004). The PPARα and PPARγ isoforms are of particular interest to the addictions field, as their receptors may be involved in modulation of dopamine and GABA transmission in mesocorticolimbic circuitries as well as providing neuroprotection against oxidative damage (Mascia et al., 2011; Melis et al., 2008; Ray et al., 2014).

PPAR agonists are anti-inflammatory compounds used to treat insulin resistance in diabetes and hyperlipidemia (Chigurupati et al., 2015) and show promise as immune therapies for AUD (see **Table 1-3**) and CNS diseases more broadly (Erickson, Grantham, et al., 2019; Le Foll et al., 2013). Early preclinical work on these targets evidenced the role of PPARs in regulating ethanol intake, stress-induced ethanol seeking, and withdrawal (Le Foll et al., 2013). The PPARγ isoform is suspected to be expressed in dopaminergic cells, as it colocalizes with tyrosine hydroxylase in the VTA (Le Foll et al., 2013; Stopponi et al., 2013). In alcohol-preferring male mice, PPAR agonists modulated treatment-response genes in the amygdala, prefrontal cortex, and liver, suggesting these AUD-relevant gene targets may mediate reductions in ethanol intake (Ferguson et al., 2014). The PPARγ agonist pioglitazone has been tested extensively in animal models of AUD. Pioglitazone affected several measures of alcohol-related behaviors in rats, including reductions in voluntary drinking, lever pressing, and reinstatement of

alcohol-seeking behavior, but not prevention of cue-induced relapse (Stopponi et al., 2011). Behavioral modifications were not due to changes in alcohol metabolism or blood glucose levels, suggesting that alcohol-related changes were not due to metabolic effects (Stopponi et al., 2011). A later investigation in rats combined pioglitazone with an FDA-approved medication for AUD, naltrexone, and revealed larger reductions for alcohol drinking with this combined administration (Stopponi et al., 2013). These findings illustrate the potential added benefit of combining neuroimmune therapies with existing, approved medications to treat AUD. Intriguingly, pioglitazone may also have anxiolytic properties involving areas of the VTA and amygdala, as it modulated yohimbine stress-induced reinstatement of alcohol seeking (Fotio et al., 2020), along with neuroprotective properties that prevent alcohol-induced neuronal and cognitive damage (Cippitelli et al., 2017).

Several other PPAR agonists have been tested in animal models. In mice, fenofibrate (PPARα), tesaglitazar (dual agonist: PPARα/γ), and bezafibrate (pan agonist: PPARα/γ/δ) were independently tested (Blednov et al., 2015; Ferguson et al., 2014). While fenofibrate and tesaglitazar produced long-lasting reductions in alcohol intake, bezafibrate produced mostly null results. Fenofibrate and tesaglitazar also reduced novelty response and increased acute withdrawal severity (Blednov et al., 2016a), but did not modify conditioned place preference for alcohol (Blednov et al., 2016b). In a rat model, fenofibrate treatment had dose-dependent effects on self-administration and reduced both the reinforcing and motivational effects of alcohol (Haile & Kosten, 2017). The mechanisms by which fenofibrate reduces alcohol consumption may be partially due to its effects on genes involved in energy metabolism, as its administration resulted in increased levels of blood acetaldehyde, which is aversive and similar to the effects of disulfiram, an FDA-approved medication for AUD. The effects of PPAR agonists may not be

uniform, with mice' responsiveness depending on drinking paradigm, sex, and genotype (Blednov et al., 2016a, 2016b; Ozburn et al., 2020). For example, Blednov and colleagues (2016a, 2016b) determined that males exhibited larger changes in alcohol consumption than females during fenofibrate and tesaglitazar administration.

Importantly, PPAR activation may exert effects on alcohol behaviors through both central and peripheral immune modulation (Erickson, Grantham, et al., 2019) and this corresponds to an increased interest in the function of peripheral inflammation in AUD. Several PPARα agonists with actions in the periphery, such as the intestinal tract, have been tested in animal models. Oleoylethanolamide (OEA) is endocannabinoid-like compound with anti-inflammatory properties mediated by PPARα activation that may reduce the permeability of intestinal cells (i.e., "leaky gut") (Anton et al., 2017; Karwad et al., 2017). In animal models, OEA, a known satiety factor, blocked cue-induced reinstatement of alcohol-seeking and reduced withdrawal severity (Anton et al., 2017; Bilbao et al., 2016). Further, OEA reduced levels of neural and peripheral proinflammatory markers, such as IL-1β and COX-2 during alcohol consumption (Anton et al., 2017). The over-the-counter medication, aspirin, has anti-inflammatory properties, which may be mediated by PPARγ activation (Yiqin et al., 2009). In rats, the co-administration of aspirin and n-acetylcysteine (NAC) inhibited chronic alcohol intake by 70% with aspirin administration alone inhibiting chronic intake by 50% (Israel et al., 2019).

Testing of several PPAR agonists has moved to human samples of heavy drinking but no randomized trial data for samples of AUD have been published. A clinical trial was conducted for a dietary supplement containing the precursor of OEA in young adult heavy drinkers (van Kooten et al., 2016). This supplement significantly improved performance on a Go/ No-Go task of inhibition, which was correlated with reductions in drinking (van Kooten et al., 2016); yet

measures of alcohol use or inflammatory markers were not collected. Importantly, an experimental medicine study (NCT01631630) of pioglitazone resulted in premature termination due to concern over myopathy risk (i.e., a neuromuscular disorder) in the active treatment group (Schwandt et al.). While several PPAR agonists are FDA-approved medications for medical conditions such as diabetes and dyslipidemia, they have shown unfavorable side-effect profiles and as a result, regulatory agencies have issued caution for future clinical trials (Wright et al., 2014). Moreover, PPARγ and dual agonists have shown concerning long-term effects on weight gain, fluid accumulation, cardiac safety, and tumor development (Amato & de Assis Rocha Neves, 2012; Wright et al., 2014). Despite these concerns, future work aims to optimize subtype interaction profiles to develop safer and more effective treatment options (Amato & de Assis Rocha Neves, 2012; Wright et al., 2014). At present, one human clinical trial is underway to test the effects of pioglitazone (45 mg/day) on alcohol use and biomarkers (NCT03864146); another trial was terminated due to the COVID-19 pandemic (NCT03860753). Researchers completed a clinical trial of fenofibrate for AUD and while trial results have yet to be published, reporting indicates that no serious adverse events occurred (NCT02158273).

Overall, evidence on PPAR agonists to date demonstrate their promising potential to reduce alcohol consumption and mitigate alcohol-related consequences in AUD. The majority of this work has been completed in animal models and shows that PPAR agonists may reduce the motivational and reinforcing features of alcohol, potentially by modulating dopaminergic signaling in the VTA and amygdala (2020). Findings across compounds have been mixed as to whether PPAR agonists' known metabolic actions, along with their anti-inflammatory properties, contribute to their effects on alcohol intake, with research suggesting that these agonists target neurons and modulate synaptic transmission more prominently than neuroimmune regulation

(Ferguson et al., 2014; Haile & Kosten, 2017; Stopponi et al., 2011). This investigation is limited by the lack of studies validating PPAR agonists' effects on markers of inflammation. Other initial findings suggest that these agonists may attenuate stress-induced alcohol consumption (Fotio et al., 2020) and exert neuroprotective benefits (Cippitelli et al., 2017). Medications mitigating alcohol-induced neural damage are highly sought after in CNS therapeutics. PPAR agonists with actions in the periphery, such as aspirin and OEA, show initial promise for reducing alcohol use and proinflammatory signaling and warrant safety and efficacy testing in humans. Human clinical trials for two of the most promising compounds, fenofibrate and pioglitazone, are emerging. However, long-term side effect profiles of certain PPAR agonists are of concern and should be tracked closely (Amato & de Assis Rocha Neves, 2012; Wright et al., 2014).

Microglia and Astrocytes

Microglia and astrocytes act as immune mediators in the brain, releasing and responding to immune signals (Nimmerjahn et al., 2005), and are implicated in alcohol-induced neuroimmune responses (Erickson, Blednov, et al., 2019; Erickson, Grantham, et al., 2019). Microglia have been shown to regulate escalation of drinking and alcohol dependence-induced changes in neuronal function (Warden et al., 2020). Activated M1 microglia are thought to secrete TNF-α, IL-6, and IL-1β, while anti-inflammatory microglia, M2, release TGF-β and IL-10 (Tang & Le, 2016). Astrocytes have the critical function of regulating synaptic glutamate levels through glutamate transporters (i.e., Glutamate Transporter 1 (GLT-1)) (Verkhratsky et al., 2015). The expression and function of astrocytic glutamate transporters are modulated by proinflammatory cytokines (Tilleux & Hermans, 2007) as well as alcohol, whereby chronic

alcohol downregulates the expression of GLT-1 (Sari, 2013). Astrocyte-specific calcium signaling can regulate ethanol intake as well as the acute stimulatory and sedative-hypnotic effects of ethanol in mice (Erickson et al., 2021). Glial cells may also play an important role in the modulation of dopamine activity relevant to addiction through the release of cytokines over dopaminergic neurons (Jimenez-Gonzalez et al., 2021). Furthermore, a recent study identifying transcriptomic patterns associated with alcohol dependence found that the largest number of cell-type specific genes with altered expression in individuals with alcohol dependence were detected in astrocytes and microglia (Brenner et al., 2020). Therefore, these glial cells represent potential new targets for medications focusing on the neuroimmune aspects of AUD (see **Table 1-4**).

To date, only one medication targeting microglia has been explored in preclinical models. Minocycline, a broad-spectrum antibiotic that crosses the BBB, is a microglial attenuator (Romero-Sandoval et al., 2008) shown to alter neuroimmune and cytokine expression in the brain and periphery (Garrido-Mesa et al., 2013). Results from minocycline studies for AUD are inconclusive. In male and female mice, minocycline modestly reduced alcohol intake in a free-choice voluntary drinking model (Agrawal et al., 2011). The effects of minocycline may be non-specific, as it reduced both alcohol and water intake in mouse models (Lainiola & Linden, 2017). Moreover, minocycline's beneficial effects on alcohol reductions were limited to adult vs. adolescent mice (Agrawal et al., 2014). However, other results suggest that minocycline modulates a host of AUD-related behaviors including reductions in alcohol-induced sedation, withdrawal-related anxiety, and alcohol reinstatement (Gajbhiye et al., 2018; Wu et al., 2011).

Medications targeting astrocytic GLT-1, which aids in the regulation of extracellular glutamate, include n-acetylcysteine (NAC), ceftriaxone, and clavulanic acid. Astrocytic compounds have been more extensively studied in animal models and are relevant to AUD as

glutamate expression is known to be dysregulated in AUD and contribute to alcohol withdrawal. NAC is an over-the-counter dietary supplement and antioxidant precursor to glutathione used to treat acetaminophen poisoning and cystic fibrosis (Ooi et al., 2011). In rat models, NAC reduced ethanol-seeking and self-administration (Lebourgeois et al., 2018) but did not prevent cue-primed ethanol reinstatement (Weiland et al., 2015). NAC may protect against chronic alcohol-induced neuroinflammation in the frontal cortex and hippocampus, as it prevented both increases in proinflammatory cytokines and decreases in anti-inflammatory cytokines in rat models (Schneider et al., 2017). Moreover, the co-administration of NAC and aspirin reduced ethanol intake and relapse binge drinking in ethanol-preferring rats (Israel et al., 2019). Ceftriaxone, a beta-lactam antibiotic, showed promising preclinical results for AUD-related behaviors as well. Ceftriaxone attenuated cue-primed reinstatement of alcohol-seeking (Weiland et al., 2015), reduced alcohol consumption (Lee et al., 2013), and attenuated relapse-like consumption across rodent models (Alhaddad et al., 2014; Qrunfleh et al., 2013). Alcohol withdrawal syndrome was alleviated in a rat model of ethanol withdrawal by ceftriaxone treatment (Abulseoud et al., 2014). Clavulanic Acid, another beta-lactam antibiotic, increased the expression of GLT-1 and attenuated ethanol consumption and preference (Hakami & Sari, 2017). Importantly, clavulanic acid attenuated alcohol consumption at a 20-40-fold lower dose than ceftriaxone and therefore shows higher potential for clinical translation, as large dose-to-body-weight ratios are unfeasible to use in human samples (Shen et al., 2019).

Among these glial targeting compounds, only minocycline and NAC have been translated into human clinical samples of addiction. A completed clinical study found no beneficial effect of a short-term minocycline treatment on inflammation or subjective response to alcohol among heavy drinkers (Petrakis et al., 2019). Currently underway is a clinical trial of minocycline

testing alcohol use, craving, and neurocognitive impairment in AUD (NCT04210713). Additionally, in a secondary analysis of a clinical trial for cannabis use disorder (CUD), NAC treatment reduced alcohol consumption by 30% (Squeglia et al., 2018). Several other clinical trials will examine the potential effectiveness of NAC in both adolescent and adult samples of AUD (e.g., NCT03216954, NCT03707951). These trials will include combination pharmacotherapy, samples with comorbid psychopathology, and neuroimaging methods that will test NAC's ability to modulate cortical levels of relevant metabolites and neural reactivity to alcohol cues.

In sum, microglia and astrocytes present promising targets for medications development for AUD. Compounds targeting astrocytes may be particularly useful in normalizing glutamate expression and treating withdrawal symptoms. The vast majority of existing studies have involved animal models, but several compounds demonstrate translational potential to clinical development. While ceftriaxone appears unlikely to translate due to its required dose size, clavulanic acid's efficacy at a much lower dose is promising for translation. Clavulanic acid has shown safety and tolerability in human clinical samples, as it is FDA-approved for clinical use in combination with an amoxicillin antibiotic. Minocycline and NAC are also FDA-approved treatments for other medical conditions and ongoing clinical trials aim to test their effects on AUD-related outcomes. While minocycline is generally well-tolerated in humans, it is less commonly prescribed than similar antibiotics because it increases risk for irreversible pigmentation, hepatotoxicity, and lupus-erythematosus-like syndrome (Garrido-Mesa et al., 2013; Smith & Leyden, 2005). Overall, NAC appears to be the most promising glia-targeting AUD treatment with multiple ongoing clinical trials. Orally administered NAC is well-tolerated with long-term use being associated with only mildly adverse effects (e.g., nausea, diarrhea)

(LaRowe et al., 2006). NAC is being tested as an AUD treatment specifically for adolescents, which represents a novel prospect, as no pharmacotherapies are currently approved for adolescents with AUD (Hammond, 2016; Winslow et al., 2016). Future research in this area can benefit from assessing biobehavioral and psychosocial factors to elucidate the mechanisms (e.g., withdrawal alleviation, neuroprotection) through which NAC and other glia-targeting neuroimmune therapies might reduce drinking and promote recovery.

Other Immune Pharmacotherapies

Compounds with specific targets differing from those covered have been explored as potential immune treatments for AUD. Indomethacin, a selective cyclooxygenase-2 (COX-2) inhibitor, has been investigated for its protective effects against alcohol-induced neuronal and cognitive damage (Pascual et al., 2015; Vetreno & Crews, 2018; Vetreno et al., 2018). Indomethacin is a potent nonsteroidal anti-inflammatory drug (NSAID) targeting COX isozymes involved in peripheral and neural inflammatory responses (Remmel et al., 2004). An initial investigation in rats reported dose-dependent reductions in alcohol self-administration (George, 1989). More recent work has focused on adolescence, a developmental period when the brain is especially sensitive to alcohol's neurotoxic effects. In adolescent rodents, indomethacin alone (Pascual et al., 2007) and in combination with exercise (Vetreno & Crews, 2018; Vetreno et al., 2018) blocked ethanol-induced neuronal cell death and behavioral deficits.

Using transcriptome-based drug discovery methods, researchers identified several novel compounds with potential for reducing excessive alcohol use (2018). Gene expression profiles of heavy drinking mice were compared with gene expression signatures of thousands of compounds and the most promising targets were selected via computational modeling. A sizeable proportion

of the compounds identified are thought to have anti-inflammatory properties, including terreic acid and pergolide, which were then validated in mice models (Ferguson et al., 2018). Terreic acid is a Bruton's tyrosine kinase (BTK) inhibitor (Kawakami et al., 1999), which is an important component in signaling pathways of B-cell receptors and malignancies, TLRs, and chemokine receptors (Kim, 2019). Pergolide is a dopamine and serotonin receptor agonist thought to have anti-inflammatory properties, yet this mechanism is poorly understood (Bendele et al., 1991). Findings showed that pergolide and terreic acid significantly reduced alcohol intake in HDID-1 mice (Ferguson et al., 2018). However, terreic acid appeared to have more selective effects on alcohol intake with pergolide decreasing water and saccharin intake as well (Ferguson et al., 2018).

Endogenous neuroactive steroids, termed "neurosteroids", are implicated in neuroimmune signaling in AUD. These steroids are synthesized in the brain that have a range of genomic and non-genomic actions, including modulation of $GABA_AR$-mediated neurotransmission, TLR-dependent signaling (i.e., blocking TLR-MyD88 binding (Balan et al., 2021)), and CRF signaling, with the potential to target complex symptomatology of AUD (Gatta et al., 2021; Morrow et al., 2020; Reddy, 2010). Neurosteroids that are positive modulators of $GABA_ARs$, such as allopregnanolone and pregnenolone, demonstrate anticonvulsant, sedative, and anxiolytic effects. Research shows that chronic alcohol exposure depletes neurosteroids in human serum and brains of rodents and monkeys; this depletion contributes to psychological and behavioral adaptations, which are further exacerbated by withdrawal and binge drinking (Finn & Jimenez, 2018; Morrow et al., 2020). Neurosteroids are being investigated as potential treatments given their ability to restore homeostasis in these functions (Morrow et al., 2020) and reduce alcohol intake (see relevant reviews (Finn & Jimenez, 2018; Giovanni & Monique, 2019;

Morrow et al., 2020)). In several preclinical studies, allopregnanolone or the precursor pregnenolone reduced ethanol intake, preference, or reinforcement in male alcohol-preferring rodents at high doses, demonstrating initial efficacy (Ford et al., 2005; Janak et al., 1998; Rezvani & Levin, 2014). However, neurosteroids may actually increase ethanol consumption and reinstatement at low doses or in non-dependent breeds (Morrow et al., 2020; Ramaker et al., 2014).

Cannabidiol (CBD), a non-psychoactive component of the cannabis plant, has received considerable attention as a possible therapeutic for illnesses including AUD (Turna et al., 2019). CBD exhibits diverse biological effects such as on learning and memory, immune system, appetitive behaviors, and neuroprotection by interacting with the body's endocannabinoid system and possibly other receptors like serotonin and opioid (Turna et al., 2019). Research supports CBD's anti-inflammatory effects with immune signaling actions in the periphery and CNS; anti-inflammatory targets of CBD include CB_1, CB_2, TRPV1, GPR55, and 5-HT_1 serotonin receptors with downstream actions on PPARγ, COX-2 enzymes, NF-κB, etc. (Burstein, 2015; Pellati et al., 2018). Several studies have tested whether CBD administration can reduce alcohol intake and related harms in preclinical models (see systematic review (Turna et al., 2019), including alcohol's neurotoxic effects, motivation and intake, and hepatoxicity). Findings consistently support CBD as a candidate pharmacotherapy for AUD. In rodent models, CBD treatment reduced voluntary alcohol consumption (Viudez-Martinez et al., 2018) and prevented cue- and stress-elicited alcohol reinstatement (Gonzalez-Cuevas et al., 2018). While, the majority of this work has yet to examine CBD's impact on immune markers, one study testing hepatoxicity found that CBD attenuated alcohol-induced increases in liver enzymes, mRNA expression of cytokines TNF-α and IL-1β, and several chemokines (Wang et al., 2017). These results suggest

that CBD's ability to prevent liver damage is partially attributable to immune processes. Other evidence from in vitro models demonstrates cannabinoids' potential to reduce intestinal permeability, which might have therapeutic implications (Alhamoruni et al., 2010).

Research on compounds reviewed in this section remains in early stages and is largely restricted to preclinical models not yet translated to human samples of AUD (see **Table 1-5**). However, one human laboratory trial of the neuroactive steroid, dutasteride, was completed and enrolled males reporting light and heavy drinking patterns. Participants were randomized in a crossover design to both placebo and 4 mg dutasteride pretreatment before alcohol administration (Covault et al., 2014). Results were encouraging, such that males with heavy drinking patterns reported fewer heavy drinking days in the two weeks following pretreatment for dutasteride vs. placebo and further, the compound was well tolerated. Clinical trials of neuroactive steroids for other psychiatric conditions similarly demonstrate safety and tolerability with no serious adverse effects reported, yet mild sedative effects may occur (Morrow et al., 2020). Continuing, animal models show that indomethacin may be a particularly promising compound for preventing alcohol-induced neurocognitive deficits. Indomethacin administration, however, can cause gastrointestinal toxicity due to its action as a partial COX-1 inhibitor and this may be particularly concerning when alcohol is concurrently consumed. Yet, analogues of indomethacin with less severe side effect profiles may become available (Blobaum et al., 2013) and may warrant safety and efficacy testing in humans. Development of medications that attenuate alcohol-related neurocognitive impairments in adults and adolescents are merited as these deficits (e.g., inhibitory control, working memory) contribute to continued alcohol use by interfering with goal-directed decision making, self-regulation, and treatment (Bates et al., 2006). Using the bioinformatic approach described above (2018), terreic acid proved to be most

selective for reducing heavy drinking in mice. While several second-generation BTK inhibitors show clinical promise, the safety profile of terreic acid (Kawakami et al., 1999) and its translatability to humans remains unclear (Kim, 2019).

As such, the next step would be replication of these promising results in additional animal models followed by research on the safety of this compound or other BTK inhibitors in humans. A major benefit of using this bioinformatics approach to select promising compounds for AUD is many of these compounds are FDA-approved for other medical conditions, thus shortening development time and reducing research costs. CBD, through its diverse biological actions, appears to advantageously target several AUD domains including liver damage, intestinal permeability, and motivation but the degree to which these effects are attributable to immune mechanisms is undetermined and warrants further research (Turna et al., 2019). CBD proves to be safe and tolerable in a range of clinical samples (Larsen & Shahinas, 2020) but translational challenges exist, including the low bioavailability of oral CBD in humans and potential contraindication with liver impairment (Turna et al., 2019). Randomized clinical trials of CBD are ongoing and will serve to translate these exciting preclinical findings to human AUD samples (NCT03252756; NCT04205682). One pilot trial will examine CBD dosing and its effects on withdrawal symptoms among inpatients. An 8-week trial of CBD will also assess changes in self-reported and biomarkers of alcohol use among treatment-seeking individuals with AUD. Moreover, randomized clinical trials of several neuroactive steroids for the treatment of AUD are also underway (NCT03872128; NCT02582905; NCT04098302; NCT04015869). These trials include crucial investigation into sex differences and the effect of neurosteroids on alcohol intake, withdrawal, stress reactivity, and mood symptoms. In sum, the complexity of the

body's immunological pathways and the phenotypic heterogeneity seen in AUD will result in the continued identification of novel immune targets.

Behavioral Interventions

In addition to the pharmacotherapies reviewed above, behavioral interventions may also mitigate heavy drinking and elevations in proinflammatory levels observed in AUD (see **Table 1**-**5**). While the anti-inflammatory effects of mind-body therapies have been explored in the context of chronic disease, depression, and aging (Bower & Irwin, 2016; Morgan et al., 2014), this area of research has only recently emerged in the context of AUD (McClintock et al., 2019). Mind-body therapies promote self-regulation and positive affect while decreasing stress reactivity and negative affectivity. Relevantly, heavy alcohol use is known to alter the body's natural biological stress system (Sinha, 2009) and stress increases alcohol craving and use. These therapies are hypothesized to interact with the neuroimmune system through downstream stress reactivity pathways, and thereby reverse activation of inflammatory mechanisms (Bower & Irwin, 2016). Existing research illustrates that mind-body therapies reduce proinflammatory gene expression profiles in healthy adults and those with medical or psychiatric conditions (Bower & Irwin, 2016). Mindfulness-Based Relapse Prevention (MBRP) is a mind-body therapy specifically designed for individuals with addiction (Grant et al., 2017). MBRP is typically delivered in 2-hour group sessions aimed to cultivate increased awareness of present-moment cognitive, emotional, and physical states, especially as they relate to cravings and withdrawal (Grant et al., 2017).

Few randomized trials of MBRP have been conducted in AUD populations and findings on its effectiveness have been mixed (Bowen et al., 2009; Zgierska et al., 2019). MBRP may be

most effective for individuals with severe AUD or comorbid mood symptomatology (Roos et al., 2017), which is supported by literature linking depression and inflammation (Miller & Raison, 2016). One trial connecting biological markers to behavior examined the impact of MBRP on peripheral proinflammatory levels in adults with alcohol dependence (McClintock et al., 2019). While significant decreases in IL-6 following MBRP were not detected, greater time spent practicing mindfulness predicted lower levels of circulating IL-6, suggesting regular mindfulness practice might reduce peripheral proinflammatory levels (McClintock et al., 2019).

One clinical trial underway will extend this research by exploring immunological, epigenetic, and neurobiological changes associated with MBRP in AUD (NCT02994043). Additional trials seek to further test MBRP efficacy, identify predictors of positive outcomes, and mechanisms of behavior change (NCT03842670; NCT0214783). Availability of behavioral interventions that serve to treat AUD maintenance factors (e.g., stress reactivity) differing from those typically targeted in existing evidence-based therapies, is a needed contribution to the field. Further, medications for AUD are largely under prescribed due to provider and patient factors and thus a group therapy option, with potentially novel anti-inflammatory actions, is critical.

Conclusions and Future Directions

A host of treatments targeting the immune system show promise for treating AUD. The guiding principle in this review is a translational focus on the biological and clinical plausibility of the immune therapies tested. We contend that, in order to push medications development forward, treatments' clinical applications and utility is equally as important as an understanding of their biological mechanisms. Considerations in the translation from preclinical to clinical medications development include dosage and target engagement. While most of the discussed

medications will be administered to humans orally, chronically, and at doses selected to prevent toxicity, most rodent studies use acute intraperitoneal injection administration with doses that produce blood levels much greater than would be achieved in humans. Relatedly, the exact peripheral and/or central mechanisms of action through which many of the discussed medications act to reduce alcohol intake remain unclear. For instance, certain compounds do not readily cross the BBB, indicative of low engagement at brain targets. Brain effects can be more easily achieved by increasing dosage in rodents, yet this is often unfeasible in humans. Along with compound availability, adverse event profile, and commercialization potential, these translational and clinical applications must be considered in order to feasibly reach, and safely and effectively treat individuals suffering from AUD (Litten et al., 2020).

Given these considerations, numerous treatments show significant promise even when held to the highest standards of clinical plausibility. For instance, two PDE4 inhibitors are in advanced stages of testing for AUD, apremilast (Blednov, Da Costa, Tarbox, et al., 2018) and ibudilast (Ray et al., 2017). Pioglitazone (Blednov et al., 2015) and fenofibrate (Haile & Kosten, 2017), both PPAR agonists, have been extensively tested for in animals models (Stopponi et al., 2013), and have moved into clinical trials for AUD. Moreover, ongoing clinical trials for NAC are wide-ranging and will test this treatment's efficacy in combination with more established AUD pharmacotherapy in adolescent samples and in adult samples with comorbid psychopathology. However, careful attention to side effect profiles and tolerability is necessary as immune research progresses into human samples with heavy alcohol use. For example, a trial of pioglitazone for AUD was halted over myopathy risk concerns (Schwandt et al.). More research testing the neuroimmune hypothesis of AUD in human samples is also needed. In addition to pharmacotherapies, mind-body therapies, particularly MBRP, show potential to

restore healthy levels of inflammation through downstream stress-reactivity pathways (McClintock et al., 2019). In brief, while in its early stages, the future of immune therapies in AUD appears bright, consistent with its application to other psychiatric disease states.

Based on the premise that immune therapies deserve careful attention for the indication of AUD, the next obstacle is establishing an effective compound screening model (L.A. Ray et al., 2021). Recognition that novel compounds and mechanisms may call for novel screening methods, is key in facilitating progression from preclinical to clinical settings. The endpoint of reduced alcohol consumption remains a gold-standard for AUD trials, yet initial efficacy testing in non-treatment seeking samples may require a broad set of endpoints, including safety and tolerability. Other important outcomes may include treatment effects on mood, neurocognition, biomarkers of peripheral and neural immune signaling, and withdrawal symptoms. Future research may also benefit from examining medication effects in the context of experimental laboratory paradigms, such as alcohol self-administration or stress- and cue-reactivity, as these methods afford efficient early efficacy testing, that is faster and less costly than full-scale clinical trials (Bujarski & Ray, 2016). Moving forward, screening should consider all aspects of medications and therapy development to advance understanding of how treatments interface with immune processes and their clinically relevant effects on brain and behavior.

After initial human testing, clinical trials should consider a broad range of factors involved in AUD recovery (Witkiewitz et al., 2020). As recently redefined, recovery from AUD is a "process by which individuals substantially reduce or eliminate AUD symptoms while enhancing one's social support and psychosocial functioning in order to build resilience to relapse" (PA 18-619). To that end, the combination of pharmacotherapy with synergistic and evidence-based behavioral therapy may be critical to reaching recovery endpoints beyond

reductions in alcohol use. Identification of optimal combinations has resulted in success across areas of medicine. Likewise, identifying subgroups of treatment responders through precision medicine approaches can boost medication effect sizes dramatically (Litten et al., 2020). Would individuals showing a particular set of vulnerabilities in their AUD presentation, such as "leaky gut", elevated peripheral proinflammatory markers, or depressive symptomatology, be best suited for therapies targeting the immune system? Further, would specific individual inflammatory profiles show better responses to these treatments targeting inflammation and disruption of immune signaling? These are the type of questions we envision having high translational value as indexed by a high potential to inform clinical care and to improve treatment efficacy. Another approach with the potential to inform medications development for AUD, including immune therapies, is the use of pharmacoepidemiology. As datasets from closed health systems become more detailed and informative, questions about the efficacy of immune therapies for heavy alcohol use may become accessible. Such approaches have already proven helpful when characterizing opioid use, a high priority area (Hudson et al., 2017). While pharmacoepidemiology offers an emerging tool in this area, one of the limitations is the fact that a full clinical picture may not emerge until treatment-seeking individuals attempting to change their drinking are considered in efficacy trials.

In closing, this qualitative review of immune therapies for AUD demonstrates optimism with regard to the biological and clinical plausibility of treatments that can restore healthy immune function as a means of promoting AUD recovery. As the field progresses with clinical testing, literature calls for adjustments in the way AUD medications are developed with a particular focus on how novel treatment mechanisms can be effectively captured in clinical samples. To that end, efficacy screening models (i.e., human laboratory trials, neuroimaging)

sensitive to the unique effect of immune modulation on psychology, behavior, and biomarkers are critical. Overreliance on models used to capture medication effects on standard phenotypes (e.g., craving or subjective response to alcohol) may result in 'missing the signal' from immune treatments on other key components of addiction (e.g., affect and neurocognition). Moving towards clinical testing and randomized controlled trials, a broad definition of recovery along with identification of predictors of treatment response are central to establishing the utility of novel immune treatments. A nuanced understanding of treatment effects in turn can advance the much-anticipated precision medicine approach to AUD.

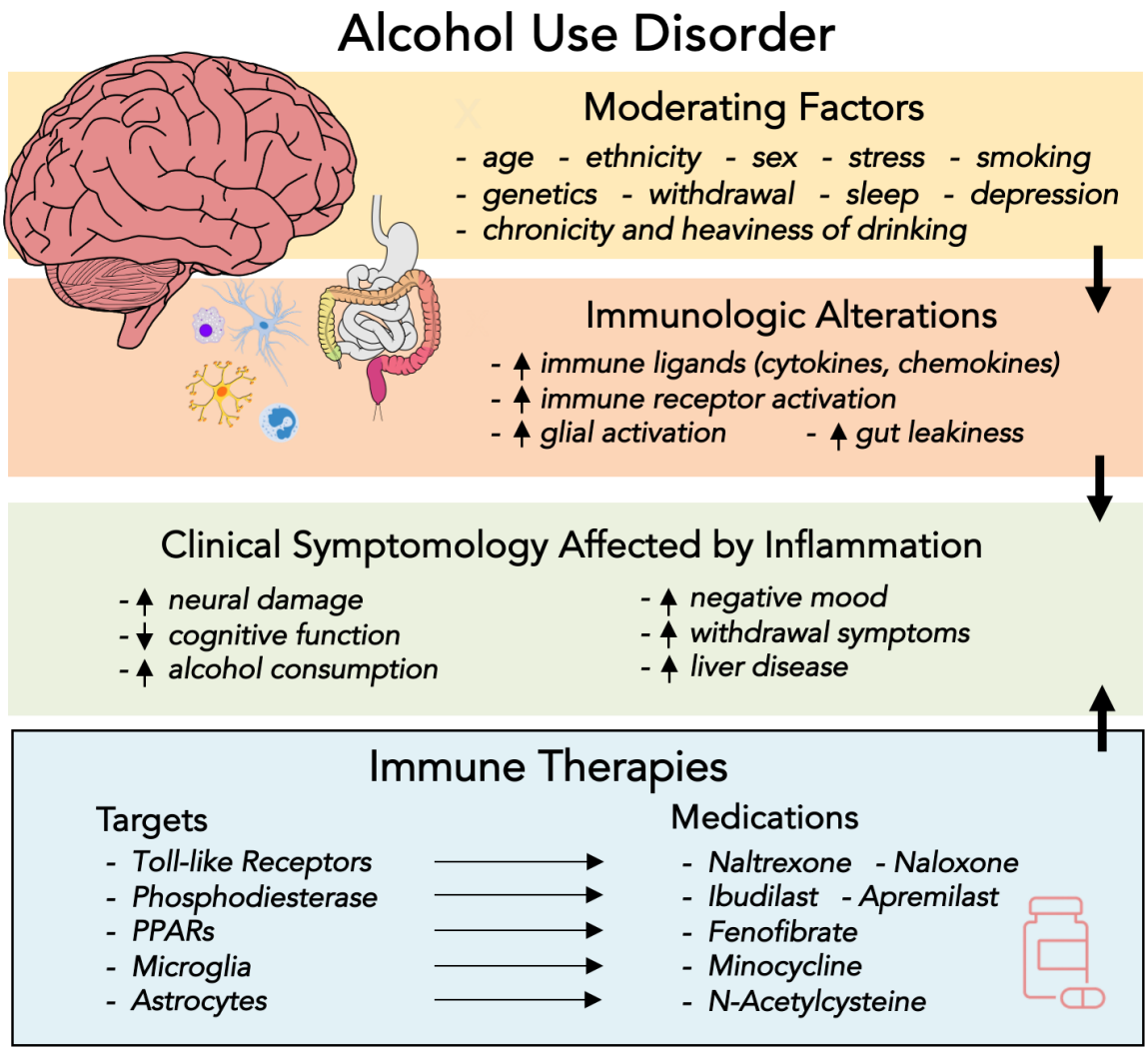

Figure 1-1. Brain-Immune Interactions in Alcohol Use Disorder. Potential moderators of the relationship between alcohol use disorder (AUD) and the immune system include factors such as age, sex, stress, sleep, and smoking. Multiple aspects of the immune system are altered by chronic alcohol consumption, including increased concentrations of proinflammatory immune ligands, increased immune receptor and glial activation, and breakdown of down lymphatic duct lining and endothelial cell junctions (i.e., gut leakiness). In return, inflammation and immune imbalance are thought to affect clinical symptoms of AUD, ranging from negative mood and cognitive dysfunction to withdrawal symptoms and liver disease. The pharmacological immune therapies discussed in the current review act on specific immune targets to potentially mitigate the effects of immunologic alterations and associated clinical symptomatology in AUD; PPARs = peroxisome proliferator activated receptors.

