Abbreviated Title: Antidepressant Effect of TS-161

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Title: An Investigation of the Antidepressant Effects of the mGlu2/3 receptor antagonist TS-161 in Treatment-Resistant Depression

NIH Principal Investigator: Carlos A. Zarate, MD
Experimental Therapeutics and Pathophysiology Branch
National Institute of Mental Health
Building 10, Rm 7-5342
9000 Rockville Pike
Bethesda, MD 20892
Phone: 301-451-0861
E-mail: zaratec@mail.nih.gov

Investigational Agents:

DRUG NAME:	TP0473292 (TS-161)
IND NUMBER:	153429
SPONSOR:	NIMH
MANUFACTURER:	Taisho Pharmaceutical Co., Ltd

Responsible Data Safety Monitoring Board (DSMB):

NIMH-IRP Data and Safety Monitoring Board

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

TABLE OF CONTENTS

1 PROTOCOL SUMMARY

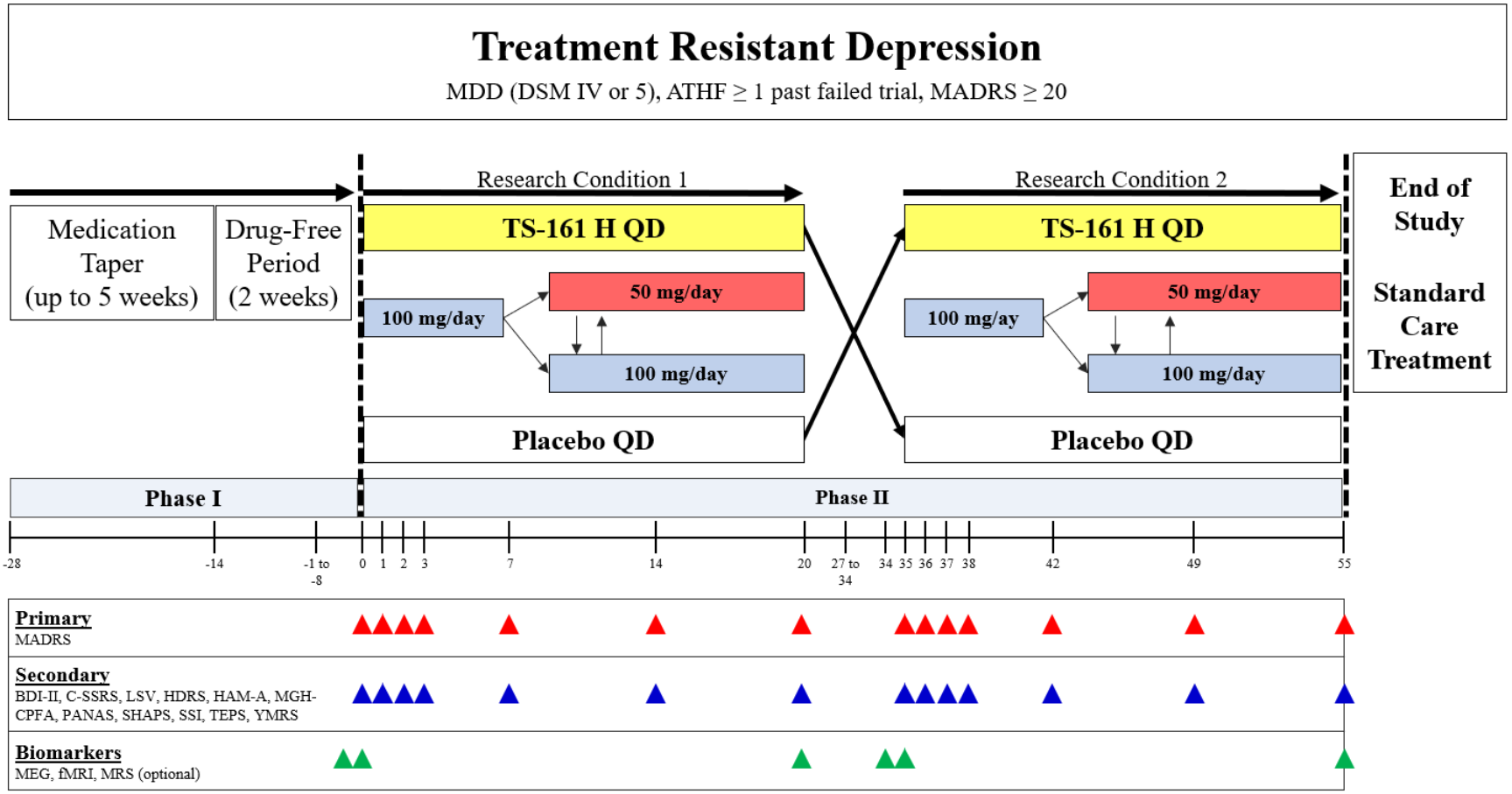
1.1 SYNOPSIS

TITLE:	An Investigation of the Antidepressant Effects of the mGlu2/3 receptor antagonist TS-161 in Treatment-Resistant Depression
STUDY DESCRIPTION:	This is a randomized, double blind, placebo-controlled, crossover, single–site study. This experimental study will assess the efficacy and safety of three weeks of 50 to 100 mg/day of TS-161, an mGlu2/3 receptor antagonist prodrug.
OBJECTIVES:	Primary Objective: The primary objective is to evaluate the ability of the mGlu2/3 receptor antagonist prodrug, TS-161, to improve overall depressive symptomatology in subjects with Major Depressive Disorder (MDD). The efficacy of a three-week course of TS-161 will be compared to three weeks of placebo in a crossover study. The Montgomery-Asberg Depression Rating Scale (MADRS) will serve as the main outcome measure. Secondary Objectives: 1. To evaluate the antidepressant efficacy of TS-161 at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing compared to placebo in a crossover study, as assessed by change from baseline on MADRS total scores. 2. To determine whether TS-161 will demonstrate a superior antidepressant efficacy compared to placebo in a crossover study, as assessed by the proportion of subjects in remission (defined as MADRS total score ≤10). 3. To determine whether TS-161 will demonstrate a superior antidepressant response compared to placebo in a crossover study, as assessed by the proportion of subjects achieving response (defined as a ≥50% reduction from baseline in MADRS total score). 4. To evaluate the antisuicidal ideation effects of TS-161 at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing compared to placebo in a cross over study, as assessed by change from baseline on item 10 (suicidality) of the MADRS, the Columbia Suicide Severity Rating Scale (C-SSRS), and the Scale for Suicidal Ideation (SSI). 5. To investigate the effects of TS-161 on mood, anxiety, and anhedonia symptoms at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing compared to placebo in a cross over study, as assessed by change

	from baseline on the Hamilton Depression Rating Scale (HDRS), Hamilton Anxiety Rating Scale (HAM-A), Positive and Negative Affect Schedule (PANAS), Snaith–Hamilton Pleasure Scale (SHAPS), and the Temporal Experience of Pleasure Scale (TEPS) scales. 6. To assess the safety and tolerability of a three-week course of TS-161 compared to placebo in a crossover study, by incidence of adverse events (AEs) and total scores using the Clinician Administered Dissociative States Scale (CADSS), Young Mania Rating Scale (YMRS), the Brief Psychiatric Rating Scale (BPRS), vital signs, changes in clinical laboratory evaluations, and electrocardiograms (ECGs).
ENDPOINTS:	<u>Primary Endpoint:</u> • Change from baseline in the MADRS total score <u>Secondary Endpoints:</u> • Proportion of subjects in remission (defined as MADRS total score ≤10). • Proportion of subjects with response (defined as ≥50% reduction from baseline in MADRS total score). • Change from baseline in HDRS, BPRS, CADSS, C-SSRS, HAM-A, PANAS, SHAPS, SSI, TEPS, YMRS total scores. • Incidence and nature of adverse events; vital signs; weight and body mass index (BMI) changes; physical examination changes; clinical laboratory evaluations; ECG. <u>Surrogate Markers of Drug Effect, Target Engagement, and Antidepressant Response</u> • Change in magnetoencephalography (MEG) spectral power (gamma power). • Change in glutamate levels in magnetic resonance spectroscopy (MRS). • Change in resting and task based functional connectivity in fMRI. • Change in peripheral biomarkers.
STUDY POPULATION:	Up to 25 individuals with Treatment-Resistant Depression (TRD) between the ages of 18 and 65 will be enrolled in the study. Participation will not be limited by gender identity or geographic location.
PHASE:	Phase 2 Proof-of-Concept (POC) Clinical Trial
DESCRIPTION OF SITES/FACILITIES	Research will be conducted at the NIH Clinical Center in Bethesda, Maryland, United States.

ENROLLING PARTICIPANTS:	
DESCRIPTION OF STUDY INTERVENTION:	This study will consist of a randomized, double-blind crossover administration of the mGlu2/3 receptor antagonist prodrug TS-161 (50 to 100 mg/day given orally) and placebo.
STUDY DURATION:	The estimated duration of this protocol is 36 months.
PARTICIPANT DURATION:	The total duration of the study, which consists of two phases, is 12 to 16 weeks.

1.2 SCHEMA


Treatment Resistant Depression
MDD (DSM IV or 5), ATHF ≥ 1 past failed trial, MADRS ≥ 20
Medication Taper (up to 5 weeks)
Drug-Free Period (2 weeks)
Research Condition 1
TS-161 H QD
100 mg/day
50 mg/day
100 mg/day
Placebo QD
Research Condition 2
TS-161 H QD
100 mg/ay
50 mg/day
100 mg/day
Placebo QD
End of Study
Standard Care Treatment
Phase I
Phase II
-28
-14
-1 to -8
0
1
2
3
7
14
20
27 to 34
34
35
36
37
38
42
49
55
Primary
MADRS
Secondary
BDI-II, C-SSRS, LSV, HDRS, HAM-A, MGH-CPFA, PANAS, SHAPS, SSI, TEPS, YMRS
Biomarkers
MEG, fMRI, MRS (optional)

1.3 SCHEDULE OF EVENTS

Timepoint[i]	Procedures
Phase I	**Medication Taper, Drug-Free Period, and Baseline Assessments**
Day -28 to Day -15	Medication Taper
Day -14 to Day -1	Drug-Free Period
Day -8 to Day -2	Clinical Rating Scales[ii] Neurocognitive Battery *Imaging :* Structural neuroimaging studies (if clinically indicated, unless completed within the past 12 months), fMRI/MRS, MEG
Day -1	Clinical Rating Scale *Sample Collection:* Safety Labs [iii,iv] AE Assessments
Phase II	**Research Conditions 1 & 2**
Research Condition 1	
Day 0	
-60 to 0 min	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood *Clinical Evaluations:* Orthostatic Vital Signs, Height and Weight
0 min	Study Drug Administration: TS-161/ Placebo (100mg)
+60 min (1 hrs.)	*Sample Collection:* PK Blood
+120 min (2 hrs.)	*Sample Collection:* PK Blood *Imaging:* MEG

Timepoint[i]	Procedures
	Clinical Evaluations: Orthostatic Vital Signs
+180 min (3 hrs.)	*Sample Collection:* PK Blood
+230 min	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood
+240 min (4 hrs.)	*Sample Collection:* PK Blood *Imaging:* fMRI/MRS *Clinical Evaluations:* Orthostatic Vital Signs
+6 hrs.	*Sample Collection:* PK Blood *Clinical Evaluations:* Orthostatic Vital Signs
+8 hrs.	*Sample Collection:* PK Blood
+10 hrs.	*Sample Collection:* PK Blood
+12 hrs.	*Sample Collection:* PK Blood
Day 1	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg)[v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 2	Clinical Rating Scales[ii] AE Assessments Neurocognitive Battery *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg)[v] *Clinical Evaluations:* Orthostatic Vital Signs

Timepoint[i]	Procedures
Day 3	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 4	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 5	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 6	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 7	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood, Safety Labs[iii, エラー! ブックマークが定義されていません。] *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs, Physical Exam
Day 8	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 9	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 10	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 11	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 12	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 13	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v]

Timepoint[i]	Procedures
	Clinical Evaluations: Orthostatic Vital Signs
Day 14	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood, Safety Labs エラー! ブックマークが定義されていません。,エラー! ブックマークが定義されていません。 *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg)[v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 15	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 16	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 17	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 18	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 19	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Sample Collection*: Urine pregnancy test *Clinical Evaluations:* Orthostatic Vital Signs
Day 20	*Sample Collection:* Research Blood, PK Blood, Safety Labs[iii] Neurocognitive Battery *Imaging:* fMRI/MRS, MEG *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs, Weight & Height, ECG
Day 21 to Day 34	Washout Period
Day 21	Clinical Rating Scales[ii] AE Assessments

