Protocol Title: An Investigation of the Antidepressant Effects of the Glycine Receptor Antagonist AV-101 (4-chlorokynurenine) in Major Depressive Disorder

Abbreviated Title: Antidepressant effect of AV101

Protocol Number: 15-M-0151

1. PRÉCIS

a. Objective

Modulation of the NMDA receptor (NMDAR) complex or other components of glutamatergic signaling is likely involved in improvement of depressive symptoms and related constructs/dimensions of observable behavior and neurobiological measures. Current standard monoaminergic pharmacological approaches for major depressive disorder (MDD) have proven to be only modestly effective during acute depressive episodes. We have systematically tested different glutamatergic modulators in patients with mood disorders in order to develop improved therapeutics. We found that the NMDAR antagonist ketamine produces rapid antidepressant effects in patients with treatment-resistant depression (in MDD and Bipolar Disorder). However, despite being highly efficacious, the proof of concept ketamine produces psychotomimetic effects.

In the present protocol, we aim to evaluate a new glutamate-mediated mechanism associated with antidepressant efficacy by targeting the glycine receptor within the NMDA receptor. Targeting the glycine co-agonist site of the (NMDA) receptor may bypass potential adverse effects that occur with ketamine without affecting the robust efficacy observed. This may then result in the "glutamate surge" that has been associated with the rapid acting antidepressant effects of ketamine.

The present Phase 2 proof-of-concept study is designed to evaluate the antidepressant effects of AV-101 (*L-4-chlorokynurenine or 4-Cl- KYN*) in MDD; this is a synthetic compound which is enzymatically converted into the selective glycine/NMDAR antagonist 7-chlorokynurine (7-Cl-KYNA) after crossing the blood brain barrier (BBB) and then reaching brain glial cells. In animal models of depression, 4-Cl-KYNA (AV-101) induced acute and prolonged antidepressant-like effects without exhibiting ketamine-like side effects as determined by the drug discrimination, conditioned place preference, and pre-pulse inhibition tests.

We will also evaluate the neurobiological mechanisms involved in the antidepressant response to AV-101. We expect that this effect may modulate glutamate transmission and reverse the clinical symptoms of depression. The demonstration that a glycine-antagonist produces antidepressant effects without psychotomimetic side effects would support the therapeutic relevance of the glycine site of the NMDAR and could direct the development of novel drug targets for the treatment of depression.

b. Design

Male and female patients, ages 18 to 65 years, with a diagnosis of MDD, currently in an episode of major depression, will be recruited for this study. This study will consist of a randomized, double-blind crossover administration of either the glycine receptor antagonist AV-101 (1,080 or 1,440 mg/day given orally) or placebo for 2 weeks. The study will assess the efficacy in improving overall depressive symptomatology and tolerability of AV-101 in treatment-resistant MDD. Other aims of the study include: 1) determining whether changes in brain neurochemicals (e.g. glutamate) and peripheral biomarkers obtained via MRS and cerebrospinal fluid (CSF) correlate with antidepressant response (decrease in Hamilton Depression Rating Scale (HDRS) total scores) to AV-101 in patients with treatment-resistant MDD, and 2) examine other potential biomarkers of response.

c. Outcome Measures

Primary: Hamilton Rating Scale (HDRS) total score.

Secondary: Proportion of subjects achieving remission (HDRS ≤7) and response (≥50% reduction from baseline in HDRS total score); change from baseline in Hamilton Anxiety Rating Scale (HAM-A), Montgomery-Asberg Depression Rating Scale (MADRS), and the Columbia Suicide Severity Rating Scale (C-SSRS) total scores. Surrogate biomarkers of drug effect/response include: changes in prefrontal glutamate levels measured with 7T ^{1}H-MRS.

2. STATISTICAL ANALYSIS

a. Analysis of data/study outcomes

The double blind will be broken and the randomization status of each participant will be revealed only after the completion of this study in all 20 individuals. An interim **blinded** analysis may be performed no more than one time (for example, at the request of the PI's Board of Scientific Counsellors) to inform ongoing scientific efforts and productivity of the Branch; however, this interim analysis will not serve as the basis for decisions to change study procedures or to terminate the study.**(Specific Aim 1)** To assess the efficacy of AV-101 compared with placebo in improving overall depressive symptomatology over a two-week course of treatment in patients with treatment-resistant MDD currently experiencing a major depressive episode.

The primary aim will be accomplished by assessing the efficacy of two weeks of oral AV-101 (1,080 or 1,440 mg/day) compared with placebo in improving overall depressive symptomatology. The primary assessment of efficacy is a comparison of HDRS scores between treatment groups.

The difference between AV-101 and placebo will be examined using a linear mixed model with restricted maximum likelihood estimation where drug and time are within subjects factors. Drug and time main effects and their interaction will be included in the model as within subjects factors. Baseline scores for each phase will be used as a time-dependent covariate. A random intercept and subject effect will be included if they contribute significantly to the model. Schwarz's Bayesian criteria will be used to examine the most appropriate covariance structure prior to formal analyses, where a compound symmetry structure will be used in another is not preferred. Bonferroni corrected post hoc tests will be used to follow up on a significant drug by time interaction. Cohen's d will be calculated to describe the effect size.

Additional follow-up analyses may examine the potential for carryover effects within the crossover study. First, an additional phase variable may be added to the original statistical model. Second, results in the first phase of the study may be examined separately to understand whether any drug effect remains in a parallel-like design.

Secondary analyses will be conducted in the same manner for BPRS, CADSS, C-SSRS, HAM-A, MADRS, PANAS, SHAPS, SSI, TEPS, and YMRS. A similar statistical model will be used to examine secondary measures. Bonferroni-like corrections will be used to determine the significance of secondary analyses.

Criteria for significance

Significance will be evaluated at $p \leq .05$, two-tailed.

Analyses will be based on the intent-to-treat (ITT) principle where all randomized subjects who receive baseline and at least one post-baseline assessment will be included. A subject will be considered a responder if the HDRS total score has decreased by 50% or more from baseline to endpoint. Response rates will be analyzed using McNemar's test for repeated measures categorical outcomes.

Safety and tolerability assessments will be based on the safety analysis set which will include all randomized subjects who are given study treatment.

The incidence rate of adverse events will be tabulated by preferred term and system organ class; serious adverse events, adverse events leading to death, and adverse events leading to withdrawal of subjects will be tabulated for each treatment group.

Analyses of surrogate neurobiological markers

Multiple logistic regression models (for categorical outcome, response vs. non-response) will evaluate candidate biological markers for independent association with outcomes. For these models, we will compute robust standard errors or associated 95% confidence intervals.

b. Power analysis

Previous studies by our group (see **Background**) demonstrated improvement in depression at 72 hours with an effect size of d=0.67. Assuming α=.05, two-tailed, 20 patients with MDD would provide 81% power to detect an effect of the same size as previously reported with a double-blind crossover study. We are primarily interested in detecting drugs with a large effect size that occurs within a short period of time (≤72 hs) as there are already many antidepressants with small effect sizes after 2 weeks of treatment. This is the reason for not studying a much larger number of patients; in addition, the current study design uses a crossover design.

c. Accrual number

If we assume that 20% of subjects will either not complete the drug free period or will drop out during the crossover double-blind portion of the study, then 25 MDD patients need to be recruited.