Table 1-1. Toll-Like Receptors

Immunotherapy	Potential Immune Target	Animal Study Findings	Human Study Findings	References
Toll-Like Receptors (TLRs):				
Naltrexone	TLR4	↓ binge drinking in adulthood ↓ alcohol preference ↓ immune-related gene mRNA expression	↓ return to drinking ↓ heavy drinking days	(Jonas et al., 2014) (Jacobsen et al., 2018a) (Jacobsen et al., 2018b)
Naloxone	TLR4	↓ alcohol-induced sedation ↓ alcohol-induced motor impairment	--	(Wu et al., 2012) (Harris et al., 2017)
Nalmefene	TLR4	↓ alcohol-induced neuroinflammation ↓ binge drinking	↓ heavy drinking days ↓ drinks per drinking day	(Karhuvaara et al., 2007) (Montesinos et al., 2017)
Sulfasalazine	IKKβ inhibition	↓ ethanol intake and preference	--	(Truitt et al., 2016)
TPCA-1	IKKβ inhibition	↓ ethanol intake and preference	--	(Truitt et al., 2016)
Amlexanox	TLR3/TRIF inhibition	↓ ethanol consumption	--	(McCarthy et al., 2018)
T5342126	TLR4	↓ ethanol consumption ↓ microglial activation marker	--	(Bajo et al., 2016)

Note. IKKβ is an inhibitor of the NF-κB kinase subunit beta; TRIF = TIR-domain-containing adapter-inducing interferon-β

Table 1-2. Phosphodiesterase Inhibitors

Immuno-therapy	Potential Immune Target	Animal Study Findings	Human Study Findings	References
Phosphodiesterase (PDE) Inhibitors:				
Ibudilast	PDE3 -4 -10 & -11	↓ ethanol consumption	↓ alcohol craving ↑ mood outcomes ↓ heavy drinking days clinical trials underway: NCT03594435 completed clinical trials: NCT03489850 NCT02025998	(Bell et al., 2015) (Ray et al., 2017) (Grodin et al., 2021)
Rolipram	PDE4	↓ ethanol intake and preference	--	(Hu et al., 2011) (Wen et al., 2012) (Blednov et al., 2014) (Franklin et al., 2015) (M. F. Gong et al., 2017) (Ozburn et al., 2020)
Mesopram	PDE4	↓ ethanol intake and preference	--	(Blednov et al., 2014)
Piclamilast	PDE4	↓ ethanol intake and preference	--	(Blednov et al., 2014)
CDP840	PDE4	↓ ethanol intake and preference	--	(Blednov et al., 2014)
Apremilast	PDE4	↑ ethanol-induced sedation and intoxication no effect on ethanol CPP or withdrawal ↓ ethanol intake and preference	clinical trial completed: NCT03175549	(Blednov, Da Costa, Tarbox, et al., 2018) (Blednov, Da Costa, Harris, et al., 2018)
Rofumilast	PDE4	↓ ethanol intake and preference	--	(Liu et al., 2017)
TP-10	PDE10	↓ relapse-like alcohol self-administration	--	(Logrip et al., 2014)

Note. CPP = conditioned place preference

Table 1-3. Peroxisome Proliferator-Activated Receptors

Immuno-therapy	Potential Immune Target	Animal Studies Findings	Human Studies Findings	References
Peroxisome Proliferator-Activated Receptors (PPARs):				
Gemfibrozil	PPARα activation	↓ ethanol intake	--	(Barson et al., 2009)
Fenofibrate	PPARα activation	↓ ethanol consumption and preference ↓ self-administration ↑ ethanol intake	clinical trial completed: NCT02158273	(Ferguson et al., 2014) (Karahanian et al., 2014) (Blednov et al., 2015) (Blednov et al., 2016a) (Blednov et al., 2016b) (Haile & Kosten, 2017) (Ozburn et al., 2020)
Pioglitazone	PPARγ activation	↓ ethanol intake and preference ↑ protection against alcohol-induced impairment ↓ ethanol self-administration ↓ ethanol cue-induced reinstatement ↓ ethanol-induced IL-6 & IL-1β expression	poor safety and tolerability clinical trial underway: NCT03864146 clinical trials halted: NCT03860753 NCT01631630	(Blednov et al., 2015) (Stopponi et al., 2011) (Stopponi et al., 2013) (Cippitelli et al., 2017) (Fotio et al., 2020) (Schwandt et al.)
Tesaglitazar	PPARα/ PPARγ activation	↓ ethanol consumption and preference no effect on ethanol intake	--	(Ferguson et al., 2014) (Blednov et al., 2015) (Blednov et al., 2016a) (Blednov et al., 2016b) (Ozburn et al., 2020)
Bezafibrate	PPARα/ PPARγ/ PPARδ activation	↓ ethanol intake and preference no effect on ethanol intake	--	(Ferguson et al., 2014) (Blednov et al., 2015)
N-acylethanolamines (OEA)	PPARα in intestinal cells	↓ ethanol cue-induced reinstatement ↓ behavioral symptoms of withdrawal ↓ ethanol-induced IL-1β, COX-2, TNF-α, iNOS, MCP-1	decreases in inhibition correlate with reduced alcohol intake	(Bilbao et al., 2016) (van Kooten et al., 2016) (Anton et al., 2017)

		↓ ethanol-induced expression of HMGB1, TLR4, NF-κB		
Aspirin	PPAR	↓ ethanol intake ↓ relapse-like binge drinking	--	(Israel et al., 2019)

Note. IL = interleukin; COX = cyclooxygenase; iNOS = inducible nitric oxide synthase; TNF-α = tumor necrosis factor-α; MCP-1 = monocyte chemotactic protein-1; HMGB1 = high mobility group box protein 1; NF-κB = nuclear factor-κB; TLR4 = toll-like receptor 4

Table 1-4. Microglia and Astrocytes

Immuno-therapy	**Potential Immune Target**	**Animal Studies Findings**	**Human Studies Findings**	**References**
Microglia:				
Minocycline	Microglial activation inhibition	↓ ethanol intake in adult mice no effect of ethanol intake in adolescent mice ↓ relapse-like ethanol consumption ↓ withdrawal-induced anxiety ↑ ethanol-induced motor impairment ↓ ethanol-induced TNF-α	no effect on subjective response to alcohol no effect on cytokine levels clinical trial completed: NCT02187211 clinical trial underway: NCT04210713	(Wu et al., 2011) (Agrawal et al., 2011) (Agrawal et al., 2014) (Lainiola & Linden, 2017) (Gajbhiye et al., 2018) (Petrakis et al., 2019)
Astrocytes:				

N-acetylcysteine	Glutamate transporter 1 (GLT-1)	↓ ethanol intake no effect on ethanol cue-induced reinstatement ↓ relapse-like binge drinking ↓ ethanol-seeking behavior ↓ ethanol-induced proinflammatory cytokines prevent ethanol-induced decreases in anti-inflammatory cytokines	↓ alcohol consumption clinical trial completed: NCT03216954 NCT02791945 NCT01214083 NCT02911285 clinical trials underway: NCT03707951 NCT03238300 NCT03879759 NCT02966873 NCT03120468	(Weiland et al., 2015) (Schneider et al., 2017) (Lebourgeois et al., 2018) (Squeglia et al., 2018) (Israel et al., 2019)
Ceftriaxone	GLT-1	↓ ethanol cue-induced reinstatement ↓ ethanol intake ↓ relapse-like ethanol intake ↓ withdrawal symptoms	--	(Sari et al., 2011) (Lee et al., 2013) (Qrunfleh et al., 2013) (Alhaddad et al., 2014) (Abulseoud et al., 2014) (Das et al., 2015) (Weiland et al., 2015) (Sari et al., 2016)
Clavulanic acid	GLT-1	↓ ethanol intake	--	(Hakami & Sari, 2017)

Note. TNF-α = tumor necrosis factor-α

Table 1-5. Other Immune Pharmacotherapies and Behavioral Therapies

Immuno-therapy	Potential Immune Target	Animal Study Findings	Human Study Findings	References
Other Potential Immune Pharmacotherapies:				
Indomethacin	cyclooxygenase (COX-2) enzyme inhibitor	↑ protection against ethanol-induced brain damage ↓ ethanol-induced NF-κB phosphorylation ↓ ethanol-induced COX-2 & iNOS expression	--	(George, 1989) (Pascual et al., 2007) (Vetreno & Crews, 2018) (Vetreno et al., 2018)
Pergolide	dopamine/ serotonin receptor agonist	↓ ethanol intake	--	(Ferguson et al., 2018)
Terreic acid	Bruton's tyrosine kinase	↓ ethanol intake	--	(Ferguson et al., 2018)

	(BTK) inhibitor			
Cannabidiol	diverse actions (e.g., COX-2 enzyme inhibition, PPARγ activation)	↓ ethanol intake ↓ cue- and stress-induced ethanol seeking ↑ protection against ethanol-induced brain damage ↓ ethanol-induced liver damaged ↓ impulsive choice ↓ ethanol-induced liver inflammation	clinical trials underway: NCT03252756 NCT04205682 NCT03248167	(Turna et al., 2019) (Wang et al., 2017)
Neuroactive steroids	toll-like receptor (TLR) 4, TLR7	↓ ethanol intake, preference, and operant responding in select rodents at high doses ↑ ethanol intake and operant responding in low doses	↓ alcohol use in males with heavy drinking patterns clinical trials underway: NCT03872128 NCT02582905 NCT04098302 NCT04015869	(Morrow et al., 2020) (Rezvani & Levin, 2014) (Covault et al., 2014)
Behavioral Therapies:				
Mindfulness-Based Relapse Prevention	downstream stress pathways	--	↑ mindfulness practice predicted ↓ IL-6 and ↓ drinking clinical trial completed: NCT01056484 clinical trial underway: NCT02994043	(McClintock et al., 2019) (Zgierska et al., 2019)

Note. IL = interleukin; COX-2 = cyclooxygenase-2; iNOS = inducible nitric oxide synthase; TNF-α = tumor necrosis factor-α; NF-κB =nuclear factor-κB; PPARγ =peroxisome proliferator-activated receptor γ

BACKGROUND FOR EMPIRICAL STUDIES OF IBUDILAST FOR AUD

Preclinical Data of Ibudilast for AUD

As reviewed above, ibudilast (MN-166) is a phosphodiesterase (PDE) inhibitor at PDE3, -4, -10, and -11 as well as a macrophage migration inhibitory factor (MIF) that crosses the blood-brain barrier. PDE4 and MIF are thought to regulate inflammatory responses in microglia and contribute to neuroinflammation (Giampa et al., 2010; Mizuno et al., 2004). In one cell culture study, ibudilast modulated several immune processes, such that it protected against microglia-induced neuronal cell death, suppressed nitric oxide production along with proinflammatory cytokine production and expression by activated microglia, and augmented the production of an anti-inflammatory cytokine (IL-10) and multiple neurotrophic factors (GDNF, NT-4; (Mizuno et al., 2004). Thus, in human samples, it is suspected that ibudilast may reduce neuroinflammation by inhibiting proinflammatory signaling, enhancing anti-inflammatory signaling, and providing neuroprotection. Importantly to AUD, PDE4 isoforms are highly expressed in neuronal and glial cells in neural regions implicated in the rewarding and reinforcing effects of alcohol, such as the amygdala and nucleus accumbens (Pérez-Torres et al., 2000). PDE enzymes play a critical role in the regulation of cAMP and its downstream signaling, which are differentially affected by acute vs. chronic alcohol exposure in specific brain regions (Logrip, 2015; Wen et al., 2018a). Ibudilast's potential to reduce drinking was initially tested in several animal models of chronic alcohol use (Bell et al., 2015). Results showed that, in two rat models (i.e., alcohol-preferring P rats, high alcohol drinking HAD1 rats), ibudilast reduced voluntary drinking by 50% during a two-bottle choice paradigm and also selectively decreased alcohol intake among mice with alcohol dependence (i.e., via intermittent ethanol vapor exposure) relative to those nondependent on alcohol (Bell et al., 2015). In addition, other PDE4 inhibitors, including rolipram and

apremilast have shown efficacy in reducing alcohol intake and even anxious-like behavior in rodent models of AUD (Blednov et al., 2014; Blednov, Da Costa, Harris, et al., 2018; Blednov, Da Costa, Tarbox, et al., 2018; Franklin et al., 2015; M. F. Gong et al., 2017; Hu et al., 2011; Ozburn et al., 2020).

Clinical Data of Ibudilast for AUD

Across healthy and clinical samples, ibudilast appears to be well-tolerated with the most common side effects being mild to moderate and including gastrointestinal distress (e.g., nausea, vomiting), depression, and headaches (DeYoung et al., 2016; Fox et al., 2018; Grodin et al., 2021; Metz et al., 2017; Ray et al., 2017; Rolan et al., 2008). Given this favorable safety profile, our laboratory and many others have sought to test ibudilast for the treatment of a wide range of mental and physical health conditions. Ibudilast is approved to treat patients with conditions such as asthma and post-stroke dizziness in Japan. Ongoing clinical trials in the United States include testing ibudilast's use for the treatment of glioblastoma, multiple sclerosis, chronic migraines, methamphetamine use disorder, and others (see clinicaltrials.gov). To date, three clinical trials of ibudilast enrolling samples with AUD have been completed, all of which were conducted in our laboratory. The first study consisted of a double-blind placebo-controlled crossover human laboratory trial of ibudilast (50 mg BID/ twice daily), which enrolled non-treatment seeking individuals with AUD (N = 24) and consisted of a 7-day intensive outpatient protocol focused on safety and initial efficacy (Ray et al., 2017). The second study involved a two-week randomized double-blind placebo-controlled experimental medication trial of ibudilast (50 mg BID), similarly enrolling non-treatment seeking individuals with AUD (N = 52). This trial collected daily electronic diary reports of mood, craving, sleep, and drinking and included an fMRI scan at the study midpoint. The primary outcomes of the trial focused on heavy drinking reduction,

negative mood improvement, and attenuation of neural cue-reactivity (Grodin et al., 2021). The third RCT involved a full-scale 12-week clinical trial, which enrolled treatment-seeking individual with AUD. The principal endpoints for this trial were focused on changes in alcohol intake. Primary outcomes show significant reductions in alcohol intake across both groups, but no medication-specific effects on alcohol. Secondary clinical and neurocognitive data were collected at multiple timepoints throughout the trial and were examined in Chapters 3 and 4 of this dissertation.

Results from the initial human safety and efficacy trial showed that ibudilast was well tolerated and reduced levels of tonic craving significantly more so than placebo (Ray et al., 2017). No severe adverse events emerged in the trial, but more participants reported at least one adverse event when taking ibudilast compared with placebo, where headaches and gastrointestinal distress were more frequent. Three human laboratory paradigms (i.e., intravenous alcohol infusion, alcohol cue-exposure, and stress exposure) were conducted for each medication condition. Results suggested that ibudilast did not significantly alter negative mood states nor phasic craving during the paradigms but there was a trend-level effect of ibudilast on promoting positive mood following stress and cue exposure (Ray et al., 2017). Thus, this trial's findings supported ibudilast safety in alcohol samples and its potential to reduce craving and improve mood outcomes in the face of stress and cue exposure during early abstinence. Continuing, the two-week experimental medication trial further supported ibudilast's safety, such that adherence rates exceeded 97% and no significant differences in adverse events were reported by symptom categories across the medication conditions (Grodin et al., 2021). Results showed that ibudilast did not significantly alter daily reports of negative mood states over the two weeks among these participants with subclinical levels of depression and anxiety. Yet, ibudilast did reduce the odds

of heavy drinking by 45% compared with placebo and attenuated bilateral neural alcohol cue-reactivity in the ventral striatum during the fMRI scan (Grodin et al., 2021). In sum, these early trials show the promise of ibudilast for reducing quantity of alcohol consumption as well as reducing tonic craving and neural cue-reactivity and improving mood in the laboratory.

Examining Potential Clinical Mechanisms of Action

Despite the promising results reviewed above on the potential for ibudilast to reduce heavy drinking and alter alcohol craving, much is left to be understood about how this new class of therapy operates in clinical samples with AUD. Treatment mechanisms of action can be established by assessing intervention-based changes in psychological measures collected during RCTs. Statistical mediation models are one of the most frequently utilized methodological approaches to test mechanisms of behavior change in order to examine why particular substance use treatments are effective within a causal inference framework (Witkiewitz et al., 2022). For instance, to understand the processes through which a specific treatment, like ibudilast, affects change, we can examine whether this treatment strongly influences a particular mechanism such as negative affectivity, and in turn if this mechanism greatly reduces the clinical outcome of interest, like drinks per drinking day. In addition, traditional laboratory-based methods, such as an alcohol administration or cue-exposure paradigms, are used to experimentally manipulate environmental conditions connected with drinking to measure psychological factors, such as subjective response to alcohol, under these conditions to likewise test potential mechanisms of novel or even established pharmacotherapies (Bujarski & Ray, 2016). While these laboratory methods are powerful and demonstrate predictive validity in clinical trials (L.A. Ray et al., 2021), they are costly and lack ecological validity. In return, novel designs, such as micro-

longitudinal, naturalistic daily reporting, have emerged to better understand how treatments work, given that these methods can enhance power and ecological validity and reduce recall error and costs (Carpenter et al., 2020).

Returning to the ANA clinical framework, through three empirical studies in this dissertation, we examined ibudilast's potential mechanisms of action aligning with the domains of incentive salience, negative emotionality, and executive function, to help fill gaps in the clinical literature on immune treatments for AUD. These domains importantly map onto the phases of the allostatic model of addiction and are supported by preclinical and human literature on immune signaling in AUD (Coleman & Crews, 2018). For instance, among individuals with AUD, several peripheral inflammatory cytokines (e.g., TNFα, IL-1β, and IL-6) have been found to correlate with alcohol craving, and may cross the BBB to exert influence on neural processes (Banks, 2015; Heberlein et al., 2014). In completed Chapter 2 below, we leverage data collected from the two-week experimental medication trial conducted in our laboratory (Grodin et al., 2021) to examine ibudilast's potential to modulate subjective response to alcohol in the natural environment. This secondary analysis included measures of alcohol craving, stimulation, sedation, and mood states that were collected each morning following a drinking day during the trial to probe daily alcohol-induced subjective effects and alcohol-related reward. We hope this works extends our understanding of how ibudilast might modulate alcohol intake in real-world contexts, such as in the face of powerful reinforcers like acute stress, alcohol cues, and one's social network. Chapters 3 and 4 utilized data collected during the 12-week randomized clinical trial of ibudilast which enrolled treatment-seeking individuals with AUD. Chapter 3 tested facets of negative affectivity and tonic craving as clinical mechanisms of treatment. Chapter 4 assessed whether ibudilast and reductions in alcohol intake improve executive functioning and other

neurocognitive domains collected through the NIH Toolbox Cognitive Battery. Both negative affectivity and neurocognitive dysfunction are suggested to be associated with greater psychiatric impairment and immune processes and historically impact treatment efficacy. The addition of these projects to an ongoing, 12-week double-blind RCT of ibudilast improve the field's understanding of how immune treatments work in the context of AUD. Identifying potential mechanisms of action is vital to advancing this line of research and informing the broader landscape on immune modulators with therapeutic potential for addiction.

CHAPTER 2:

The Effect of Neuroimmune Modulation on Subjective Response to Alcohol in the Natural Environment

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ABSTRACT

Background: Despite the promising implications for novel immune therapeutics, few clinical trials have tested these therapies to date. An understanding of how immune pharmacotherapies influence complex alcohol use disorder (AUD) profiles, including subjective response to alcohol is very limited. Initial findings show that ibudilast, a neuroimmune modulator, reduces rates of heavy drinking and measures of alcohol craving.

Methods: This study serves as a secondary analysis of a two-week clinical trial of ibudilast that enrolled a non-treatment seeking sample with AUD. Eligible participants (N = 52) were randomized to ibudilast or matched placebo and completed daily diary assessments (DDAs) during the two-week period. Each morning, participants retrospectively reported on their mood and craving levels both before and during the previous day's drinking episode, as well as stimulation and sedation levels during the previous day's drinking episode. Multilevel models compared the effects of ibudilast and placebo on subjective alcohol response. Exploratory analyses tested whether ibudilast moderated the relationship between daily stimulation/ sedation and alcohol intake and whether withdrawal-related dysphoria moderated ibudilast's effects on subjective response.

Results: Ibudilast did not significantly alter mean levels of stimulation or sedation (p's > .05). It did, however, moderate the effect of daily stimulation on drinking (p = .045). Ibudilast attenuated alcohol-induced increases in craving compared with placebo (p = .047), but not other subjective response measures. Only among individuals without withdrawal-related dysphoria did ibudilast significantly temper daily alcohol-induced changes in urge to drink and positive mood.

Conclusions: Ibudilast's effects on subjective alcohol responses appear to be nuanced and perhaps most salient for individuals drinking for positive reinforcement versus to feel normal.

Consistent with previous findings, reductions in alcohol craving may represent a primary mechanism of ibudilast. The ecologically valid nature of DDAs provide a clinically useful window into how individuals experience alcohol's effects while taking ibudilast.

INTRODUCTION

Harmful use of alcohol is the leading risk factor for premature disability and mortality globally among individuals aged 15 to 49 years (2018). Excessive drinking, along with biological and environmental risk factors, can progress to alcohol use disorder (AUD), which is characterized by repeated alcohol use despite negative consequences (Gilpin & Koob, 2008; Kranzler & Soyka, 2018). Notwithstanding the wide range of health and psychological consequences associated with AUD, a large treatment gap remains, with less than 8% of persons with past-year AUD receiving alcohol care and even fewer receiving evidence-based care (SAMSHA, 2019). Multi-system strategies are needed to advance treatments and increase utilization rates among the diverse set of people with AUD. Development of novel, effective pharmacotherapies is one approach likely to help (Litten et al., 2012). To support people in recovery, medications must target factors sustaining drinking. Identifying mechanisms of action, such as through randomized controlled trials (RCTs), human laboratory paradigms, and collection of real-world data represents a vital step in medications development (Carpenter et al., 2020).

Behavioral pharmacology has established subjective response to alcohol as a reliable, multi-faceted phenotype serving as a central biobehavioral marker of positive and negative reinforcement from alcohol (Bujarski & Ray, 2014). Individual variability in alcohol's acute subjective effects, specifically greater stimulation and reward and lower sedation, predict liability for AUD, including escalation of alcohol use and AUD symptomatology (King et al., 2021; Quinn & Fromme, 2011; Schuckit et al., 2007). Positive mood, negative mood, and craving are acutely modulated by alcohol use, such that individuals typically experience an increase in positive mood and craving and decrease in negative mood along rising breath alcohol

concentrations (BrACs), serving as reinforcers of alcohol intake (Bujarski & Ray, 2014). Thus, researchers routinely assess whether pharmacotherapies can effectively modulate subjective response to alcohol through experimental human laboratory paradigms (Bujarski & Ray, 2016; Ray et al., 2016). Importantly, a recent meta-analysis has shown that medication effects on subjective responses to alcohol in the laboratory predict their efficacy in clinical trials for AUD (L. A. Ray et al., 2021). In an initial safety and efficacy trial, our laboratory used an intravenous alcohol administration paradigm to test whether the novel pharmacotherapy, ibudilast, modulated subjective response in a clinical sample of AUD (Ray et al., 2017). While ibudilast did not significantly alter subjective response, subjective effects of mood were dependent on participant's degree of depression symptomatology.

Novel designs testing alcohol's subjective effects are emerging, such as daily diary methods and ecological momentary assessment (EMA) in which participants report on their drinking experiences in real-world settings (Miranda et al., 2014; Trela et al., 2016). For instance, using EMA in an RCT that enrolled adolescents with problematic drinking, Miranda Jr. et al. (2014) found that naltrexone attenuated alcohol-induced increases in stimulation and enhanced alcohol-induced sedation, as compared to placebo. These naturalistic reports are consistent with findings on naltrexone's subjective effects in human laboratory settings (Ray et al., 2019). Although less temporally precise than EMA, daily diary methods, which typically include data collection once daily, have lower participant burden and can enhance compliance. While assessment of medication effects on acute subjective response to alcohol via daily diary assessments (DDAs) is limited, past work has utilized these designs to assess daily relationships among urge, mood, and drinking (Helstrom et al., 2016; Kranzler et al., 2013). In a trial of naltrexone for heavy drinking among young adults, morning DDAs revealed that higher daily

urge was associated with a greater likelihood of taking the medication, which in return, predicted a lower likelihood of same-day intoxication among the treatment group (Bold et al., 2016). The current study consists of a secondary analysis of a two-week experimental medication RCT of ibudilast, which demonstrated treatment-related reductions in rates of heavy drinking, as reported through daily diary methods, and reduced neural alcohol cue-reactivity (Grodin et al., 2021). This study seeks to further test ibudilast's effects on subjective response to alcohol in the natural environment via DDA. When comparing participant report of drink quantity between these two methods (i.e., EMA vs. DDA), estimates of alcohol consumption are largely consistent, such that 75% of reports fell within 1 standard drink (Stevens et al., 2020). Similarly, research from affective science suggests that DDA versus EMA do not meaningfully alter estimates of emotion variability in the real-world nor their associations with health outcomes (Schneider et al., 2020). In sum, micro-longitudinal, naturalistic daily reporting is a valuable and highly complementary method to clinical trials, as they can increase power and ecological validity, reduce recall error, and result in more cost-effective RCTs (Carpenter et al., 2020).

Despite a mounting body of work connecting the immune system with the development and maintenance of AUD (Crews et al., 2019) and the important implications for the development of these novel therapeutics (Meredith, Burnette, et al., 2021), few RCTs have tested immunotherapies in the context of AUD to date. Thus, our understanding of how these medications influence complex AUD profiles, including subjective response, is limited (Ray et al., 2017). Alcohol is believed to alter immune signaling and contribute to neuroinflammation indirectly through systemic inflammation and directly via events in the brain that stimulate release of inflammatory molecules, induce neural damage, and alter neural signaling (Crews & Vetreno, 2016). In preclinical models, an inflammatory state alters ethanol intake, preference,

and behavioral responses to ethanol (Blednov et al., 2014; Blednov, Da Costa, Harris, et al., 2018; Liu et al., 2011; Northcutt et al., 2015). In human AUD samples, peripheral proinflammatory markers are consistently elevated and correlate with alcohol use (Adams et al., 2020; Crews, Lawrimore, et al., 2017). As such, considerable interest exists for novel treatments that can restore healthy levels of inflammation and immune signaling to promote recovery from AUD (Erickson, Grantham, et al., 2019; Meredith, Burnette, et al., 2021).

Phosphodiesterase (PDE) inhibitors are a class of immune therapies tested extensively in preclinical models of AUD (Blednov, Da Costa, Harris, et al., 2018; Franklin et al., 2015; Hu et al., 2011; Logrip et al., 2014; Ozburn et al., 2020). PDEs are enzymes that play a central role in the regulation of intracellular levels of cyclic adenosine monophosphate (cAMP), along with its downstream signal transductions (Wen et al., 2018b). Acute alcohol exposure activates cAMP signal transduction, while chronic exposure to alcohol attenuates cAMP signaling pathways in specific brain regions (Logrip, 2015). PDE4 isoforms are expressed in neuronal and glial cells in brain regions implicated in the rewarding and reinforcing effects of alcohol, such as the nucleus accumbens and amygdala (Pérez-Torres et al., 2000). Ibudilast is a selective PDE inhibitor and macrophage migration inhibitory factor (MIF) inhibitor that crosses the blood-brain barrier (Gibson et al., 2006), attenuates astrocyte and microglial activation, and increases anti-inflammatory cytokine expression (Mizuno et al., 2004). Notably, preclinical work demonstrated that ibudilast reduced voluntary ethanol intake in three different rodent models of AUD (Bell et al., 2015). Thus, ibudilast represents a promising pharmacotherapy for AUD, but its mechanisms of action remain largely unknown in clinical samples.

To date, our laboratory has tested ibudilast in two clinical samples with AUD. In an initial safety and efficacy trial, ibudilast improved mood resilience following stress exposure and

reduced tonic levels of craving (Ray et al., 2017). Mood resilience was defined as a faster recovery of positive mood to baseline levels in the treatment condition following exposure to a stressful personal narrative. However, as noted above, ibudilast did not robustly alter subjective response during an alcohol administration paradigm. Yet, this study had a relatively small sample size (N = 24), and findings could not be extended to subjective effects of alcohol in real-world settings, as participants were required to maintain abstinence during the trial for safety reasons. Extending medications development to naturalistic settings, particularly for novel pharmacotherapies like ibudilast, is needed, as it enables researchers to assess medication effects with far greater ecological validity and to examine dynamic within-person processes through repeated assessments. Electronic real-world data capture is a cost-effective way to collect numerous occasions of alcohol self-administration and related subjective effects in participants' natural environment (Carpenter et al., 2020). As such, work testing ibudilast's ability to modulate subjective response in naturalistic drinking settings has the potential to further our understanding of its biobehavioral mechanisms, particularly in the context of powerful natural reinforcers and cues. For this reason, the present study will extend findings published from a two-week clinical trial of ibudilast in our laboratory, which utilized daily diary methods (Grodin et al., 2021). DDAs of subjective alcohol response were collected during this trial to identify biobehavioral mechanisms of ibudilast, but had yet to be analyzed.

The present study sought to test the effect of neuroimmune modulation by ibudilast on subjective response to alcohol in the naturalistic environment. This secondary analysis leveraged DDAs from a two-week experimental medication RCT of ibudilast, stratified on sex and withdrawal-related dysphoria, that enrolled non-treatment seeking participants with AUD. The DDAs included reports of alcohol use and subjective response measures of stimulation, sedation,

mood, and craving. Each morning, participants retrospectively reported on their mood and craving levels both before and during the previous day's drinking episodes, as well as stimulation and sedation levels during the previous day's drinking episodes. We hypothesized that ibudilast would significantly reduce average levels of alcohol-related stimulation and increase average levels of alcohol-related sedation compared with placebo during participant naturalistic drinking episodes. Second, we hypothesized that ibudilast would significantly attenuate daily alcohol-induced changes (i.e., from before to during drinking timepoints) in craving and mood compared with placebo. Two sets of exploratory analyses were also undertaken in which we tested (1) if ibudilast moderated the effect of alcohol-related stimulation and sedation on same-day number of drinks consumed and (2) if the presence of withdrawal-related dysphoria moderated ibudilast's effects on daily alcohol-induced changes in mood and craving.

MATERIALS AND METHODS

Trial Design

The current study is a secondary analysis of data collected during a two-week clinical trial of ibudilast for heavy drinking reduction and negative mood improvement in a sample of non-treatment seeking individuals with AUD (ClinicalTrials.gov identifier: NCT03489850). Primary trial findings and full study procedures were previously published (see (Grodin et al., 2021)). Fifty-two eligible participants were randomized to either ibudilast (50 mg BID) or matched placebo (see **Figure 2-1**). Randomized participants were asked to attend in-person study visits on Day 1 (randomization), Day 8 (midpoint), and Day 15 (final assessment), and complete electronic DDAs to report on previous day craving, mood, and alcohol and cigarette use. When

participants endorsed previous day alcohol consumption, they also reported on levels of stimulation and sedation. Participants completed a neuroimaging scan at study midpoint. The clinical trial was approved by the University of California, Los Angeles Institutional Review Board [UCLA IRB#17-001741]. Prior to completing study procedures, all participants provided written informed consent after receiving a full study explanation.

Participants

A community-based sample of individuals with current DSM-5 AUD was recruited for the trial through social media and mass transit advertisements in the greater Los Angeles area. Study inclusion criteria were: (a) between 21 and 50 years of age; (b) meet current DSM-5 diagnostic criteria for mild-to-severe AUD (First et al., 2015); and (c) report heavy drinking levels 30 days prior to their screening visit, as defined by the National Institute on Alcohol Abuse and Alcoholism as >14 drinks per week for men and >7 drinks per week for women. Exclusion criteria were: (a) currently receiving or seeking treatment for AUD; (b) current DSM-5 diagnosis of another substance use disorder (excluding alcohol or nicotine); (c) lifetime DSM-5 diagnosis of bipolar disorder or any psychotic disorder; (d) current use of psychoactive drugs, other than cannabis, as verified by a urine toxicology screen; (e) if female: pregnancy, nursing, or decision to not use a reliable method of birth control; (f) presence of nonremovable ferromagnetic objects, claustrophobia, serious head injury, or prolonged period of unconsciousness (>30 min; due to neuroimaging protocol); (g) medical condition that could interfere with safe study participation; and (h) recent use of medications contraindicated with ibudilast treatment (e.g., alpha or beta agonists, theophylline, or other sympathomimetics). Participants were also required to have reliable internet access to complete electronic DDAs.

A total of 190 individuals consented to participate in the initial in-person screening visit. Of those, 81 individuals were deemed clinically eligible and were invited to complete a physical screening to determine medical eligibility. A total of 52 participants were randomized to study medication (n = 24) or placebo (n = 28). Included in the present analyses are 50 participants (n = 23 ibudilast; n = 27 placebo) who completed at least one daily diary report after randomization. Participants were compensated up to $250 for their participation in the study and received an additional $100 bonus if all study visits and ≥80% of DDAs were completed.

Screening and Trial Procedures

The clinical trial was conducted at an outpatient research clinic in an academic medical center. Interested individuals completed an initial telephone-screening interview and eligible callers were then invited to the laboratory for an in-person behavioral screening visit. At the start of all in-person visits, participants were required to have a BrAC of 0.00g/dl and a urine toxicology test negative for all drugs excluding cannabis. Eligible participants were asked to complete an in-person physical screening visit consisting of laboratory tests and physical exam by a study physician. Participants meeting all study eligibility criteria who attended the in-person randomization visit were randomly assigned to receive either 50 mg BID of ibudilast or matched placebo. Randomization was stratified by sex and participant report of experiences with withdrawal-related dysphoria. This a-priori stratification variable was intended to capture the "dark side of addiction" (Koob & Mason, 2016), whereby individuals reporting withdrawal-related dysphoria were estimated to experience greater dysfunction of the immune system. MediciNova, Inc. (La Jolla, CA, USA) supplied ibudilast and placebo for the trial but did not provide any financial support for the study. The UCLA Research pharmacy prepared and

dispensed all study medication in blister packs. Research staff, participants, and providers remained blind to medication condition during the trial. Participants were titrated on ibudilast as follows: 20 mg BID during days 1-2 and 50 mg BID during days 3-14. Target medication dose was selected based on safety considerations as well as preclinical and clinical data (Beardsley et al., 2010; Cho et al., 2010; Hutchinson et al., 2009; Worley et al., 2016). Medication compliance was monitored through pill counts and self-report via DDA. Side effects were closely monitored and reviewed by study physicians.

Screening Assessments

During the in-person screening visit, participants completed a set of assessments for individual differences and eligibility screening. Assessments included collection of demographic information (e.g., age, sex, race, income), substance use characteristics and history, and psychological functioning and diagnoses. Surveys used to characterize the sample (see **Table 2.1**) included the Beck Depression Inventory (Beck et al., 1996) (BDI-II) to assess levels of depression symptomatology, the Snaith-Hamilton Pleasure Scale (SHAPS) to measure anhedonia (Snaith et al., 1995), the Alcohol Use Disorders Identification Test (Saunders et al., 1993) (AUDIT) to capture alcohol problem severity, Penn Alcohol Craving Scale (Flannery et al., 1999) (PACS) to measure tonic craving levels, and the Reasons for Heavy Drinking Questionnaire (Adams et al., 2016) (RHDQ) to capture one's motivations for heavy drinking. In addition, the RHDQ determined the presence of withdrawal-related dysphoria for randomization stratification as follows: raw scores ranging from 0 - 10 on the RHDQ question #6: "I drink because when I stop, I feel bad (I am nervous, irritable, and I sleep poorly)", were dichotomized into *yes /no,* based on a cut-off of 6+ points. Interviews used to determine eligibility criteria and

determine baseline quantity and frequency of alcohol use were administered by clinical graduate students or trained research staff and included the Timeline FollowBack (TLFB) measuring alcohol, cigarette, and cannabis use over the previous 30 days (Sobell & Sobell, 1992; Sobell et al., 1986), the Clinical Institute Withdrawal Assessment for Alcohol Scale - Revised (Sullivan et al., 1989) (CIWA-Ar) assessing clinically significant alcohol withdrawal, and the Structured Clinical Interview for DSM-5 (First et al., 2002) (SCID-5) to determine current AUD diagnosis and severity and to screen for exclusionary psychiatric diagnoses.