Timepoint[i]	Procedures
Day 28 to Day 33	Neurocognitive Battery *Imaging:* Clinical MRI, fMRI/MRS, MEG
Day 34 エラー! ブックマークが定義されていません。	Clinical Rating Scales[ii] *Sample Collection:* Safety Labs [iii, エラー! ブックマークが定義されていません。] AE Assessments
Research Condition 2	
Day 35	
-60 to 0 min	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood *Clinical Evaluations:* Orthostatic Vital Signs, Height and Weight
0 min	*Study Drug Administration:* TS-161/ Placebo (100mg)
+60 min (1 hrs.)	*Sample Collection:* PK Blood
+120 min (2 hrs.)	*Sample Collection:* PK Blood *Imaging:* MEG *Clinical Evaluations:* Orthostatic Vital Signs
+180 min (3 hrs.)	*Sample Collection:* PK Blood
+230 min	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood
+240 min (4 hrs.)	*Sample Collection:* PK Blood *Imaging:* fMRI/MRS *Clinical Evaluations:* Orthostatic Vital Signs
+6 hrs.	*Sample Collection:* PK Blood *Clinical Evaluations:* Orthostatic Vital Signs

Timepoint[i]	Procedures
+8 hrs.	*Sample Collection:* PK Blood
+10 hrs.	*Sample Collection:* PK Blood
+12 hrs.	*Sample Collection:* PK Blood
Day 36	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 37	Clinical Rating Scales[ii] AE Assessments Neurocognitive Battery *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 38	Clinical Rating Scales[ii] AE Assessments *Sample Collection*: Research Blood *Study Drug Administration*: TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 39	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 40	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 41	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs

Timepoint[i]	Procedures
Day 42	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood, Safety Labs[iii,エラー! ブックマークが定義されていません。] *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs, Physical Exam
Day 43	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 44	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 45	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 46	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 47	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 48	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 49	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Research Blood, PK Blood, Safety Labs[iii,エラー! ブックマークが定義されていません。] *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) [v] *Clinical Evaluations:* Orthostatic Vital Signs
Day 50	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 51	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 52	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg)

Timepoint[i]	Procedures
	Clinical Evaluations: Orthostatic Vital Signs
Day 53	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 54	*Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Sample Collection*: Urine pregnancy test *Clinical Evaluations:* Orthostatic Vital Signs
Day 55	*Sample Collection:* Research Blood, PK Blood Neurocognitive Battery *Imaging:* fMRI/MRS, MEG *Study Drug Administration:* TS-161/ Placebo (100mg or 50mg) *Clinical Evaluations:* Orthostatic Vital Signs
Day 56 (or End of Study)	Clinical Rating Scales[ii] AE Assessments *Sample Collection:* Safety Labs[iii,エラー! ブックマークが定義されていません。] *Clinical Evaluations:* Urine Toxicology, Orthostatic Vital Signs, Weight, Height, ECG, Physical Exam
Standard Treatment	
Up to 2 months	Standard Treatment

[i] Allow up to 3 hours for procedures scheduled on the same day. Allow up to 3 days on procedures scheduled on different days to accommodate changes to the schedule. Some procedures may not be performed due to unforeseen technical or scheduling problems.

[ii] See the Schedule of Clinical Rating Scales for specific scales used.

[iii] See the Safety Labs section for a list of labs conducted.

[iv] A urine pregnancy test (HCG) will be obtained as indicated and no more than 24 hours prior to start of each research condition and imaging procedure. A urine toxicology screen will be obtained within 24 hrs of administration of study drug.

[v] See Dosing and Administration for more details on dose adjustments.

1.4 SCHEDULE OF CLINICAL RATING SCALES

Timepoint[i]	C-SSRS-lifetime, FHS	BPRS, YMRS	CADSS	C-SSRS, HDRS, HAM-A, PANAS, SHAPS, SSI, TEPS	MADRS
Study Phase I					
Days -8 to -2	X[ii]				
Day -1	X[ii]	X	X	X	X
Study Phase II					
Research Condition 1					
Day 0					
-60 min					X
+230 min		X	X	X	X
Day 1		X	X	X	X
Day 2			X	X	X
Day 3			X	X	X
Day 7			X	X	X
Day 14			X	X	X
Day 21		X	X	X	X
Research Condition 2					
Day 34		X	X	X	X
Day 35					
-60 min					X
+230 min		X	X	X	X
Day 36		X	X	X	X
Day 37			X	X	X
Day 38			X	X	X
Day 42			X	X	X
Day 49			X	X	X
Day 56 (or End of Study)		X	X	X	X

[i] Allow up to 3 hours on same day for procedures scheduled on the same day.

[ii] These will be completed once during Phase I, if not previously done under protocol 01-M-0254.

2 INTRODUCTION

2.1 STUDY RATIONALE

Modulation of glutamatergic signaling is implicated in improvement of depressive symptoms and related constructs/dimensions of observable behavior and neurobiological measures with treatment. Current standard monoaminergic pharmacological approaches for major depressive disorder (MDD) have proven to be only modestly effective during acute major depressive episodes (MDEs). We have systematically tested different glutamatergic modulators in subjects with mood disorders in order to develop improved therapeutics. We found that the *N*-methyl-D-aspartate receptor (NMDAR) antagonist, ketamine, produces rapid antidepressant effects in patients with treatment-resistant depression (TRD in MDD, Bipolar Disorder) and in suicidal ideation. However, despite being highly efficacious, ketamine produces psychotomimetic effects and has the risk of abuse. The antidepressant effects of mGlu2/3 receptor antagonists are worthy of pursuit, since the antidepressant profile in preclinical assays as well as the synaptic/neural cellular and molecular mechanisms involved in their actions are comparable to those of ketamine, but without the side effects and abuse potential of ketamine.

In the present protocol, we aim to evaluate a new glutamate-mediated mechanism associated with antidepressant efficacy by targeting the mGlu2/3 receptor with a potent and selective antagonist. Targeting the mGlu2/3 receptor with an antagonist is anticipated to, and similar to ketamine, result via pre-synaptic mechanisms in a "glutamate surge" with subsequent α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) activation and gamma power increases but without potential adverse effects that occur with ketamine.

The present Phase 2 proof-of-concept (POC) clinical trial is designed to evaluate in subjects with MDD, the antidepressant effects of TS-161, the prodrug of a potent and selective mGlu2/3 receptor antagonist TP0178894 that readily crosses the blood brain barrier (BBB). In animal model assays of antidepressant efficacy, TS-161 induced acute and prolonged antidepressant-like effects without exhibiting ketamine-like side effects as determined by the lack of increase in locomotor activity or abuse potential.

We will also evaluate the putative neurobiological mechanisms involved in the antidepressant response to TS-161. We expect that this effect may modulate glutamate transmission and reverse the clinical symptoms of depression. The demonstration that an mGlu2/3 receptor antagonist produces antidepressant effects without psychotomimetic side effects would support the therapeutic relevance of the mGlu2/3 receptor and could direct the development of novel drug targets for the treatment of depression.

2.2 BACKGROUND

2.2.1 Ketamine Induces Rapid Antidepressant Effects, but Produces Dissociative Side Effects and has the Potential for Abuse

Mood disorders (MDD and bipolar disorder [BD]) are common, chronic, recurring, serious mental illnesses associated with significant morbidity, and often become life-threatening. A variety of antidepressants have been developed; unfortunately, these medications take weeks to months to achieve their full effects and in the meantime, patients continue to suffer from their symptoms and continue to be at risk of self-harm as well as harm to their personal and professional lives. Although a significant proportion of patients usually respond to currently available medications within three

to four weeks, remission rates are usually low at this point and may take several more weeks to become manifest (Entsuah, Huang et al. 2001). Moreover, up to one-third of patients still do not respond with four consecutive trials of antidepressants. A more rapidly acting antidepressant medication in TRD would significantly impact the treatment of depression and have major health care implications.

In subjects with TRD, ketamine is the only pharmacological treatment that has antidepressant effects that both occur within hours and are sustained for one week or more with a single-administration (Zarate, Singh et al. 2006, Kishimoto, Chawla et al. 2016). In the 2006 study, of 17 subjects, 71% of participants met criteria for antidepressant response (a 50% decrease in HDRS scores), and 29% achieved remission (≤7 HDRS) at 24 hours following infusion of ketamine. Six (35%) subjects maintained response to ketamine for at least one week; in contrast, no subject on placebo responded at Day 1 or Day 7. In a recent study, we found that the antidepressant efficacy of ketamine lasted at least 11 days with single administration in subjects with TRD (Nugent, Ballard et al. 2019). Within six hours to one day, a single ketamine infusion led to response rates comparable to those seen following eight weeks of treatment with monoaminergic based antidepressants. Furthermore, ketamine induced remission in approximately one-third of subjects within one day, which is in stark contrast to that of monoaminergic-based approaches, which usually require 10-14 weeks of chronic daily usage to produce similar remission rates (Trivedi, Rush et al. 2006).

A recent meta-analysis that included seven clinical trials examining the antidepressant response to ketamine in subjects with depression (several of these studies were from our group) found that ketamine produced an antidepressant effect at 24 hours post-infusion with odds ratios for response of 9.87 (4.37-22.29) and transient remission of 14.47 (2.67-78.49) (Newport, Carpenter et al. 2015). In addition, considerable evidence demonstrates that ketamine also has rapid antisuicidal ideation, anxiolytic, and anti-anhedonic effects in individuals with treatment-resistant MDD (Zarate, Singh et al. 2006, DiazGranados, Ibrahim et al. 2010, Murrough, Iosifescu et al. 2013, Lally, Nugent et al. 2014). Furthermore, preliminary evidence suggests that acute ketamine administration has salutary effects on a number of immune and inflammatory and bone biological indices associated with heart disease and osteopenia, which are potentially modifiable risk factors and have often been found to be altered in depression (Kadriu, Gold et al. 2017). Whether acutely rectifying these indices in addition to improving depressive symptoms could have long-term benefits on brain and body health, particularly as one ages, remains unknown. However, it is quite clear that treatments capable of producing a rapid (within hours), robust, and sustained antidepressant effect would be of major importance to individuals with depression. Such an intervention could also potentially minimize the cumulative morbid effects of depression and altered immune and inflammatory states that place MDD subjects at increased risk of premature death from either medical conditions or suicide (Zivin, Ilgen et al. 2012, Choi, DiNitto et al. 2017). Taken together, the evidence gleaned from these studies have introduced a new paradigm for the research into the mechanisms implicated in antidepressants with a rapid onset of action.

Synaptic plasticity changes involving AMPA receptors (AMPARs) may underlie the acute and sustained antidepressant actions of ketamine (Koike and Chaki 2014, Duman, Aghajanian et al. 2016, Zanos, Moaddel et al. 2016). Ketamine's NMDAR blockade is hypothesized to be the first step in a cascade of events ("disinhibition hypothesis") that includes rapid increases in presynaptic glutamate release ("glutamate burst"), enhanced regional activity in excitatory networks and, ultimately, changes in synaptic plasticity and connectivity, thus rapidly restoring

the impaired synaptic and circuit homeostasis associated with depression (Krystal, Karper et al. 1994). We first demonstrated in antidepressant assays (Maeng, Zarate et al. 2008) that the administration of AMPAR antagonist (NBQX) blocks the antidepressant-like effects of ketamine, which indicates that the neurotransmission at AMPARs is involved in these effects. This finding has now been replicated by multiple labs (Li, Lee et al. 2010, Autry, Adachi et al. 2011, Zanos, Moaddel et al. 2016).

Unfortunately, the widespread clinical use of ketamine for the treatment of major depression is restricted to certain subgroups (e.g., TRD, severe suicidal ideation) as ketamine requires close monitoring when administered, due to its side effects, including dissociation, psychotomimetic properties, and abuse potential(Acevedo-Diaz, Cavanaugh et al. 2019). Consequently, alternative medications that share the robust antidepressant actions of ketamine, but lack its side effects and abuse potential, are urgently needed. mGlu2/3 receptor antagonists have a similar antidepressant profile and share similar mechanisms implicated in antidepressant response to ketamine, including AMPAR stimulation (Maeng, Zarate et al. 2008, Dong, Zhang et al. 2017), but without the limitations of ketamine (Witkin, Monn et al. 2016).

2.2.2 Modulating mGlu2/3 Receptor Results in Antidepressant-like Properties in Antidepressant Assays

The metabotropic glutamate receptor family is comprised of eight different receptor subtypes (mGlu1–mGlu8 receptors), which are divided into three main groups (group I: mGlu1 and mGlu5 receptors; group II: mGlu2 and mGlu3 receptors; group III: mGlu4, mGlu6, mGlu7, and mGlu8 receptors). Of these, group II mGlu receptors (mGlu2 and mGlu3 receptors) are coupled to Gi/Go to negatively regulate adenylyl cyclase activity, and are highly expressed within the cortical and limbic regions of the brain, the brain areas associated with emotion and cognition.

Preclinical studies have reported the efficacy of group II metabotropic glutamate receptor (mGlu2/3 receptor) antagonists in reducing behavioral despair in the acute forced-swim test at 30–60 min following drug administration (Chaki, Yoshikawa et al. 2004, Bespalov, van Gaalen et al. 2008, Witkin, Monn et al. 2016) and decreasing escape failures in the learned helplessness paradigm (Yoshimizu, Shimazaki et al. 2006). mGlu2/3 receptors are expressed in hippocampal, synaptic, mossy fiber-CA3 pyramidal cells and at excitatory synapses in the prefrontal cortex (PFC) (Ohishi, Ogawa-Meguro et al. 1994). mGlu2 receptors are primarily localized peri-synaptically in close proximity to the pre-synaptic terminals (Petralia, Wang et al. 1996, Shigemoto, Kinoshita et al. 1997), where they act as auto-receptors to decrease synaptic glutamate transmission when activated, presumably serving as a homeostatic mechanism to prevent excessive glutamate release (Tzounopoulos, Janz et al. 1998, Chen, Huang et al. 2001). In contrast, mGlu3 receptors are primarily localized to glial cells (Ohishi, Shigemoto et al. 1993) and their activation inhibits astrocyte growth (Ciccarelli, Sureda et al. 1997) and increases levels of glutamate transporter proteins (Aronica, Gorter et al. 2003), thus indirectly decreasing extracellular glutamate levels.

mGlu2/3 receptor inhibition elicits rapid antidepressant actions in preclinical studies, similar to ketamine. In particular, a single administration of an mGlu2/3 receptor antagonist reduced immobility time in the 24-h forced-swim test (Dwyer, Lepack et al. 2012), decreased time delay until food consumption in the novelty-suppressed feeding test (Koike, Kasamatsu et al. 2013, Fukumoto, Iijima et al. 2014), rapidly reversed chronic stress-induced decreases in sucrose preference, which was sustained for at least 10 days (Dwyer, Lepack et al. 2013), and reversed

chronic corticosterone-induced behavioral deficits(Ago, Yano et al. 2013, Koike, Iijima et al. 2013) in rodents. In addition, mGlu2/3 receptor blockade reversed the decrease in sucrose preference and increased behavioral despair produced by chronic social defeat stress in mice within 24 hours after a single administration (Dong, Zhang et al. 2017). While a large (n = 310 subjects) clinical trial of a negative allosteric modulator of mGlu2/3 receptor (RG1578; decoglurant) failed to demonstrate antidepressant responses compared with placebo, no measure of target engagement was included in this trial and therefore we cannot conclude that the target was engaged. Additional studies are needed to determine the potential of mGlu2/3 receptor antagonists in the treatment of TRD (Quiroz, Tamburri et al. 2016). Both orthosteric and allosteric modulator for mGlu2/3 receptor have been shown to exert antidepressant effects, however the potency of allosteric modulators was demonstrated to be weaker than that of the orthosteric antagonists (Campo, Kalinichev et al. 2011). For this reason, we are pursuing investigation with a potent and selective mGlu2/3 receptor orthosteric antagonist.

Our work challenges the existing hypothesis that *N*-methyl-D-aspartate receptor (NMDAR) inhibition is ketamine's primary mechanism of antidepressant action; rather, we found that ketamine's antidepressant effects may be due to its metabolite (2*R,*6*R*)-hydroxynorketamine (HNK), whose mechanism may be NMDAR-independent (Zanos, Moaddel et al. 2016, Lumsden, Troppoli et al. 2019). We recently found that (2*R,*6*R*)-HNK induces synaptic plasticity as shown by increases in AMPAR-mediated EPSPs (Zanos, Moaddel et al. 2016). We subsequently found that this effect appears to be due to pre-synaptic mechanisms, as (2*R,*6*R*)-HNK induced paired-pulse depression (Zanos, Highland et al. 2019). In addition, whole-cell patch clamp data showed increased mEPSC frequencies, but not amplitude, in the presence of tetradotoxin (TTX), indicating pre-synaptic regulation of synaptic strengthening. mGlu2 receptor specifically acts as an autoreceptor and, when inhibited, increases the probability of glutamate release, similar to (2*R,*6*R*)-HNK. Our preclinical data indicate that administration of the mGlu2/3 receptor agonist LY379268 to mice blocked ketamine and (2*R,*6*R*)-HNK's antidepressant actions and that, together, sub-effective doses of an mGlu2/3 receptor antagonist and sub-effective doses of ketamine exerted antidepressant effects, suggesting synergistic mechanisms. Furthermore, the antidepressant actions of ketamine and (2*R,*6*R*)-HNK were also blocked in mGlu2 receptor, but not mGlu3 receptor, knockout mice (Zanos, Highland et al. 2019). Notably, recent studies have also indicated important roles of mGlu3 receptor in the antidepressant effects, presumably through postsynaptic mechanisms (Joffe, Santiago et al. 2020). Studies suggest that mGlu2/3 receptor antagonists exert antidepressant effects in multiple antidepressant assays in rodents (Yoshimizu, Shimazaki et al. 2006, Palucha-Poniewiera, Wieronska et al. 2010, Dong, Zhang et al. 2017); these appeared within 24 hours after a single administration in the chronic unpredictable stress (Dwyer, Lepack et al. 2013) and chronic social defeat stress models (Dong, Zhang et al. 2017). These compounds have also been associated with sustained antidepressant effects lasting ~ one week after a single administration (Dwyer, Lepack et al. 2013, Dong, Zhang et al. 2017). It is notable that mGlu2/3 receptor antagonists share mechanisms implicated in the antidepressant response to ketamine, including AMPAR stimulation (Maeng, Zarate et al. 2008), brain derived neurotrophic factor (BDNF)/TrkB signaling, mechanistic target of rapamycin complex 1 (mTORC1) signaling, and synthesis of synaptic proteins (such as GluA1) in the PFC (Dong, Zhang et al. 2017, Chaki 2019).

In addition to synaptic mechanisms, serotonergic and dopaminergic systems have also been suggested to have important roles in the antidepressant actions of the mGlu2/3 receptor antagonists. The mGlu2/3 receptor antagonists increased the firing of the 5-HT neurons in the dorsal raphe nucleus and 5-HT release in the mPFC, indicating activation of the serotonergic system.

Furthermore, the antidepressant actions of mGlu2/3 receptor antagonists were blocked by depletion of 5-HT and a 5-HT_{1A} receptor antagonist, suggesting the serotonergic system is necessary for mGlu2/3 receptor antagonists to exert their antidepressant actions (Fukumoto, Iijima et al. 2014, Fukumoto, Iijima et al. 2016, Fukumoto, Iijima et al. 2018). Likewise, an mGlu2/3 receptor antagonist has been demonstrated to increase number of spontaneously active dopamine neurons in the ventral tegmental area and to increase extracellular dopamine level in the nucleus accumbens and medial prefrontal cortex (Witkin, Monn et al. 2016), suggesting the dopaminergic system is also implicated in the antidepressant effects of mGlu2/3 receptor antagonists. These serotonergic and dopaminergic mechanisms are also shared with ketamine (Dong, Zhang et al. 2017, Chaki 2019, Chaki and Fukumoto 2019). In terms of safety, mGlu2/3 receptor antagonists did not induce side effects similar to those of ketamine in mouse tests, nor do they appear to have abuse potential (Witkin, Monn et al. 2017). Furthermore, mGlu2/3 receptor antagonists increase gamma oscillations (Ahnaou, Ver Donck et al. 2014) which is linked to gamma activation (see below) thus, this gives us a putative biomarker for our POC studies. The candidate drug we selected, TS-161, is a prodrug of TP0178894, an orthosteric mGlu2/3 receptor antagonist. The Phase 2 POC study will be conducted on our research unit.

2.2.3 Surrogate Neurobiological Markers of mGlu2/3 receptor antagonist TS-161's Mechanism of Action and/or Antidepressant Therapeutic Response

An impediment to progress in the discovery and testing of novel medications for serious psychiatric disorders is the lack of established surrogate neurobiological markers capable of predicting therapeutic response or identifying mechanisms of action. Identifying such markers may greatly facilitate drug discovery. The proposed project examines the utility of several potential neurobiological surrogate markers. A surrogate biomarker is most useful in that the number of subjects required to demonstrate an effect is less than that needed to prove clinical efficacy. Therefore, by assessing the same number of subjects with the neurobiological markers as those being evaluated for clinical efficacy, the proposed study should have sufficient power to identify surrogate neurobiological markers of practical significance. For example, previous studies from our laboratory found that gamma power changes, as determined by MEG, may be a putative biomarker to identify a subgroup of subjects with TRD who will respond favorably to ketamine's antidepressant effects (Cornwell, Salvadore et al. 2012, Nugent, Ballard et al. 2019).

In the proposed project, we will evaluate the following approaches as potential surrogate markers of drug effects and/or treatment response:

1. Gamma oscillatory power as well as other spectral analyses with MEG
2. Resting and task based functional connectivity with fMRI. Resting and task based functional
3. Prefrontal glutamate levels (and other brain metabolites) measured with 7T 1H-MRS
4. Plasma/serum/Lymphocytes/mRNA for neurotrophic biomarkers

2.2.4 Convergent Evidence that Enhancing AMPAR Activity is Critical to the Mechanism of Action of Rapid Acting Antidepressants

Understanding the mechanism of action by which ketamine exerts its antidepressant effects could lead to advances in the development of the next generation of antidepressants. As noted above, preclinical data from our laboratory indicates that ketamine's antidepressant effects are mediated through AMPARs. We demonstrated blockage of the antidepressant-like properties of

ketamine in the forced swim test (FST) with a relatively selective AMPAR antagonist (NBQX) (Maeng, Zarate et al. 2008). Other investigators subsequently replicated this finding (Li, Lee et al. 2010, Autry, Adachi et al. 2011, Andreasen, Gynther et al. 2013, Beurel, Grieco et al. 2016, Zanos, Moaddel et al. 2016).

The present study proposes to expand our preclinical findings by evaluating whether an mGlu2/3 receptor antagonist also produces the same effects of ketamine in subjects with TRD (i.e., antidepressant effects, increases in glutamate release and gamma power).

2.2.5 Gamma Oscillations as a Putative Cross-Species Biomarker of Synaptic Plasticity and AMPAR Throughput

One theory posits that the rapid antidepressant effects of ketamine are probably initiated by its antagonistic effects on NMDARs; these effects regulate glutamate/GABA and/or AMPAR/NMDAR activity. Preclinical studies have shown that ketamine rapidly induces synaptogenesis and reverses synaptic deficits caused by chronic stress, thus restoring network connectivity (Duman and Aghajanian 2012), while clinical studies support enhanced plasticity in response to ketamine (Cornwell, Salvadore et al. 2012, Nugent, Wills et al. 2019). Animal studies have begun to elucidate ketamine's downstream effects that might underlie its beneficial effects in depressed subjects. Ketamine's NMDAR blockade is hypothesized to be the first step in a cascade of events that includes rapid increases in presynaptic glutamate release, enhanced regional activity in excitatory networks and, ultimately, changes in synaptic plasticity and connectivity. In rodents, low-dose ketamine administration rapidly triggers three successive events: first, presynaptic disinhibition of glutamatergic neurons, which leads to a glutamate burst; second, increased activation of the AMPAR, combined with the blockade of extrasynaptic NMDARs; and third, postsynaptic activation of neuroplasticity-related signaling pathways involving BDNF and mTORC1, which results in overall synaptogenesis and synaptic potentiation (Li, Lee et al. 2010, Autry, Adachi et al. 2011, Liu, Lee et al. 2012). Both animal (Pinault 2008, Zhang, Yoshida et al. 2012, Anderson, Pinault et al. 2014, Jones, Anderson et al. 2014) and human (Rivolta, Heidegger et al. 2015, Shaw, Saxena et al. 2015) studies indicate that acute, subanesthetic ketamine infusion is associated with robust increases in gamma power. Multiple synaptic mechanisms play a role in regulating gamma oscillations, including AMPAR-mediated depolarization and $GABA_A$ receptor-mediated inhibition. Ketamine may influence both of these systems, both by silencing GABAergic inhibitory synapses and by increasing glutamate release, thereby activating AMPARs (Ren, Pribiag et al. 2016). The decreased activity in GABAergic interneurons and the disinhibition of excitatory pyramidal neurons (Homayoun and Moghaddam 2007) presumably provide the mechanism for increased gamma oscillations (Carlen, Meletis et al. 2012) and corresponding measured power. The antidepressant mechanism is likely more complex, however, given that blockade of NMDARs on interneurons and the subsequent disinhibition of pyramidal neurons does not consistently produce an antidepressant effect (Miller, Moran et al. 2016).