Electronic Daily Diary Assessments

Each morning throughout the two-week trial, participants were asked to retrospectively report on their previous day experiences by completing an electronic DDA survey (see Appendix). Study staff provided instructions on DDA completion and participants practiced filling out the survey at their randomization visit. Daily text messages or emails containing links to DDAs were sent to participants at 8am each morning during their 14-day medication period. Additional telephone or text reminders were sent by study staff as needed. At the start of each daily survey, participants were asked, "Did you drink any alcohol yesterday?" If participants endorsed alcohol use the previous day, they reported on drink type and quantity, and then completed two sets of items: 1) ratings of mood, craving, and urge *before* drinking, and 2) ratings of mood, craving, urge, stimulation, and sedation *while* drinking. For example, participants were asked: "*Before you drank*, how strong was your urge to drink alcohol yesterday?" and "*While drinking*, how strong was your urge to drink alcohol yesterday?" The current analyses focus primarily on drinking days, given our interest in medication-related changes in subjective response to alcohol.

Mood states were assessed via the short form of the Profile of Mood States (POMS-SF) survey (Curran et al., 1995; McNair et al., 1971). POMS-SF is a standard, validated psychological rating scale that measures dimensions of transient mood states by asking subjects to indicate how well each item describes their mood on a 5-point Likert scale (Not at All = 0 to Extremely = 4). To keep the survey brief and thus reduce the burden on participants, only select items from POMS-SF were chosen for DDAs (see Statistical Analyses). Reports of stimulation and sedation were assessed via the validated Brief Biphasic Alcohol Effects Scale (Rueger et al., 2009) (B-BAES). The B-BAES is a six-item measure on the acute stimulant and sedative effects of alcohol on an 11-point scale (Not at All = 0 to Extremely = 10 points). Urge to drink was captured via the item, "How strong was your urge to drink alcohol yesterday" (No Urge = 0 to Strongest Ever = 10), in line with previously published reports (Ray et al., 2007; Ray et al., 2010). Phasic craving was assessed using the first and last items from the validated Alcohol Urge Questionnaire (Bohn et al., 1995; MacKillop, 2006) (AUQ) on a 7-point Likert scale (Strongly Disagree = 0 to Strongly Agree = 6). Participants reported the quantity of standard alcoholic drinks consumed according to established guidelines and provided details about non-standard drinks (e.g., malt liquor, sake, hard seltzers, etc.). Drink entries were reviewed and verified by study staff.

Statistical Analyses

DDA Item Scoring

All descriptive and statistical analyses were completed in SAS Version 9.4 on the sample of participants who completed at least one DDA. Select items from the POMS-SF tension (i.e., Anxious, Uneasy) and depression subscales (i.e., Downhearted, Discouraged), were summed to

form a negative mood state score (range 0 - 16) and select items from the vigor subscale (i.e., Joyful, Cheerful, Energetic, Lively) were summed to form a positive mood state score (range from 0 - 16) for each timepoint, consistent with previous reports (Bujarski et al., 2015; Sheets et al., 2015). The two AUQ items were summed to form a craving score (range 0 - 12). Stimulation and sedation subscales from B-BAES were calculated using standard methods (range 0 - 30).

Multilevel Models

Models were fit in SAS using the MIXED procedure and a multilevel framework, unstructured covariance matrix, residual maximum likelihood (REML) estimation, and random intercepts with observations nested within subjects to account for clustering and to preserve suitable Type-1 error rates (Raudenbush & Bryk, 2002). Kenward-Rogers degrees of freedom were chosen to reduce bias and obtain more accurate *p*-value estimates. Main and simple effects were probed by recentering dichotomous variables (e.g., medication condition, time) and using the simple slopes approach. Daily alcohol use quantity, mood states, craving, and urge data from non-drinking days were treated as missing. Comparable three-level models were fit for variables having both before and during drinking observations (i.e., positive mood, negative mood, urge, and craving), such that these observations were nested within day and days were nested within subjects. All models were tested with the following level-2 covariates: sex, AUD severity (DSM-5 symptom count), and baseline drinks per drinking day (DPDD). In addition, daily number of drinks consumed during the trial was included as a predictor with random effect in all subjective response models to account and control for potential day-level drink quantity effects on subjective response. To examine both between- and within-subject effects and interactions, covariates were centered at the grand mean (CGM) and focal within-subject variables were centered within cluster (CWC) (Enders & Tofighi, 2007).

Specifically, to assess the effect of medication on the acute stimulant and sedative effects of alcohol, one model for each B-BAES subscale was estimated in which stimulation or sedation served as the outcome and medication condition (ibudilast vs. placebo) served as the focal predictor. To assess the effect of medication on alcohol-induced changes in mood and craving, three-level models were run for each positive mood, negative mood, craving, and urge scores, as predicted by medication condition, time (before vs. during drinking) and a medication × time interaction.

Two sets of exploratory analyses were conducted. First, to explore how medication effects might impact drinking outcomes, we tested whether ibudilast moderated the effect of stimulation/ sedation on same-day drinking during the trial, given support for these variables as strong predictors of alcohol use (King et al., 2021; Schuckit et al., 2007). As such, a within-subject cross-level interaction of medication × stimulation or sedation was added with random slopes, and same-day number of drinks served as the outcome. In a similar fashion, we also tested whether ibudilast moderated the effect on stimulation/ sedation on next-day drinking (yes/ no) using cross-lagged logistic models; this analysis served to test whether subjective response predicted future drinking behaviors. Second, given the trial's *a priori* interest in a withdrawal-related dysphoria characteristic, we tested whether dysphoria would moderate ibudilast's effects on alcohol-induced changes in mood and craving. A three-way interaction was added to models estimating the outcomes- positive mood, negative mood, urge, and craving (i.e., withdrawal-related dysphoria × medication × time). Stimulation and sedation variables were limited to a single timepoint and were thus excluded from analyses testing before to during drinking changes.

To assess the effect of medication on alcohol-induced changes in mood and craving, three-level models were run for each positive mood, negative mood, craving, and urge scores, as

predicted by medication condition, time (before vs. during drinking) and a medication × time interaction. One set of exploratory analyses were conducted. Given the trial's *a priori* interest in a withdrawal-related dysphoria characteristic, we tested whether dysphoria would moderate ibudilast's effects on alcohol-induced changes in mood and craving. A three-way interaction was added to models estimating the outcomes- positive mood, negative mood, urge, and craving (i.e., withdrawal-related dysphoria × medication × time).

RESULTS

Participant Characteristics

The final sample of randomized participants who completed at least one DDA, consisted of 50 non-treatment seeking individuals with current AUD (n = 23 ibudilast; n = 27 placebo). Overall, 66% of the sample reported their sex as male, 68% reported an annual household income < $60,000, and the average age was 32.7 years (see **Table 2-1**). Regarding race, participants most frequently identified as White (56%), followed by 14% Black or African American, and 12% mixed race. In addition, 24% of the sample identified as Hispanic/Latinx. Participants had an average of 5.6 DPDD in the month prior to their baseline visit. Medication adherence was high, as both medication groups exceeded 97% adherence rates. Adverse events by symptom category did not significantly differ between medication groups.

Daily Diary Assessments

In total, 653 DDAs were completed (92.6% completion rate) with participants missing between 0 to 4 days of reports during the two-week trial. Participants completed an average of 13.06 (*SD* = 1.14) DDAs, comprised of 7.92 (*SD* = 3.49) drinking days on average (range 1-14

days; total n = 396) and 5.14 (*SD* = 3.62) non-drinking days on average (range 0-12; total n = 257). Interclass correlations (ICCs) from unconditional subjective response models support the use of multilevel nested models. For instance, ICCs from three-level models on positive and negative mood, urge and craving accounting for day- and subject-level clustering ranged from .594 to .776, indicating that approximately 59% to 78% of the total variability is attributable to clustering observations. See **Table 2-2** for estimated marginal means of subjective response and DPDD variables during the two-week trial by medication condition.

Effect of Ibudilast on Stimulation and Sedation

Stimulation. Only daily number of drinks was a significant covariate of stimulation ($p < .001$), such that greater alcohol intake was associated with higher daily stimulation. After accounting for covariates, medication condition did not significantly predict average levels of stimulation ($b = -2.93$, $p = .108$).

Sedation. Only AUD severity was a significant covariate of sedation, such that greater AUD severity was associated with higher mean sedation ($p = .035$). After accounting for covariates, medication condition did not significantly predict mean sedation during the trial ($b = -2.41$, $p = .103$).

Effect of Ibudilast and Subjective Response on Drinking Outcomes

Ibudilast × Stimulation. When testing whether medication condition moderated the effect of stimulation on same-day drinking during the trial, two covariates were significant: DPDD at baseline ($p < .0001$) and sex ($p = .023$), where baseline drinking and male sex were associated with greater mean DPDD during the trial. While medication condition was not a significant

predictor of mean trial DPDD in this full model (b = -0.67, p = .232), a significant cross-level medication × stimulation interaction was detected (b = 0.23, p = .045). As such, medication moderated the effect of daily stimulation on same-day number of drinks consumed. When probing for simple effects, results showed that only participants taking ibudilast (b = 0.30, p = .001), but not placebo (b = 0.07, p = .350), reported a significant, positive relationship between daily stimulation and same-day alcohol use (see **Figure 2-2**). This suggests that for a day with a 1-point higher score on stimulation, we would expect a 0.30-point relative within-person increase in number of drinks among those taking ibudilast. However, for the logistic model, a cross-level medication × daily stimulation interaction did not significantly predict likelihood of next-day drinking (p = .646), nor did medication condition predict likelihood of next-day drinking (p = .721).

Ibudilast × Sedation. When testing whether medication condition moderated the effect of sedation on same-day drinking during the trial, two covariates were significant: DPDD at baseline (p < .0001) and sex (p = .035), where baseline drinking and male sex were associated with greater mean DPDD during the trial. In this full model, medication condition (b = -1.93, p = .054) and the cross-level medication × sedation interaction (b = 0.14, p = .059) were only marginally significant. When probing simple effects, results again showed that participants on ibudilast (b = 0.20, p < .001), but not placebo (b = 0.05, p = .278), reported a significant positive relationship between daily sedation and same-day alcohol use (see **Figure 2-2**). This suggests that for a day with a 1-point higher score on sedation, we would expect a 0.20-point relative within-person increase in number of drinks among those taking ibudilast. However, for the logistic model, a cross-level medication × daily sedation interaction did not significantly predict

likelihood of next-day drinking (p = .858), nor did medication condition predict likelihood of next-day drinking (p = .730).

Effect of Ibudilast on Alcohol-Induced Changes in Mood and Craving

Positive and Negative Mood. In testing the impact of medication on alcohol-induced changes in mood, daily number of drinks during the trial was a significant within-subject predictor of positive mood, where greater daily drinking predicted higher same-day positive mood (p = .004). No other covariates were significant. Neither the medication × time interaction (b = 0.04, p = .895), nor medication condition significantly predicted same-day positive mood (b = -0.54, p = .545). Participant report of positive mood was significantly greater at the during drinking timepoint (b = 1.20, p < .0001). Similarly, neither the medication × time interaction (b = -0.05, p = .817), nor medication condition significantly predicted same-day negative mood (b = 0.55, p = .429). As expected, participant report of negative mood was significantly lower at the during drinking timepoint (b = -0.88, p < .0001).

Urge and Craving. In testing the impact of medication on alcohol-induced changes in urge and craving, baseline DPDD and daily number of drinks consumed during the trial were significant predictors of urge and craving (p's < .005); AUD severity was also a significant predictor of craving (p = .037). For these significant covariates, higher scores were associated with greater urge and craving. Neither the medication × time interaction (b = -0.27, p = .173), nor medication condition (b = -0.27, p = .667) significantly predicted daily urge. Participant report of urge was significantly greater at the during drinking timepoint (b = 0.59, p < .0001). While medication condition was not significantly associated with average daily craving, the cross-level medication × time interaction (b = 0.46, p = .047) did significantly predict craving. When probing simple

effects by recentering, results show that only for the placebo condition (b = 0.69, p < .0001), but not ibudilast (b = 0.24, p = .167), did same-day craving significantly increase during alcohol intake (see **Figure 2-3**), showing that ibudilast attenuated within-subject alcohol-induced increases in craving.

Moderating Role of Withdrawal-Related Dysphoria

In total, 19 participants were categorized as having withdrawal-related dysphoria (n = 8 ibudilast; n = 11 placebo). Given the modestly sized subgroupings, this set of analyses are exploratory and should be interpreted as such. In testing our exploratory models on whether the presence of withdrawal-related dysphoria moderated the effect of ibudilast on same-day alcohol-induced changes in mood, craving, and urge, two significant covariates emerged. For models predicting craving and urge, baseline DPDD (p's < .005) and daily number of drinks during the trial (p's < .0005), were positively associated with craving and urge; daily number of drinks was also positively associated with same-day positive mood (p = .004). Several three-way interactions were detected, such that for daily changes in craving (b = -1.64, p = .0004; see **Figure 2-4**), urge (b = -0.88, p = .028; see **Figure 2-4**), and positive mood (b = -3.40, p < .0001; see **Figure 2-5**), a significant dysphoria × medication × time interaction emerged after accounting for relevant covariates. Yet, this three-way interaction term was not significant for the model predicting negative mood (p = .300; see **Figure 2-5**). A consistent pattern emerged when probing these interactions, such that among participants without reported withdrawal-related dysphoria, the medication × time interactions were significant for craving (p = .0002), urge (p = .021), and positive mood (p = .001). Participants without withdrawal dysphoria and randomized to ibudilast reported smaller and non-significant increases in subjective responses

(p's range from .190 to .952) compared with placebo (p's <.0001). For instance, while taking placebo, participants had an average daily alcohol-induced increase in positive mood of 1.31 points, while those on ibudilast displayed an average daily alcohol-induced decrease in positive mood by 0.02 points. For those reporting presence of withdrawal-related dysphoria, no significant medication × time interactions were detected for craving (p = .110) nor urge (p = .301), but the interaction was significant for positive mood (p < .0001). Unexpectedly, participants with withdrawal dysphoria on ibudilast reported greater same-day alcohol-induced increases in positive mood (p < .0001) than placebo (p = .005).

DISCUSSION

In this secondary analysis, we tested biobehavioral mechanisms of ibudilast, a neuroimmune modulator, through naturalistic daily reporting of subjective response to alcohol collected during a two-week RCT enrolling 50 non-treatment seeking participants with AUD. Electronic DDAs were administered each morning to participants to capture their previous day drinking behaviors and subjective alcohol response measures. First, we were interested in understanding whether ibudilast altered average levels of stimulation and sedation during drinking episodes. Results showed that ibudilast treatment did not significantly change average levels of stimulation nor sedation during the trial compared with placebo. These findings are consistent with an initial safety trial in which ibudilast did not significantly affect any subjective response variables during an experimentally controlled alcohol infusion in the laboratory (Ray et al., 2017). Relatedly, a trial combining laboratory and EMA methods showed that topiramate reduced drinking-related craving but not the stimulant or sedative effects of alcohol (Miranda et al., 2016). However, animal literature shows that apremilast, another PDE inhibitor, did alter a

wide range of ethanol-induced effects in mice, such as reducing acute functional tolerance and increasing the sedative, intoxicating effects, and aversive properties of ethanol (Blednov, Da Costa, Harris, et al., 2018). Perhaps unlike certain pharmacotherapies for AUD such as naltrexone, neuroimmune modulators, like ibudilast may not reduce drinking by robustly suppressing alcohol's stimulant properties or amplifying its sedative effects. Rather, ibudilast may more directly alter other central mechanisms like alcohol craving or may exert a wider range of effects on multiple mechanisms that cumulatively impact drinking outcomes.

Second, we tested a related exploratory aim examining the moderating effect of ibudilast on alcohol-related stimulation and sedation and same-day number of drinks consumed. Participants on ibudilast reported a significant, positive relationship between their stimulation and sedation ratings and same-day drinking levels, neither of which was observed in the placebo condition. This suggests that participants randomized to ibudilast consumed more alcohol on days when they retrospectively reported feeling more stimulated (or sedated) during a drinking episode than on days when they felt less stimulated (or sedated). Yet for those on placebo, we did not detect a significant relationship between one's feelings of stimulation or sedation and alcohol use. These findings are consistent with EMA data showing that naltrexone potentiated participant's subjective "high" across rising levels of estimated BrAC (Miranda et al., 2014). Similarly, topiramate was shown to strengthen the association between mean positive affect and frequency of cannabis use (Emery et al., 2021). These results are also in line with a secondary analysis of our lab's initial efficacy trial, whereby ibudilast potentiated the association between mood states and one's craving for alcohol following a stress exposure paradigm compared with placebo (Meredith, Green, et al., 2021). Mechanistically, PDE4 inhibitors attenuate alcohol-induced neuroimmune activation and dysregulation of GABAergic signaling (Avila et al., 2017;

Blednov, Da Costa, Harris, et al., 2018; Carlson et al., 2016). These important processes are connected to behavioral responses to ethanol (Crews, Lawrimore, et al., 2017; Liang & Olsen, 2014). Thus, micro-longitudinal reports collected during the current trial helped to elucidate dynamic, day-to-day associations between within-person subjective effects and drinking, such that ibudilast seemed to moderate these relationships for a given individual, rather than by altering average subjective response levels across participants.

For our second primary aim, we assessed whether ibudilast, compared with placebo, attenuated daily alcohol-induced changes in positive mood, negative mood, urge, and craving (i.e., from pre-drinking to during drinking levels). Among the full sample, we found that ibudilast significantly dampened within-person alcohol-induced increases in craving seen under the placebo condition, but not other subjective response indicators. This suggests that one of the mechanisms by which ibudilast exerts its effects on drinking outcomes, such as reductions in heavy drinking (see (Grodin et al., 2021)), may be by diminishing one's desire to continue drinking during an episode. Considering its immunomodulatory actions, ibudilast may reduce the acute and chronic proinflammatory effects of alcohol, either indirectly through suppression of peripheral inflammation or directly by altering cAMP signaling pathways and suppressing cytokine expression and in the brain (e.g., rewards regions relevant to craving) (Avila et al., 2017). In return, acute alcohol-induced increases in craving are blunted. Supporting these findings is research on methamphetamine use disorder (MUD). An RCT for inpatients with MUD showed that ibudilast (50 mg BID) significantly blunted the rewarding effects of methamphetamine during an infusion in the laboratory (Worley et al., 2016) and similarly diminished drug-induced increases in proinflammatory levels during infusion (Li et al., 2020). Continuing, previous results from our group implicate ibudilast in the reduction of tonic craving

(Ray et al., 2017) and neural alcohol-cue reactivity, as evidenced by attenuation of cue-elicited activation in the ventral striatum compared with placebo (Grodin et al., 2021). It is thus plausible that reductions in alcohol craving and reward, across these contexts, represent a primary mechanism of action of ibudilast for AUD. Craving likely represents a more proximal determinant of alcohol use than stimulation and sedation, which are shown to indirectly influence alcohol self-administration through craving (Green et al., 2019; Wardell et al., 2015).

An additional exploratory aim was to test whether a characteristic of AUD severity, withdrawal-related dysphoria, moderated ibudilast's effects on daily alcohol-induced changes in mood and craving. Notably, we found that individuals without a reported history of withdrawal-related dysphoria who were treated with ibudilast showed attenuation of alcohol-induced changes in craving, urge, and positive mood when compared to placebo. This tempering of alcohol's effects may reflect ibudilast's enhancement of anti-inflammatory and neurotrophic factors suspected to impact dopaminergic signaling in rewards regions, such as the nucleus accumbens, where PDE4 and PDE10 are highly expressed (Bland et al., 2009; Ramirez & Smith, 2014). However, individuals who endorsed this withdrawal-dysphoric profile did not appear to benefit from treatment via this mechanism, such that ibudilast did not significantly blunt acute rewarding and reinforcing effects of alcohol. Although intriguing, these moderation findings should be interpreted with caution given the limited sample size, particularly the subgroup of individuals reporting experiences with withdrawal-related dysphoria (n = 19). Despite these findings, preliminary analyses from this two-week RCT show that withdrawal dysphoria did not moderate clinical response to ibudilast regarding rates of heavy drinking or drinks per drinking day. Notably, these subjective response results are somewhat in contrast to what might be expected for individuals with a history of withdrawal and experiencing the "dark side of

addiction", such that these individuals may potentially show greater dysfunction of the immune system and thus may be predicted to have better response to an anti-inflammatory treatment, such as ibudilast. However, it is suspected that other mechanisms may be central to the maintenance of AUD among individuals with withdrawal dysphoria, beyond the enhancing effects of alcohol. Namely, these individuals may primarily drink to feel 'normal' and alleviate physiological or psychological distress, particularly during early abstinence (Adams et al., 2016; Koob & Mason, 2016), which was not the focus on the current study. The present findings also differ somewhat from our laboratory's initial efficacy trial of ibudilast, in which individuals with higher levels of depression (e.g., experiencing the "dark side of addiction") showed attenuation of alcohol-induced increases in positive mood and 'wanting' during intravenous alcohol administration (Ray et al., 2017). A relevant difference between these studies is that participants enrolled in the efficacy trial were likely in a state of early abstinence, as they were asked to refrain from drinking for safety reasons; yet those enrolled in the present trial were not asked to change their drinking behaviors and consumed alcohol on roughly 60% of trial days and around 6 DPDD on average. In preclinical models, withdrawal increases the expression of innate immune markers in brain regions regulating autonomic and emotional states (Freeman et al., 2012) and while speculative, may thus represent a unique condition with the potential to impact ibudilast's therapeutic effects. For instance, ibudilast reduced opioid withdrawal symptoms among individuals with heroin dependence (Cooper et al., 2016) and another PDE4 inhibitor, rolipram, diminished withdrawal-induced behaviors indicative of negative affect in rodents (M.-F. Gong et al., 2017). Future research evaluating the impact of withdrawal states on immune signaling in larger clinical samples is needed to advance understanding of these complex processes and immune intervention.

These findings should be considered in the context of the study's strengths and limitations. One limitation is that DDAs were reported retrospectively once daily, which is less temporally accurate than EMA designs. As such, items on subjective response and drinking were reported by participants concurrently the morning following a drinking episode and did not capture one's subjective response level at a specific BrAC or blood alcohol curve limb. As such, this weakens our ability to draw a causal link between the effect of subjective response on alcohol intake and may introduce recall bias. Next, participants with more non-drinking days and incomplete DDAs during the trial are suspected to have greater error variance in their data given the lower number of observations with subjective response data. The lack of daily pre-drinking data on stimulation and sedation prevented us from examining daily changes in these variables, such that we could not account for pre-drinking levels. The sample was comprised of non-treatment seeking individuals with moderate AUD on average and the majority (62%) did not fall in the withdrawal-related dysphoria category. Future work with ibudilast in more diverse and treatment-seeking samples with more significant experiences of withdrawal-related dysphoria is needed. This study's strengths include a clinical AUD sample enrolled in a rigorous double-blind RCT testing a promising novel pharmacotherapy. This trial displayed strong medication adherence rates and tolerability. Further, DDAs had high completion rates and the data comprise a substantial number of drinking episodes (e.g., ~400 DDAs). Morning reports are also less likely to be affected by the intoxicating effects of alcohol that may lend to reporting errors, as could be seen with EMA or nightly reports. Finally, to our knowledge, this is the first study on the effect of immune modulation on subjective alcohol response in the natural environment.

In closing, this daily diary study complements findings from our previous reports of ibudilast treatment for AUD by examining medication effects on subjective response during real-world

drinking episodes. The nuanced nature of the findings, including the distinction among those with and without withdrawal-related dysphoria and within vs. between person subjective response effects, speak to the heterogeneity of AUD and dynamic mechanisms maintaining alcohol use. Ibudilast's effects on subjective alcohol responses, such as positive mood and craving, appear to be nuanced and perhaps most salient for individuals drinking for positive reinforcement as opposed to normalizing. Treatment with ibudilast potentiated the within-person relations between stimulation/ sedation and alcohol intake in this trial, such that an individual's quantity of consumption on a given day appears to be more tightly connected to subjective response. The ecologically valid nature of these DDA, through retrospective reports of past day drinking and subjective responses to alcohol, provide a clinically useful window into how individuals experience and recall alcohol's effects while taking ibudilast, compared to placebo. Novel medications and novel biological targets call for careful assessment of mechanisms beyond the "usual suspects", such as changes in mean levels of subjective response and alcohol craving. Ultimately, the combination of multiple scientific approaches, including human laboratory, DDAs, neuroimaging, and biomarker assessment, offer complementary and clinically useful findings that can inform the development of ibudilast, and immune treatments for AUD more broadly.

Table 2-1. Participant characteristics by treatment condition

Variable	Combined (N = 50)	Ibudilast (n = 23)	Placebo (n = 27)
Demographic			
Age (Years)	32.66 (8.52)	34.13 (9.30)	31.41 (7.75)
Sex (No., %)			
Male	33 (66.0%)	16 (69.6%)	17 (63.0%)
Female	17 (34.0%)	7 (30.4%)	10 (37.0%)
Education (Years)	15.16 (2.11)	15.13 (2.49)	15.18 (1.78)
Race (No., %)			
White	28 (56.0%)	16 (69.6%)	12 (44.4%)
Black or African American	7 (14.0%)	5 (21.7%)	2 (7.4%)
Asian	4 (8.0%)	0 (0.0%)	4 (14.8%)
Pacific Islander	1 (2.0%)	0 (0.0%)	1 (3.7%)
Mixed Race	6 (12.0%)	1 (4.4%)	5 (18.5%)
Another Race	4 (8.0%)	1 (4.4%)	3 (11.1%)
Ethnicity (No., %)			
Not Hispanic/ Latinx	38 (76.0%)	18 (78.3%)	20 (74.1%)
Hispanic/ Latinx	12 (24.0%)	5 (21.7%)	7 (26.0%)
Annual Household Income (No., %)			
$0 - $29,999	19 (38.0%)	10 (43.4%)	9 (33.3%)
$30,000 - $59,999	15 (30.0%)	5 (21.7%)	10 (37.0%)
$60,000 - $89,999	9 (18.0%)	4 (17.4%)	5 (18.5%)
$90,000 - $119,999	2 (4.0%)	1 (4.3%)	1 (3.7%)
> $120,000	5 (10.0%)	3 (13.0%)	2 (7.4%)
Substance Use			
Drinks per drinking day[a]	5.61 (3.26)	5.83 (2.61)	5.42 (3.76)
% heavy drinking days[a]	45.92 (31.22)	48.08 (29.68)	44.09 (32.93)
Positive THC screen (No., %)	15 (30.0%)	7 (30.4%)	8 (29.6%)
Smokes cigarettes (No., %)	27 (54.0%)	16 (59.3%)	11 (47.8%)
PACS Total	12.38 (6.29)	12.87 (5.24)	11.96 (7.13)
SCID AUD symptom count	5.02 (2.33)	5.30 (2.42)	4.78 (2.26)
AUDIT total	16.42 (6.02)	16.26 (6.00)	16.56 (6.48)
Withdrawal-related dysphoria (No., %)	19 (38.0%)	8 (34.8%)	11 (36.7%)
Mental Health			
BDI-II Total	10.74 (8.23)	12.83 (8.42)	8.96 (7.78)
SHAPS Total	1.66 (2.92)	1.87 (1.84)	1.48 (3.63)

Note. [a] determined by Timeline FollowBack collected on the 30 days prior to baseline visit;

PACS = Penn Alcohol Craving Scale; SCID = Structured Clinical Interview for the DSM-5;

AUD = alcohol use disorder; AUDIT = Alcohol Use Disorders Identification Test; BDI-II = Beck Depression Inventory II; SHAPS = Snaith-Hamilton Pleasure Scale.

Table 2-2. Estimated marginal means for subjective response variables by medication condition

Variable	**Ibudilast (n = 23)**		**Placebo (n = 27)**	
	Before Drinking Mean (SE)	**During Drinking** Mean (SE)	**Before Drinking** Mean (SE)	**During Drinking** Mean (SE)
Positive mood[a]	5.85 (0.63)	7.05 (0.63)	6.41 (0.58)	7.57 (0.58)
Negative mood[a]	3.05 (0.44)	2.15 (0.44)	2.56 (0.41)	1.71 (0.41)
Urge[b]	3.41 (0.41)	3.87 (0.41)	3.95 (0.38)	4.67 (0.38)
Craving[c]	4.46 (0.49)	4.70 (0.49)	4.93 (0.45)	5.63 (0.45)
Stimulation[d]	-	13.30 (1.30)	-	16.23 (1.20)
Sedation[d]	-	5.81 (1.05)	-	8.22 (0.97)
Daily number of drinks per drinking day[e]	-	5.42 (0.41)	-	6.08 (0.38)

Note: Estimated marginal means drawn from subjective response models; [a] subscale drawn from the Profile of Mood States- Short Form, possible range: 0 - 16; [b] single-item Urge rating, possible range 0 - 10; [c] subscale drawn from the Alcohol Urge Questionnaire, possible range 0 - 12; [d] subscales from Brief Biphasic Alcohol Effects Scale, possible range 0 - 30; [e] raw average number of drinks per drinking day during the trial according to participant report on daily diaries.

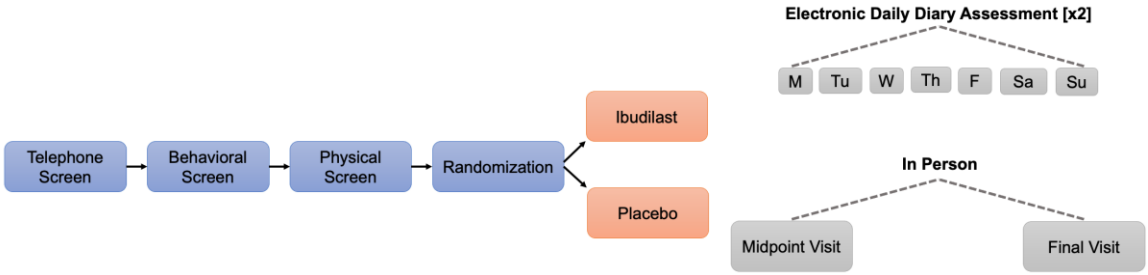


Figure 2-1. Study design for two-week randomized controlled trial of ibudilast for alcohol use disorder with daily diary assessments.

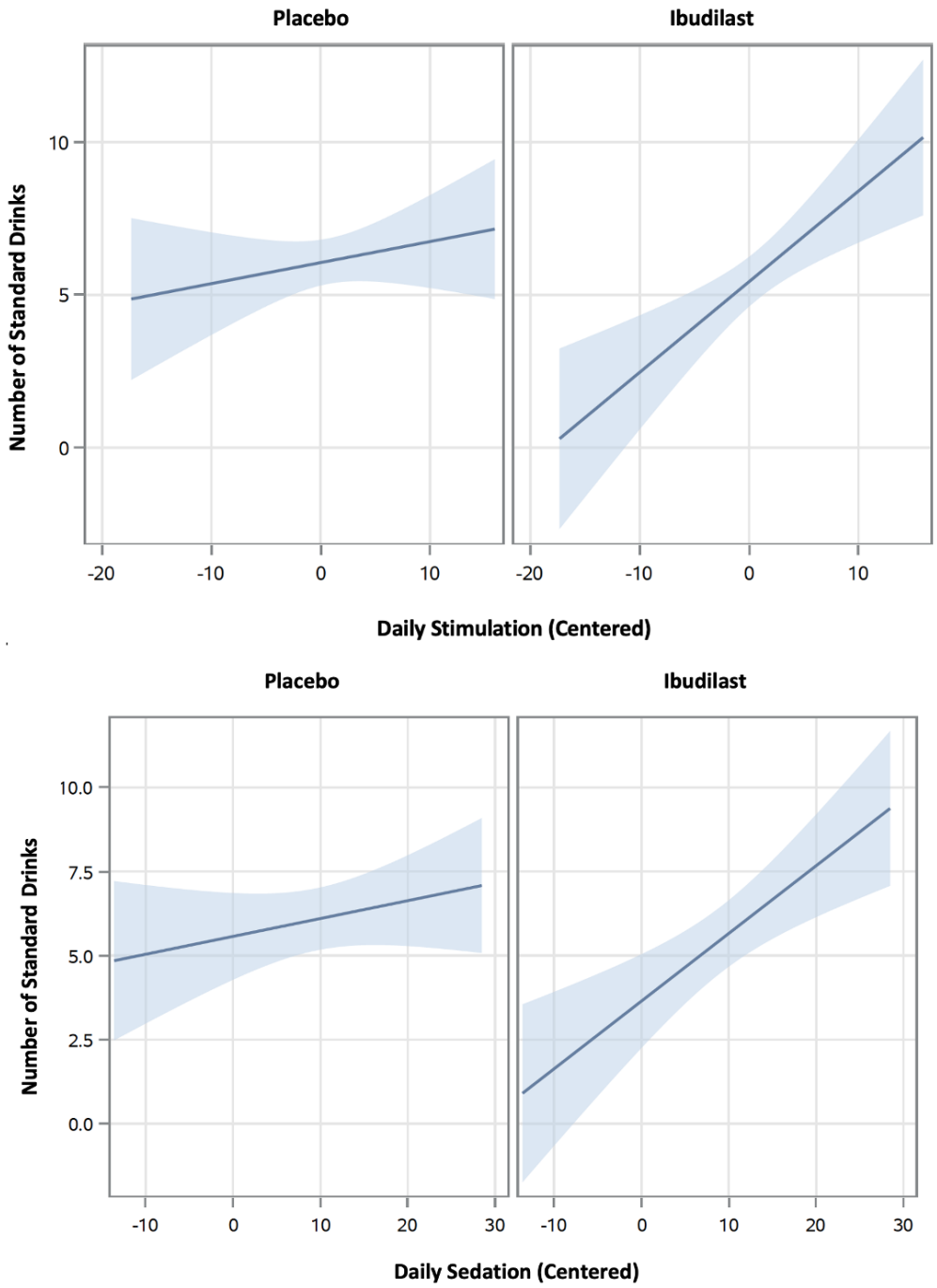


Figure 2-2. Moderating effect of ibudilast treatment on daily stimulation/ sedation and number of drinks.

Note. Visual shows that participants on ibudilast (*p's* < .001), but not placebo's (*p's* > .250), reported a signficant positive relationship between daily stimulation/ sedation and same-day alcohol use.

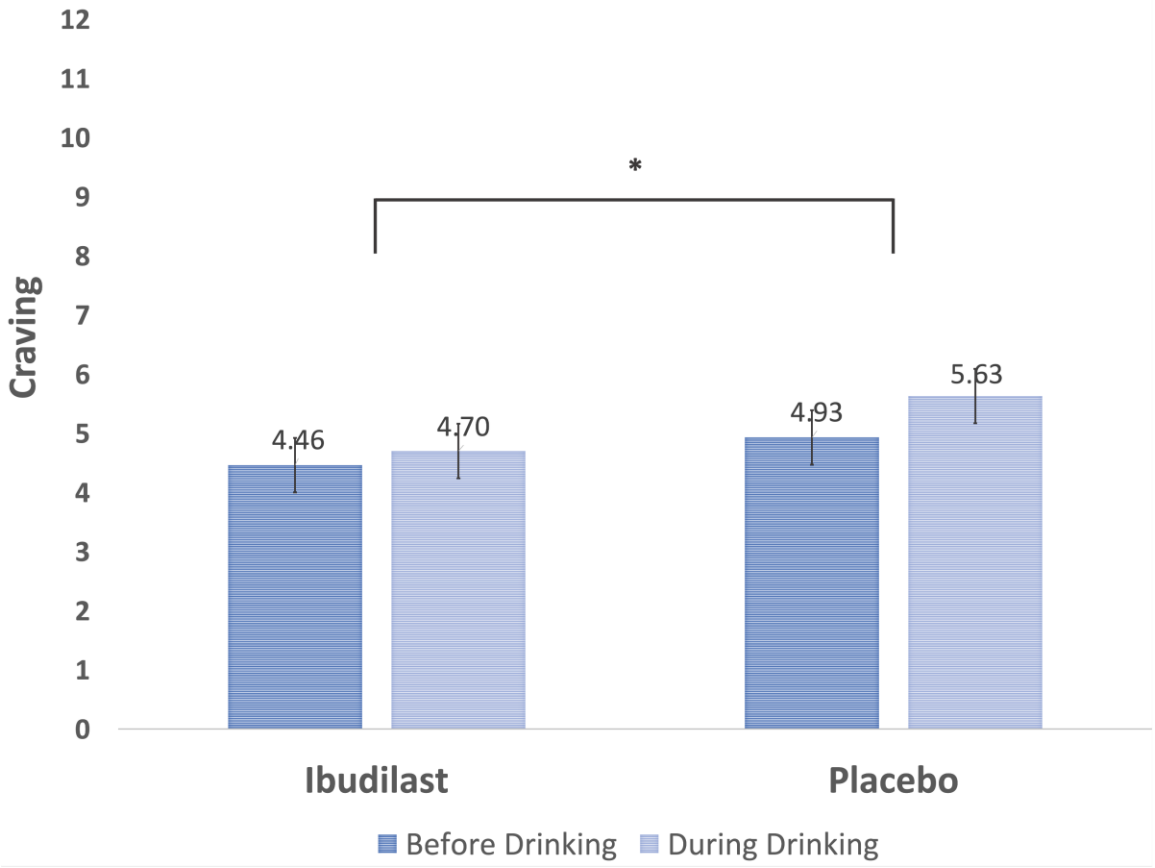
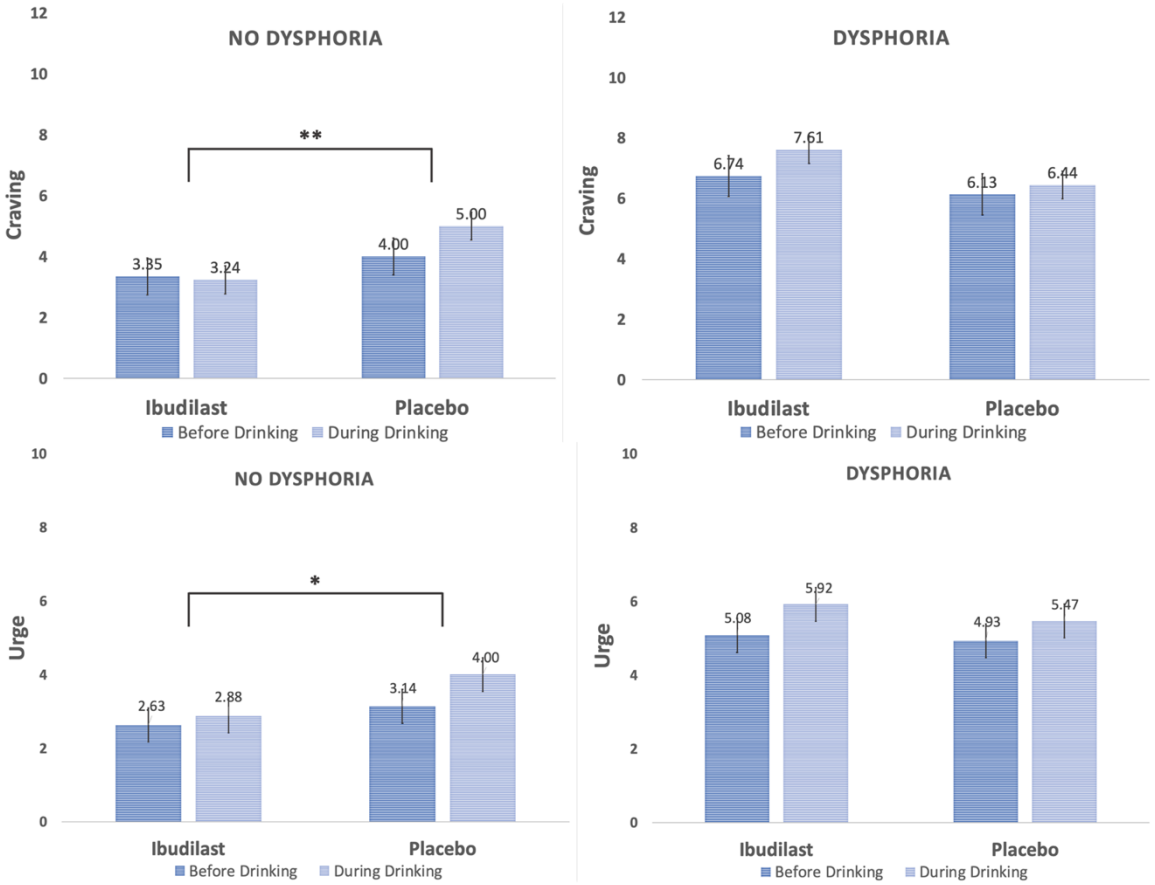


Figure 2-3. Ibudilast attenuates alcohol-induced increases in craving compared to placebo. Note: * indicates a significant (p = .047) medication (ibudilast vs. placebo) x time (before vs. during drinking) interaction for alcohol craving reported on daily diary assessments.

Figure 2-4. Alcohol-induced changes in craving and urge by medication condition and presence of withdrawal-related dysphoria.



Note: Three-way interactions (medication x time x withdrawal-related dysphoria) were detected for daily changes in craving (p = .0004) and urge (p = .028); ** indicates a significant (p < .001) medication (ibudilast vs. placebo) x time (before vs. during drinking) interaction for alcohol craving among those without reported withdrawal-related dysphoria; * indicates a significant (p = .021) medication x time interaction for alcohol urge among those without reported withdrawal-related dysphoria.

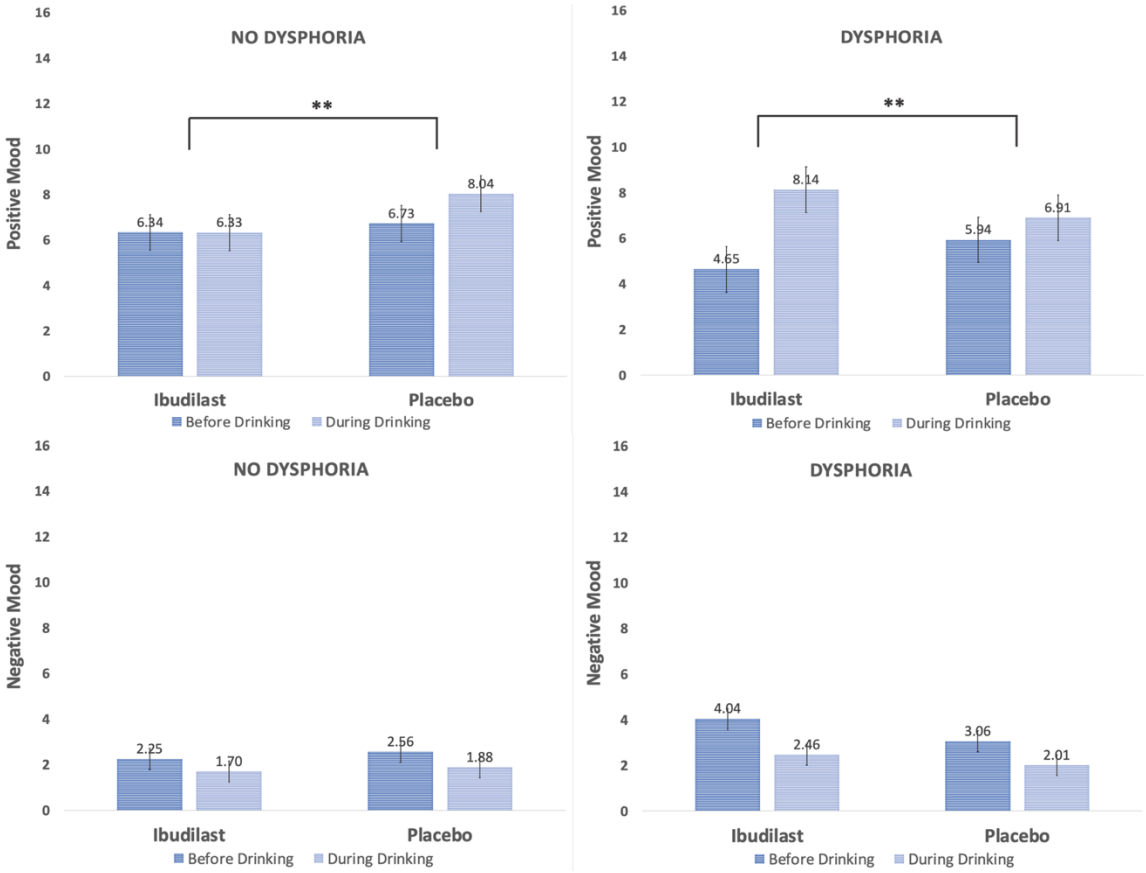


Figure 2-5. Alcohol-induced changes in mood by medication condition and presence of withdrawal-related dysphoria.

Note: Three-way interaction (medication x time x withdrawal-related dysphoria) was detected for daily changes in positive mood ($p < .001$); ** indicates a significant ($p < .01$) medication (ibudilast vs. placebo) x time (before vs. during drinking) interaction for positive mood, whereby the ibudilast group showed smaller alcohol-related changes in mood among those without withdrawal-related dysphoria, yet greater changes in mood among those with withdrawal-related dysphoria.

Appendix 2-1. Daily Diary Assessment

Did you drink any alcohol yesterday?

No	Yes
○	○

You reported that you drank alcohol yesterday. Please confirm that this is correct.

○ I did drink yesterday
○ I did not drink yesterday

How did you feel **yesterday before drinking**?

	Not at all	A little	Moderately	Quite a bit	Extremely
Downhearted	○	○	○	○	○
Discouraged	○	○	○	○	○
Uneasy	○	○	○	○	○
Anxious	○	○	○	○	○

How did you feel **yesterday before drinking**?

	Not at all	A little	Moderately	Quite a bit	Extremely
Joyful	○	○	○	○	○
Cheerful	○	○	○	○	○
Energetic	○	○	○	○	○
Lively	○	○	○	○	○

Before drinking, I craved a drink yesterday.

Strongly Disagree 0	1	2	3	4	5	Strongly Agree 6
○	○	○	○	○	○	○

Before drinking, all I wanted to do yesterday was have a drink.

Strongly Disagree 0	1	2	3	4	5	Strongly Agree 6
○	○	○	○	○	○	○

Before you drank, how strong was your urge to drink alcohol yesterday?

No urge 0	1	2	3	4	5	6	7	8	9	Strongest ever 10
○	○	○	○	○	○	○	○	○	○	○

How many **12-ounce beers** did you have yesterday?

How many **5-ounce glasses of wine** did you have yesterday?

How many **1.5-ounce shots of liquor** did you have yesterday - **alone or in a mixed drink**?

If you drank something not listed above, please indicate below the alcohol type (e.g. sake, soju, malt liquor, etc.) and number of drinks (e.g. two shots of sake, 24oz malt liquor, etc.): **Enter "none", if not applicable.**

How did you feel **yesterday while drinking**?

	Not at all	A little	Moderately	Quite a bit	Extremely
Downhearted	○	○	○	○	○
Discouraged	○	○	○	○	○
Uneasy	○	○	○	○	○
Anxious	○	○	○	○	○

How did you feel **yesterday while drinking**?

	Not at all	A little	Moderately	Quite a bit	Extremely
Joyful	○	○	○	○	○
Cheerful	○	○	○	○	○
Energetic	○	○	○	○	○
Lively	○	○	○	○	○

While drinking, I craved a drink yesterday.

Strongly Disagree 0	1	2	3	4	5	Strongly Agree 6
○	○	○	○	○	○	○

While drinking, all I wanted to do yesterday was have a drink.

Strongly Disagree 0	1	2	3	4	5	Strongly Agree 6
○	○	○	○	○	○	○

While drinking, how strong was your urge to drink alcohol yesterday?

No urge 0	1	2	3	4	5	6	7	8	9	Strongest ever 10
○	○	○	○	○	○	○	○	○	○	○

The following adjectives describe feelings. Please rate how you were feeling yesterday **while you were drinking:**

	Not at all 0	1	2	3	4	5	6	7	8	9	Extremely 10
Energized	○	○	○	○	○	○	○	○	○	○	○
Excited	○	○	○	○	○	○	○	○	○	○	○
Up	○	○	○	○	○	○	○	○	○	○	○
Sedated	○	○	○	○	○	○	○	○	○	○	○
Slow thoughts	○	○	○	○	○	○	○	○	○	○	○
Sluggish	○	○	○	○	○	○	○	○	○	○	○

12-WEEK CLINICAL TRIAL OF IBUDILAST FOR AUD

TRIAL OVERVIEW

The 12-week randomized clinical trial was conducted by our laboratory with dissertation chair, Dr. Ray serving as the Principal Investigator. The registered trial clinical trial (NCT03594435) was the first full-scale randomized clinical trial of ibudilast recruiting a treatment-seeking sample with AUD (see **Figure 3-0**). The primary aims of the RCT were to compare alcohol outcomes between treatment groups [ibudilast (50 mg BID) vs. matched placebo]. The primary outcome was percent heavy drinking days and secondary outcomes were as follows: drinks per day, drinks per drinking day, percent days abstinent, percent of participants with no heavy drinking days, and percent of participants abstinent. The full study procedures and primary trial aims will be reported in a manuscript that is currently in preparation.

Chapters 3 and 4 of the dissertation explored the effect of medication condition on other clinical measures capturing monthly changes in craving, depression, and anxiety as well as neurocognitive performance at post-treatment.

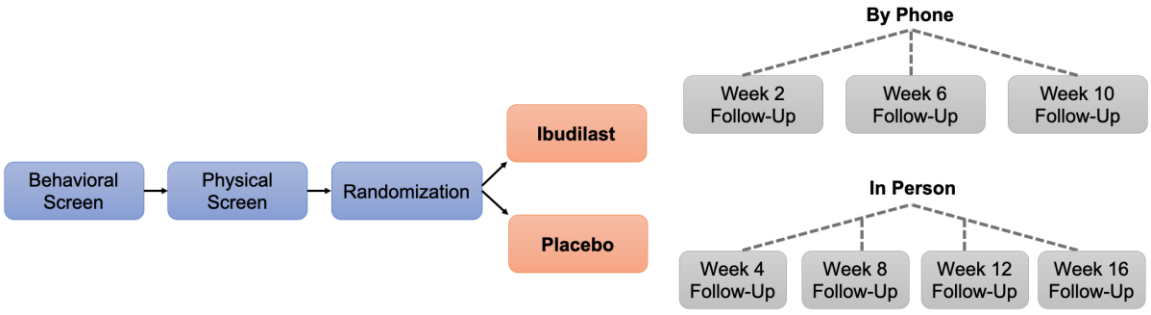


Figure 3-0. Ibudilast for AUD Clinical Trial Design Overview

MATERIALS AND METHODS

Clinical Trial Design

The current project is a secondary analysis of data collected for a phase 2, 12-week randomized, double-blind clinical trial of ibudilast for the treatment of AUD (ClinicalTrials.gov identifier: NCT03594435). In the parent trial, 102 eligible treatment-seeking participants with current AUD were randomized to either ibudilast (50 mg BID) or matched placebo. Participants were asked to attend in-person follow-up visits at 4, 8, and 12 weeks post-randomization. During these visits, participants reported on their alcohol and substance use and completed clinical measures of craving for alcohol, depression, and anxiety. Telephone visits were also conducted at weeks 2, 6, and 10 to collect interim alcohol and substance use data. At randomization and week 12 visits, participants completed the NIH Toolbox Cognition Battery (Hodes et al., 2013). Additionally, the NIAAA-developed behavioral platform "Take Control" was administered at each in person visit (Devine et al., 2016). This intervention consists of 11 computer-based modules that deliver evidence-based information to individuals with alcohol problems and provides suggestions for making changes in their drinking habits.

The study was approved by the University of California, Los Angeles Institutional Review Board and was monitored by a Data and Safety Monitoring Board. All participants provided written informed consent after discussing the study procedures and medication with a licensed physician. Participants were compensated up to $385 for their time and effort according to a specified schedule.

Participants

Inclusion criteria were: (1) meet current DSM-5 diagnostic criteria for moderate or severe AUD; (2) between the ages of 18 to 65; (3) seeking treatment for AUD; and (4) report drinking

at least 14 drinks per week if male or 7 drinks per week if female in the 28 days prior to consent.

Exclusion criteria were: (1) meet current DSM-5 diagnostic criteria for a SUD specific to any psychoactive substances aside from cannabis (mild use disorder allowed), nicotine, or alcohol); (2) a lifetime diagnosis of schizophrenia, bipolar disorder, or psychotic disorder; (3) positive urine toxicology screen for narcotics, amphetamines, or sedative hypnotics; (4) clinically significant alcohol withdrawal symptoms; (5) if female: pregnant, nursing, or declining use of reliable method of birth control; (6) a medical condition that may interfere with safe study participation (e.g., unstable cardiac, renal, or liver disease, uncontrolled hypertension or diabetes); (7) abundant liver enzymes, AST, ALT, or GGT >3x upper normal limit, signaling potential liver disease; (8) attempted suicide in past year or reported serious suicidal intention or plan in the past month; (9) current prescription medication that contraindicates use of ibudilast, including alpha or beta agonists, theophylline, or other sympathomimetic; (10) current prescription for any medications for AUD or any psychotropic medications (e.g., psychostimulants and benzodiazepines) with the exception of stable antidepressants (> 4 weeks); (11) have any other circumstances that, in the opinion of the investigators, compromises participant safety.

Screening Procedures

The clinical trial was conducted at an outpatient research facility in an academic medical center. Recruitment methods included radio, social media, print, and transit advertisements inviting individuals who wish to change their drinking to reach out for participation in a treatment study. Interested individuals completed an online or telephone screening interview and, if eligible, were then invited for an in-person intake visit consisting of clinical interviews and measures. To continue with study procedures, participants needed a BrAC of 0.00g/dl and a

urine toxicology test negative for all drugs excluding cannabis at the start of the in-person visit. Following, eligible participants were asked to complete an in-person physical screening visit consisting of vital signs, laboratory tests and physical exam and medical history interview by a study physician.

Randomization and Medication

Participants who met all eligibility criteria and who attended the in-person randomization visit were randomly assigned to receive either 50 mg BID of ibudilast or matched placebo in a 1:1 ratio. The allocation was carried out using a stratified block randomization procedure with sex and heavy drinking as the stratification factors. MediciNova, Inc. (La Jolla, CA, USA) supplied ibudilast medication and placebo for the trial but did not provide any financial support. The UCLA Research pharmacy prepared and dispensed study medication in blister packs according to the stratification list. Research staff, providers, and participants remained blind to randomization condition during the trial. Participants were titrated on ibudilast to minimize nausea, as follows: participants started at 20 mg BID for 2 days and increased 50 mg BID on day 3. For the last three days of treatment, ibudilast dose was reduced to 20 mg BID prior to stopping the medication. Compliance was monitored by study staff using participant self-report and verified by the pill count method at each in-person follow-up visit. Study physicians closely monitored and reviewed side effects.

This target dosage was chosen based on preclinical data, clinical data, and safety considerations (Beardsley et al., 2010; Cho et al., 2010; Hutchinson et al., 2009; Worley et al., 2016). All in vitro, in vivo animal, and clinical neuropharmacology studies of ibudilast, found efficacy to be dose- or concentration-incremental - at least up to 80-100 mg/day doses. The target dose reflects experience in MediciNova safety trials, where 50 mg BID has been well tolerated,

and adverse events were easily managed. This 50mg BID dosing representing the upper limit of what the manufacturer believes is the maximal tolerated and potentially efficacious dose for an addiction indication. The half-life of ibudilast is estimated at approximately 19 hours, justifying BID dosing.

Baseline Assessments Measures

At baseline, demographic information, including race, ethnicity, age, income, employment status, smoking status, and biological sex, as well as various self-report measures of substance use, mental health, and chronic pain were collected. Severity of AUD was quantified using the Alcohol Use Disorders Identification Test (AUDIT) total score (Saunders et al., 1993). The AUDIT is the most widely used alcohol screening instrument for hazardous drinking and is a well-validated measure of severity in AUD samples (Saunders et al., 1993). The Structured Clinical Interview for DSM-5 (SCID-5; (First et al., 2015) is semi-structured interview used to assess the inclusionary and exclusionary psychiatric diagnoses. Interviews were performed by a master's level clinician or trained research staff under the supervision of a licensed psychologist. Other exclusionary criteria were assessed by study physician/ nurse practitioners at the in-person medical screening visit as well as through urine drug screening, breathalyzer test, concomitant medications form, pregnancy test, and the Clinical Institute Withdrawal Assessment for Alcohol Scale – Revised (CIWA-Ar; (Sullivan et al., 1989) to capture clinically significant alcohol withdrawal.

Alcohol Use

Covering the entire study timeframe (i.e., 30 days prior to baseline screening through 12 weeks post-randomization), participants reported on their daily alcohol use and provided details about drink volume, quantity, and type, in line with standard practice. Standard drinks were

calculated in line with the Timeline FollowBack (TLFB) interview (Sobell et al., 1986), which was administered by trained research staff either in person or over the phone. For analyses, bi-weekly drinks per drinking day were calculated for each participant for timeframes of interest. This drinking variable served as a secondary clinical outcome of interest for the primary trial aims.

CHAPTER 3:

Exploring Negative Affect and Alcohol Craving as Clinical Mechanisms of Ibudilast for the Treatment of Alcohol Use Disorder

Lindsay R. Meredith, MA, Craig K. Enders, PhD & Lara A. Ray, PhD

ABSTRACT

Background: Several immune medications have shown early potential in mitigating alcohol intake in preclinical models and small-scale clinical trials for alcohol use disorder (AUD). To elucidate the clinical utility and clinical mechanisms of this new class of therapies, research needs to carefully assess medication-related changes in complex AUD symptomatology, including craving and negative affectivity, over time.

Methods: This project is a secondary analysis of a 12-week randomized clinical trial of the neuroimmune modulator, ibudilast, for AUD. The trial enrolled 102 individuals (40% female, average age 44 years) seeking treatment for AUD who were randomized to ibudilast (50 mg BID) or matched placebo. Throughout the trial, participants reported on their alcohol use bi-weekly and clinical symptoms of alcohol craving, depression, and anxiety monthly. Linear growth models compared rates of change in clinical symptomatology between ibudilast and placebo groups. Exploratory analyses tested whether rates of change were moderated by biological sex.

Results: Participants consumed an average of 7.5 drinks per drinking day and had minimal levels of depression and anxiety at baseline. Throughout the 12-week trial, participants in the ibudilast group had significantly steeper declines in alcohol craving (1.31 points per month) as compared with placebo (.48 points per month; $p = .021$). Male and female participants showed similar rates of change in craving but at treatment endpoint, female participants taking ibudilast had significantly lower craving levels than females taking placebo ($p < .001$). Rates of change for depression and anxiety scores did not differ between medication conditions. Depression levels significantly decreased over time across all participants regardless of medication condition

(p's < .005), while anxiety levels remained relatively stable. Similarly, biological sex did not moderate changes in anxiety or depression between medication groups.

Conclusions: Consistent with findings from pilot trials of ibudilast for AUD, this medication appears to reduce one's craving for alcohol. Despite shared neuroimmune correlates between negative affectivity and addiction, ibudilast did not decrease depression or anxiety levels beyond the effects of placebo. However, participants in the sample reported non-clinically elevated levels of negative affectivity at pre-treatment, possibly reducing power to detect medication effects.

INTRODUCTION

Alcohol use disorder (AUD) is a highly prevalent mental health condition with a substantial public health burden. Less than 8% of people with past-year AUD received any alcohol treatment, demonstrating a significant care gap (SAMHSA, 2023). Currently, only three medications are FDA-approved for abstinence or drinking reduction and the numbers needed to treat are quite high (McPheeters et al., 2023). Contraindications for taking these medications include commonly co-occurring health conditions, such as acute hepatitis, opioid use, or kidney dysfunction (Fairbanks et al., 2020). Two of the available medications are approved only for patients who have already established abstinence from alcohol. Further, despite concerted efforts, the alcohol field has limited understanding of how existing treatments work from the neurobiological to psychosocial level, termed mechanisms of behavior change (Magill et al., 2023; Meisel et al., 2023).

Alternative pharmacological treatments, including novel and repurposed agents with a range of biobehavioral targets, have been tested in animal and clinical samples with AUD (Burnette et al., 2022). Among the agents tested are medications that modulate the immune system. Several of these immune medications have shown early potential in mitigating alcohol intake in preclinical models (Coleman & Crews, 2018; Crews, Lawrimore, et al., 2017; Crews et al., 2015; Erickson, Grantham, et al., 2019). A small proportion of these medications, including ibudilast, apremilast, minocycline, and *N*-acetylcysteine, have moved forward in the translational pipeline to testing in human laboratory trials and clinical trials for AUD, but with mixed results (Mason et al., 2024; Meredith, Burnette, et al., 2021). As such, current evidence is lacking on the clinical efficacy of immune medications among clinical samples with AUD.

Interest in exploring immune compounds as treatments for AUD comes from research suggesting that the immune system is involved in the development and maintenance of AUD (Coleman & Crews, 2018). Sustained heavy alcohol use contributes to altered immune processes and neural functioning, along with an excessive inflammatory response (Adams et al., 2023). For example, preclinical research shows that sustained alcohol exposure alters immune signaling in the central nervous system (CNS) through multiple pathways, such as by activating toll-like receptors (TLR; (Alfonso-Loeches et al., 2010) and modifying astrocytic and microglial expression (Bachtell et al., 2017). Meta-analytic work indicates that individuals with AUD have consistent elevations in peripheral inflammatory markers, such as TNF-α, IL-6, and IL-8, compared with healthy individuals without AUD (Adams et al., 2020; Moura et al., 2022).

Alcohol-related inflammation is suspected to reinforce heavy drinking behavior and worsen salient maintenance factors of AUD, including negative emotionality and craving (Coleman & Crews, 2018). As clinical data emerge from randomized trials testing immunomodulators for AUD, it will be important not only to determine clinical efficacy but also to thoughtfully test hypothesized treatment mechanisms (Grigsby et al., 2023). This research will help the field better understand how these interventions may work and for which individuals (Meisel et al., 2023). In particular, it will be important to study how treatment efficacy and clinical mechanisms may differ across biological sex, a traditionally under researched area (Kirsch et al., 2024). Sex differences in response to immune treatment is supported by findings in several fields (Martinez-Muniz & Wood, 2020; Rainville & Hodes, 2019), including preclinical work showing that female rodents are more sensitive to ethanol-induced neuroimmune responses than male rodents (Barton et al., 2017; Pascual et al., 2017; Wilhelm et al., 2016). To elucidate

the clinical utility and clinical mechanisms of this new class of therapies, we need to carefully assess medication-related changes in complex AUD symptomatology over time.

Negative affective states, a common feature of addiction (Lannoy et al., 2021), are likely suggestive of overall greater psychiatric impairment and are consistently linked with poorer treatment outcomes, disorder severity, and suicide attempts (Cano et al., 2017; Pavkovic et al., 2018; Swan et al., 2020). In the context of alcohol treatment, changes in negative affect are dynamically associated with changes in alcohol intake (Witkiewitz & Villarroel, 2009). Individuals with AUD are more vulnerable to negative affect in part because chronic alcohol exposure sensitizes immune signaling pathways (Coleman & Crews, 2018; Crews, Lawrimore, et al., 2017; Tynan et al., 2010). Alterations in neuroimmune signaling are hypothesized to be shared by a range of medical and psychiatric conditions like addiction and depression (Miller & Raison, 2016; Neupane, 2016). For instance, a low-grade proinflammatory state is known to impact various neurotransmitter systems in the CNS (e.g., dopamine, glutamate, and serotonin pathways) and contribute to 'sickness behavior,' encompassing symptoms of fatigue, poor concentration, and reduced social interaction. Anti-inflammatory therapies have been shown to significantly reduce negative affect, including measures of anhedonia (De Berardis et al., 2017; De Berardis et al., 2015; Lee et al., 2018) and anxiety (De Berardis et al., 2015; Stein et al., 2012; Stein et al., 2014), in psychiatric disorders such as major depression, bipolar disorder, and schizophrenia (Adzic et al., 2018; Andrade, 2016; Park et al., 2018; Rosenblat et al., 2016). Negative affect, an important feature of addiction, may be a potential treatment target of neuroimmune modulation for AUD that improves clinical outcomes (Zhang et al., 2002; Zhang et al., 2008).

Another key maintenance factor of AUD is alcohol craving, which predicts alcohol intake, severity of AUD, and relapse (Martins et al., 2022; Mayhugh et al., 2018; McHugh et al., 2016; Schneekloth et al., 2012; Vafaie & Kober, 2022). Craving may also be modulated by proinflammatory signaling in the brain and gut (Coleman & Crews, 2018). In preclinical models, administration of anti-inflammatory compounds is thought to mitigate the rewarding properties of ethanol, as evidenced by reductions in ethanol self-administration and conditioned place preference (see a translational review paper, see: (Meredith, Burnette, et al., 2021)). In two clinical studies, self-reported alcohol craving and intake were significantly associated with multiple peripheral markers of inflammation among predominantly male participants with AUD who enrolled in a detoxification program (Heberlein et al., 2014; Leclercq, Cani, Neyrinck, Starkel, et al., 2012). However, alcohol craving in humans is complex, extending to craving provoked by alcohol-related cues, acute craving induced by alcohol intake, subjective craving over time (i.e., tonic craving), and stress-induced craving (Schacht et al., 2013). In pharmacotherapy trials for substance use disorders (SUDs), craving is one of the most commonly tested mechanisms of actions; this is typically assessed through repeated self-report measures and experimental paradigms (e.g., cue exposure; (Meredith et al., 2023). However, a limited number of studies have tested the effects of immune treatments on alcohol craving in clinical samples with AUD (Meredith, Burnette, et al., 2021).

Ibudilast is one potential immune treatment for AUD that has been tested in randomized clinical trials. It is a phosphodiesterase (PDE) inhibitor at PDE3, -4, -10, and -11 as well as an allosteric macrophage migration inhibitory factor (MIF) that crosses the blood-brain barrier. Both PDE4 and MIF are thought to regulate inflammatory responses in microglia and contribute to neuroinflammation (Giampa et al., 2010; Mizuno et al., 2004). In human samples, it is

suspected that ibudilast may reduce neuroinflammation by inhibiting proinflammatory signaling, enhancing anti-inflammatory signaling, and providing neuroprotection. Our laboratory has conducted three randomized trials with ibudilast for AUD: (1) one-week human laboratory trial focused on safety, (2) two-week experimental medicine trial, and (3) most recently, a full-scale 12-week clinical trial.

In the 12-week clinical trial of ibudilast for AUD, the principal endpoints were focused on reductions in alcohol intake. While results showed significant drinking reductions across both medication groups, we observed no medication-specific effects in support of ibudilast efficacy. Central to the current project, we also collected secondary data on clinical measures of negative affect and craving at several timepoints. Results from our earlier trials of ibudilast demonstrated its potential to reduce alcohol craving and modulate affective states. Specifically, following a stress exposure paradigm, ibudilast promoted a stronger recovery of positive affect (Ray et al., 2017). Ibudilast also attenuated both neural cue-reactivity (Grodin et al., 2021) and alcohol-induced craving but it did not alter daily reports of negative affect state (Meredith et al., 2022). While these initial results show that ibudilast can modulate transient affective states under experimental conditions and multiple facets of phasic craving, little is known about how ibudilast might temper clinical measures of negative affect and craving in AUD over a longer timeframe time, which are characteristic of symptoms of AUD.

In this project, we tested whether clinical markers of self-reported alcohol craving and negative affectivity changed over the course of a 12-week randomized trial of ibudilast for AUD and whether the rate of change differed between treatment groups. In an exploratory analysis, we tested whether biological sex moderated these changes in clinical symptomatology. We hypothesized that levels of depression, anxiety, and alcohol craving would decrease over the 12-

week trial and that those randomized to ibudilast would show significantly steeper declines. This project helps to fill an important gap in the literature by serving as an investigation into ibudilast's potential clinical mechanisms, namely facets of negative affect and craving.

MATERIALS AND METHODS

Clinical Self-Report Measures

At baseline and at 4, 8, and 12 weeks post-randomization participants completed three self-report measures of interest, the Beck Depression Inventory-II (BDI-II; (Beck et al., 1996) to capture levels of depressive symptomatology, the Beck Anxiety Inventory (BAI; (Beck et al., 1988) to capture levels of anxiety symptomatology, and the Penn Alcohol Craving Scale (PACS; (Flannery et al., 1999) to capture levels of tonic craving for alcohol.

Negative Affect. The BDI-II and BAI are commonly used measures that assess the intensity and severity of depression or anxiety symptoms in a variety of clinical populations (Moore et al., 2016; Seignourel et al., 2008). They are highly reliable and display good sensitivity and validity (Wang & Gorenstein, 2013). The BDI-II has 21 items reflecting a range of symptoms, thoughts, and attitudes frequently displayed among individuals with depression that are summed to create a score ranging from 0 to 63 points. Traditional cut-off scores to fall in the mild, moderate, and severe range are 14, 20, and 29, respectively, but can vary depending on the clinical sample. Changes in these self-reported BDI-II scores are shown to reflect clinician ratings (Hershenberg et al., 2020). Participants are asked to indicate the statement that best describes how they have been feeling in the past two weeks (ranging from 0 to 3 points).

Similarly, the BAI has 21 items reflecting a variety of somatic and cognitive symptoms commonly displayed among individuals with anxiety that are summed to create a score ranging

from 0 to 63 points (Beck et al., 1988). Traditional cut-off scores for mild, moderate, and severe are 8, 16, and 26, respectively. BAI scores are shown to be positively associated with AUDIT scores and alcohol craving (McCaul et al., 2017). Participants are asked to report on how much they have been bothered by each symptom in the past week from 'not at all' (score = 0) to 'severely' (score = 3).

Alcohol Craving. The PACS 5-item self-report measure assesses the frequency, intensity, and duration of past-week alcohol craving (Flannery et al., 1999). This measure is shown to have high internal consistency and validity and can be used for longitudinal monitoring of alcohol craving, particularly in treatment contexts. Research suggests that PACS scores significantly predict future drinking behavior and relapse (Flannery et al., 2003; Stohs et al., 2019). For each of the five items (range from 0 to 6 points), participants are asked to indicate the statement that best describes their craving in the past week. Items are summed to create a score from 0 to 30 points.

Data Analytic Plan

To evaluate whether ibudilast treatment reduced symptoms of craving, depression, and anxiety over the 12-week clinical trial, linear multilevel growth models with random intercepts and random effect of time were carried out using the full sample of randomized participants (in SAS Version 9.4). Exploratory models examined the moderating effect of biological sex on medication-related changes in these outcomes over time. Descriptive statistics including mean and standard deviation were computed for continuous variables, and frequencies and percentiles were computed for categorical variables to summarize the baseline and post-randomization study data (**see Table 3-1** and **Table 3-2**). In line with best practices, covariates were centered at the grand mean (CGM; (Enders & Tofighi, 2007). For all models, the following covariates were

added: biological sex (male or female; level 2, CGM in main models), baseline AUDIT score (level 2, CGM), and drinks per drinking day (DPDD) in the preceding 2 weeks (4 timepoints, level-1, log transformed, CGM).

The initial longitudinal analyses consisted of one linear trajectory per medication group spanning from pre-treatment to treatment endpoint. The main model for each outcome was:

$$Y_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)X_{1ti} + \beta_2 X_{2i} + \beta_3(X_{1ti})(X_{2i}) + \beta_4 X_{4i}^{cgm} + \beta_5 X_{5i}^{cgm} + \beta_6 X_{6ti}^{cgm} + \varepsilon_{ij}$$

For example, the model for craving was:

$$CRAVING_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)(TIME_{1ti}) + \beta_2(MED_{2i}) + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4\left(Sex_{4i}^{cgm}\right) + \beta_5\left(AUDIT_{5i}^{cgm}\right) + \beta_6\left(logDPDD_{6ti}^{cgm}\right) + \varepsilon_{ti}$$

where CRAVING$_{ti}$ is outcome score at occasion *t* for individual *i*, MED_{2i} is a binary variable for medication condition (0 = placebo, 1 = ibudilast), $TIME_{1ti}$ is a temporal predictor that codes the four repeated measurements as integers from 0 to 3. This defines the model parameters as follows: β_0 as the predicted placebo group average at pre-treatment and β_2 as the medication group mean difference at pre-treatment; β_1 is the placebo group's linear trend during the trial; β_3 represents medication group differences in change rate during the trial; b_0 and b_1 are normally distributed random effects that allow change trajectories to vary across individuals. Additionally, β_4 is a binary variable for biological sex (0 = male, 1= female) and β_5 is a continuous variable to represent pre-treatment AUD severity, both of which serve as covariates. Coefficient, β_6 is a log transformed, repeated measures variable capturing DPDD in the two weeks prior to collection of each clinical outcome measure during the trial; it serves as a covariate for quantity of alcohol intake. See **Figure 3-10** for a set of model formulas.