Notably, acute administration of the ketamine metabolite (2*R*,6*R*)-HNK also increases gamma oscillations likely via mGlu2 receptor signaling, despite the fact that the metabolite does not inhibit NMDARs at concentrations that increase gamma power (Zanos, Moaddel et al. 2016, Zanos, Highland et al. 2019). Because AMPAR blockade can attenuate (2*R*,6*R*)-HNK-induced gamma oscillations, enhanced AMPAR activity is likely to be the mechanism by which (2*R*,6*R*)-HNK and mGlu2/3 receptor antagonists increase gamma power, although the mechanism of increased AMPAR activity is not known (Bartos, Vida et al. 2007). Taken together, these findings

underscore the close relationship between gamma oscillations and inhibition/excitation balance (Kehrer, Maziashvili et al. 2008, Gandal, Sisti et al. 2012).

Previous studies from our laboratory also used cortical excitability as a plasticity measure of antidepressant response to ketamine. Twenty unmedicated subjects with treatment-resistant MDD received a single, open-label intravenous infusion of ketamine hydrochloride (0.5 mg/kg) (Cornwell, Salvadore et al. 2012). MEG recordings were performed at baseline (prior to) and approximately seven hours after the ketamine infusion. At these two timepoints, subjects passively received tactile stimulation (somatosensory evoked potentials) to the right and left index fingers while they rested with eyes closed. Subjects exhibiting robust improvements in depressive symptoms 230 minutes post-ketamine infusion (responders) had increased cortical excitability within this antidepressant response window. Specifically, we found that stimulus-evoked somatosensory cortical responses (i.e., gamma-band power) increased after infusion relative to pretreatment responses in treatment responders but not in nonresponders. Spontaneous somatosensory cortical gamma-band activity during rest did not change within the same time frame after ketamine in either responders or nonresponders. These findings suggest that NMDAR antagonism does not directly lead to increased cortical excitability hours later and thus might not be sufficient for the therapeutic effects of ketamine to take hold. Rather, increased cortical excitability as depressive symptoms improve is consistent with the hypothesis that enhanced non-NMDAR-mediated glutamatergic neurotransmission via synaptic potentiation is central to the antidepressant effects of ketamine.

These results support the hypothesis that ketamine's rapid antidepressant effects are due to enhanced AMPAR throughput in cortex.

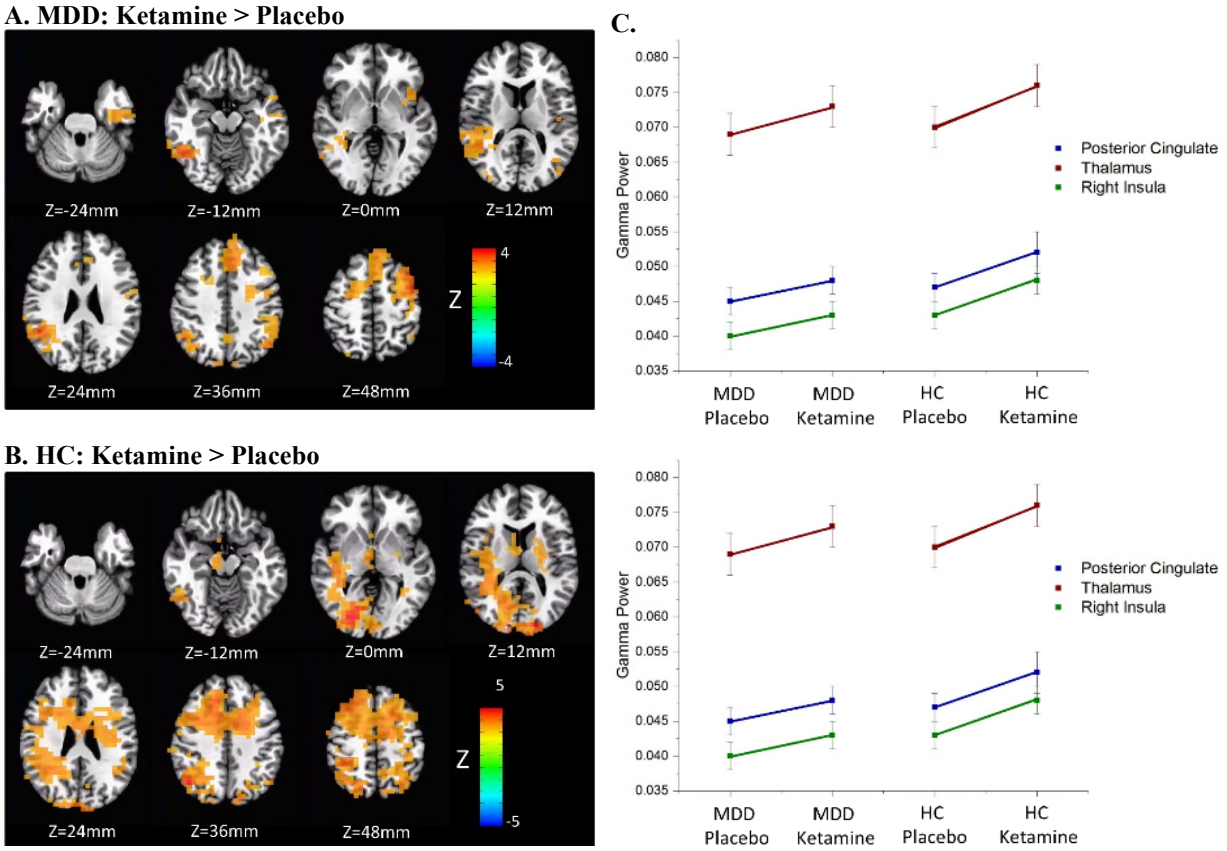


Figure 1A shows the contrasts for Ketamine-Day 0 versus Placebo-Day 0 for the MDD group. **Figure 1B** shows the same contrast in the healthy control group. Regions include those involved in the central executive (CEN), salience (SN), and default mode (DMN) networks. **Figure 1C** illustrates the estimated marginal means from mixed models of RMS gamma power extracted from selected regions of interest defined on the Ketamine-Day 0 vs. Placebo-Day 0 contrast across both groups. MDD subjects exhibited increases post-ketamine to levels commensurate with those seen in healthy control subjects following placebo infusion.

In a follow-up study, we used the same task (passive tactile stimulation) but with a more rigorous design (Nugent, Wills et al. 2019), again finding increased gamma power in responders compared with nonresponders. In a double-blind, placebo-controlled study of 35 unmedicated, TRD- MDD subjects and 25 healthy controls, we also assessed ketamine's mechanism of action using gamma power with MEG as a proxy measure for homeostatic balance (Nugent, Ballard et al. 2019). MDD subjects showed significant improvements in depressive symptoms, and unexpectedly, healthy subjects exhibited modest but significant increases in depressive symptoms for up to one day after ketamine administration. Both groups showed increased resting gamma power six to nine hours following ketamine. In controls, increased gamma power was associated with increased depressive symptoms. In MDD subjects, baseline gamma power moderated the relationship between post-ketamine gamma power and antidepressant response. While higher post-ketamine gamma power was associated with better response in MDD subjects with lower baseline gamma, the relationship was inverted in MDD subjects with higher baseline gamma (Nugent, Ballard et al. 2019). We next sought to replicate previous findings of increased gamma response

to a somatosensory stimulus at 230 min and Day 1 in ketamine responders versus non-responders in 31 depressed subjects and 25 healthy controls (Nugent, Wills et al. 2019). A significant difference in peak gamma power was seen in the depressed ketamine responders versus non-responders. These results implicate AMPAR throughput in ketamine's mechanism of antidepressant action. While potentially promising, it is important to note that MEG gamma power as a biomarker is still in the exploratory stages. No dose-dependent gamma power changes have been yet demonstrated with ketamine. [redacted] It is important to emphasize that the SAD TS-161 study was conducted in healthy volunteers and as such the results might be different in depressed subjects. For this reason, we will examine all MEG frequency band changes with TS-161.

The blood oxygen level dependent (BOLD) signal measured using functional MRI (fMRI) has been shown to be modulated by other mGluR2 agonists in the striatal, dorsolateral prefrontal cortex and ACC (Krystal 2013, Wolf, Ruparel et al. 2013). In this study we plan to use resting state fMRI to probe disruption of neural circuits connected to these regions. Additionally, we plan to use simple cognitive tasks, such as working memory, continuous performance and emotion identification to localize any effects of TS-161.

2.2.6 Prefrontal Glutamate Levels Measured with 7T 1H-MRS and fMRI

Several recent preclinical studies have demonstrated the need of a "glutamate surge" and the accompanying AMPA activation in generating the antidepressant-like response of ketamine and other NMDA antagonists. However, blocking NMDAR receptors likely results in its psychomimetic properties and contributes to its abuse potential. We hypothesized that TS-161 will also rapidly increase glutamate release by selectively inhibiting mGlu2 receptors thus activating AMPA receptors and intracellular neuroprotective cascades including AKT and mTORC1.

In a recent study (Evans, Lally et al. 2018), we found only a trend-level increase in glutamate levels in the pgACC using ^{1}H-MRS 7T in MDD subjects 24 hours post-ketamine, suggesting that the changes (i.e., glutamate burst) could have been more immediate to the infusion of ketamine. In this study, we plan to measure glutamate levels in the pgACC using ^{1}H-MRS 7T in MDD subjects at plasma Cmax of active metabolite of the initial dose of TS-161 (100 mg) at a timeframe that would presumably coincide with the glutamate surge.

2.2.7 Neurochemical Indices as Putative Biomarkers of Synaptic Plasticity, Drug Effects, and Antidepressant Response of Ketamine

BDNF is the most abundant neurotrophin in the brain, and its activation of tropomyosin-related kinase B (TrkB) receptors stimulates neural growth, survival, and plasticity. In preclinical models of despair, ketamine activates mTORC1, which is a critical hub of cellular growth and proliferation (Hay and Sonenberg 2004). Ketamine was found to increase mTORC1 phosphorylation and other downstream molecular targets critical for transcriptional activation within one hour of administration (Li, Lee et al. 2010). Twenty-four hours following ketamine exposure, greater numbers of mature dendritic spines were observed. These molecular and cellular effects were lost if the rodent was pretreated with rapamycin, an mTORC1 antagonist. These findings suggest that mTORC1 activation is necessary for ketamine's rapid-acting antidepressant effects in a rodent model of despair, which may occur via BDNF. After initial studies from our group suggested that peripheral BDNF levels do not correlate with antidepressant response to

ketamine without genotypic stratification (Machado-Vieira, Yuan et al. 2009, Laje, Lally et al. 2012), several subsequent studies with larger samples (including ours) reported a positive association between baseline serum BDNF and ketamine's antidepressant effects. One study found that in a sample of 22 TRD subjects, plasma BDNF levels at 4 hours were associated with greater antidepressant improvement at the same timepoint 24 hours and 72 hours post-ketamine infusion (Haile, Murrough et al. 2014). In a subsequent study, we found that changes in plasma BDNF were proportional to changes in EEG slow wave activity (SWA), and that this link was present only in subjects who responded to ketamine treatment, suggesting enhanced synaptic plasticity (Duncan, Sarasso et al. 2013). In order to identify pathways that are responsible for ketamine's effect, we carried out a targeted metabolomic approach using a double-blind, placebo-controlled crossover design, with infusion order randomized with medication-free patients with TRD- MDD (29 subjects) and healthy controls (25 subjects). Ketamine treatment resulted in a general increase in circulating sphingomyelins, levels which were not correlated with response. Ketamine response resulted in more pronounced effects in the kynurenine pathway and the arginine pathway at 4 h post-infusion, where a larger decrease in circulating kynurenine levels and a larger increase in the bioavailability of arginine were observed in responders to ketamine treatment, suggesting possible mechanisms for response to ketamine treatment (Moaddel, Shardell et al. 2018).