The exploratory moderation model for each outcome included the addition of a medication × sex × time three-way interaction (and inclusion of lower order two-level interaction terms), as follows:

$$Y_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)X_{1ti} + \beta_2 X_{2i} + \beta_3(X_{1ti})(X_{2i}) + \beta_4 X_{4i} + \beta_5 X_{5i}^{cgm} + \beta_6 X_{6ti}^{cgm} + \beta_7(X_{1ti})(X_{4i}) + \beta_8(X_{2i})(X_{4i}) + \beta_9(X_{1ti})(X_{2i})(X_{4i}) + \varepsilon_{ti}$$

For example, the model for craving was:

$$Craving_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)TIME_{1ti} + \beta_2 MED_{2i} + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4 SEX_{4i} + \beta_5 AUDIT_{5i}^{cgm} + \beta_6 logDPDD_{6ti}^{cgm} + \beta_7(TIME_{1ti})(SEX_{4i}) + \beta_8(MED_{2i})(SEX_{4i}) + \beta_9(TIME_{1ti})(MED_{2i})(SEX_{4i}) + \varepsilon_{ti}$$

Notably, β_7 represents sex differences in change rate during the trial. For these models, we also tested for differences in simple slopes (i.e., change rate) for the lower-order terms.

Longitudinal data often violate independence assumptions because within-person observations are more similar than observations across subjects, requiring nested models in order to preserve suitable Type I error rates (Raudenbush & Bryk, 2002); nesting was supported by interclass correlations (ICCs) from unconditional models ranging from .61 to .63. Normality assumptions and misspecifications (e.g., using QQ plot and Kernel Density) were checked. Several variables, namely, DPDD, BDI-II, and BAI were log-transformed (with an added constant of 1) to minimize skewness and kurtosis and account for a substantial number of zeros. Kenward-Rogers degrees of freedom was used to reduce bias and obtain more accurate *p*-value estimates. Models were fit using an unstructured covariance matrix. The linear growth models assumed a conditionally missing at random process, using residual maximum likelihood (REML) estimation, where an individual's missingness is fully determined by their observed data (i.e., treatment assignment, covariates, and outcome scores from previous waves).

Based upon a visual inspection of change trajectories for log transformed BDI-II and BAI scores during the trial, we ran piecewise linear mixed models to improve model fit. These models included two linear trajectories per medication group with the first spanning pre-treatment to 1 month post-randomization, and the second covering the remaining trial period (months 2-3). The main piecewise model for BDI-II and BAI outcomes are below.

$$Y_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)X_{1ti} + (\beta_2 + b_2)X_{2ti} + \beta_3 X_{3i} + \beta_4(X_{1ti})(X_{3i}) + \beta_5(X_{2ti})(X_{3i}) + \beta_6 X_{6i}^{cgm} + \beta_7 X_{7i}^{cgm} + \beta_8 X_{8ti}^{cgm} + \varepsilon_{ij}$$

For example, the model for depression was:

$$logDEPRESSION_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)(TIME_{1ti}) + (\beta_2 + b_2)(TIMEDIF_{2ti}) + \beta_3(MED_{3i}) + \beta_4(TIME_{1ti})(MED_{3i}) + \beta_5(TIMEDIF_{2ti})(MED_{3i}) + \beta_6\left(Sex_{6i}^{cgm}\right) + \beta_7\left(AUDIT_{7i}^{cgm}\right) + \beta_8\left(logDPDD_{8ti}^{cgm}\right) + \varepsilon_{ti}$$

This coding scheme defines the model parameters as follows: β_0 as the predicted placebo group average at one month post-randomization, β_3 as the medication group mean difference at one month; β_1 and β_2 are the placebo group's linear trend during the first phase and the change to that linear trend in the second phase, respectively; and β_4 and β_5 represent medication group differences in the two change rates. The exploratory moderation models were also rerun according to this piecewise approach (i.e., included medication × sex × time and medication × sex × timedif three-way interactions and lower order terms).

RESULTS

Participants

Participants (N = 102) from the 12-week clinical trial had the following characteristics: average age of 44 years, 40% female, and 51% identifying as White, 24% as Black or African

American, 13% as mixed race, 7% as another race, and 31% as Latina/o/x (**see Table 3-1**). Regarding employment status, approximately a third of the sample were unemployed (32%), a third were working full-time (34%), and a quarter were working part time (26%). Nearly one half of the sample (45%) reported household incomes < $30,000 and 21% had household income $90,000 or greater. In the 30 days prior to their baseline visit, participants had an average of 23 drinking days and 7.54 DPDD. At baseline, approximately a quarter (23%) of the sample had a positive urine toxicology screen for THC and a third (34%) reported at least 1 day of cigarette use in the prior 30 days. The average DSM-5 AUD symptom count was 6.73, indicating severe AUD at baseline. In regard to mental health symptomatology, participants reported an average raw BDI-II score of 11.38 points (below mild depression cutoff), average raw BAI score on 7.76 points (below mild anxiety cutoff), and average raw PACS score of 13.78 points. At week 12, 86 participants provided data on clinical measures (ibudilast n = 47, placebo n = 39), representing a retention rate of 84.3%.

Craving by Medication Condition

In total, participants contributed to 350 observations out of a possible 408 (i.e., 102 participants with 4 repeated measurements), resulting in a missing data rate of 14.2% for craving outcomes. Raw mean craving scores and standard deviations are reported in **Table 3-2** and estimated means and standard errors from the full linear growth model are reported in **Table 3-3**. The dashed lines in **Figure 3-1** show mean craving scores (PACS total) by medication group and occasion (estimated with missing data handling) and the solid lines show the predicted trajectories from a linear growth model prior to adding covariates. Results from the full covariate-adjusted linear growth model (**see Table 3-4a)** showed that the placebo group's mean craving scores decreased by .48 points per month during the trial (β_1 = -.48(*.28*), t = -1.72, p =

.087), and the ibudilast group's monthly change rate was statistically steeper (β_3 = -.83(*.35*), t = -2.33 p = .021; **see Figure 3-4**). Thus, mean craving scores for the ibudilast group decreased by 1.31 points per month during the trial, t = -5.23, p < .0001. At treatment endpoint, the estimated mean for ibudilast group was 8.51 points and for placebo group was 11.75 points. All three covariates were significantly associated with craving, such that being female (β_4 = 3.22(*.96*), t = 3.37, p = .001), having a higher baseline AUDIT score, (β_5 = .36(*.06*), t = 5.55, p < .0001), and having greater bi-weekly log DPDD (β_6 = 1.89(*.40*), t = 4.66, p < .0001), in the preceding two weeks was associated with greater craving scores on average. The R^2 value indicating proportion of variance explained by model predictors increased by .02 after adding focal predictors of medication and medication × time interaction. This shows that medication effects explained 2% of the total variance in craving outcomes, representative of a small effect size according to Cohen's benchmarks for multiple regression (Cohen, 1988).

Craving by Medication Condition and Biological Sex

To explore the potential moderating effect of biological sex on medication-related changes in craving over time, we added a three-way interaction of medication × sex × time, along with the corresponding lower order two-way interaction terms to the linear growth model. No significant difference between simple slopes for males compared with females was detected (t = .49, p = .629), suggesting that males and females had similar change rates for craving during the trial (**see Figure 3-5a**). Similarly, neither the simple slope difference between ibudilast and placebo groups for females (t = -.64, p = .526) nor males (t = -.09, p = .526) were significant.

Upon visual inspection there were clear distinctions at treatment endpoint by sex. Thus, the model was run with time centered at week 12. Results showed a significant medication × sex interaction, β_8 = -5.10(*2.23*), t = -2.26, p = .026; **see Figure 3-5b**). Estimated means for

craving at treatment endpoint were then computed per medication groups and sex; simple effects were compared. A simple effect of sex for the placebo group showed that females (estimated mean = 14.89) had significantly higher craving scores than males (estimated mean = 9.16) at treatment endpoint (t = 3.64, p < .001). However, for the ibudilast group, males (estimated mean = 8.29) and females (estimated mean = 9.01) had similar craving scores (t = 1.28, p = .202). In addition, a simple effect of medication for females showed that those in the ibudilast group had significantly lower craving scores than females in the placebo group at (t = -3.45, p < .001), while males had similar craving scores across medication groups.

Depression by Medication Condition

In total, participants contributed to 354 observations out of a possible 408, resulting in a missing data rate of 13.2% for depression outcomes. Raw mean depression scores and standard deviations are reported in **Table 3-2** and estimated means and standard errors from the full piecewise growth model (prior to log transformation) are reported in **Table 3-3**. The dashed lines in **Figure 3-2a** show mean depression scores (BDI-II total) by medication group and occasion (estimated with missing data handling) and solid lines show the predicted trajectories from a linear growth model prior to adding covariates. Similarly, **Figure 3-2b** presents BDI-II scores after a log transformation was completed to improve normality. **Figure 3-2c** presents BDI-II scores after log transformation and implementation of piecewise growth model to improve fit.

Results from the full covariate-adjusted linear growth model (**see Table 3-4a**) showed that the placebo group's mean log depression scores decreased by .16 points per month during the trial (β_1 = -.16 (*.06*), t = -2.94, p = .004), and the ibudilast group's monthly change rate was not statistically steeper (β_3 = -.03(*.07*), t = -.03, p = .632; **see Figure 3-6a**). Mean log depression score for the ibudilast group decreased by .20 points per month during the trial (t = -3.97, p =

.0001). One covariate was significantly associated with depression, such that having greater log DPDD in the preceding two weeks was associated with greater depression scores on average, β_6 = .18(*.07*), t = 2.47, p = .014. Results from the piecewise model were similar (**see Table 3-4b**) but show that change rates were faster from pre-treatment until 1-month post-randomization, with placebo group log depression scores decreasing by .49 points and ibudilast group by .38 points and change flattened during the last two months. The medication groups did not have statistically different change rates during either period, but the placebo group had nominally faster change rates during the first time period, while the ibudilast group had nominally faster changes rates during the second time period (**see Figure 3-6b)**.

Depression by Medication Condition and Biological Sex

To explore the potential moderating effect of biological sex on medication-related changes in depression over time, we added a three-way interaction of medication × sex × time, along with the corresponding lower order two-way interaction terms to the linear growth model. No significant difference between simple slopes for males compared with females was detected (t = -1.18, p = .242), suggesting that males and females had similar change rates for depression during the trial (**see Figure 3-7a**). Similarly, neither the simple slope difference between males and females in the ibudilast group (t = .13, p = .899) nor the placebo group (t = 1.48, p = .143) was significant. For the piecewise model, there was a significant sex × time interaction from pre-treatment to 1-month post-randomization (t = 2.20, p = .029; **see Figure 3-7b**), where males in the placebo group had a significantly faster decline in depression scores than females in the placebo group. This same sex difference was not present among the ibudilast group.

Anxiety by Medication Condition

In total, participants contributed to 350 observations out of a possible 408, resulting in a missing data rate of 14.2% for anxiety outcomes. Raw mean depression scores and standard deviations are reported in **Table 3-2** and estimated means and standard errors from the full piecewise growth model are reported in **Table 3-3** (prior to log transformation). The dashed lines in **Figure 3-3a** show mean anxiety scores (BAI total) by medication group and occasion (estimated with missing data handling) and solid lines show the predicted trajectories from a linear growth model prior to adding covariates. Similarly, **Figure 3-3b** presents BAI scores after a log transformation was completed to improve normality. **Figure 3-3c** presents BAI scores after log transformation and implementation of the piecewise growth model to improve fit.

Results from the full covariate-adjusted linear growth model (**see Table 3-4a)** showed that the placebo group's mean log anxiety scores decreased by .01 points per month during the trial (β_1 = -.01(*.05*), t = -.21, p = .830), and the ibudilast group's monthly change rate was not statistically steeper (β_3 = -.06(*.06*), t = -.99, p = .324; **see Figure 3-8a**). Mean log anxiety scores for the ibudilast group decreased by .07 points per month during the trial (t = -1.66, p = .101). One covariate was significantly associated with anxiety, such that higher baseline AUDIT score was associated with greater anxiety scores on average (β_5 = .05(*.009*), t = 4.88, p < .0001). Results from the piecewise model (**see Table 3-4b**) show that anxiety scores significantly increased from pre-treatment until 1-month post-randomization for the placebo group (log anxiety scores increased by .29 points, t = 2.31, p = .023) and increased at a slower rate for the ibudilast group (.14 points). The placebo group's change rate significantly differed during the second trial period, representing a decline in anxiety scores (t = -2.70, p = .008). The medication groups did not have statistically different change rates during either period (**see Figure 3-8b**).

Greater quantity of recent alcohol use was also significantly associated with higher anxiety scores during the trial (t = 2.01, p = .045) in the piecewise model.

Anxiety by Medication Condition and Biological Sex

To explore the potential moderating effect of biological sex on medication-related changes in anxiety over time, we added a three-way interaction of medication × sex × time, along with the corresponding lower order two-way interaction terms to the linear growth model. No significant difference between simple slopes for males compared with females was detected (t = .93, p = .355), suggesting that males and females had similar change rates for anxiety during the trial (**see Figure 3-9a**). Similarly, neither the simple slope differences between males or females in the ibudilast group (t = -1.53, p = .129) nor the placebo group (t = .13, p = .897) was significant. Again, for the piecewise models, there were no sex × time or sex × medication interactions for either time period (**see Figure 3-9b**).

DISCUSSION

In this secondary analysis of a 12-week randomized clinical pharmacotherapy trial enrolling 102 treatment-seeking individuals with AUD, we explored whether the study medication, ibudilast, had beneficial effects on a range of clinical symptoms. As immune treatments for AUD have only been tested in a limited number of randomized trials to date, we were interested in probing clinical mechanisms of action to better understand maintenance factors potentially targeted by this new class of medications. To this end, we implemented linear growth models to test whether individuals randomized to ibudilast showed steeper declines in clinical symptoms compared to individuals randomized to placebo. The PACS, BDI-II, and BAI self-report measures were collected monthly throughout the trial to capture symptom changes in

craving, depression, and anxiety, respectively. Consistent with other craving outcomes from prior randomized trials of ibudilast for AUD, participants in the medication group experienced significantly faster reductions in tonic craving levels over the 12-week treatment period. Contrastingly, rates of change for depression and anxiety were similar among individuals in the ibudilast and placebo groups. While participants in both conditions reported significant reductions in depression levels during the trial, anxiety symptoms were low and decreased slightly from pre- to post-treatment. As an exploratory step, we tested whether biological sex moderated changes in clinical symptoms. Rates of change for the three clinical outcomes did not differ by sex during the trial. Yet, at treatment endpoint, there was a significant medication by sex interaction for craving with females taking ibudilast show significantly lower BDI-II scores than females taking placebo.

As a PDE inhibitor, ibudilast helps normalize the cAMP signaling pathway, which is disrupted with chronic alcohol intake in specific brain regions. Ibudilast may reduce tonic alcohol craving by restoring healthy neural transmission and reducing inflammation in relevant regions, such as the nucleus accumbens, amygdala, and hippocampus (Grigsby et al., 2023; Grodin et al., 2022; Pérez-Torres et al., 2000; Wen et al., 2018a). Our current results are supported by a set of multimodal findings capturing facets of craving from two previous clinical trials of ibudilast in our laboratory. Across these projects, ibudilast reduced tonic craving levels over one week, blunted subjective response to alcohol in the natural environment, and reduced neural activation to alcohol cue exposure. In the present trial, ibudilast unfortunately did not improve drinking outcomes compared to placebo, but these data provide consistent evidence that ibudilast reduces multiple types of alcohol craving across clinical samples with AUD. A recent pilot trial of a comparable PDE inhibitor, apremilast, showed medication-related reductions in

alcohol intake across 11 days, which contributed to a large effect size (Grigsby et al., 2023). Taken together, these findings support continued interest in PDE inhibitors for the treatment of AUD and warrant additional trialing in other research laboratories and clinical AUD samples.

In regard to the clinical relevance of these craving levels, researchers have proposed a benchmark for the PACS, where scores of 15 points or higher indicate clinically significant levels (Hartwell et al., 2019). Raw scores at pre-treatment started around 13.8 points for both conditions and by treatment endpoint, estimated craving means fell to 8.5 points for the ibudilast condition and 11.8 points for the placebo condition. Relatedly, 42% of participants in the placebo group and 40% in the ibudilast group reported scores above the 15-point cutoff at pre-treatment. By the end of the trial, 26% of the placebo group and only 15% of the ibudilast group endorsed scores of at least 15 points. Ibudilast-related benefits on craving outcomes were estimated as a small effect size. A recent qualitative review synthesizing craving outcomes from 60 randomized pharmacotherapy trials for AUD found that the PACS measure was commonly administered for assessment of tonic craving (i.e., in 20% of trials; (Marin et al., 2023). Among studies clearly reporting means, PACS scores ranged between 15–19 points at baseline, slightly higher than the present project, and fell to 6–12 points by treatment endpoint (Foa et al., 2013; Harel et al., 2022; Litten et al., 2013; Morley et al., 2006), in line with our results. For instance, a trial testing combined prolonged exposure therapy and naltrexone for comorbid AUD and PTSD reported naltrexone group PACS scores at 6.6 points and placebo group scores at 9.7 after 24 weeks of treatment (Foa et al., 2013), which is similar to the 3-point estimated difference between conditions found in the present study. Taken together, craving reductions found during this trial appear clinically meaningful and comparable to other pharmacotherapy trials, including those

testing naltrexone, which is an FDA-approved medication for AUD with a prosed primary mechanism of craving reduction.

Despite literature supporting shared neuroimmune correlates between negative affectivity and addiction (Coleman & Crews, 2018; Miller & Raison, 2016; Neupane, 2016), we did not detect a beneficial effect of ibudilast on depression or anxiety scores during the trial. Ibudilast is thought to alter neurotrophic expression and microglial activation, which are dysregulated in both depression and AUD (Cho et al., 2010; Grodin et al., 2022; Mizuno et al., 2004). Animal research has shown antidepressant and anxiogenic effects of PDE4 inhibitors similar to ibudilast (Zhang et al., 2002; Zhang et al., 2008). However, findings from our laboratory have not supported a strong effect of ibudilast treatment on mood. For example, ibudilast did not alter alcohol-induced changes in positive or negative mood symptoms (Meredith et al., 2022), nor did it improve mood on daily report over two weeks (Grodin et al., 2021). It is notable that depression levels for participants enrolled in our laboratory's three trials have been minimal. At pre-treatment only 7% of our sample were experiencing a current major depressive episode and average BDI-II scores fell below the cutoff score for even mild depression (i.e., 13 points). Likewise, average BAI scores fell below the mild cutoff score of 8 points. As such, these low scores likely made it more difficult to detect medication effects on mood. Positively, participants taking ibudilast had reduced depressive and anxiety symptoms throughout the trial, indicating that ibudilast was not iatrogenic to mood. It is interesting the anxiety scores increased slightly from baseline to 1-month post-randomization. This effect could potentially be due to heightened awareness to physiological symptoms after starting a new medication (e.g., being attuned to potential side effects).

Supporting prior literature, quantity of recent alcohol consumption at each timepoint was positively related to higher depression, anxiety, and craving scores in the present study (Witkiewitz & Villarroel, 2009). A meta-analysis of acamprosate trials found that participants were 7.5 times more likely to have depression remit when they were able to achieve continued abstinence (Lejoyeux & Lehert, 2011). It may be useful to test a more dynamic model in the future, particularly when mood symptomatology is modest. For example, assessing whether ibudilast alters the relationship between daily negative affectivity, craving and alcohol use using momentary assessment in the context of a full clinical trial. This would allow for a more fine-grain analysis of whether medication effects on mood act as a clinical mechanism.

As an important exploratory aim, we tested whether medication-related changes in clinical symptoms were moderated by sex. Our sample had 41 participants who reported their biological sex as female (40%), which was slightly higher than anticipated based on previous pharmacotherapy trials in our laboratory. Preclinical research suggests that females may be more sensitive to alcohol's effects on neuroimmune signaling and inflammation (Barton et al., 2017; Pascual et al., 2017; Wilhelm et al., 2016) but human research is limited. We hypothesized that females may show a stronger response to anti-inflammatory treatment. However, our results did not find significant differences between male and female participant change trajectories for the two treatment groups. Visually, females taking ibudilast tended to have faster reductions in craving, depression, and anxiety symptoms than females taking placebo pills, but these differences were minimal. At treatment endpoint, female participants randomized to ibudilast were estimated to have PACS craving scores nearly 6 points lower than females randomized to placebo, whereas males had more similar scores between medication groups. Male participants displayed a more robust placebo response across trial outcomes. These results correspond to a

sex by medication interaction detected for one of the trial's primary alcohol outcomes, drinks per day, where female participants taking ibudilast had lower scores than placebo group participants, but the reverse was true for males. These results show that it will be important to continue probing how treatment efficacy and clinical mechanisms among immune treatments for AUD might differ by sex. This will likely require intentional efforts to recruit female participants.

This project should be considered in the context of its strengths and limitations. Notable strengths include the analysis of pertinent clinical data collected during one of the first full-scale double-blind randomized controlled trials of a neuroimmune compound the treatment for AUD. The clinical measures chosen are widely used and validated and capture relevant maintenance factors of heavy alcohol use including negative affect and craving. These analyses took advantage of the longitudinal nature of the data to test rates of change in symptoms severity from baseline to treatment endpoint, including the implementation of piecewise models. Further, the sample is fairly diverse in regard to socioeconomic status and the distribution of race and ethnicity, with half of participants reporting a race other than White and over 30% identifying as Hispanic or Latina/o/x. However, the proportion of individuals identifying as Asian or Pacific Islander was very low (3%). This was also a treatment-seeking sample with pre-treatment drinking rates, AUD severity, and comorbid tobacco and cannabis use that likely corresponds to individuals seeking outpatient treatment in the community. A previously noted limitation for these analyses is the minimal symptoms of depression and anxiety at baseline, which impacted our ability to detect medication effects on negative mood. As anticipated, several participants dropped out of the trial or were lost to follow-up or did not reliability take the study medication. It will be important to conduct sensitivity analysis to better control for medication adherence and missing data, especially in circumstances where participants dropped out due to poor response to

ibudilast or placebo (i.e., continued heavy drinking). It may also be helpful to rerun these analyses for a modified intention-to-treat sample. Lastly, due to pandemic-related disruptions, the sample size was smaller than anticipated and this likely decreased power to detect medication effects.

Table 3-1. Participant Characteristics by Treatment Condition

Variable	Combined (N = 102)	Ibudilast (n = 53)	Placebo (n = 49)
Demographic			
Age (Years)	44.26 (10.81)	42.74 (10.01)	45.92 (11.49)
Sex (No., %)			
Male	61 (59.8%)	32 (60.4%)	29 (59.2%)
Female	41 (40.2%)	21 (39.6%)	20 (40.8%)
Race (No., %)			
White	52 (51.0%)	25 (47.2%)	27 (55.1%)
Black or African American	24 (23.5%)	13 (24.5%)	11 (22.4%)
Mixed Race	13 (12.7%)	7 (13.2%)	6 (12.2%)
Another Race	7 (6.9%)	5 (9.4%)	2 (4.1%)
American Indian or Alaska Native	3 (2.9%)	2 (3.8%)	1 (2.0%)
Pacific Islander	2 (2.0%)	1 (1.9%)	1 (2.0%)
Asian	1 (1.0%)	0 (0.0%)	1 (2.0%)
Ethnicity (No., %)			
Not Hispanic/ Latina/o/x	70 (68.6%)	37 (69.8%)	33 (67.3%)
Hispanic/ Latina/o/x	32 (31.4%)	16 (30.2%)	16 (32.7%)
Annual Household Income (No., %)			
$0 - $29,999	46 (45.1%)	23 (46.9%)	23 (43.4%)
$30,000 - $59,999	20 (19.6%)	16 (30.2%)	4 (3.9%)
$60,000 - $89,999	15 (14.7%)	5 (9.4%)	10 (20.4%)
$90,000 - $119,999	9 (8.8%)	3 (5.7%)	6 (12.2%)
> $120,000	12 (11.8%)	6 (11.3%)	6 (12.2%)
Employment Status			
Full Time	35 (34.3%)	16 (30.2%)	19 (38.8%)
Part Time	26 (25.5%)	14 (26.4%)	12 (24.5%)
Retired or on Disability	8 (7.8%)	5 (9.4%)	3 (6.1%)
Unemployed	33 (32.4%)	18 (34.0%)	15 (30.6%)
Alcohol and Substance Use			
Drinks per drinking day[a]	7.54 (5.36)	7.57 (4.30)	7.52 (3.36)
% heavy drinking days[a]	66.6% (34.55)	67.5% (34.55)	65.7% (34.89)
# of drinking days[a]	22.56 (7.40)	22.42 (7.46)	22.71 (7.41)
Positive THC screen (No., %)	23 (22.5%)	12 (22.6%)	11 (22.4%)
Mild CUD (No,. %)[b]	12 (11.8%)	6 (11.3%)	6 (12.2%)
Any cigarette use (No., %)[a]	35 (34.3%)	18 (34.0%)	17 (34.7%)
Smokes cigarettes (FTND)	40 (39.2%)	20 (37.7%)	20 (40.8%)
PACS Total	13.78 (6.17)	13.83 (5.85)	13.73 (6.56)
SCID AUD symptom count*	**6.73 (1.93)**	**7.15 (2.05)**	**6.27 (1.69)**
AUDIT total	20.24 (7.35)	21.23 (8.24)	19.16 (6.14)
Relief /Habit Drinking (No., %)[c]	52 (51.0%)	27 (50.9%)	25 (51.0%)
Mental Health			
Current Depression (No., %)[b]	7 (6.9%)	4 (7.5%)	3 (6.1%)
BDI-II Total	11.38 (7.89)	11.98 (8.55)	10.73 (7.13)
BAI Total	7.76 (7.51)	8.53 (7.87)	6.94 (7.08)
ISI Total	8.51 (5.80)	9.00 (6.29)	7.98 (5.23)

* and **bold** indicate significance difference ($p < .011$); nmiss = 1 for AUD symptom count

Table 3-1 Legend:

Note. [a] based on Timeline FollowBack collected on the 30 days prior to baseline visit; [b] based on DSM-5 criteria for current Cannabis Use Disorder or current Major Depressive Episode, respectively; [c] based on UCLA Reward Relief Habit Drinking scale (all other participants reported reward drinking); THC = tetrahydrocannabinol; PACS = Penn Alcohol Craving Scale; SCID = Structured Clinical Interview for the DSM-5; AUD = alcohol use disorder; AUDIT = Alcohol Use Disorders Identification Test; BDI-II = Beck Depression Inventory II; BAI = Beck Anxiety Inventory; ISI = Insomnia Severity Index;

Table 3-2. Raw Means and Standard Deviations for Clinical Symptomatology by Treatment Group and Timepoint

	Penn Alcohol Craving Scale Total Score Mean (Standard Deviation)					
Timepoint	*Ibudilast*	*n*	*Placebo*	*n*	*Total Sample*	*Total N*
Baseline	13.83 (5.85)	53	13.73 (6.56)	49	13.78 (6.17)	102
Week 4	10.93 (6.31)	44	11.34 (6.34)	38	11.21 (6.29)	82
Week 8	10.13 (6.55)	45	11.17 (6.87)	35	10.59 (6.67)	80
Week 12	8.91 (6.33)	47	10.15 (6.35)	39	9.48 (6.34)	86

	Beck Depression Inventory-II Total Score Mean (Standard Deviation)					
Timepoint	*Ibudilast*	*n*	*Placebo*	*n*	*Total Sample*	*Total N*
Baseline	11.98 (8.55)	53	10.73 (7.13)	49	11.38 (7.88)	102
Week 4	9.00 (8.30)	44	6.67 (5.66)	39	7.90 (7.23)	83
Week 8	8.63 (8.88)	46	6.70 (7.11)	37	7.77 (8.15)	83
Week 12	7.94 (9.98)	47	6.02 (5.64)	39	7.07 (8.31)	86

	Beck Anxiety Inventory Total Score Mean (Standard Deviation)					
Timepoint	*Ibudilast*	*n*	*Placebo*	*n*	*Total Sample*	*Total N*
Baseline	8.53 (7.87)	53	6.94 (7.08)	49	7.76 (7.51)	102
Week 4	8.00 (7.55)	44	7.63 (7.05)	38	7.83 (7.28)	82
Week 8	7.89 (8.74)	45	6.09 (5.16)	35	7.10 (7.40)	80
Week 12	7.13 (8.73)	47	5.33 (6.20)	39	6.31 (7.70)	86

Table 3-3. Estimated Means and Standard Error for Clinical Symptomatology by Treatment Group and Timepoint from Full Linear or Piecewise Growth Models.

	Penn Alcohol Craving Scale Total Score Mean (Standard Error)	
Timepoint	*Ibudilast*	*Placebo*
Baseline	12.43 (0.73)	13.19 (0.76)
Week 4	11.12 (0.65)	12.71 (0.68)
Week 8	9.81 (0.66)	12.23 (0.71)
Week 12	8.51 (0.76)	11.75 (0.84)

	Beck Depression Inventory-II Total Score Mean (Standard Error)	
Timepoint	*Ibudilast*	*Placebo*
Baseline	11.98 (1.09)	10.73 (1.13)
Week 4	8.94 (1.03)	6.90 (1.09)
Week 8	8.43 (1.02)	6.61 (1.10)
Week 12	7.93 (1.18)	6.33 (1.27)

	Beck Anxiety Inventory Total Score Mean (Standard Error)	
Timepoint	*Ibudilast*	*Placebo*
Baseline	8.53 (1.03)	6.94 (1.07)
Week 4	8.24 (1.01)	7.88 (1.07)
Week 8	7.68 (0.92)	6.76 (0.99)
Week 12	7.13 (1.09)	5.65 (1.19)

Note. Depression and anxiety total scores are estimated means and standard errors derived from fitted piecewise models with two timepoints

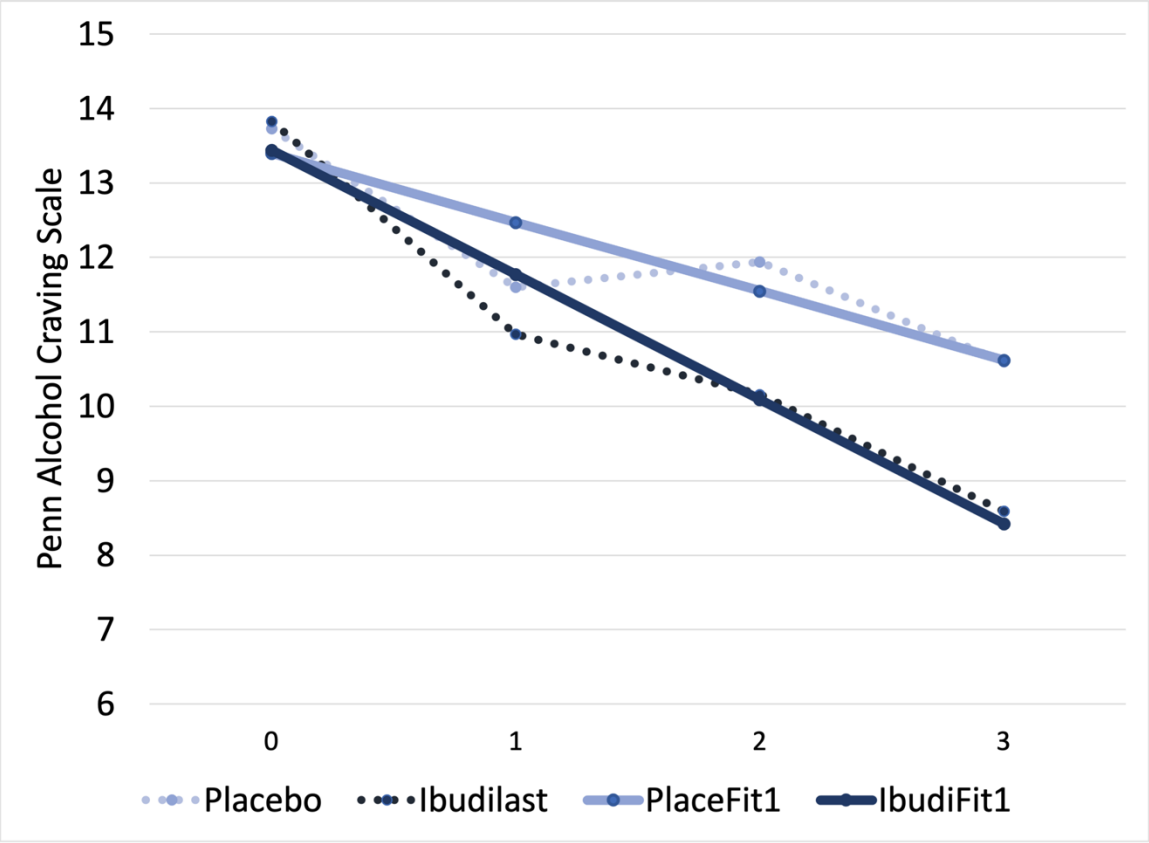
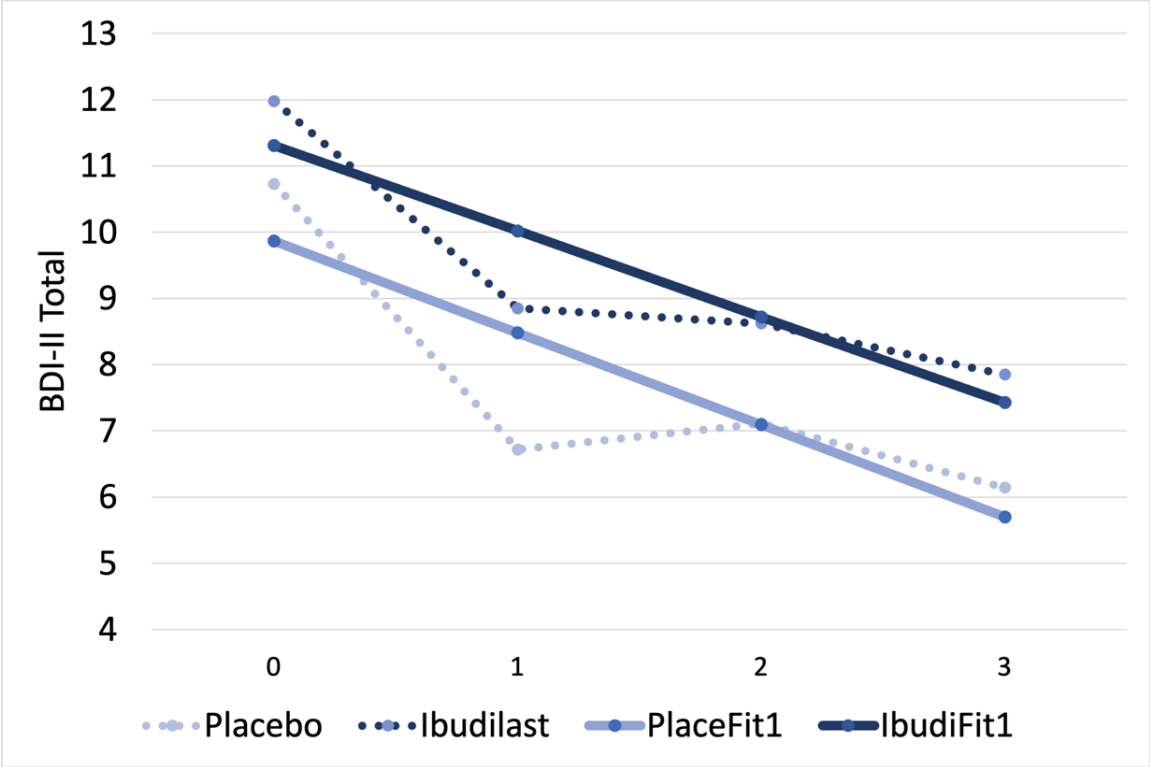
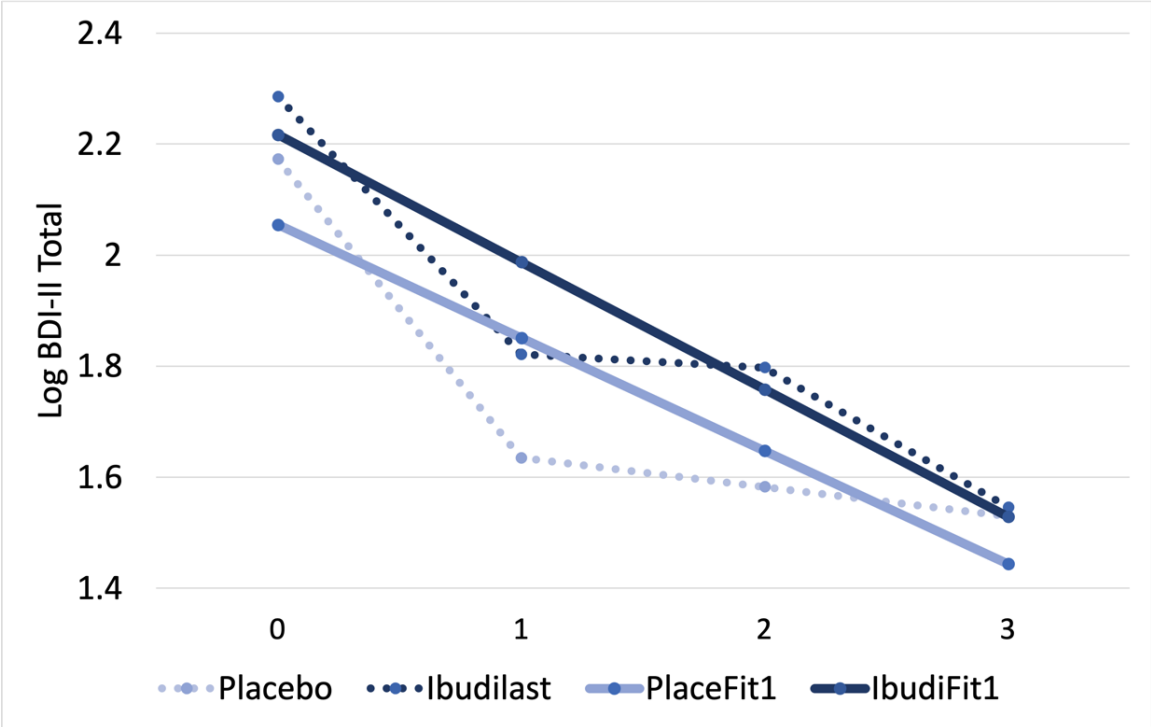