We will thus be collecting whole blood for the purpose of transcriptional profiling, metabolomics, proteomics, and the measurement of inflammatory measures, oxidative stress markers and neurotrophic factors, as well as glycine/serine levels, all directly implicated in the pathophysiology and treatment response in mood disorders (reviewed in (Machado-Vieira, Soeiro-De-Souza et al. 2014)). We will also explore inflammatory markers such as cytokines and how they may be related to drug effects or treatment response.

2.2.8 RDoC, Synaptic Plasticity, and Antidepressant Response to Ketamine

Depression is a multidimensional disorder characterized by a variety of symptoms that include depressed mood, anhedonia, negative cognitive biases, and altered activity levels. The focus on diagnostic entities rather than the component constructs has likely limited attempts to understand the biology of mental illness. A more dimensional approach, like that described by the Research Domain Criteria (RDoC) initiative, is a potential solution to this issue. Indeed, in an exploratory factor analysis of a variety of depression ratings collected in 119 subjects with either MDD or bipolar depression, we found that the best solution comprised eight unidimensional factors: *Depressed Mood, Tension, Negative Cognition, Impaired Sleep, Suicidal Thoughts, Reduced Appetite, Anhedonia,* and *Amotivation*. Importantly, a differential pattern of response, both to ketamine and placebo, was observed across these unidimensional constructs (factors) (Ballard, Yarrington et al. 2018). In other words, the use of these unidimensional constructs may reveal patterns not observed with traditional scoring of individual instruments. The empirical identification of unidimensional constructs creates more refined scores that may elucidate the connection between specific symptoms and underlying pathophysiology. For this reason, we plan to conduct an exploratory analysis to measure the different constructs derived from our exploratory factor analysis (i.e., *Depressed Mood, Tension, Negative Cognition, Impaired Sleep, Suicidal Thoughts, Reduced Appetite, Anhedonia,* and *Amotivation*).

2.2.9 Neurocognition and Glutamatergic Modulation

The acute cognitive effects of ketamine treatment for depression have been minimally studied. In healthy participants, acute infusions using larger ketamine doses have been associated with significant short-term deficits particularly in working memory, source memory and episodic memory, as well as subjective cognitive effects (e.g., impaired memory, confusion) (Morgan, Mofeez et al. 2004).

Further, few studies have examined possible cumulative cognitive effects of repeated ketamine treatment in depressed subjects (Blier, Zigman et al. 2012, Irwin, Iglewicz et al. 2013, Diamond, Farmery et al. 2014, Shiroma, Albott et al. 2014, George, Galvez et al. 2017, Galvez, Li et al. 2018). Cognitive deficits following repeated use of NMDAR antagonists have been reported in animal studies (Jentsch, Tran et al. 1997, Mandillo, Rinaldi et al. 2003) and in recreational users of ketamine (Morgan, Muetzelfeldt et al. 2010). In humans, these deficits have been observed primarily on measures of verbal fluency, episodic memory and attention/working memory (Morgan, Mofeez et al. 2004, Morgan, Muetzelfeldt et al. 2010). A prior study showed that cognitive functioning was associated with subsequent antidepressant response to ketamine at 24 hours (Murrough, Wan et al. 2013). Individuals who demonstrated a positive antidepressant response to ketamine had slower processing speed at baseline, compared with individuals who did not respond. Furthermore, the authors did not find adverse cognitive effects of ketamine 7 days post treatment.

The precise mechanism for the cognitive side effects of ketamine is unclear but could possibly be linked to enhanced glutamate release. Aspects of the disinhibition hypothesis was originally proposed by Moghaddam and colleagues where they suggested that hyperactivation of non-NMDA receptors, as opposed to a "glutamatergic deficiency" from NMDAR blockade may account for some of the cognitive deficits and schizophrenia-like symptoms of NMDA receptor antagonists (Moghaddam, Adams et al. 1997). Thus, it stands that the side effect of ketamine, dissociation, could be related to the mechanism of antidepressant action as they appear to share important mechanistic processes implicated in ketamine's therapeutic properties. In support, as was previously discussed, there is increasing evidence to suggest that the primary mechanism of ketamine for its antidepressant effects is NMDAR-independent (Zanos, Moaddel et al. 2016). Since mGlu2/3 receptor antagonists would also be linked to an increase in glutamate release, it is imperative that we monitor cognition in subjects exposed to such drugs. Preclinical evidence however, does not suggest that cognitive deficits will occur with mGlu2/3 receptor antagonists (Witkin, Monn et al. 2017). In fact, mGlu2/3 receptor antagonists do appear to have pro-cognitive effects as demonstrated in the social recognition test (Higgins, Ballard et al. 2004, Shimazaki, Kaku et al. 2007). We will obtain a detailed neurocognitive battery during the study following dose administration.

2.2.10 Current Proposal

In Study Phase I, subjects will be 2-weeks drug-free prior to their Study Phase II randomization except if subjects were taking a medication with a long half-life such as fluoxetine, aripiprazole, or brexpiprazole in which case the washout will be five weeks. The MADRS will be the primary outcome measure for depression severity. Other measures obtained will include behavioral measures, peripheral biomarkers, MEG, fMRI, and ^{1}H-MRS. Male and female subjects, ages 18 to 65 years, with a diagnosis of MDD, currently in an episode of major depression, will be recruited for this study, which will take place at the NIH Clinical Center in Bethesda, MD.

2.2.10.1 The Candidate Drug: TS-161, an mGlu2/3 Receptor Antagonist Prodrug

TP0473292 (TS-161), an orally bioavailable prodrug of TP0178894 (a potent and selective mGlu2/3 receptor antagonist), is being developed as a novel antidepressant by Taisho Pharmaceutical Co., Ltd. Preclinical evaluation of TP0473292 (TS-161) showed rapid conversion into the pharmacologically active metabolite TP0178894 on oral administration in rats and monkeys. The pharmacokinetic profile of TP0178894 was consistent with once-a-day oral dosing in humans. In animal models of depression and behavior, including those refractory to current medications, TP0178894 or its prodrug TP0473292 (TS-161) demonstrated potent antidepressant-like effects. [redacted]

2.2.10.2 Nonclinical Pharmacological Profiles of TP0178894 and TP0473292

TP0178894 showed high affinity for human mGlu2 receptor (Ki=4.27 nM), and human mGlu3 receptor (Ki=2.83 nM). TP0178894 also had potent antagonist activity for human mGlu2 receptor (IC50=23.3 nM), and human mGlu3 receptor (IC50=20.9 nM). In contrast, TP0178894 had a negligible antagonistic effect for mGlu4, 6 and 8 receptors. Moreover, TP0178894 at 10 μM showed no significant affinity for other 66 molecules including receptors, transporters and ion channels, indicating that TP0178894 is a selective mGlu2/3 receptor antagonist. In addition, TP0178894 also showed potent antagonist activity for rat mGlu2 receptor (IC50=23.2 nM), and rat mGlu3 receptor (IC50=27.8 nM), indicating that TP0178894 has no species difference in antagonist activity for mGlu2/3 receptor.

Oral administration of TP0473292 was converted into the active metabolite TP0178894 in rodents and monkeys. TP0473292 per se had a negligible affinity for human mGlu2 receptor and mGlu3 receptor. Moreover, TP0473292 at 10 μM did not show significant affinity for other 65 molecules including receptors, transporters and ion channels. Oral administration of TP0473292 exerted antidepressant effects in a rat FST (the lowest effective dose: 1 mg/kg) at concentration of TP0178894 in rat cerebrospinal fluid (CSF) of 1.87 ng/mL. In addition, TP0473292 also exhibited the antidepressant effect in a repeated corticosterone treatment model (the lowest effective dose: 1 mg/kg, p.o.), which is refractory to current medications such as fluvoxamine and imipramine (Iijima, Ito et al. 2010). In contrast, TP0473292 did not affect spontaneous locomotor activity in rats up to 10 mg/kg, p.o., indicating that TP0473292 may not cause sedation or excess excitation. In addition, TP0473292 counteracted an mGlu2/3 receptor agonist, LY379268-induced hypolocomotion, indicating that TP0473292 has antagonist activity for mGlu2/3 receptor in vivo. These results show that TP0473292 is an orally active mGlu2/3 receptor antagonist with antidepressant potential.

2.2.10.3 Preclinical Pharmacokinetic Profiles of TP0178894 and TP0473292

TP0473292, a prodrug of TP0178894, an mGlu2/3 receptor antagonist, is orally bioavailable, and presystemically converts into TP0178894 in rats and monkeys.

Absorption, distribution, metabolism, excretion, and pharmacokinetic drug-drug interaction of TP0178894 and TP0473292 were investigated in *in vivo* in rats and monkeys, and in *in vitro* studies. The summary of results was as follows: After single oral administration of TP0473292 to

rats (1 mg/kg) and monkeys (1.63 mg/kg), plasma levels of the active form TP0178894 reached a maximum concentration (Cmax) within 3 hours post-dose, and decreased with an elimination half-life ($T_{1/2}$) of 1 to 5 hours. The oral bioavailability as TP0178894 was 58.4% (rats) and 56.6% (monkeys). The prodrug TP0473292 was not measurable in plasma at all the sampling time points, indicating complete presystemic hydrolysis of TP0473292 to TP0178894 after gastrointestinal absorption.

TP0178894 was found to cross the BBB into rat [redacted] CSF after a single oral administration of TP0473292 (1 mg/kg). The TP0178894 levels in [redacted] CSF reached Cmax of [redacted] 2.78 ng/mL at 3 hours post-dose, respectively. [redacted] The [redacted] CSF-to-plasma exposure (area under the concentration-time curve: AUC) ratios of TP0178894 were [redacted] 0.0149, [redacted]

TP0473292 was almost completely hydrolyzed to TP0178894 in cryopreserved hepatocytes of rats, monkeys, and humans, and no metabolites related to TP0178894 were detected. A metabolite derived from an ester side-chain moiety [redacted] was also released from the prodrug TP0473292 via hydrolysis, which was further metabolized to glucuronide [redacted] via glucuronidation. No human specific metabolites were found in *in vitro* studies. In the human recombinant carboxylesterase (CES) system, TP0473292 was hydrolyzed by both CES1 and CES2 which express predominantly in liver and intestine, respectively.

Following single intravenous administration of TP0178894 to rats (1 mg/kg) and monkeys (1 mg/kg), 24-hour-urinary excretion rates of TP0178894 were 76.6% (rats) and 97.4% (monkeys) of the dose, indicating renal excretion as the main route of clearance of TP0178894.

TP0473292 and its metabolites (TP0178894, [redacted] showed no inhibitory potential on seven major CYP isoforms. TP0473292 and TP0178894 had no induction potential on the CYP1A2, CYP2B6, and CYP3A4 messenger ribonucleic acids (mRNA) expression in human hepatocytes. Under the conditions of the CYP induction study, TP0473292 would likely undergo degradation to yield TP0178894, [redacted] therefore, the metabolites derived from modifications of the ester side-chain moiety were also considered to have no induction potential for CYP1A2, CYP2B6, and CYP3A4 mRNA expression. [redacted]

2.2.10.4 Nonclinical Toxicology of TP0473292 and TP0178894

[redacted]

[redacted]

[redacted]

2.2.10.5 Prior Human Experiences - Single and Multiple Ascending Dose Studies (TS161-US101)

A first-in-human, randomized, double-blinded, placebo-controlled, single and multiple ascending dose study was conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of TS-161 administered orally to healthy male and female participants.

Methods

The study was composed of three parts; Part A (SAD, Single Ascending Dose), Part B (CSF), and Part C (MAD, Multiple Ascending Dose).

A total of 70 healthy participants received TS-161 (15 mg to 400 mg; n=54) or placebo (n=16) in the study.

In Part A, 40 participants were enrolled to receive TS-161 (15 mg to 400 mg; n=30) or placebo (n=10). Eight participants were randomized (6 active and 2 placebo) in each cohort to receive a single dose of TS-161 (15, 50, 100, 200, or 400 mg) or placebo. All participants were dosed under a fasted condition except for participants in Cohort 2, who were first dosed under a fasted condition (Cohort 2 [Fasted]), and then dosed again under a fed condition (Cohort 2 [Fed]).

In Part B, six participants were enrolled to receive a single dose of 100 mg TS-161 under a fasted condition to evaluate CSF concentrations of TP0178894 (active metabolite).

In Part C, 24 participants were enrolled to receive TS-161 (50 mg to 150 mg; n=18) or placebo (n=6). Eight participants were randomized (6 active and 2 placebo) in each cohort to receive a 10-day multiple dose of TS-161 (50, 100, or 150 mg) or placebo.

Safety

There were no deaths or serious adverse events (SAEs) during the study. Thirty-nine (72.2%) out of 54 participants in the total TS-161 group reported 85 adverse events (AEs) and 7 (43.8%) out of 16 participants in the total placebo group reported 20 AEs. In Part C (MAD), 2 participants in the 150 mg TS-161 group and 1 participant in the placebo group discontinued from the study by participants' request due to AEs. Overall, the frequently experienced AEs with exposure-related increases in their incidence were nausea, vomiting, and dizziness (including dizziness postural).

Single Ascending Dose

In Part A (SAD), 16 (53.3%) out of 30 participants in the fasted TS-161 group reported 25 AEs and 4 (40.0%) out of 10 participants in the fasted placebo group reported 7 AEs. In Cohort 2, one (16.7%) out of 6 participants in the fasted TS-161 group reported 4 AEs, whereas 3 (50.0%) out of 6 participants in the fed TS-161 group reported 7 AEs. In Part B (CSF), 6 (100.0%) of 6 participants reported 8 AEs.

In Part A, the most frequent AEs included orthostatic heart rate response increased (13.3%), orthostatic hypotension (13.3%), and somnolence (10.0%) during fasted and fed combined TS-161 treatment. The incidences of these AEs were not dose-related. All AEs were mild in intensity. The total number of AEs and participants with AEs increased with increase in dose levels; however, there were no other apparent dose-related or meal condition-related trends in the incidences of individual AEs. In Part B, the most frequent AE was post lumbar puncture syndrome (83.3%).

In Part A, 15 (50.0%) out of 30 participants in the fasted TS-161 group reported 24 AEs that were considered possibly or probably related to the study drug and 1 participant reported 1 AE that was considered not related to the study drug (skin laceration). In the fed TS-161 group, 3 (50.0%) out of 6 participants reported 6 AEs that were considered possibly or probably related to the study drug and 1 participant reported 1 AE that was considered not related to the study drug (blood creatine phosphokinase increased). In Part B, 2 (33.3%) out of 6 participants reported 2 AEs that

were considered possibly related to the study drug and 5 participants reported 6 AEs that were considered not related to the study drug (post lumbar puncture syndrome and anxiety).

There was no apparent treatment- or dose-related trends in clinical laboratory results, vital sign measurements, 12-lead ECG measurements or physical/neurological examination findings. The results from Parts A and B indicate that single oral doses of up to 400 mg TS-161 under fasted conditions were safe and well-tolerated in healthy participants.

Multiple Ascending Dose

In Part C (MAD), 15 (83.3%) out of 18 participants in the TS-161 group reported 45 AEs and 3 (50.0%) out of 6 participants in the placebo group reported 9 AEs. Two subjects who received TS-161 had discontinued early from the study by subjects' request due to AEs. One subject experienced AEs of dissociation, nausea, vomiting and anxiety, which were moderate in intensity, as well as mild headache, constipation, panic attack, abnormal dreams and dizziness. The second subject experienced nausea, headache and dizziness postural, which were moderate in intensity, and mild constipation.

The system organ classes (SOCs) with high incidences of AEs in TS-161 treatment included Nervous System Disorders (10 participants [55.6%]) and Gastrointestinal Disorders (8 participants [44.4%]).

The total number of AEs increased with increase in dose levels. The most frequent AEs in TS-161 treatment included nausea (44.4%), headache (22.2%), somnolence (22.2%), vomiting (16.7%), and abnormal dreams (16.7%). All AEs were mild in intensity, except for the moderate AEs observed in participants who discontinued early from the study and one moderate AE of vomiting which was observed in a participant who completed the study. The AEs with dose-related increases in incidences were nausea, vomiting, dizziness (including dizziness postural), headache, and abnormal dreams.

Fifteen (83.3%) out of 18 participants in the TS-161 group reported 45 AEs that were considered possibly or probably related to study drug.

There were no apparent treatment- or dose-related trends in clinical laboratory results, vital sign measurements, 12-lead ECG measurements or physical/neurological examination findings.

The results from Part C indicate that TS-161 was safe and well-tolerated up to 100 mg when administered daily for 10 days under fed conditions to healthy participants.

In summary, TP0473292 an orally bioactive prodrug of TP0178894, a potent and selective mGlu2/3 receptor antagonist, was well-tolerated with a favorable pharmacokinetic profile. TP0473292 (TS-161) exerts ketamine-like antidepressant effects in relevant animal models of depression. Based on these results TP0473292 (TS-161) was selected as a candidate drug for clinical development. The daily clinically efficacious dose of TP0473292 was estimated to be 50–100 mg/day by predicting the human dose required to give an efficacious CSF exposure of TP0178894.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

This protocol involves more than minimal risks to subject. By agreeing to participate in this study, subjects will be temporarily forgoing the opportunity to receive routine clinical care in the community. This will be clearly explained to all patients, along with the treatment strategies that are generally used in patients with MDD. Including the two-week medication-free period before Phase II, participants with MDD may not receive traditional standard treatment for MDD during the study. If subjects discontinue the study, we will provide short-term treatment at the NIMH as long as they remain here on a voluntary basis. However, if subjects do not remain on a voluntary status, then they may have to be transferred to a local hospital for further treatment. If subjects are hospitalized elsewhere, the NIH will not cover the costs associated with that hospitalization.

2.3.1.1 TS-161 Administration

None of the adverse events reported for TS-161 in the Phase 1 study were considered serious. The most common side effects associated with the doses used in this study include: nausea, increased heart rate, sleepiness, and decreased blood pressure. Overall, the clinical laboratory assessments indicated that there were no meaningful changes in laboratory parameters as a result of TS-161 treatment, and the number of abnormal results did not present a safety concern during the study. In addition, there were no apparent trends in the vital signs, physical examinations, and 12-lead ECGs.

Clinical studies on drug interactions between TS-161 and other products have not been conducted. Results from in vitro cytochrome P-450 (CYP) inhibition and induction studies showed that the prodrug (TP0473292) and its metabolites (TP0178894, [redacted] were not potent inhibitors or inducers of the major human CYP isoforms evaluated. Thus, it is unlikely that TS-161 will demonstrate drug-drug interaction through CYP inhibition or induction in humans. [redacted] Therefore, concomitant use of TS-161 with substrates of these transporters should be avoided. **Appendix B** provides a list of allowable and not allowable concomitant medications.

Pulse, blood pressure, adverse events, and laboratory values will be monitored throughout the study.

2.3.1.2 Medication Taper/Drug-Free Period

Hospitalization may be somewhat upsetting to patients. It is essential to ensure patient safety while they are being withdrawn from concomitant medication. There may be a worsening of depressive symptoms including suicidal ideation as the patient's concomitant medications are being discontinued. Subjects with MDD may not receive standard treatment for 12-16 weeks.

All patients will be admitted to the 7SE Mood and Anxiety Disorders Research Unit at the NIMH for supervision. In order to assess if a patient should be withdrawn from/remain off medications to participate, the following criteria must be met: a) subject is experiencing a MDE of sufficient severity (see Study Population for inclusion/exclusion criteria); and, b) the subject does not appear to have significantly benefited from their current medication regimen. We will attempt to verify the latter with the treating clinician(s). Subjects will be told to immediately inform

research staff if they develop suicidal ideation, intent, a suicidal plan, or arrangements for suicide during withdrawal and the medication-free period. Risk management procedures performed during the medication-taper period will continue during this and future periods of the study. With subjects off medication, there may be a worsening of symptoms if it is necessary that a patient discontinue medication/s and during the medication-free period. Such worsening could include increased depressive symptoms, sleep disorder, and/or suicidal ideation. The inpatient nursing staff has extensive experience handling voluntary adult patients with severe mental illness and is adept at maintaining patient safety and comfort during inpatient stays. Furthermore, patients will be assigned a Clinical Research Advocate (CRA) from the Human Subjects Protection Unit (HSPU). The CRA will monitor subjects during research participation from initial consent until study completion. All participants will be hospitalized for the duration of the protocol.

2.3.1.3 Worsening of Symptoms/Delay in Treatment

There could be a delay in treatment for as long as 16 weeks from the initial screening visit; this period of time includes the taper off medications, the drug free period, and the double-blind study phase. There may be a worsening of depressive and anxiety symptoms, including suicidal ideation, as the patient's psychotropic medications are discontinued. Subjects will be told to immediately inform their research physician if they develop suicidal ideation or symptoms of mania, or a worsening of depressive symptoms. We have included safety measures such as discontinuation of the study drug and transition to standard clinical treatment should subjects worsen to a sufficient degree. These precautions are likely to be effective in minimizing risks.

2.3.1.4 Blood Draw

Venipuncture or intravenous lines may be associated with the momentary discomfort of the needle stick, as well as a small potential risk of hematoma (bruise) formation.

The total amount of research blood drawn during the whole study will not be more than 370mL during the 14-16 week study.

2.3.1.5 Clinical Rating Scales

The risks and discomforts of clinical ratings or psychological assessments are minimal. No discomfort is expected to occur during clinical interviews or psychological assessments, other than the potential emotional stress caused by discussing difficult psychiatric topics.

2.3.1.6 Neurocognitive Battery

There are also minimal risks associated with the neurocognitive tasks, beyond possible stress or frustration. Participants will be informed that they can stop participation in these tasks at any time.

2.3.1.7 MEG

MEG is associated with minimal medical risk or discomfort. Remaining still for the duration of the scan may result in mild muscle stiffness or fatigue.

The potential risks associated with MEG will be minimized as follows:

- In addition to a Basic Life Support (BLS)-certified staff member, ancillary staff will be present throughout in case of emergency.

2.3.1.8 MRI

There is minimal risk associated with exposure to magnetic waves during MR imaging, though the potential risk of heart rhythm disturbances exists for patients with a history of heart rhythm abnormalities or those who have certain types of pacemakers. A substantial risk to persons who have metallic objects inside their bodies exists, as the magnet in the scanner can cause these to move. Pregnant women should not undergo MRI because of the possible harmful effects to the fetus. People with claustrophobia may find this procedure uncomfortable as it involves having one's head confined to a relatively small space. A small amount of discomfort may be associated with having to be still for the 90 minutes required for the MR scan, possibly resulting in mild muscle stiffness and fatigue. In addition, very high field magnets (above 3 Tesla) may cause additional side-effects of peripheral nerve stimulation. This is experienced as twitching in the muscles, or a tingling sensation. Additionally, some subjects report dizziness, mild nausea, headache, a metallic taste in their mouth, or a sensation of flashing lights. These sensations are not considered harmful and desist upon cessation of scanning. On very rare occasions, subjects may experience some eye discomfort. No biological hazards to humans have been associated with high static magnetic fields. Finally, the MRI scanning procedure is loud, and may affect hearing; we will place earplugs to wear during the scan to minimize discomfort and prevent adverse effects on hearing.

The potential risks associated with MRI will be minimized as follows:

- The potential for claustrophobia will be mitigated by explaining the nature of the procedure in detail prior to entering the MR scanner.
- In addition to a BLS-certified staff member, ancillary staff will be present throughout in case of emergency.
- A member of the research team will place hearing protection, such as earplugs, in each subject to be worn during the scan to minimize discomfort and prevent any adverse effects on hearing.
- A history of any intraocular, intra-aural, intracranial, or intra-thoracic ferromagnetic implants or devices is an exclusion criterion. Ferromagnetic objects can be heated and/or moved in a strong magnetic field (3T and 7T), which may result in bodily harm.
- Women of child-bearing potential will undergo urine pregnancy testing to rule out pregnancy no more than 24 hours prior to each imaging session.
- A power meter will be used to ensure that experimental sequences fall within the accepted guidelines for radio frequency (RF) energy exposure. Applied RF power will be limited so that the specific absorption averaged over the head does not exceed 3W/kg for 10 minutes.
- While subjects are in the scanner, it is standard procedure to provide them with a squeeze bulb. Subjects are instructed to squeeze it in case of emergency (e.g. eye discomfort, nausea, emesis), after which the scan will stop immediately. If subjects experience eye discomfort or desire to stop for any reason, the scan will stop immediately.