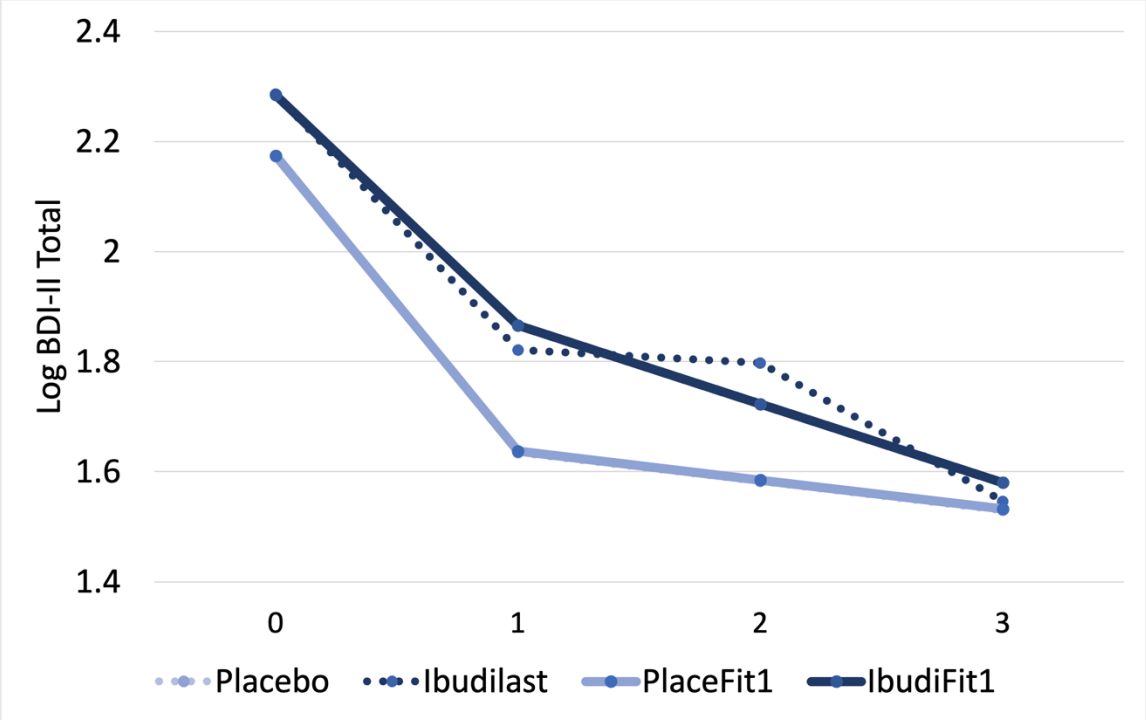
Figure 3-1. Penn Alcohol Craving Scale (PACS) raw means accounting for missing data handling methods (dotted lines) overlayed on reduced linear growth model-adjusted means (solid lines) by medication condition


13
12
11
10
9
8
7
6
5
4
BDI-II Total
0
1
2
3
Placebo
Ibudilast
PlaceFit1
IbudiFit1

A

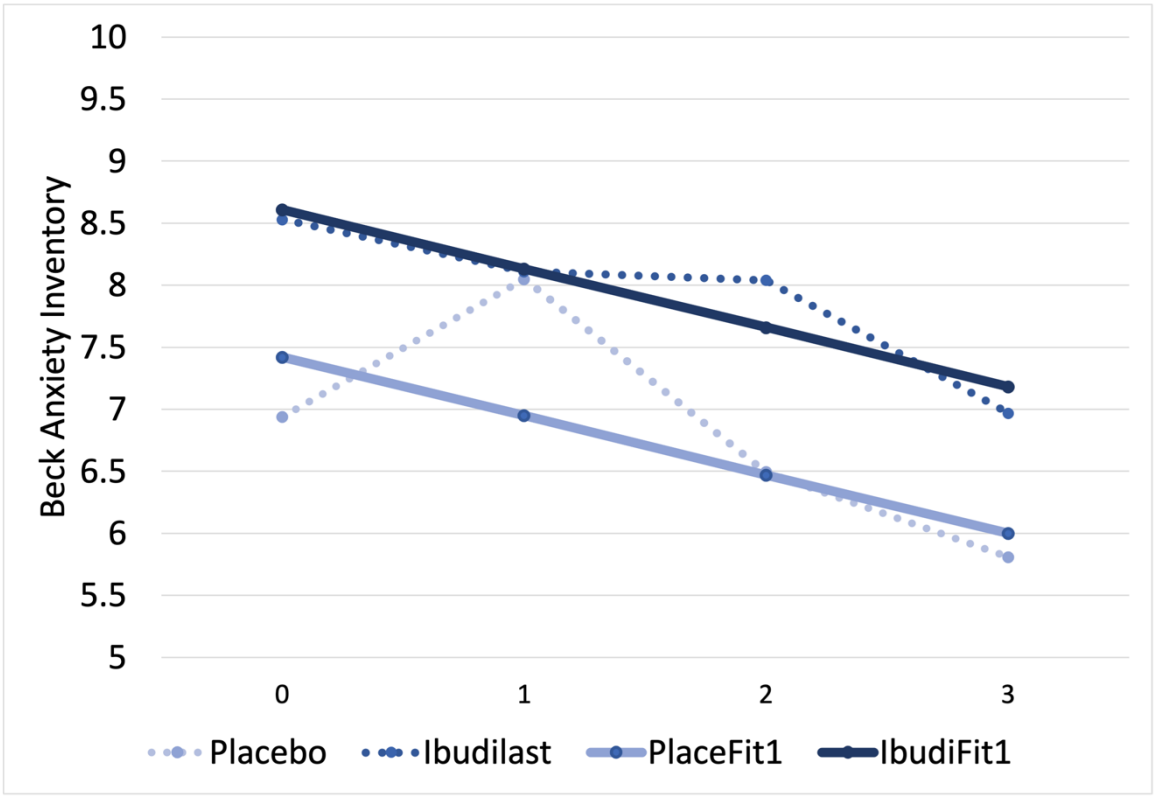

2.4
2.2
2
1.8
1.6
1.4
Log BDI-II Total
0
1
2
3
Placebo
Ibudilast
PlaceFit1
IbudiFit1

B

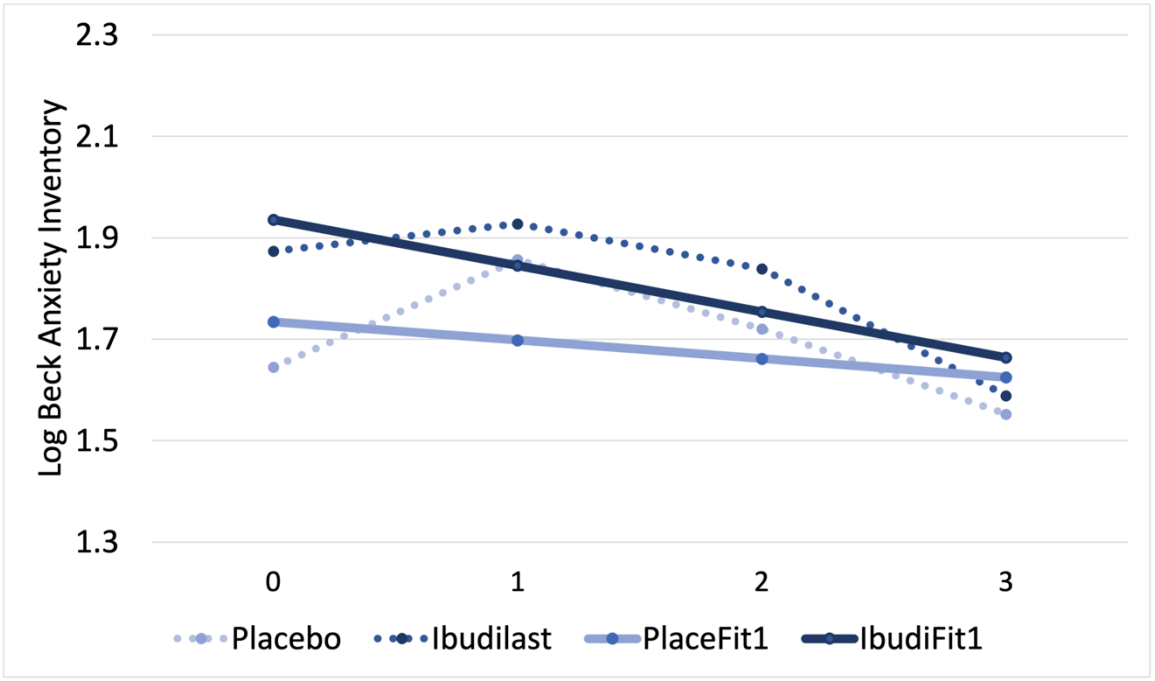


C

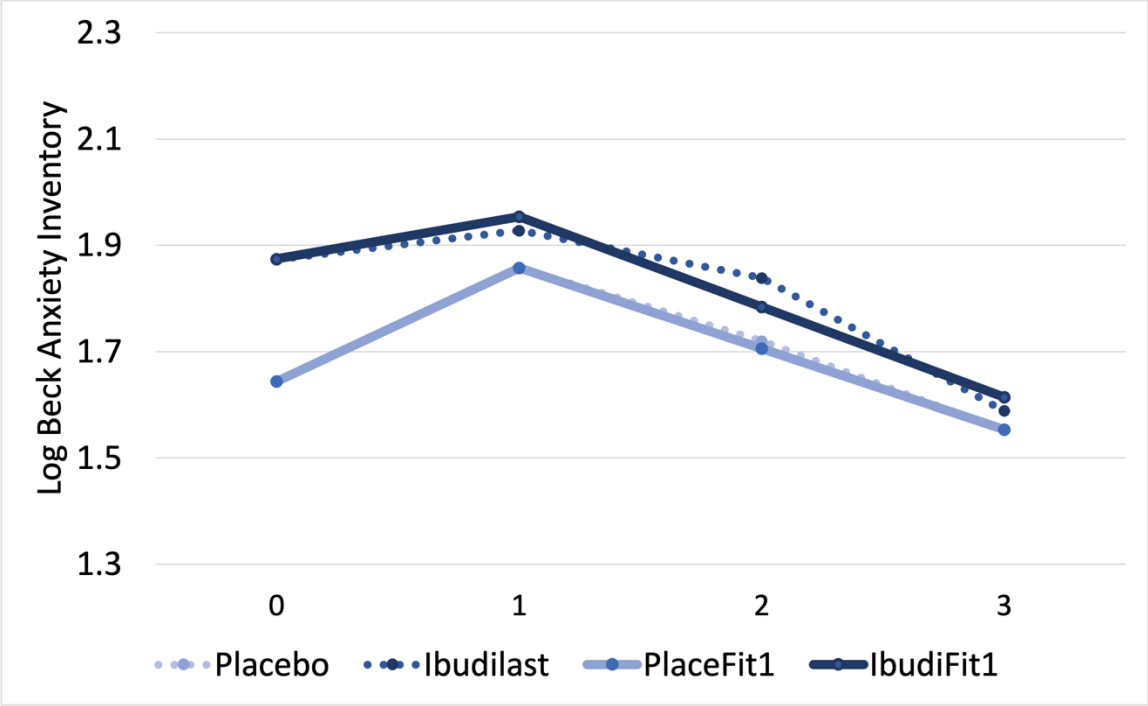
Figure 3-2. Raw means (dotted lines) accounting for missing data handling methods overlayed on reduced linear growth model-adjusted means (solid lines) by medication condition for Beck Depression Inventory-II (BDI-II; **A**), log transformed BDI-II (**B**), and 2 timepoint piecewise log transformed BDI-II (**C**)


Beck Anxiety Inventory
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
0
1
2
3
Placebo
Ibudilast
PlaceFit1
IbudiFit1

A


Log Beck Anxiety Inventory
2.3
2.1
1.9
1.7
1.5
1.3
0
1
2
3
Placebo
Ibudilast
PlaceFit1
IbudiFit1

B



C

Figure 3-3. Raw means (dotted lines) accounting for missing data handling methods overlayed on reduced linear growth model-adjusted means (solid lines) by medication condition for Beck Anxiety Inventory (BAI, **A**), log transformed BAI (**B**), and 2 timepoint piecewise, log transformed BAI (**C**)

Table 3-4a. Linear Growth Model Results for Monthly Changes in Clinical Scores of Alcohol Craving, Depression, and Anxiety During the Randomized Clinical Trial of Ibudilast

Fixed Effects					
	b	**SE**	**K.R. DF**	**t-value**	**p-value**
Craving Model (PACS)					
Intercept	13.23	0.76	103	17.46	< **.0001*****
Medication-Ibudilast vs. Placebo	-0.77	1.04	98	-0.74	.463
Time	-0.48	0.28	255	-1.72	.087
Time × Medication	-0.83	0.35	249	-2.33	**.021***
Pre-treatment AUDIT (CGM)	0.36	0.06	92	5.55	**<.0001*****
Sex (CGM)	3.22	0.96	93	3.37	**.001****
Log DPDD Past 2 Weeks (CGM)	1.89	0.40	288	4.66	< **.0001*****
Depression Model (Log BDI-II)					
Intercept	2.01	0.13	102	15.91	< **.0001*****
Medication-Ibudilast vs. Placebo	0.14	0.17	97	0.78	.437
Time	-0.16	0.06	97	-2.94	**.004****
Time × Medication	-0.03	0.07	86	-0.48	.632
Pre-treatment AUDIT (CGM)	0.007	0.01	99	0.60	.548
Sex (CGM)	-0.14	0.17	98	-0.81	.419
Log DPDD Past 2 Weeks (CGM)	0.18	0.07	301	2.47	**.014***
Anxiety Model (Log BAI)					
Intercept	1.76	0.12	104	14.77	< **.0001*****
Medication-Ibudilast vs. Placebo	0.11	0.16	99	0.65	.517
Time	-0.01	0.05	101	-0.21	.830
Time × Medication	-0.06	0.06	90	-0.99	.324
Pre-treatment AUDIT (CGM)	0.05	0.009	96	4.88	**<.0001*****
Sex (CGM)	0.20	0.14	97	1.45	.149
Log DPDD Past 2 Weeks (CGM)	0.08	0.06	291	1.33	.183

Note. ***$p \leq .0001$, * *$p \leq .01$, *$p \leq .05$;** SE = standard error; K.R. DF = Kenwood Rogers Degrees of Freedom; PACS = Penn Alcohol Craving Scale; AUDIT = Alcohol Use Disorders Identification Test; DPDD = drinks per drinking day (log transformed, level 1 variable covering the two weeks prior to each collection of clinical measure); CGM = centered at grand mean; BDI-II = Beck Depression Inventory-II; BAI = Beck Anxiety Inventory; Placebo is the reference group

Table 3-4b. Piecewise Linear Growth Model Results for Monthly Changes in Clinical Scores of Depression and Anxiety During the Randomized Clinical Trial of Ibudilast

Fixed Effects					
	b	**SE**	**K.R. DF**	**t-value**	**p-value**
Depression Model (Log BDI-II)					
Intercept	1.66	0.16	95	10.43	**< .0001*** **
Medication-Ibudilast vs. Placebo	0.20	0.22	94	0.92	.358
Time 1	-0.49	0.14	139	-3.48	**<.001** **
Time 1 × Medication	0.11	0.18	131	0.59	.558
Time 2	0.45	0.17	169	2.69	**.008** **
Time 2 × Medication	-0.20	0.22	165	-0.91	.364
Pre-treatment AUDIT (CGM)	0.01	0.01	100	0.51	.610
Sex (CGM)	-0.18	0.17	99	-1.05	.300
Log DPDD Past 2 Weeks (CGM)	0.10	0.07	285	1.35	.180
Anxiety Model (Log BAI)					
Intercept	1.93	0.11	93	17.43	**< .0001*** **
Medication-Ibudilast vs. Placebo	-0.02	0.15	92	-0.14	.890
Time 1	0.29	0.12	100	2.31	**.023***
Time 1 × Medication	-0.15	0.17	91	-0.93	.356
Time 2	-0.43	0.16	91	-2.70	**.008** **
Time 2 × Medication	0.13	0.21	87	0.62	.539
Pre-treatment AUDIT (CGM)	0.04	0.01	95	4.82	**<.0001*** **
Sex (CGM)	0.19	0.14	96	1.37	.173
Log DPDD Past 2 Weeks (CGM)	0.13	0.06	265	2.01	**.045***

Note. ***$p \leq .0001$**, * ***$p \leq .01$, *$p \leq .05$;** SE = standard error; K.R. DF = Kenwood Rogers Degrees of Freedom; AUDIT = Alcohol Use Disorders Identification Test; DPDD = drinks per drinking day (log transformed, level 1 variable covering the two weeks prior to each collection of clinical measure); CGM = centered at grand mean; BDI-II = Beck Depression Inventory-II; BAI = Beck Anxiety Inventory; Placebo is the reference group

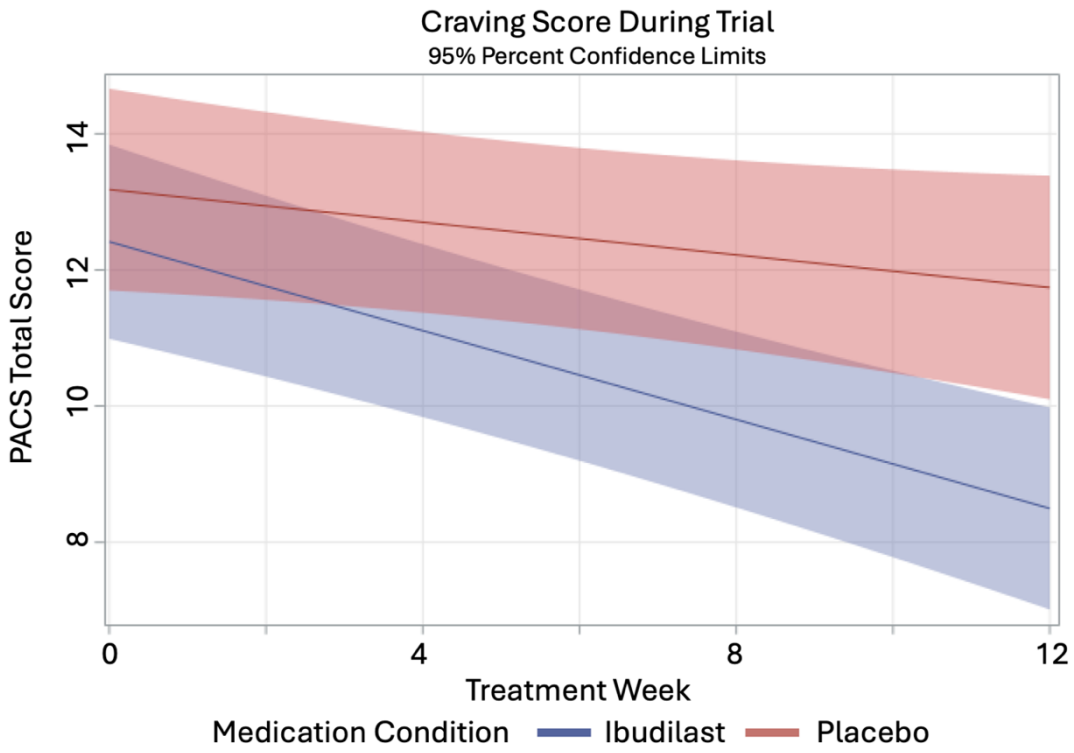
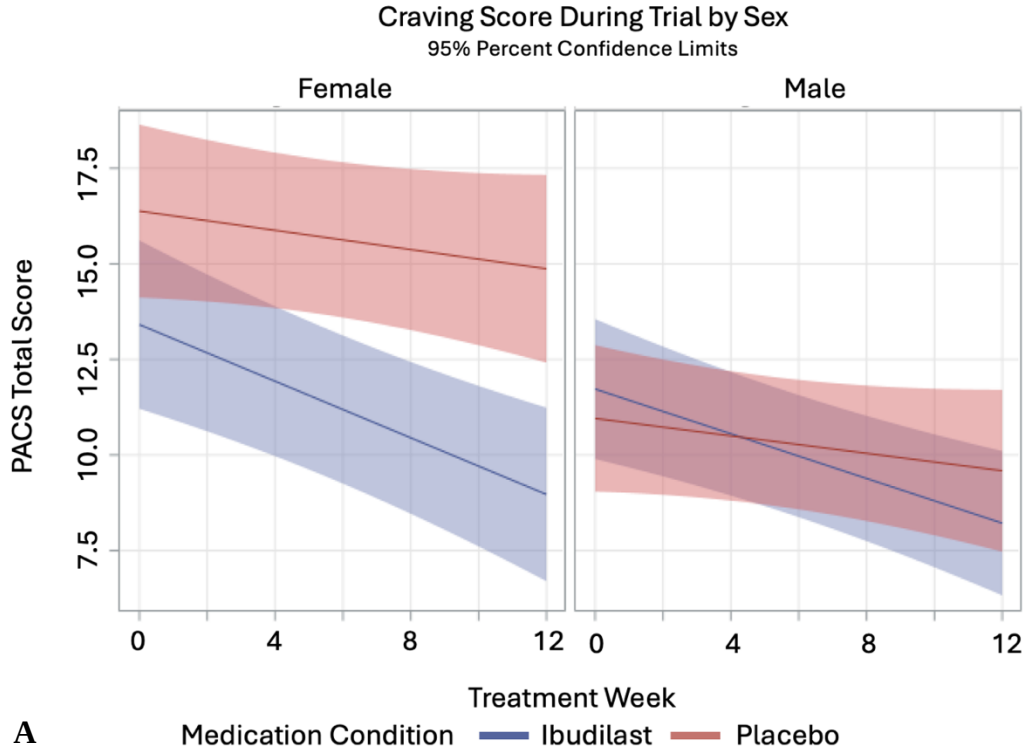
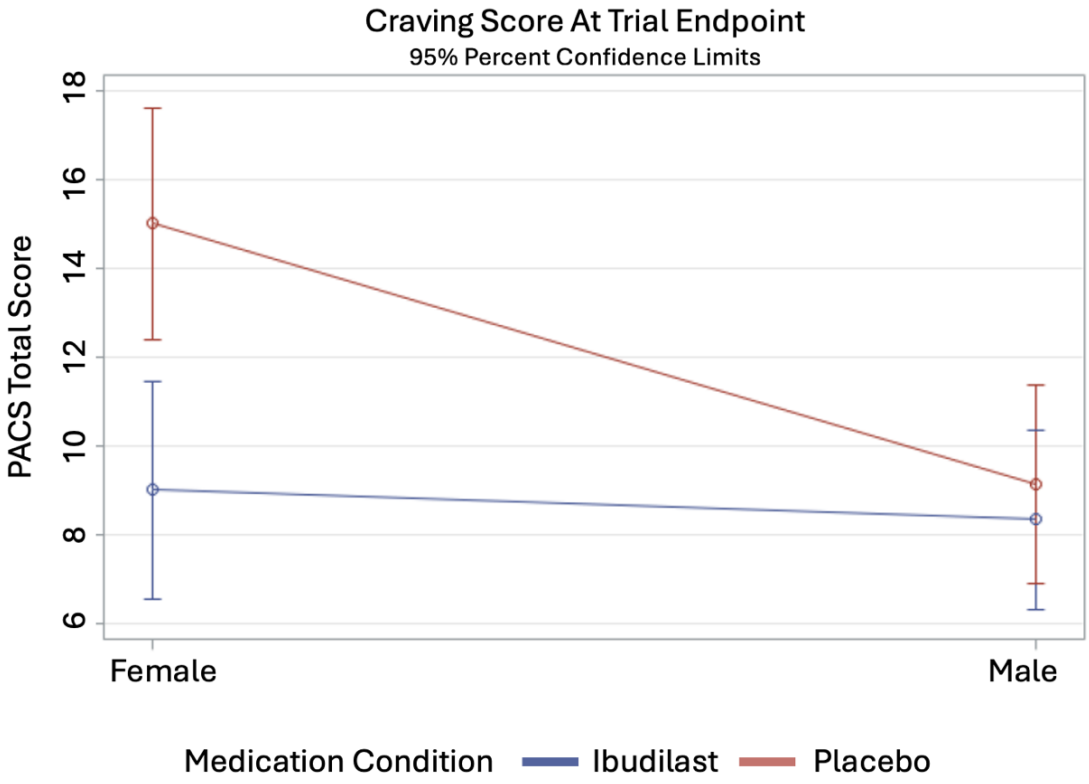


Figure 3-4. Changes in Penn Alcohol Craving Scale (PACS) score during the trial by medication condition. Ibudilast group displayed a significantly steeper decline in craving than placebo group over the course of the trial (p = .020). At treatment endpoint, estimated means for ibudilast group = 8.51 points and placebo group = 11.75 points

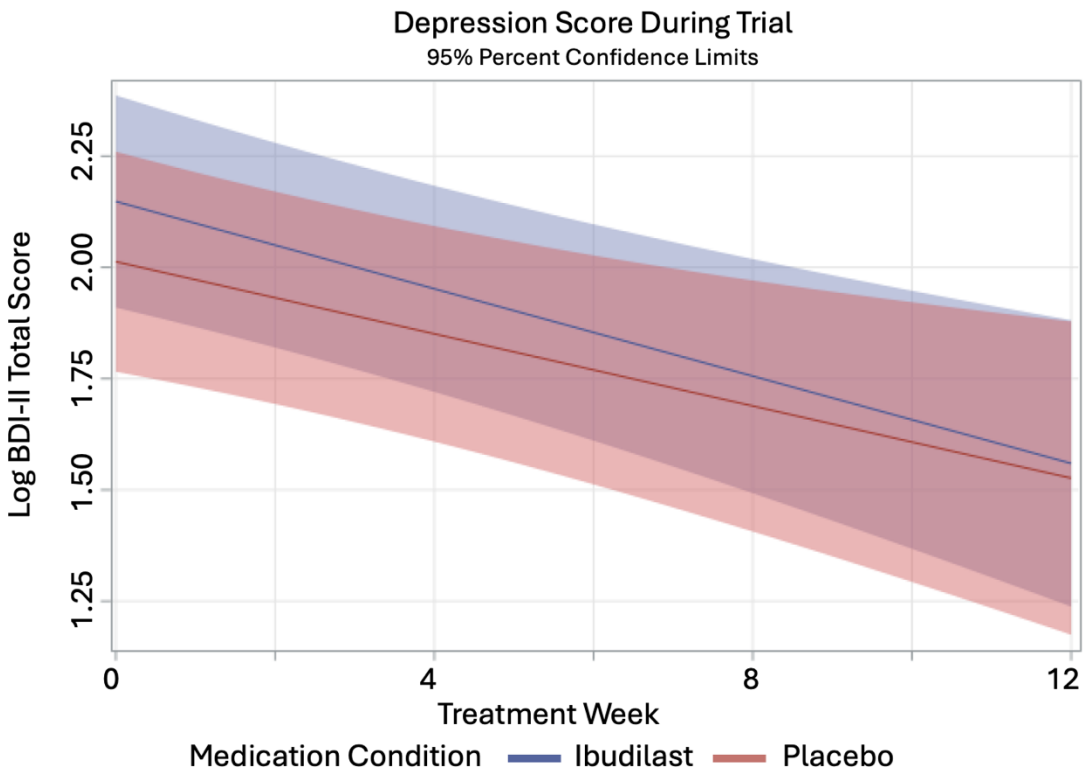


A

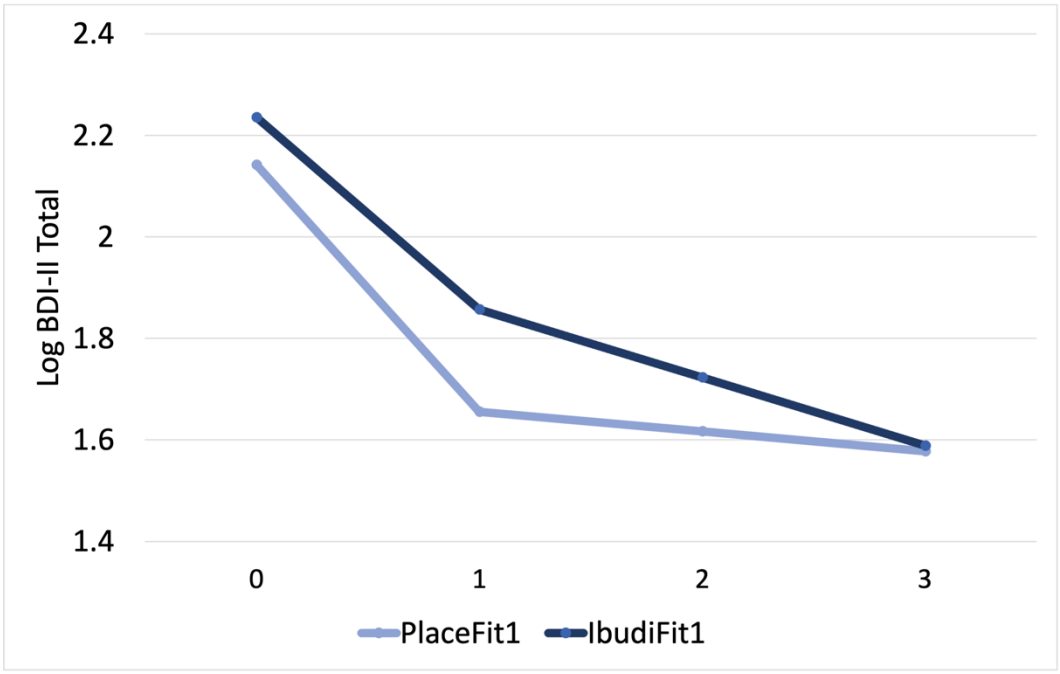


B

Figure 3-5. Penn Alcohol Craving Scale (PACS) scores during the trial by biological sex and medication condition. No significant differences in changes over time between males and females (**A**). However, at treatment endpoint, females on ibudilast had significantly lower scores than females on placebo ($p < .001$; **B**). At treatment endpoint, estimated means for ibudilast group females = 9.01 points and males = 8.29 points. At treatment endpoint, estimated means for placebo group females = 14.98 points and males = 9.16 points



A



B

Figure 3-6. Changes in Beck Depression Inventory-II (BDI-II) score (logged transformed) during the trial by medication condition for linear growth model (**A**) and piecewise model (**B**). No significant differences in changes over time between medication groups.