2.3.1.9 Standard Treatment Phase

During the optional traditional standard treatment phase, participants may have worsening of symptoms, including suicidal ideation, as medications and dosages are adjusted. In addition, pharmacologic treatment may result in side effects.

At the end of study participation, patients with MDD will be offered short-term traditional standard treatment (up to two months). During this period, patients will be assessed and discussed at daily morning rounds by nursing staff and licensed independent practitioners (MD, APRN, part of the research team) for changes in psychiatric symptomatology and medication side effects. Additional interventions will be made depending on their status/progress. Additionally, our branch holds several weekly meetings to discuss our inpatients in the standard treatment phase, including an interdisciplinary treatment team meeting. During this meeting, ancillary team members not typically present at morning rounds (e.g., physical therapy, occupational therapy, pharmacy) will also have the opportunity to present their observations and weigh in on patient progress. If issues arise - for instance, psychiatric decompensation or intolerable side effects- medications may be adjusted/discontinued and/or additional medications or therapeutic interventions initiated based on a risk-benefit discussion between the inpatient treatment team and the patient. Additionally, if the patient expresses suicidal or homicidal thinking in the treatment phase, is judged to be an imminent risk of harm to self and/or others, and is not amenable to additional voluntary treatment on 7SE, he or she may be involuntarily psychiatrically hospitalized for his or her safety at an outside hospital.

2.3.2 Known Potential Benefits

This study offers no direct benefits to participants but is likely to yield important and generalizable knowledge about the development of new treatments for MDD.

2.3.3 Assessment of Potential Risks and Benefits

The primary risks of study participation include possible adverse events associated with TS-161, discomfort during the medication taper and drug-free periods, and mild discomfort resulting from blood draws, imaging procedures. This study is likely to yield generalizable knowledge about the antidepressant effects of TS-161. Overall, the risks are reasonable in relation to anticipated benefit.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the ability of the mGlu2/3 receptor antagonist prodrug, TS-161, to improve overall depressive	Montgomery-Asberg Depression Rating Scale (MADRS) change from baseline	The MADRS is a tool used for the evaluation of depressive symptoms in adults and for the assessment

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
symptomatology in subjects with MDD. The efficacy of a three-week course of TS-161 will be compared to three weeks of placebo in a crossover study. The Montgomery-Asberg Depression Rating Scale (MADRS) will serve as the main outcome measure. Hypothesis: Among TRD-MDD subjects, the active drug (TS-161; 50-100 mg/day) will result in less severe depressive symptoms than placebo after three weeks of treatment.		of any changes to those symptoms.
Secondary		
1. To evaluate the antidepressant efficacy of TS-161 230 min and 1, 2, 3, 7, and 14 days post-initial dosing compared to placebo in a crossover study. Hypothesis: Among TRD-MDD subjects, the active drug (TS-161; 50-100 mg/day) compared to placebo will result in less severe depressive symptoms than placebo 230 min, 1, 2, 3, 7, and 14 days post-initial dosing.	1. Change from baseline on MADRS total scores	1. The MADRS is a tool used for the evaluation of depressive symptoms in adults and for the assessment of any changes to those symptoms.
2. To determine whether TS-161 will demonstrate a superior antidepressant efficacy compared to	2. Proportion of subjects in remission (defined as MADRS total score ≤10)	2. The MADRS is a tool used for the evaluation of depressive symptoms in adults and for the

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
placebo in a crossover study Hypothesis: Among TRD-MDD subjects, the active drug compared to placebo will result in a greater proportion of subjects in remission at 230 min, 1, 2, 3, 7, and 14 days post-initial dosing.		assessment of any changes to those symptoms.
3. To determine whether TS-161 will demonstrate a superior antidepressant response compared to placebo in a crossover study. Hypothesis: Among TRD-MDD subjects, the active drug compared to placebo will result in a greater proportion of responders at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing.	3. Proportion of subjects achieving response (defined as a ≥50% reduction from baseline in MADRS total score).	3. The MADRS is a tool used for the evaluation of depressive symptoms in adults and for the assessment of any changes to those symptoms.
4. To evaluate the antisuicidal ideation effects of TS-161 at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing compared to placebo in a cross over study. Hypothesis: Among TRD-MDD subjects, the active drug will result in less severe suicidal ideation than placebo at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing.	4. Change from baseline on item 10 (suicidality) of the MADRS and total score on the C-SSRS, and the Scale for Suicidal Ideation (SSI).	4. Item-10 of the MADRS used to assess suicidal ideation.
5. To investigate the effects of TS-161 on mood,	5. Change from baseline on the HDRS, HAM-A, the	5. These rating scales evaluate various aspects of

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
anxiety, and anhedonia symptoms at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing compared to placebo in a cross over study. Hypothesis: Among TRD-MDD subjects, TS-161 will result in less severe mood, anxiety, and anhedonia symptoms than placebo at 230 min, 1, 2, 3, 7, 14, and 21 days post-initial dosing.	PANAS, Snaith–Hamilton Pleasure Scale (SHAPS), and the Temporal Experience of Pleasure Scale (TEPS) scales.	mood, anxiety, and anhedonia.
6. To assess the safety and tolerability of a three-week course of TS-161 compared to placebo in a crossover study. Hypothesis: Among TRD-MDD subjects, the active drug will result in comparable safety and tolerability to placebo at the different timepoints measured during the 3 weeks of treatment.	6. Incidence of AEs and total scores using the Clinician Administered Dissociative States Scale (CADSS), Young Mania Rating Scale (YMRS), the Brief Psychiatric Rating Scale (BPRS), vital signs, changes in clinical laboratory evaluations, and ECGs.	6. These measures and evaluations assess various aspects of clinical condition, adverse events, and mood and anxiety symptomology.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Surrogate Markers of Drug Effect, Target Engagement, and Antidepressant Response 1. To determine whether the glutamate peak in the medial PFC (mPFC) correlates with changes in depression scores to TS-161. <u>Hypothesis 1:</u> Among TRD-MDD subjects, pre-treatment mPFC glutamate levels will predict the magnitude of clinical improvement to TS-161 in subjects with MDD, but not to placebo. <u>Hypothesis 2:</u> Among TRD-MDD subjects, post-treatment increases in glutamate levels will predict the magnitude of clinical improvement to TS-161 (but not placebo).	1. ^{1}H-MRS correlates with changes in MADRS score	1. The MADRS is a tool used for the evaluation of depressive symptoms in adults and for the assessment of any changes to those symptoms. MRS is used to measure glutamate levels.
2. To determine whether gamma power increases with TS-161are associated with treatment response to TS-161. <u>Hypothesis 1:</u> Among TRD-MDD subjects, pre-treatment gamma power levels will predict the magnitude of clinical improvement to TS-161, but not to placebo.	2. Gamma power measured via MEG	2. MEG scanning is used to measure gamma power.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Hypothesis 2: Among TRD-MDD subjects, post-treatment increases in gamma power will be positively associated with antidepressant response to TS-161 (but not placebo).		
3. To determine whether TS-161 modulates task-dependent and resting-state activity in the frontolimbic circuitry and whether changes in activity in the frontolimbic circuitry are correlated with antidepressant response to TS-161 Hypothesis 1: Among TRD-MDD subjects, TS-161 administration will increase pgACC activity and decrease amygdala activity to emotional faces more than placebo. Similar effects will be observed in activity associated with the resting-state networks. TS-161 will also decrease pgACC activity during the tasks more than placebo.	3. Changes in activity in the frontolimbic circuitry	3. fMRI is used to measure frontolimbic circuitry
Tertiary/Exploratory		
1. To determine whether TS-161 will increase plasma/serum BDNF and vascular endothelial growth factor (VEGF) and decrease glycine/serine and alter oxidative stress/inflammatory parameters in peripheral	1. Plasma/serum BDNF, vascular endothelial growth factor (VEGF), glycine/serine, and oxidative stress/inflammatory parameters in peripheral leukocytes	1. To identify potential biomarkers of TS-161 antidepressant activity.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
leukocytes compared to placebo and/or baseline levels.		
1. To collect blood samples for optional exploratory studies focusing on identification of genes, proteins, and metabolic responses that may influence the disposition, efficacy, safety and tolerability of TS-161.	2. Blood samples for the identification of genes, proteins, and metabolic responses	2. To identify potential biomarkers of TS-161 antidepressant activity.
3. To evaluate neurocognitive functioning on TS-161 compared to placebo and/or baseline levels.	3. Neurocognitive functioning	3. To evaluate any neurocognitive effects of TS-161

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a Phase 2 randomized, double blind, placebo-controlled, crossover, single–site, approximately 16-week inpatient experimental study that will assess the efficacy and safety of three weeks of 50 to 100 mg/day of TS-161, an mGlu2/3 receptor antagonist prodrug. Subjects will meet diagnostic criteria for MDD without psychotic features, according to DSM-IV or DSM-5 guidelines and confirmed by the SCID-P. Approximately 25 subjects with MDD will be recruited for this study to allow 20 subjects to crossover and complete the study. TS-161 and placebo will be taken orally in capsule form as a single daily dose for 3 weeks in each of two crossover Research Conditions.

This single site, inpatient study will be conducted at the NIH Clinical Center in Bethesda, MD.

The protocol will not include interim analyses, substudies, or stratifications.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study will use a double-dummy blind dosing design, all subjects will receive two capsules in the AM approximately 30 minutes after start of breakfast, and dose adjustment, if required due to intolerance, will be done blindly. A flexible dose design (50 or 100 mg/day) will be used in this study since this type of dosing permits better assessment of safety and efficacy than does a fixed dose, especially when this compound has not been previously tested in subjects with major depression. The maximum daily dose of TS-161 administered in this study will be 100 mg/day. All subjects except those who are responders at the end of the first treatment condition

will cross over. To avoid carry-over effects between the different test sessions, there will be an interval of 14-21 days, pending response to Research Condition 1. Subjects will then be blindly crossed over to the second treatment condition (either TS-161 or placebo) for another three weeks. Subjects maintaining response to Research Condition 1 after two weeks will receive an additional one-week washout before being crossed over to Research Condition 2. Subjects maintaining response (50% improved from baseline on the MADRS) after this additional week will be withdrawn from the study and will receive standard clinical treatment.

4.3 JUSTIFICATION FOR DOSE

The dose of TS-161 was arrived at based on toxicology studies in animals, as well as from Single Ascending and Multiple Ascending Dose Studies conducted in humans. The daily clinically efficacious dose of TS-161 was estimated to be 50–100 mg/day by predicting human dose required for giving an efficacious CSF exposure of TP0178894 based on preclinical studies.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Ability of subject to understand and the willingness to sign a written informed consent document. To verify this, subjects must score ≥ 80% on the consent quiz (Appendix A).
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Aged 18 to 65.
4. Subjects must fulfill DSM-IV or DSM-5 criteria for MDD, single episode or recurrent without psychotic features, based on clinical assessment and confirmed by a structured diagnostic interview (SCID-P). Subjects must be experiencing a current major depressive episode of at least 4 weeks duration.
5. Subjects must have an initial score of ≥ 20 on the MADRS and a YMRS score of <12 within one week of the start of Phase I and upon entry into Phase II.
6. Ability to take oral medication and be willing to adhere to the TS-161 regimen.
7. Subjects must have a current or past history of lack of response to at least one adequate antidepressant trial (may be from the same chemical class) in the current major depressive episode, operationally defined using the modified-Antidepressant Treatment History Form (ATHF)); a failed adequate trial of ECT [or TMS] would count as an adequate antidepressant trial.
8. For females of reproductive potential: use of contraception starting at the time of enrollment and agreement to use such a method during study participation and for an additional 4 weeks after the end of Phase II (see Contraception Guidance, Appendix D).
9. For males of reproductive potential: use of condoms or other methods from the time of enrollment to ensure effective contraception with partner, and for an additional 90 days after the end of Phase II (see Contraception Guidance, Appendix D).
10. Agreement to adhere to Lifestyle Considerations throughout study duration