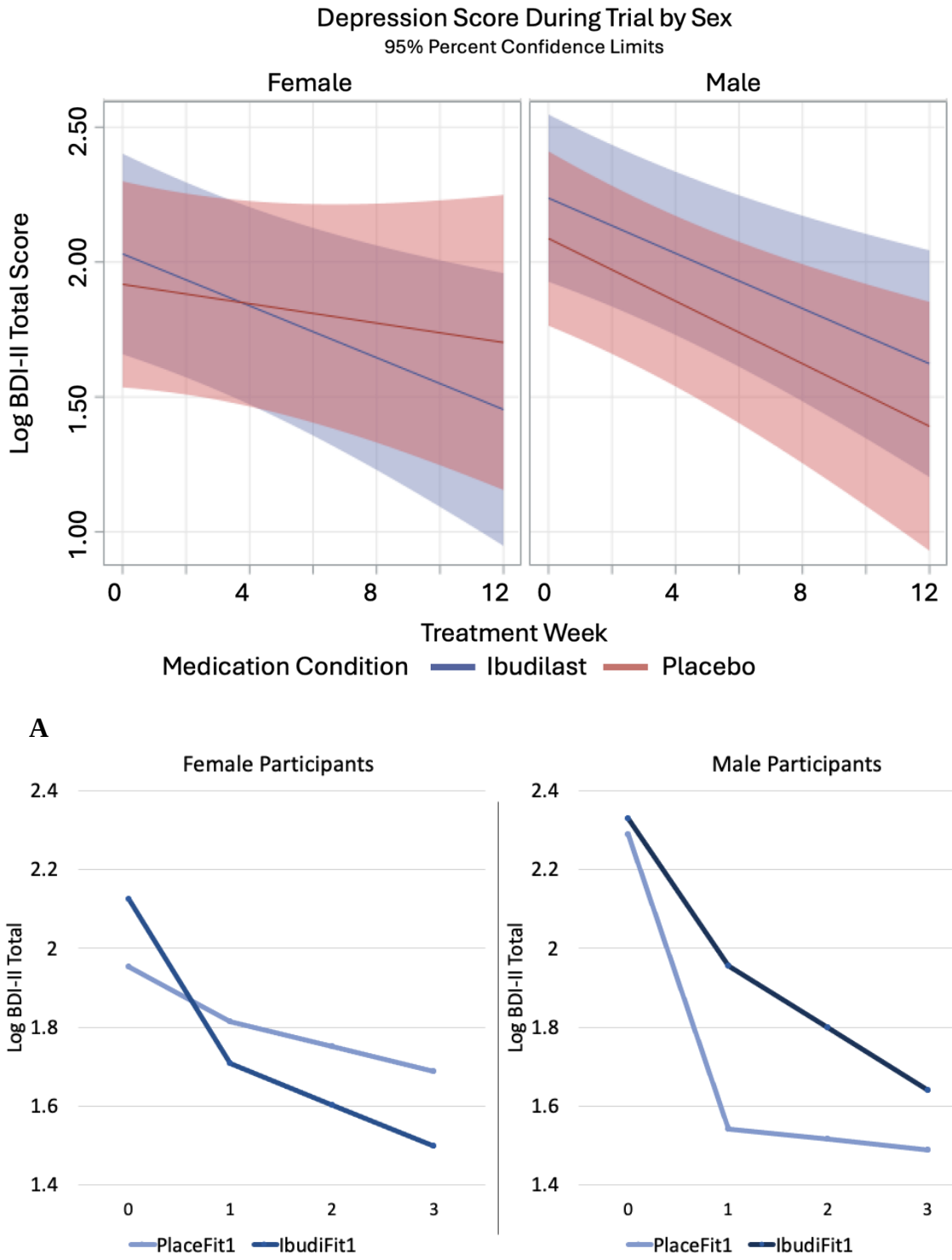
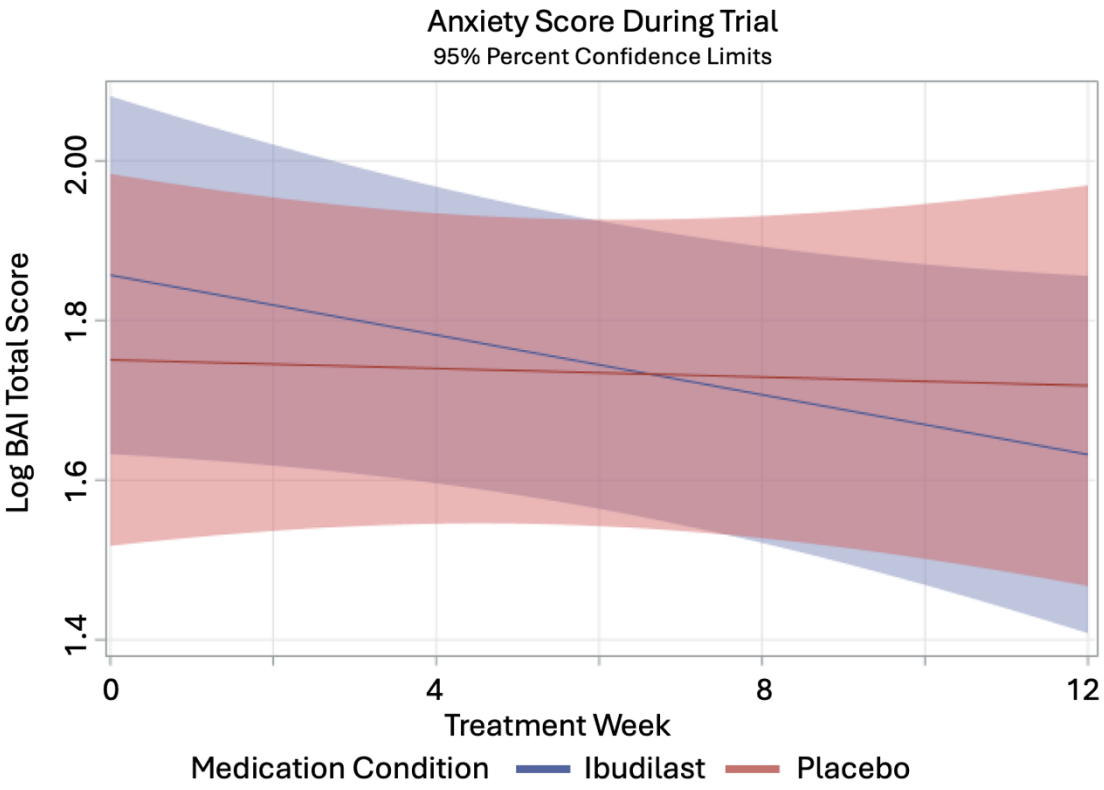
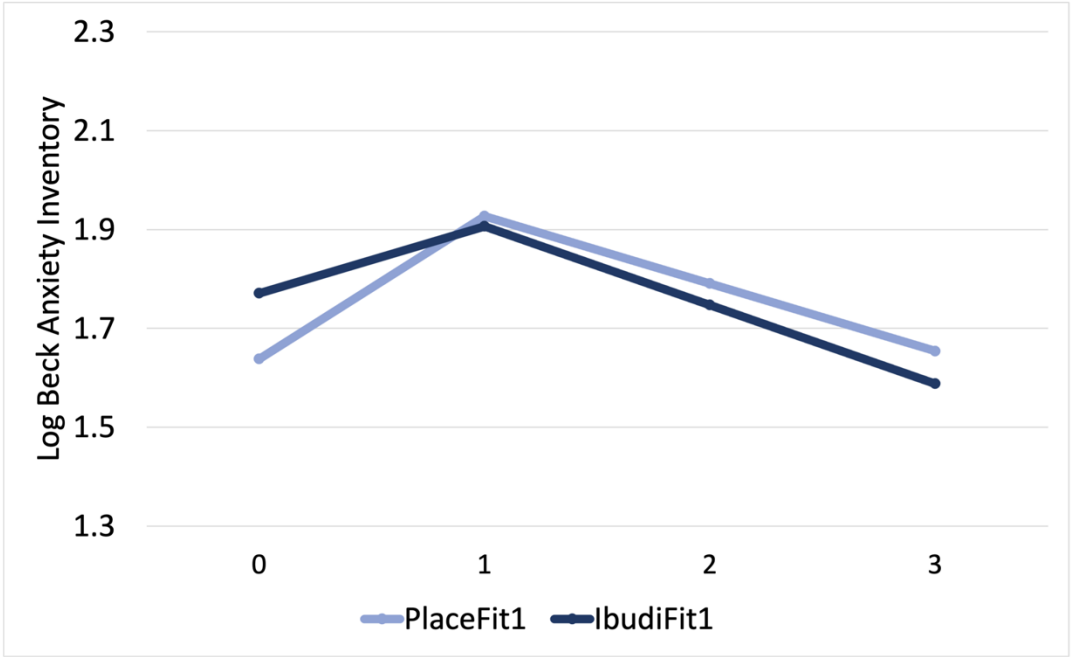


Figure 3-7. Changes in Beck Depression Inventory-II (BDI-II) score (logged transformed) during the trial by medication condition and biological sex for linear growth model (**A**) and piecewise model (**B**). Per the piecewise model, males in the placebo group had a significantly faster decline in depression scores than females in the placebo group in the first month (p = .029)

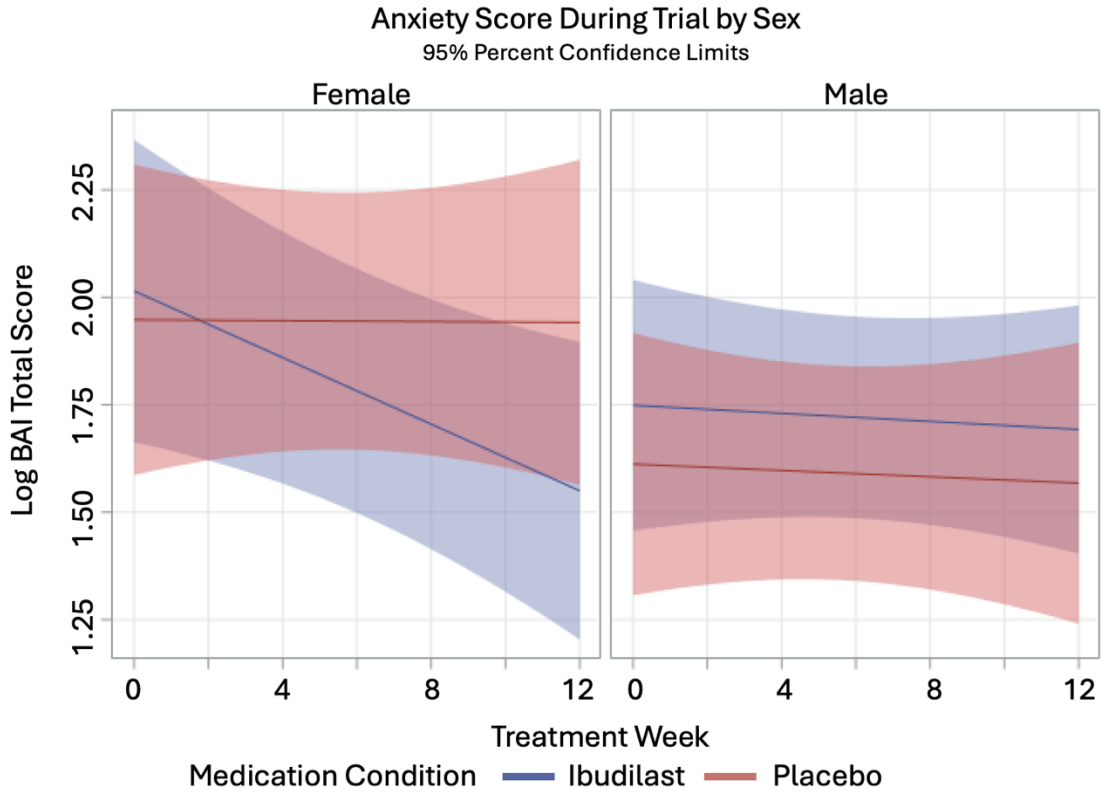


A

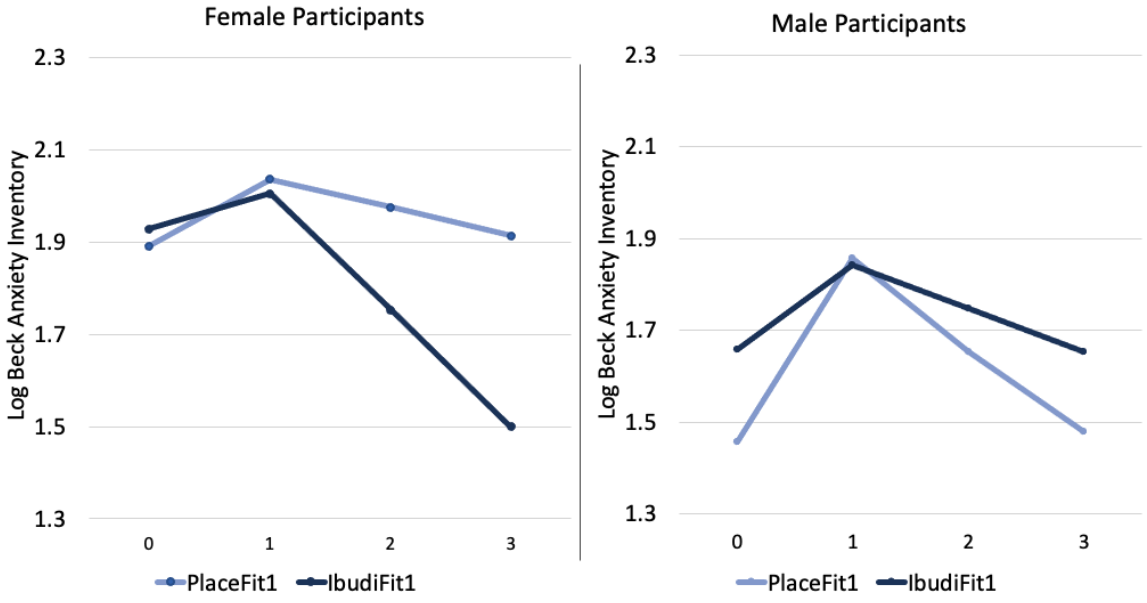


B

Figure 3-8. Changes in Beck Anxiety Inventory (BAI) score (logged transformed) during the trial by medication condition for linear growth model (**A**) and piecewise model (**B**). No significant differences in changes over time between medication groups.



A



B

Figure 3-9. Changes in Beck Anxiety Inventory (BAI) score (logged transformed) during the trial by medication condition and biological sex for linear growth model (**A**) and piecewise model (**B**). No significant differences in changes over time between medication groups or between males and females

Primary Models:

$$Y_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)X_{1ti} + \beta_2 X_{2i} + \beta_3(X_{1ti})(X_{2i}) + \beta_4 X_{4i}^{cgm} + \beta_5 X_{5i}^{cgm} + \beta_6 X_{6ti}^{cgm} + \varepsilon_{ti}$$

$$CRAVING_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)(TIME_{1ti}) + \beta_2(MED_{2i}) + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4\left(Sex_{4i}^{cgm}\right) + \beta_5\left(AUDIT_{5i}^{cgm}\right) + \beta_6\left(logDPDD_{6ti}^{cgm}\right) + \varepsilon_{ti}$$

$$logBDI_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)(TIME_{1ti}) + \beta_2(MED_{2i}) + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4\left(Sex_{4i}^{cgm}\right) + \beta_5\left(AUDIT_{5i}^{cgm}\right) + \beta_6\left(logDPDD_{6ti}^{cgm}\right) + \varepsilon_{ti}$$

$$logBAI_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)(TIME_{1ti}) + \beta_2(MED_{2i}) + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4\left(Sex_{4i}^{cgm}\right) + \beta_5\left(AUDIT_{5i}^{cgm}\right) + \beta_6\left(logDPDD_{6ti}^{cgm}\right) + \varepsilon_{ti}$$

Moderation Models:

$$Y_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)X_{1ti} + \beta_2 X_{2i} + \beta_3(X_{1ti})(X_{2i}) + \beta_4 X_{4i} + \beta_5 X_{5i}^{cgm} + \beta_6 X_{6ti}^{cgm} + \beta_7(X_{1ti})(X_{4i}) + \beta_8(X_{2i})(X_{4i}) + \beta_9(X_{1ti})(X_{2i})(X_{4i}) + \varepsilon_{ti}$$

$$Craving_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)TIME_{1ti} + \beta_2 MED_{2i} + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4 SEX_{4i} + \beta_5 AUDIT_{5i}^{cgm} + \beta_6 logDPDD_{6ti}^{cgm} + \beta_7(TIME_{1ti})(SEX_{4i}) + \beta_8(MED_{2i})(SEX_{4i}) + \beta_9(TIME_{1ti})(MED_{2i})(SEX_{4i}) + \varepsilon_{ti}$$

$$logBDI_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)TIME_{1ti} + \beta_2 MED_{2i} + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4 SEX_{4i} + \beta_5 AUDIT_{5i}^{cgm} + \beta_6 logDPDD_{6ti}^{cgm} + \beta_7(TIME_{1ti})(SEX_{4i}) + \beta_8(MED_{2i})(SEX_{4i}) + \beta_9(TIME_{1ti})(MED_{2i})(SEX_{4i}) + \varepsilon_{ti}$$

$$logBAI_{ti} = (\beta_0 + b_{0i}) + (\beta_1 + b_1)TIME_{1ti} + \beta_2 MED_{2i} + \beta_3(TIME_{1ti})(MED_{2i}) + \beta_4 SEX_{4i} + \beta_5 AUDIT_{5i}^{cgm} + \beta_6 logDPDD_{6ti}^{cgm} + \beta_7(TIME_{1ti})(SEX_{4i}) + \beta_8(MED_{2i})(SEX_{4i}) + \beta_9(TIME_{1ti})(MED_{2i})(SEX_{4i}) + \varepsilon_{ti}$$

Figure 3-10. Detailed formulas for one timepoint linear growth models predicting clinical symptoms of craving, depression, and anxiety during the 12 week trial of ibudilast for AUD.

Note. Superscript of 'cgm' indicates variable was centered at the grand mean; log indicates that the variable was log transformed to improve normality.

CHAPTER 4:

Neurocognitive Function during a Clinical Trial of Ibudilast for Alcohol Use Disorder

Lindsay R. Meredith, MA, Wave-Ananda Baskerville, MA & Lara A. Ray, PhD

RATIONALE FOR NEUROCOGNITIVE FUNCTION AS CLINICAL MECHANISM

Neurocognitive dysfunction is one of the harms associated with AUD (Koob & Volkow, 2016) and it is well-documented in literature enrolling participants receiving inpatient alcohol treatment (Stavro et al., 2013). As outlined in my master's thesis, it is unclear the extent to which neurocognitive differences among individuals with AUD vs. healthy comparison individuals precede the onset of the disorder or whether these impairments emerge during the disease course of addiction (Meredith et al., 2020). However, initial research shows that one's degree of neurocognitive impairment is significantly related to disorder severity, lifetime and recent quantity of alcohol consumption, and age of initiation (Duka et al., 2003; Horner et al., 1999; Lim et al., 2017; Nguyen-Louie et al., 2017; Sullivan et al., 2002; Woods et al., 2016). Health factors associated with more severe impairment include malnutrition, withdrawal severity, altered liver function, and thiamine metabolism (Ritz et al., 2016). These impairments are related to brain changes suggestive of accelerated brain aging (Chanraud et al., 2007; Guggenmos et al., 2017; Pfefferbaum et al., 1997). For example, mice undergoing short-term abstinence from chronic ethanol exposure, displayed impaired reversal learning (i.e., cognitive flexibility), which is thought to be driven by ethanol-induced orbitofrontal cortex damage (Badanich et al., 2011).

The immune system is implicated in neurocognitive processes like learning and memory and is thought to facilitate the progression of diseases and brain aging (Yirmiya & Goshen, 2011). As an example, peripheral injection of *E. coli* in older rats induced retrograde and anterograde impairments in memory, which was thought to be driven by IL-1β proinflammatory responses in the hippocampus (Barrientos et al., 2006; Pugh et al., 2001). These findings suggest that adults with enhanced proinflammatory states may be particularly vulnerable to memory impairments. In rodent and human samples, proinflammatory cytokines, particularly IL-6, TNF-

α, and IL-1β, serve as mediators between cellular processes -- synaptic plasticity, neurogenesis, and neurotransmission-- and neurocognitive functioning, which contribute to the pathogenesis of conditions such as dementia and major depression (McAfoose & Baune, 2009; Wilson et al., 2002). Continuing, TLRs, which are strongly implicated in AUD-related immune signaling, are thought to negatively regulate hippocampal plasticity and memory retention (Okun et al., 2010). In an experimental study, 20 healthy adults received a typhoid vaccination to provoke mild peripheral inflammation, and then completed a positron emission tomography scan and performed memory tasks (Harrison et al., 2014). This inflammatory challenge reduced glucose metabolism in the medial temporal lobe and acutely impaired spatial memory. In addition, neurotrophic factors, such as BDNF and GDNF, which are secreted by various immune cell types, are shown to be essential regulators of cellular and neural plasticity, and can mediate the beneficial health effects of the immune system (Yirmiya & Goshen, 2011). While much progress has been made in understanding the impact of the immune system on learning, memory, and other cognitive processes, research in this area enrolling human samples with mental health conditions, like AUD and major depression, is still in its infancy.

As discussed in detail above, chronic alcohol consumption induces neuroinflammation and modulates immune signaling. Research focused on the mechanistic role of the immune system in cognitive processes and initial preclinical research on animal models of AUD, support the potential contribution of AUD-related immunomodulation on neurotoxicity / neuronal damage and cognitive impairment (He & Crews, 2008). To start, TLR4 signaling may play an important role in the cognitive and behavioral consequences of alcohol-induced inflammatory damage (Pascual et al., 2011). Pascual and colleagues (2011) conducted a preclinical study showing that mice who underwent chronic ethanol exposure and then underwent a withdrawal

period had TLR4-related immune activation (i.e., astroglia, microglia) in the frontal cortex and striatum, which in turn was correlated with cognitive and behavioral impairments not seen in TLR4-deficient mice (TLR4 $^{-/-}$ KO). These impairments were captured at 2-weeks of ethanol withdrawal via measures of short- and long-term object memory recognition, conditioned taste aversion extinction, and anxiety-related behaviors during a dark and light box paradigm.

Second, another rodent study focused on PPARγ receptor activation in adult Wistar rats, demonstrated the benefits of pioglitazone (i.e., PPARγ agonist). This compound attenuated alcohol-induced neuroinflammation, neurodegeneration, and cognitive damage in the form of reversal and spatial learning (Cippitelli et al., 2017). Initial findings have also been detected for PPARs agonists with actions in the periphery, such as OEA (see **Chapter 1**). A pilot trial conducted for a dietary supplement containing the precursor of OEA in young adults with heavy drinking patterns showed OEA-related improvements in performance on a Go/ No-Go task of inhibition (van Kooten et al., 2016). Taken together, these findings provide insight into potential mechanisms of chronic alcohol intake and immune system interactions, particularly their impact on neuronal damage and cognitive functions, and suggest the promising potential for pharmacological interventions targeting the immune system to help ameliorate these AUD-related consequences.

The relevance of testing the impact of immune modulating therapies on cognitive deficits associated with alcohol use has been highlighted in several review articles on the topic (Coleman & Crews, 2018; Crews, Lawrimore, et al., 2017; Erickson, Grantham, et al., 2019).

ABSTRACT

Background: Neurocognitive deficits in clinical samples with AUD are heterogenous but well-documented in the literature for a broad set of domains. Therapeutics that can improve neurocognitive functioning are highly desirable, as this may increase engagement in goal-directed actions and self-regulation, resulting in reduced alcohol intake. Medications targeting the immune system show particular promise in enhancing neurocognition, given the known relationship between the immune system and neurocognitive processes like memory.

Methods: This study is a secondary analysis of a 12-week randomized clinical trial of ibudilast (50 mg BID) enrolling treatment-seeking participants with AUD. During the trial, a subsample of participants (n = 66, 39% female, average age 45 years) had the opportunity to complete the NIH Toolbox Cognition Battery at pre- and post-treatment. Linear regression models tested whether medication group predicted neurocognitive performance at post-treatment for four different domains, after accounting for relevant covariates, including pre-treatment scores, baseline severity, and biological sex. Exploratory analyses tested whether recent alcohol use impacted performance.

Results: Participants consumed an average of 7.9 drinks per drinking day in the 30 days before baseline assessment and 46% smoked cigarettes. At pre-treatment neurocognitive scores ranged from the 17th to 69th percentile, with inhibitory control and attention being the domain with lowest performance and reading decoding skills and crystalized abilities being the domain with highest performance. Among the 50 participants providing data at both timepoints, neurocognitive performance improved from pre- to post-treatment for three of the four repeated tests, including inhibitory control and attention, processing speed, and working memory. However, the ibudilast group did not have significantly higher scores than the placebo group for

any neurocognitive domain. Number of drinks per drinking day in the two weeks prior to post-treatment testing was not associated with performance scores.

Conclusions: While neurocognitive scores improved from pre- to post-treatment across both conditions, ibudilast did not beneficially enhance neurocognition during this trial for AUD. Interestingly, by the end of the trial, participants performed above normed averages on all subtests, aside from the Flanker task measuring inhibitory control and attention. Difficulties with inhibition is a known risk and maintenance factor for AUD.

INTRODUCTION

Alcohol use disorder is a prevalent mental health condition with varied symptom presentations, including craving, depression, liver disease, relationship problems, and impairments in neurocognition (Koob & Volkow, 2016). Among individuals with AUD, neurocognitive deficits are heterogenous but well-documented in the literature for a broad set of domains, including executive function, memory, and processing speed (Stavro et al., 2013). The neurotoxic effects of alcohol contribute to structural and functional brain abnormalities, which are correlated with declines in cognition (Chanraud et al., 2007; Guggenmos et al., 2017; Pfefferbaum et al., 1997; Spindler et al., 2021). Thus, neurocognitive impairments are suggested to be graded correlates of chronic alcohol intake but there is also evidence of pre-existing vulnerability, such as higher impulsivity and reduced inhibitory control prior to alcohol initiation (Duka et al., 2003; Horner et al., 1999; Lim et al., 2017; Nguyen-Louie et al., 2017; Sullivan et al., 2002; Woods et al., 2016).

Lowered neurocognitive abilities are relevant to clinical outcomes in AUD, as they interfere with engagement in goal-directed actions, planning, and self-regulation, which maintain alcohol-seeking behavior (Bates et al., 2006). The Addictions Neuroclinical Assessment, which is an important neuroscience-informed framework, highlights executive function as one of the three functional domains most relevant to AUD (Gunawan et al., 2023). Research on neurocognitive recovery with alcohol abstinence is limited, but findings are optimistic that these abilities do improve (Le Berre et al., 2017; Stavro et al., 2013). Improvements in neurocognition during abstinence are consistently correlated with changes in brain volume (Durazzo et al., 2015; Yeh et al., 2007). Individual factors, such as cigarette smoking status and co-morbid bipolar disorder may diminish neurocognitive recovery in AUD (Staudt et al., 2023). However, research has not

found consistent evidence that one's quantity of previous alcohol intake, biological sex, nor age markedly impact recovery. Even more sparse is research on changes in neurocognitive performance during active treatment, such as in the context of a clinical trial for AUD (Butler & Le Foll, 2019; Roten et al., 2015). Existing research on cognitive enhancing medications has focused primarily on severe cognitive deficits, such as Wernicke-Korsakoff's syndrome (Mistarz et al., 2021). Therapeutics that can improve neurocognitive function are highly desirable in the field, as they may help individuals with AUD better reduce their alcohol intake and improve overall quality of life.

Novel medications that target the immune system show particular promise in improving neurocognition in AUD, as neurocognitive processes are impacted by the immune system. Proinflammatory cytokines serve as mediators between cellular processes and neurocognitive functioning, and contribute to the pathogenesis of inflammatory conditions, such as addiction (Harrison et al., 2014; McAfoose & Baune, 2009; Wilson et al., 2002). Chronic alcohol intake is shown to alter neuroimmune signaling and contribute to inflammation in the brain and body (Coleman & Crews, 2018; Erickson, Grantham, et al., 2019; Shafiee et al., 2023), which may in turn impact neurocognitive processes. For instance, preclinical research on animal models of AUD supports the contribution of alcohol-related immunomodulation on neurotoxicity, neuronal damage, and cognitive impairment (He & Crews, 2008; Jabaris et al., 2015; Pascual et al., 2011). Despite this mechanistic research on chronic alcohol intake, immune system interactions, and their impact on neurocognitive functions, no published clinical AUD studies to date have assessed whether immune intervention alters neurocognitive processes over time.

Ibudilast is a novel neuroimmune modulator that has been testing in several randomized trials for AUD. Initial results found that the medication was well-tolerated and it reduced neural

activation to alcohol cues, attenuated alcohol-induced craving, and lowered levels of neurometabolite markers of inflammation (Grodin et al., 2021; Grodin et al., 2022; Meredith et al., 2022; Ray et al., 2017). Ibudilast is a phosphodiesterase (PDE) inhibitor and an allosteric macrophage migration inhibitory factor (MIF), which enhances brain-derived neurotrophic factor (BDNF) expression (Yirmiya & Goshen, 2011). Much work on BDNF suggests that augmentation of BDNF signaling confers immune-related benefits on learning, memory, long-term potentiation, and neurogenesis (Derecki et al., 2010; Ziv et al., 2006). Additionally, PDE inhibitors are thought to facilitate long-term potentiation and memory through blockade of cAMP degradation and promotion of neuronal survival and plasticity (Barad et al., 1998; Frey et al., 1993). Preclinically, PDE4 inhibitors have improved memory deficits in object recognition among hypertensive rats (Jabaris et al., 2015). Clinically, preliminary findings suggests that ibudilast has the potential to improve cognition in individuals with methamphetamine use disorder (MUD). Individuals with MUD randomized to ibudilast demonstrated improvements in sustained attention compared to those who received placebo during early abstinence (Birath et al., 2017). Taken together, it is hypothesized that ibudilast may promote neurocognitive recovery, such as response inhibition and attention, during early abstinence from alcohol through an anti-inflammatory mechanism.

Most recently, our laboratory conducted a 12-week randomized clinical trial of ibudilast, which enrolled treatment-seeking individuals with AUD. Primary aims were focused on drinking outcomes and findings showed reductions in alcohol over time, but individuals taking ibudilast did not have more favorable outcomes than those taking placebo. Neurocognitive performance was assessed prior to randomization and at 12 weeks post-treatment initiation using the NIH Toolbox Cognition Battery. For this present study, we explored changes in neurocognition

during the clinical trial of ibudilast. We tested whether participants randomized to ibudilast showed greater improvements across four domains of neurocognitive performance, as compared to placebo. In an exploratory set of analyses, we assessed whether neurocognitive performance at trial endpoint was related to recent alcohol use.

MATERIALS AND METHODS

Participant Subsample

For this project, only a subsample of the 102 randomized participants were included, as not all participants had the opportunity to complete the NIH Toolbox assessment (Hodes et al., 2013). Protocol changes were made during the COVID-19 pandemic to minimize participant and staff burden and face-to-face interactions. In addition, the NIH Toolbox was not collected at the end of the clinical trial due to lack of research staffs' access to the assessment battery (i.e., subscription ended). In total 36 randomized participants were excluded due to these protocol adjustments, resulting in 66 participants who had the opportunity to complete NIH Toolbox cognition battery at both baseline (i.e., pre-treatment) and 12-week follow-up (i.e., post-treatment).

NIH Toolbox

To examine potential ibudilast-related improvements in neurocognitive functioning over the course of the trial, trained study staff or graduate students administered a portion of the NIH Toolbox Cognition Battery at the screening or randomization visit and again at the 12-week follow-up visit (treatment endpoint).

Validation. The NIH Toolbox is a standardized cognitive battery developed through NIH's *Blueprint for Neuroscience Research initiative* (Hodes et al., 2013). The Cognition battery

measures domains of attention, inhibitory control, episodic memory, working memory, language, and processing speed (Weintraub, Bauer, et al., 2013). Validation and standardization procedures on the NIH Toolbox showed good convergent (range of r = .48 to r = .93) and discriminant (range of r = .05 to r = .30) validity when being compared to "gold standard" tests in the field of neurocognitive assessment (Weintraub, Dikmen, et al., 2013). Relevantly to this trial, this NIH battery showed high test-retest reliability (range of r = .72 to r = .96) and robust age-related performance results. The sample used to develop norms included diverse individuals aimed to match the U.S. demographics (Beaumont et al., 2013). This cognition battery has been implemented in several studies enrolling clinical samples with AUD/SUDs (Frazer et al., 2018; Jett et al., 2023; Sanborn et al., 2022; Sepe-Forrest et al., 2024), including the candidate's thesis which utilized data collected at baseline from a portion of the clinical trial participants (Meredith et al., 2020).

Scoring. The NIH Toolbox Cognition battery is a brief, multidimensional assessment tool lasting 45 to 60 minutes. The raw scores are conveniently normed electronically to provide three scores of performance: Age-Corrected Standard Scores, Uncorrected Standard Scores, and Fully Corrected Standard Scores. For the current project, the Fully Corrected Standard Scores (T-score metric, Mean = 50, Standard Deviation = 10) were used in which raw scores were normed based on a nationally representative sample, while adjusting for demographic variables, including age, biological sex, educational attainment, and race/ ethnicity (Weintraub, Dikmen, et al., 2013).

Neurocognitive Domains. Participants enrolled in the trial competed five subtests (**see Figure 4-1**). The administered subtests included: [1] List Sorting Working Memory Test, [2] Pattern Comparison Processing Speed Test, [3] Picture Sequence Memory Test, [4] Flanker Inhibitory Control and Attention Test, and [5] Oral Reading Recognition Test (pre-treatment timepoint

only). The List Sorting test measures working memory (i.e., processing and storage of information). Food and animal items were presented visually along with simultaneous audio recordings stating item name. Participants were then asked to repeat item names back in size order. The Pattern Comparison test measures processing speed. Participants were asked to respond as quickly as possible to indicate whether two simple pictures were the same or different. The Picture Sequence test measures episodic memory. Participants were presented with a sequence of events (visually and via audio recording) and then attempted to place scrambled pictures into the correct temporal order. The Flanker test measures attention and inhibitory control domain of executive functioning. Participants were asked to focus on the middle arrow stimulus while inhibiting attention to other nearly arrows during both congruent and incongruent trials. The Oral Reading test measures language, such as reading decoding skills and crystalized abilities. Words were presented visually, and participants were asked to pronounce and read words accurately.

Data Analytic Plan

Descriptive statistics including mean and standard deviation were computed for continuous variables, and frequencies and percentiles were computed for categorical variables to summarize baseline demographic, substance use and mental health data (**see Table 4-1**). Means, standard deviations, and percentiles for Fully Corrected Standard Scores of neurocognitive performance are presented in **Table 4-2**. Simple independent samples t-tests were conducted to determine whether neurocognitive performance scores for each domain significantly differed between medication groups at pre-treatment or post-treatment. Simple paired sample t-tests were conducted to determine whether neurocognitive performance scores for each of the four domains significantly changed from pre-treatment to post-treatment.

To evaluate the hypothesis that ibudilast treatment would improve neurocognitive performance during the 12-week trial, a linear regression model was run for each of the four neurocognitive performance scores. Specifically, each model included medication condition (ibudilast or placebo) as the focal predictor, post-treatment neurocognitive performance score as the outcome, and relevant covariate predictors centered at the grand mean (CGM; (Enders & Tofighi, 2007), namely biological sex (male or female), baseline AUDIT total score (continuous), and corresponding pre-treatment neurocognitive performance score (continuous). An exploratory analysis was conducted to assess whether recent drinking influenced neurocognitive performance. For this, DPDD in the two weeks prior to neurocognitive testing was added as a predictor variable in each of the four models.

All continuous variables were verified as normally distributed using the Shapiro-Wilk test for normality and visual inspection of Q-Q plot and distribution plots. After this inspection, DPDD at treatment endpoint was log-transformed (with an added constant of 1) to minimize skewness and kurtosis and account for a substantial number of zeros. The only outliers that emerged across continuous variables were three scores in the 97-99th percentile for the Picture Sequence Memory test at pre-treatment, but these values were not implausible, and the mean percentile for the sample was around the 50th percentile (i.e., 48%). Additional baseline covariates were considered, including cigarette smoking status, cannabis use days, and positive THC screen, along with rates of alcohol use (e.g., percent change in drinking from pre- to post-treatment, abstinent status at treatment endpoint). However, none of these variables were consistently correlated with the neurocognitive outcome scores and thus were not included in the final models.

RESULTS

Participants

The subsample of participants from the 12-week clinical trial with neurocognitive data (N = 66; ibudilast group n = 35; placebo group n = 31) had the following characteristics: average age of 45 years, 39% female, 50% identifying as White, 30% identifying as Black or African American, 9% identifying as mixed race, and 30% identifying as Latina/o/x (**see Table 4-1**). In the 30 days prior to their baseline visit, participants had an average of 7.87 drinking per drinking day, 5.48 drinks per day, and 6 days of cannabis use. Approximately a quarter (24%) of the sample had a positive urine toxicology screen for THC at baseline and 46% reported that they smoked cigarettes. The average DSM-5 AUD symptoms count was 7, indicating severe AUD at baseline. In regard to mental health symptomatology, participants reported an average BDI-II score of 10.5 points, average BAI score of 7 points, and average ISI score of 8.5 points. In addition, the average Oral Reading Recognition fully-corrected standard score at baseline, was 55 points (69th percentile).

Neurocognitive Standard Score Means

Pre-treatment neurocognitive scores for the full subsample (N = 66) ranged from the 17th percentile to 69th percentile (**see Table 4-2** for full details). Post-treatment neurocognitive scores (n = 50) ranged from the 27th percentile to 70th percentile. At both timepoints, participants scored lowest on the Flanker task, which measured inhibitory control and attention, with a mean standard score of 40.47 points at pre-treatment and 43.94 points at post-treatment. For the Oral Reading Recognition test collected only at pre-treatment, scores were slightly above normed averages, falling at 55.05 points. Aside from this measure of crystalized abilities, participants scored highest at pre-treatment on memory tasks, namely the Picture Sequence Memory task

measuring episodic memory at 49.68 points and List Sorting task measuring working memory at 49.58 points. At post-treatment, participants scored highest on the Picture Comparison task measuring processing speed at 54.10 points.

According to simple paired t-tests for participants providing data at both timepoints (n = 50), neurocognitive performance significantly improved from pre- to post-treatment for the three of the four domains tested, including those measuring processing speed, working memory, and inhibitory control and attention (**see Table 4-2**), *p's* < .05. According to simple independent samples t-tests, no performance differences were detected between medication groups at pre-treatment or post-treatment. (*p*'s > .120). At pre-treatment, the largest distinction between medication groups was for processing speed skills with groups differing by over 4.5 points or by 18 percentile points (ibudilast group performing higher). At post-treatment the largest distinction was for inhibitory control and attention with groups differing by over 2 points or by 7 percentile points (ibudilast group performing higher).

Linear Regression Models

Using a linear regression model predicting post-treatment neurocognitive performance on the Flanker task, medication condition did not significantly predict post-treatment scores, $t(45) = -0.61$, $p = .545$; **see Table 4-3**. The only significant covariate was pre-treatment Flanker performance, $t(45) = 5.87$, $p < .0001$. At post treatment, the model-adjusted estimated mean for placebo condition was 43.10 and the estimated mean for ibudilast condition was 44.60, after accounting for covariates (**see Figure 4-2**).

For the Pattern Comparison Processing Speed Task, medication did not significantly predict post-treatment score, $t(45) = 1.02$, $p = .312$; **see Table 4-3**. The only significant covariate was pre-treatment task performance, $t(45) = 4.37$, $p < .0001$. At post treatment, the model-

adjusted estimated mean for placebo condition was 56.04 and the estimated mean for ibudilast condition was 52.58, after accounting for covariates (**see Figure 4-2**).

For the List Sorting Working Memory task, medication did not significantly predict post-treatment score, $t(45) = 1.59$, $p = .120$; **see Table 4-3**. The only significant covariate was pre-treatment task performance, $t(45) = 6.84$, $p < .0001$. At post treatment, the model-adjusted estimated mean for placebo condition was 53.16 and the estimated mean for ibudilast condition was 50.20, after accounting for covariates (**see Figure 4-3**).

For the Picture Sequence Memory task, medication did not significantly predict post-treatment score, $t(45) = -0.03$, $p = .974$; **see Table 4-3**. The only significant covariate was pre-treatment task performance, $t(45) = 4.91$, $p < .0001$. At post treatment, the model-adjusted estimated mean for placebo condition was 51.95 and the estimated mean for ibudilast condition was 52.04, after accounting for covariates (**see Figure 4-3**).

For the second set of models that included recent (past 2 week) drinking as a predictor of performance, interpretation of findings did not meaningfully change (**see Table 4-4)**. In these models, log DPDD was not a significant predictor of post-treatment performance for any neurocognitive domain (p's > 165). However, for all neurocognitive domains aside from episodic memory, higher DPDD in the two weeks before testing was associated with lower neurocognitive performance.

DISCUSSION

In the present study, we tested the hypothesis that ibudilast, a neuroimmune modulator, would promote a faster recovery of neurocognitive functioning compared with placebo over the course of a 12-week randomized clinical trial that enrolled treatment-seeking participants AUD.

Neurocognitive performance across four domains was assessed using the NIH Toolbox Cognition Battery (Weintraub, Bauer, et al., 2013) at pre-treatment and again at treatment endpoint among a subsample of participants. In total, 66 participants completed the battery at pre-treatment and 50 participants also provided post-treatment data. Results from the primary models showed no significance differences in neurocognitive scores at post-treatment between medication conditions for the four domains assessed, including inhibitory control and attention, working memory, episodic memory, and processing speed. Averaging across medication groups, neurocognitive performance significantly improved during the trial for three domains: processing speed, working memory, and inhibitory control and attention. This change is likely attributable to participants' marked drinking reductions over the course of the trial in regard to both quantity and frequency of alcohol use. At baseline, participants were consuming an average of 8 DPDD and after 12 weeks were consuming 4 DPDD on average. Percent days abstinent from alcohol increased from around 25% to over 50%. However, exploratory models did not find recent drinking or reductions in drinking to significantly predict neurocognitive performance on the individual level. As noted in Chapter 3, depression levels also decreased during the trial, and improvements in mood can positively impact performance on these batteries.

Initial interest in this research question came from literature suggesting that medications targeting the immune system show promise in improving neurocognitive recovery among individuals with AUD, as neurocognitive processes like learning and memory are modulated by the immune system (Yirmiya & Goshen, 2011). Further, ibudilast's immune mechanisms in particular, such that it enhances BDNF (Derecki et al., 2010; Ziv et al., 2006) and inhibits PDE4 expression (Barad et al., 1998; Frey et al., 1993), are linked with memory, neurogenesis, and long-term potentiation. Yet, in this study we found no clear benefit of ibudilast treatment on

neurocognitive performance. The sample size for this analysis was smaller than anticipated and ultimately impacted our ability to detect group differences. Another reason for these null findings may be that this sample did not have clinically significant impairments in neurocognitive functioning prior to enrollment in the trial. This is evidenced by scores on tests of memory and processing speed falling around average (i.e., *t*-score = 50). The candidate's master's thesis supports the notion that neurocognitive deficits may be less apparent for outpatient samples with AUD, as most of the research in this area comes from inpatient samples (Meredith et al., 2020).

The largest group difference at post-treatment was found for the domain of inhibitory control and attention (Flanker Task), where the ibudilast group scored at the 30th percentile on average, while placebo group scored at the 23rd percentile according to the 'raw' fully-adjusted standard scores. Pre-treatment performance scores were low and very similar between groups, within 1-2 percentile points. A prior pilot study trialing ibudilast for methamphetamine use disorder (MUD) showed that participants taking ibudilast had greater improvements in sustained attention than those taking placebo (Birath et al., 2017). While group differences in the present study were modest and non-significant, it is noteworthy that participants performed much lower in this neurocognitive domain than others, approximately 1 standard deviation below the mean (~15th percentile), suggesting this is an area of relative weakness. Thus, participants may benefit most from enhanced inhibitory control skills. Difficulties with impulsivity and inhibition are known risk factors for AUD and are also thought to be impaired by chronic drinking. For example, research has found that moderate alcohol use can impact inhibitory control, even in young adulthood (Lopez-Caneda et al., 2014; Wetherill et al., 2013). Alternatively, results from the regression models, show that the largest medication group difference was for the task measuring working memory in which the placebo group actually performed better. By the end of

the trial, participants performed above normed averages on all subtests, aside from the Flanker task measuring inhibitory control and attention.

This study has several strengths including the administration of a well-validated assessment battery developed by the NIH and designed for longitudinal assessments. Given that this battery includes only one subtest per domain, however, it is less robust than other comprehensive neuropsychological batteries. However, the NIH Toolbox did appear to detect small improvements in neurocognitive performance during the trial, alongside drinking reductions. Thus, it would be helpful in future research to capture its sensitivity to alcohol-related impairment and recovery in a much larger sample of participants with AUD. For instance, a recent study using a subset of 346 participants from the Adolescent Brain and Cognitive Development study did detect performance differences on the NIH Toolbox measure of episodic memory between youth who did and did not use cannabis (Wade et al., 2024). Another strength of this study is the novelty of (1) assessing changes in neurocognitive functioning during a clinical trial for AUD and (2) exploring the potential for an immune treatment to improve performance. The sample was well-characterized and included a fairly diverse group of participants with heavy drinking patterns and who were seeking treatment for AUD. In regard to other limitations, the COVID-19 pandemic disrupted the collection of neurocognitive data during the trial. The goal was to collect data from approximately 100 participants, but the final sample size of participants with complete data was only 50 participants. Neurocognitive performance was only collected at one timepoint after starting study medication. Future trials may benefit from multiple repeated assessment (e.g,. monthly) to improve power and precision.

Table 4-1. Participant Characteristics by Treatment Condition for NIH Toolbox Subsample

Variable	Combined (N = 66)	Ibudilast (n = 35)	Placebo (n = 31)
Demographic			
Age (Years)	44.85 (10.09)	43.46 (9.18)	46.42 (10.97)
Sex (No., %)			
Male	40 (60.6%)	22 (62.9%)	18 (58.1%)
Female	26 (39.4%)	13 (37.1%)	13 (41.9%)
Race (No., %)			
White	33 (50.0%)	15 (42.9%)	18 (58.1%)
Black or African American	20 (30.3%)	10 (28.6%)	10 (32.3%)
Mixed Race	6 (9.1%)	5 (14.3%)	1 (3.2%)
Another Race	5 (7.8%)	4 (11.4%)	1 (3.2%)
American Indian or Alaska Native	1 (1.5%)	1 (2.9%)	0 (0.0%)
Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)
Asian	1 (1.5%)	0 (0.0%)	1 (3.2%)
Ethnicity (No., %)			
Not Hispanic/ Latina/o/x	46 (69.7%)	23 (65.7%)	23 (74.2%)
Hispanic/ Latina/o/x	20 (30.3%)	12 (34.3%)	8 (25.8%)
Alcohol and Substance Use			
Drinks per drinking day[a]	7.87 (6.05)	7.53 (4.61)	8.25 (7.42)
% heavy drinking days[a]	69.9% (34.6)	66.6% (36.7)	73.8% (32.1)
Drinks per day[a]	5.48 (3.66)	5.68 (4.24)	5.24 (2.93)
Cannabis use days[a]	6.05 (10.91)	5.29 (10.46)	6.90 (11.50)
Positive THC screen (No., %)	16 (24.2%)	8 (22.9%)	8 (25.8%)
Smokes cigarettes (FTND)	30 (45.5%)	14 (40.0%)	16 (51.6%)
PACS Total	14.55 (6.45)	15.06 (6.04)	13.97 (6.93)
SCID AUD symptom count*	**7.02 (1.97)**	**7.57 (1.95)**	**6.37 (1.83)**
AUDIT total	21.76 (7.26)	22.94 (8.26)	20.42 (5.78)
Relief /Habit Drinking (No., %)[b]	36 (54.5%)	19 (54.3%)	17 (54.8%)
Mental Health			
Oral Reading Recognition Score	55.05 (11.69)	55.06 (12.62)	55.03 (10.75)
BDI-II Total	10.49 (8.31)	10.31 (8.61)	10.68 (8.08)
BAI Total	7.02 (7.62)	8.60 (8.59)	5.23 (6.00)
ISI Total	8.45 (6.19)	9.46 (7.06)	7.35 (4.90)

* and **bold** indicate significance difference (p = .013); number missing = 1

Table 1 Legend:
Note. [a]based on Timeline FollowBack collected on the 30 days prior to baseline visit; [b]based on UCLA Reward Relief Habit Drinking scale (all other participants reported reward drinking); THC = tetrahydrocannabinol; PACS = Penn Alcohol Craving Scale; SCID = Structured Clinical Interview for the DSM-5; AUD = alcohol use disorder; AUDIT = Alcohol Use Disorders Identification Test; BDI-II = Beck Depression Inventory II; BAI = Beck Anxiety Inventory; ISI = Insomnia Severity Index;

Table 4-2. NIH Toolbox Battery Neurocognition Fully Corrected Standard T-Scores by Treatment Group and Timepoint

	Flanker Inhibitory Control & Attention Mean (Standard Deviation) Percentile		
Timepoint	*Ibudilast*	*Placebo*	*Total Sample*
Baseline (n = 66)	40.40 (8.45)	40.55 (9.97)	40.47 (9.13)
	16.85%	17.23%	17.03%
Baseline (n = 50)	41.39 (8.68)	40.36 (8.76)	40.94 (8.64)
	19.46%	16.75%	18.25%
Week 12 (n = 50)	44.86 (10.59)	42.77 (11.00)	43.94 (10.71)*
	30.36%	23.48%	27.23%

	Pattern Comparison Processing Speed Mean (Standard Deviation) Percentile		
Timepoint	*Ibudilast*	*Placebo*	*Total Sample*
Baseline (n = 66)	49.63 (14.40)	44.97 (12.47)	47.44 (13.63)
	48.52%	30.75%	39.90%
Baseline (n = 50)	50.93 (14.30)	45.18 (11.67)	48.40 (13.39)
	53.70%	31.49%	43.64%
Week 12 (n = 50)	53.79 (13.22)	54.50 (14.91)	54.10 (13.85)*
	64.77%	67.36%	65.91%

	Picture Sequence Memory Mean (Standard Deviation) Percentile		
Timepoint	*Ibudilast*	*Placebo*	*Total Sample*
Baseline (n = 66)	50.31 (10.13)	48.97 (7.50)	49.68 (8.95)
	51.24%	45.90%	48.72%
Baseline (n = 50)	50.07 (10.89)	49.23 (8.16)	49.70 (9.70)
	50.28%	46.93%	48.80%
Week 12 (n = 50)	52.50 (11.72)	51.36 (9.37)	52.00 (10.66)
	59.87%	55.41%	57.93%

	List Sorting Working Memory Mean (Standard Deviation) Percentile		
Timepoint	*Ibudilast*	*Placebo*	*Total Sample*
Baseline (n = 66)	50.46 (9.75)	48.58 (8.61)	49.58 (9.21)
	51.83%	44.35%	48.32%
Baseline (n = 50)	50.68 (10.27)	47.27 (8.90)	49.18 (9.75)
	52.71%	39.24%	46.73%
Week 12 (n = 50)	51.11 (9.21)	52.00 (9.30)	51.50 (9.17)*
	54.42%	57.93%	55.96%

	Oral Reading Recognition[+] Mean (Standard Deviation) Percentile		
Timepoint	*Ibudilast*	*Placebo*	*Total Sample*
Baseline (n = 66)	55.06 (12.61)	55.03 (10.75)	55.05 (11.69)
	69.36%	69.25%	69.32%
Baseline (n = 50)	56.00 (12.30)	54.59 (11.64)	55.38 (11.91)
	72.57%	67.69%	70.47%

*represents a significant positive change in performance from baseline to week 12 among participants with complete data (n = 50; per paired sample t-test *p*'s <.05)

[+]This assessment was administered at baseline only as it serves as a measure of crystalized abilities.

Note. No significant differences between medication groups were detected at baseline or at week 12 according to simple independent samples t-test *p*'s > .120)

Cognition Subtest	Domain Assessed
Flanker Test:	*Inhibitory control and attention*
List Sorting Working Memory Test:	*Working memory*
Pattern Comparison Test:	*Processing speed*
Picture Sequence Memory Test:	*Episodic memory*
Oral Reading Recognition:	*Reading decoding skills and crystalized abilities*

Figure 4-1. NIH Toolbox Cognition Battery subtests administered and corresponding domains

Table 4-3. Regression Model Results for Post-Treatment Neurocognition Fully Corrected T-Scores

Fixed Effects					
	b	**SE**	**DF**	**t-value**	**p-value**
Attention & Inhibitory Control Model					
Intercept	44.19	1.61	45	27.54	**< .0001***
Medication-Ibudilast vs. Placebo	-1.49	2.45	45	-0.61	.545
Pre-treatment performance (CGM)	0.82	0.14	45	5.87	**< .0001***
Pre-treatment AUDIT (CGM)	-0.05	0.16	45	-0.33	.744
Sex (CGM)	-2.20	2.45	45	-.90	.374
Processing Speed Model					
Intercept	52.17	2.22	45	23.50	**< .0001***
Medication-Ibudilast vs. Placebo	3.46	3.39	45	1.02	.312
Pre-treatment performance (CGM)	0.56	0.13	45	4.37	**< .0001***
Pre-treatment AUDIT (CGM)	-0.17	0.22	45	-0.81	.423
Sex (CGM)	6.17	3.42	45	1.80	.078
Working Memory Model					
Intercept	50.51	1.20	45	42.19	**< .0001***
Medication-Ibudilast vs. Placebo	2.96	1.87	45	1.59	.120
Pre-treatment performance (CGM)	0.69	0.10	45	6.84	**< .0001***
Pre-treatment AUDIT (CGM)	-0.08	0.12	45	-0.68	.499
Sex (CGM)	1.12	1.86	45	0.60	.549
Episodic Memory Model					
Intercept	52.01	1.72	45	30.18	**< .0001***
Medication-Ibudilast vs. Placebo	-0.09	2.63	45	-0.03	.974
Pre-treatment performance (CGM)	0.66	0.13	45	4.91	**< .0001***
Pre-treatment AUDIT (CGM)	0.13	0.17	45	0.74	.464
Sex (CGM)	1.17	2.63	45	0.45	.657

Note. ***p* < .0001**; AUDIT = Alcohol Use Disorders Identification Test; CGM = centered at grand mean

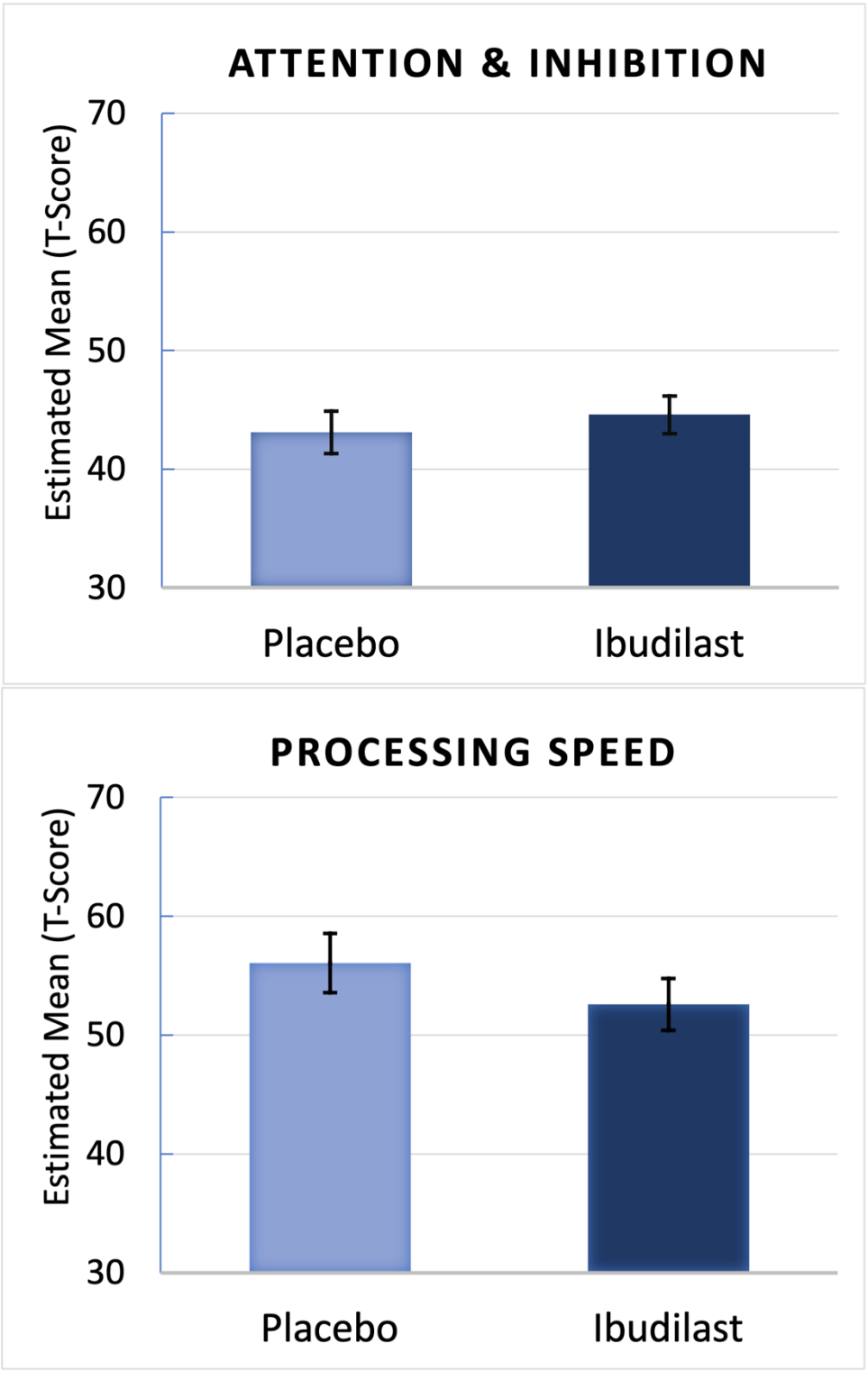


Figure 4-2. Model-adjusted standard scores by treatment condition for neurocognitive domains measuring attention and inhibitory control (Flanker Task) and processing speed (Pattern Comparison). Treatment condition did not significantly predict differences in post-treatment performance (*p*'s > .120)

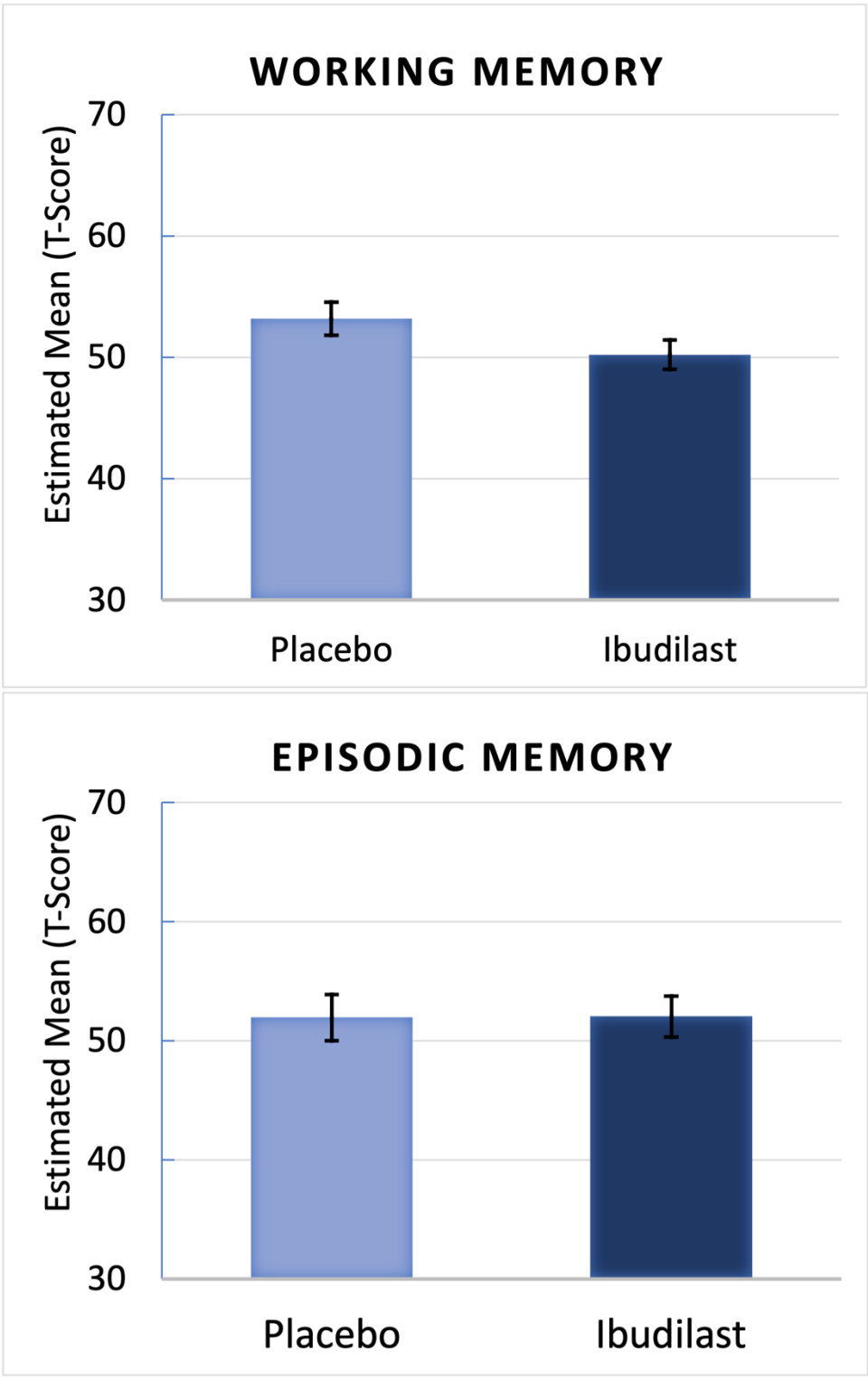


Figure 4-3. Model-adjusted standard scores by treatment condition for neurocognitive domains measuring working memory (List Sorting) and episodic memory (Picture Sequence Memory). Treatment condition did not significantly predict differences in post-treatment performance (*p*'s > .300)

Table 4-4. Exploratory Regression Model Results for Post-Treatment Neurocognition Fully Corrected T-Scores with Recent Alcohol Use Added as Predictor

Fixed Effects					
	b	**SE**	**DF**	**t-value**	**p-value**
Attention & Inhibitory Control Model					
Intercept	44.26	1.64	43	27.02	**< .0001***
Medication-Ibudilast vs. Placebo	-1.53	2.54	43	-0.60	.552
Pre-treatment performance (CGM)	0.81	0.14	43	5.69	**< .0001***
Pre-treatment AUDIT (CGM)	-0.05	0.16	43	-0.31	.758
Sex (CGM)	-1.98	2.57	43	-.77	.445
Log DPDD Weeks 10 – 12 (CGM)	-0.91	1.69	43	-0.54	.594
Processing Speed Model					
Intercept	52.37	2.25	43	23.24	**< .0001***
Medication-Ibudilast vs. Placebo	3.12	3.51	43	0.89	.380
Pre-treatment performance (CGM)	0.54	0.13	43	4.21	**.0001***
Pre-treatment AUDIT (CGM)	-0.17	0.22	43	-0.81	.423
Sex (CGM)	6.88	3.58	43	1.92	.061
Log DPDD Weeks 10 – 12 (CGM)	-2.24	2.30	43	-0.97	.336
Working Memory Model					
Intercept	50.58	1.22	43	41.36	**< .0001***
Medication-Ibudilast vs. Placebo	2.67	1.96	43	1.36	.180
Pre-treatment performance (CGM)	0.68	0.10	43	6.56	**< .0001***
Pre-treatment AUDIT (CGM)	-0.09	0.13	43	-0.70	.486
Sex (CGM)	1.46	1.96	43	0.75	.458
Log DPDD Weeks 10 – 12 (CGM)	-0.73	1.25	43	-0.58	.564
Episodic Memory Model					
Intercept	51.85	1.71	43	30.39	**< .0001***
Medication-Ibudilast vs. Placebo	-0.03	2.66	43	-0.01	.990
Pre-treatment performance (CGM)	0.66	0.13	43	4.96	**< .0001***
Pre-treatment AUDIT (CGM)	0.12	0.17	43	0.70	.489
Sex (CGM)	0.61	2.68	43	0.23	.820
Log DPDD Weeks 10 – 12 (CGM)	2.50	1.77	43	1.41	.165

Note. ***$p \leq .0001$**; AUDIT = Alcohol Use Disorders Identification Test; DPDD = drinks per drinking day (log transformed); CGM = centered at grand mean

OVERALL DISCUSSION

Less than 8% of individuals with past-year AUD received any alcohol treatment and much fewer received evidence-based care, such as FDA-approved pharmacotherapies. Among those receiving front-line pharmacological and behavioral treatments, relapse and heavy drinking is common, as existing interventions are only moderately effective. As such, the development of novel and more efficacious treatments is a high research priority in the alcohol research field. Relatedly, the body of literature implicating the critical role of the immune system in the development and maintenance of addiction has grown dramatically in the past few decades and has led to interest in the use of immune compounds as treatments for AUD. Despite progress in understanding the connection between the peripheral and neuroimmune system with AUD, few clinical trials testing immune treatments for AUD have been conducted to date. As such, little is known about which of these compounds or interventions might be most effective in human samples with AUD and how these immune therapies influence various salient maintenance factors of AUD, such as reward, craving, executive function, and negative emotionality.

To address this research gap, this dissertation pursued an examination of neuroimmune treatments for AUD across four chapters, including one qualitative review, followed by three empirical projects drawing from randomized controlled trials testing a neuroimmune modulator, ibudilast, for the treatment AUD. A common thread throughout this dissertation was a focus on exploring clinical mechanism of immune treatments. This approach was informed by a dominant neurobiological therapy of addiction, the allostatic model, which suggests that individuals with AUD experience three stages of the addiction cycle: (1) binge and intoxication, (2) withdrawal/ negative affect, and (3) preoccupation/ anticipation. To bridge from neurobiology to clinical psychology, the Addictions Neuroclinical Assessment (ANA; (Kwako et al., 2016) served as a

relevant transdiagnostic framework. This framework complements the phases of the addiction cycle to help guide measurement of relevant clinical domains among individuals with AUD. As such, Chapters 2 - 4 involved secondary data analyses exploring ibudilast's potential to alter maintenance factors falling into the three phases of the addiction cycle, namely alcohol craving/ reward, negative emotionality, and neurocognitive functioning. The ultimate goal of this work is to improve the field's understanding of how immune treatments might successfully alter factors sustaining AUD among individuals with diverse symptom profiles and backgrounds.

In Chapter 1, we started with a comprehensive, qualitative review of both existing preclinical and clinical literature on the development of immune treatments for AUD. We sought to provide a translational perspective supporting the safe and effective clinical application of candidate immune therapies for AUD. For instance, we covered topics such as safety profiles, approval status, and highlighted existing findings from completed trials. We were surprised to find that very few clinical trials had already been completed. Additionally, the majority of these trials were pilot studies enrolling a small sample of non-treatment seeking individual with AUD. Because of this, we added a list of ongoing clinical trials within each section, to allow readers to follow-up with those trial findings as they are reported. The lack of existing clinical research data increased our interest in making the most of the valuable randomized controlled trial data available in our laboratory to better understand how these immune medications may work on the psychological level. The review also covered translational limitations, such as the fact that animal studies typically administer compounds via an injection, which results in much higher blood levels than is often feasible in humans. We discussed the need to use novel screening methods, such as capturing potential treatment effects on mood, neurocognition, biomarkers of inflammation/ immune signaling, and withdrawal symptoms. We concluded the review by

highlighting the most promising interventions from our synthesis of the literature, including apremilast, ibudilast, fenofibrate, *N*-acetylcysteine, and mind-body therapies.

To explore arguably one of the most well-researched and important antecedent of alcohol use, i.e., craving, as a clinical mechanism of immune interventions, Chapters 2 and 3 of the dissertation utilized data from two clinical trials of ibudilast for AUD. In a two-week experimental medicine trial, which enrolled 52 non-treatment seeking participants with AUD, electronic daily diary reports were collected to capture individuals' subjective response to alcohol use in real-world contexts. Chapter 2 emphasized the merit in using naturalistic reports, such that they serve as ecologically valid measures of craving and drinking in participants' own environment, where powerful, personal cues and reinforcers of drinking are present. Across 653 daily diary assessments using multilevel models, results showed that ibudilast blunted alcohol-induced increases in craving during drinking episodes when compared to the placebo group. This suggested that ibudilast may exert its effects on drinking outcomes by diminishing one's desire to continuing drinking during a drinking episode. In this sense, alcohol is less rewarding and reinforcing. These findings are supported by neuroimaging results from the same trial, where participants randomized to ibudilast had blunted neural activation in the ventral striatum following exposure to visual alcohol cues (Grodin et al., 2021). However, ibudilast did not alter subjective feelings of stimulation or sedation during naturalistic drinking episodes.

Chapter 3 focused on data derived from a recently completed 12-week clinical pharmacotherapy trial of ibudilast enrolling 102 treatment-seeking individuals with AUD. We hoped to show the durability of ibudilast's beneficial effects on craving. To do so, we collected monthly clinical measures of tonic alcohol craving and tested whether the ibudilast group had significantly steeper declines in craving over the 3-month treatment period. Findings supported

this hypothesis and by treatment endpoint, participants on ibudilast were estimated to have Penn Alcohol Craving Scores 3 points lower than the placebo group. As such, this dissertation supports consistent evidence that ibudilast reduces multiple facets of alcohol craving and reward and this likely represent a central mechanism of action.

Continuing, to explore negative affectivity as a target for immune treatment, we similarly tested multiple measures of emotionality in Chapters 2 and 3. Using the same daily diary approach, we tested whether ibudilast altered acute alcohol-related changes in positive and negative mood. Experimental methods have shown that alcohol initially enhances positive mood and reduces negative mood, and this can serve as a strong driver of chronic alcohol use. While alcohol-induced mood alterations were feasibly replicated through the daily reports, ibudilast did not dampen these changes in mood among the full sample. Through an exploratory aim, however, we did find that ibudilast blunted alcohol-induced increases in positive mood among a subsample of participants-- those who were motivated to drink for positive reinforcement (i.e., to feel good). Consistent with Chapter 2's main results on mood, ibudilast did not improve levels of general mood across the two-week medication period, as reported in the primary trial manuscript (Grodin et al., 2021).

The introduction of Chapter 3 provides a detailed review on the shared immune correlates among negative affective states and addiction. For instance, anti-inflammatory therapies have been shown to reduce negative mood in multiple clinical trials for other psychological disorders. Thus, using data from the 12-week clinical trial of ibudilast, we tested whether the study medication reduced symptoms of depression and anxiety over three months. Across analyses testing changes in mood, there were no consistent differences across treatment conditions. Both groups had modest reductions in symptoms of depression and relatively stable levels of anxiety.

It is notable that depression levels for participants enrolled in our laboratory's trials have been minimal with BDI-II scores ranging from 8–11 points at pre-treatment (below cutoff for mild depression). We expected participants enrolled in 12-week trial to have higher levels of depression given that they identified as treatment-seeking for AUD, and treatment-seeking samples consistently have greater AUD severity and higher rates of comorbid mental health conditions (Venegas et al., 2021). Yet only 7% of participants met diagnostic criteria for a current major depressive episode. It would be interesting to see whether immune treatment influences mood in a sample with comorbid depression and AUD, with the added support of a behavioral intervention. Tracking changes in measures of positive mood and well-being, such as eudaimonia, would also be worthwhile and help inform a broader definition of recovery.

Lastly, Chapter 4 represents an exploratory aim assessing whether ibudilast improved neurocognitive functioning during the 12-week trial. Neurocognitive dysfunction is one of the harms associated with AUD, particularly severe AUD. Impaired cognition is clinically relevant as it interferes with one's ability engage in self-regulation, flexible decision-making, and work toward long-term goals. As such, the field has a strong interest in therapeutics that can improve these functions to promote abstinence. There is a wealth of basic science literature documenting the influence of the immune system on neurocognitive processes like learning and memory and disease progression. We were thus enthusiastic to assess whether a neuroimmune modulator might promote faster recovery of neurocognitive performance among individuals actively reducing their drinking. To test this hypothesis, the NIH Toolbox Cognition Battery was administered to a subset of participants enrolled in the 12-week clinical trial at pre- and post-treatment. Across four domains of neurocognition including inhibitory control / attention, working memory, processing speed, and episodic memory, we found no significant performance

differences between treatment conditions at week 12. Interestingly, participants across groups tended to have higher performance scores by study endpoint, suggesting some recovery of premorbid neurocognitive functions with drinking reductions. Our results were limited by a small sample size with only 50 participants providing data at both timepoints. Assessment of neurocognitive data in future trials would benefit from more frequent assessments (e.g., monthly) to improve precision and power. Collection of multimethod measures within each domain, such as self-report survey of impulsivity in addition to a behavioral measure, would improve our ability to draw strong conclusions.

Finally, we wish to highlight future directions for this research. It will be important to contextualize results from Chapters 3 and 4 with side effects and medication adherence data from the primary trial. As more clinical data emerges in the alcohol field, it is critical to better understand the time course of immune treatment effects on drinking and clinical outcomes. For instance, when can researchers or patients expect to see a clinical benefit? When considering SSRIs, the full therapeutic effects can take 1-2 months, while medications like naltrexone have much faster effects, with therapeutic effectiveness thought to be achieved within hours of the first dose (Center for Substance Abuse Treatment, 2009). Sensitivity analyses are also planned for Chapters 3 and 4 to better capture how trial dropout may have impacted findings. In Chapter 3 for example, the missing data handling methods assumed a conditionally missing at random process. While this is a standard assumption, it necessitates the untestable proposition that uncaptured clinical data (i.e., post-dropout) carry no additional information about missingness (Enders et al., 2020). To explore this issue, we will consider a missing not at random processes that links dropout to the unseen score values. This is notable given the robust placebo response,

particularly among males and higher dropout rates in the placebo group. Lastly, we will conduct a modified-intent-to-treatment analysis.

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