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current use of disallowed concomitant medications (see Appendix B: Drugs Allowed and Not Allowed) or transcranial magnetic stimulation (TMS) less than 2 weeks prior to the start of Phase II.
2. Treatment with a reversible monoamine oxidase inhibitor (MAOI) less than 4 weeks prior to the start of Phase II.
3. Treatment with fluoxetine, aripiprazole, or brexpiprazole less than 5 weeks prior to the start of Phase II.
4. Treatment with clozapine or electroconvulsive therapy (ECT) less than 4 weeks prior to the start of Phase II.
5. Lifetime history of deep brain stimulation.
6. Previous antidepressant non-response to ketamine or esketamine.
7. No structured psychotherapy will be permitted during the total duration of the study. Subjects unable or unwilling to stop psychotherapy will be unable to participate in the study.
8. Pregnancy or lactation
9. Current psychotic features or a diagnosis of schizophrenia or any other psychotic disorder as defined in the DSM-IV or DSM-5.
10. Subjects with a history of DSM-IV drug or alcohol dependency or abuse (or "alcohol use disorder" per DSM-5), except for caffeine or nicotine dependence, within the preceding 3 months.
11. Subjects with a DSM-IV or DSM-5 Axis II diagnosis of borderline or antisocial personality disorder.
12. Subjects with a history of a head injury that resulted in loss of consciousness exceeding 5 minutes (for the imaging component of the study).
13. Serious, unstable illnesses including hepatic, renal, gastroenterologic, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease.
14. Subjects with any current or chronic history of liver disease, hepatic, or other biliary abnormalities.
15. Subjects with a history of persistent orthostatic hypotension or other cardiac conditions where orthostatic hypotension would be a safety concern
16. Subjects with unstable clinical hyperthyroidism or hypothyroidism.
17. Subjects with one or more seizures without a clear and resolved etiology.
18. Clinically significant abnormal laboratory tests. In particular, participants with the following based on baseline liver safety values will be excluded:
    - Alkaline phosphatase (Alk Phos) > 150 U/L
    - Alanine aminotransferase (ALT) > 55 U/L
    - Aspartate aminotransferase (AST) > 34 U/L

- Total bilirubin (TB)> 1.2 mg/dL
- Direct bilirubin (DB) > 0.5 mg/dL

19. Subjects who, in the Principal Investigator's judgment, pose a current serious suicidal or homicidal risk.
20. Positive HIV test.
21. For those participating in imaging procedures, contraindications to imaging (metal in body, claustrophobia, etc.)
22. Participants with COVID-19, or suspected COVID-19
23. A current NIMH employee/staff or their immediate family member.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Children

We are excluding minors as we feel preliminary safety and efficacy data should be obtained in adults before proceeding to vulnerable populations.

5.3.2 Pregnant Women, Fetuses, or Neonates

The safety of TS-161 during pregnancy and breast-feeding is unknown. In addition, the safety profiles for MRI in pregnant women are unknown.

5.3.3 Prisoners

Prisoners will be excluded from participation.

5.3.4 Decisionally Impaired Adults

We are excluding cognitively impaired persons as we feel that preliminary safety and efficacy data should be obtained in non-impaired adults before proceeding to vulnerable populations. Given that this study presents no direct clinical benefit for the participant and the research involves more than a minor increase in minimal risk, the risks will outweigh the benefits for this population. We also want to ensure that all participants understand the consenting process as well as all study procedures, including the study tests and measures. Therefore, cognitively impaired persons, who may not be able to provide their own consent, will not be enrolled.

5.3.5 Participation of NIH Staff or family members of study team members

NIH staff and family members of study team members may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable and an unidentifiable manner.

The NIH Information Sheet on NIH Staff Research Participation will be made available. Please see Informed Consent Process for consent of NIH Staff.

5.3.6 Patients with Comorbid Anxiety or Other Psychiatric Disorders, Besides Those Listed Under Inclusion/Exclusion Criteria

Exclusion of patients with comorbid anxiety or other psychiatric disorders would affect the generalizability of our findings, given that a substantial percentage of patients with MDD may have additional diagnoses. Allowing the participation of patients with a history of comorbid disorders broadens the inclusion criteria to more closely approximate patients seen in real world settings. Thus, we will exclude patients with comorbid diagnoses only if it has been the primary focus of treatment in the past six months.

5.3.7 HIV/AIDS Positive Subjects

The drug used in this study is experimental and may have unknown interactions with medications used to control HIV. In addition, the effects of HIV on the brain may be a confound to our examination of brain biomarkers. For experimental therapeutic depression studies, we have historically excluded HIV-positive individuals.

5.4 Persons over 65 Years of Age

Subjects older than 65 will be excluded to prevent age-related changes in brain structure and function from confounding our analyses. In addition, experimental TS-161 use in older and/or medically compromised patients has not been explored due to greater theoretical risk of serious adverse events.

5.5 Non-English Speakers

The majority of the required monitoring and rating instruments are not validated in languages other than English, and we do not have the ability to provide clear written and verbal communication with those individuals.

5.6 Lifestyle Considerations

During this study, participants are asked to:

1. Abstain from alcohol and drug use (except for caffeine and nicotine) while in the study.
    - For individuals of reproductive potential: use of highly effective contraception starting at the time of enrollment and agreement to use such a method during study participation and for an additional 4 weeks after the end of Phase II.
    - For males of reproductive potential: use of condoms or other methods from the time of enrollment to ensure effective contraception with partner, and for an additional 90 days after the end of Phase II.
    - Agree to remain inpatient, unless authorized by the Principal or Lead Associate Investigator. Passes will be permitted for inpatients if the subject is clinically assessed and deemed psychiatrically and medically stable, including not an imminent danger to self or others and the pass does not interfere with the study or unit procedures.
    - The taper off of medications and drug-free period of the study may be done as an outpatient if clinically appropriate.

5.7 SCREEN FAILURES

Screening for inclusion into the study will be done under protocol 01-M-0254, "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers."

5.8 STRATEGIES FOR RECRUITMENT AND RETENTION

Our recruitment program seeks to increase awareness of our studies among a broad demographic (e.g. patients and families, scientific community, mental health clinicians) through a range of recruitment materials and venues. Participants will be recruited under Screening Protocol 01-M-0254: "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers" or under this protocol using IRB approved recruitment materials.

NIH employees/staff will not be directly recruited by or through their supervisors to participate in this study but are eligible for participation. NIMH employees/staff and their immediate family members cannot participate in this protocol.

We will use the following recruitment methods:

1. Non-Paid Advertisements
    a. Internal NIH media (e.g. NIH Record)
    b. NIH Internet (e.g. http://patientinfo.nimh.nih.gov/)
    c. Listservs:

        With the permission of listserv administrators, we will distribute information regarding our studies. We will not post directly on listservs. We will distribute information to the administrators of listservs such as the: NIMH Outreach Partnerships Updates, NIH research assistant listserv, Community Services Announcements- City of Gaithersburg, Montgomery County Providers, Howard County Providers, and Frederick Providers Council listservs. We will request that the following disclaimer be included with any distributed information:

        "You are receiving this message because your email address is included in the above listserv. The purpose of this message is to inform you of our NIMH research studies. The moderator of the listserv has permitted its use for this distribution."

    d. Short Text Descriptions:

        Short Text Descriptions of this study will appear on social networking sites as Facebook, Instagram, and Twitter. Only official NIH accounts will be used. These may also be publicized using Community Resource Listings (e.g. Community Calendars, Newsletters, electronic and print- twenty-five word text summary).

2. Paid Advertisements:
    a. Local print publications (e.g. Washington Post Express, The Gazette)
    b. National print publications (e.g. Washington Post, NAMI Advocate Magazine)
    c. Public transit advertisements (e.g. posters on Washington Metropolitan Area Transit Authority Metrobuses/Metrorail)

d. Radio advertisements (e.g. WMZQ, WASH, WLZL/CBS Radio)

3. Brochures:

   a. Mental Health Clinics, (e.g. Threshold Services, Adventist Potomac Ridge Behavioral Health, Northern VA Mental Health Institute), Hospitals (e.g. Suburban Hospital, Walter Reed National Military Medical Center), and local Clinicians

   b. Professional Conferences (e.g. American Psychiatric Association and American College of Neuropsychopharmacology annual conferences), Symposiums (e.g. Challenging Depression: New Insights into Research and Treatment (Suburban Hospital)), and other professional settings (e.g. Latino Behavioral Health Institute, Frederick Providers Council, DC Department of Mental Health)

   c. Mental Health Advocacy Conferences (e.g. NIMH Outreach Partnership Annual Meeting, Maryland's Annual Suicide Prevention Conference)

   d. Self-Help and Advocacy Groups (e.g. local NAMI and DBSA chapters, Jewish Social Service Agency, Active Minds)

4. NIH Referrals:

   a. Investigators from other NIH inpatient units and outpatient clinics, including those from the NIMH, may refer patients to the screening protocol, as they may be eligible for additional studies.

   b. CenExcelCBH, a recruitment and screening database developed by the Centers for Behavioral Health (CBH), will be added as an additional referral/recruitment source for this protocol. CBH is a local research facility that recruits and enrolls participants in psychiatric studies. Subjects that present at CBH sign an IRB-approved CBH consent form to be enrolled in their database and collect screening information. The medical and psychiatric history information collected will assist in determining if an individual is a potential candidate for this protocol. If the individual meets the initial eligibility for this study, a CBH team member will give them the contact information for the NIMH staff along with IRB approved recruitment materials for the study. If the potential referral is interested in participating in the study, it will be up to them to contact the NIMH research team. None of the individuals' information will be given to the NIMH for recruitment purposes. If they are interested in learning more about the NIH study, they will call and complete a brief telephone screening with NIMH staff. If they meet study criteria, we will invite them for an in-person screening visit under protocol 01-M-0254. We may ask the individual to sign a CBH release of information form to share existing diagnostic information from their CBH records with NIH.

Brochures and other IRB-approved recruitment materials or study information may be given directly to individuals interested in our research in both hard copy and electronic formats, depending on request. Associate Investigators and members of the NIMH Marketing & Community Relations Unit will distribute recruitment materials to individuals/groups, such as mental health clinics, hospitals, self-help and advocacy groups, and local clinicians. Clinicians who are contacted will be provided with information to disseminate to patients as they see fit. We

will explain to them that individuals interested in participating in our studies will need to initiate contact with our group and that we will not make this initial contact.

5.8.1 Costs

There are no costs to participants enrolled in this study.

5.8.2 Compensation

All participants will be compensated for time and research-related inconveniences. Compensation will be in the form of a check payment and will be given at the end of each study phase. Payment will be prorated for parts completed if subjects do not complete the study. Escorts will not be compensated.

The Designation of Reimbursement for Travel and Subsistence (DRTS) form for this protocol, which details the compensation for hotel and transportation, is attached in iRIS.

NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

PHASE I			
Procedure	**Number**	**Pay per Procedure**	**Total Pay**
Clinical Rating Scales	2	20	40
Blood Draw	1	10	10
Neurocognitive Testing	1	80	80
Clinical MRI	1	40	40
fMRI/MRS	1	80	80
MEG	1	100	100
Phase I Total			**$ 350**

PHASE II			
Procedure	**Number**	**Pay per Procedure**	**Total Pay**
Clinical Rating Scales	17	20	340
Blood Draws	26	10	260
Neurocognitive Testing	5	80	400
Additional Safety Monitoring (study drug days)	42	48	2,016
fMRI/MRS	5	80	400
MEG	5	100	500
Maximum Phase II Total			**$3,916**

The maximum compensation, including Phases I and II is $4,266.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention: TP0473292 (IND)

The use of TP0473292 (TS-161) in this study has been approved by the FDA under IND #153429. The study drug will be supplied by Taisho Pharmaceutical Co., Ltd. The sponsor of the IND is the National Institute of Mental Health. The IB is attached in iRIS.

TS-161 is not available for commercial use.

6.1.1.1 Study Intervention: MRI Machines (NSR Device)

MRI are non-significant risk (NSR) devices that will be used for investigational research under this protocol. Under this protocol, we are using a 7T Magnetom MRI Prototype Siemens System manufactured by Siemens Medical Solutions USA, Inc. Images generated by the MRI differ from what are created in a routine clinical scan. However, all studies done under this protocol will be performed within FDA safety guidelines. The use of research coils in the MRI has not been approved by the FDA and is considered investigational. Additionally, some of the MR machines used in this study are considered investigational (not yet approved by the FDA for this use) but are used within the FDA safety guidelines.

Criteria establishing MRI scanners as NSR devices:

a. is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject

   An MRI scanner is not an implantable device.

b. is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject

An MRI scanner is not for use in supporting or sustaining human life. It does not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

c. is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject

   An MRI scanner, as used under this protocol, is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

d. otherwise presents a potential for serious risk to the health, safety or welfare of a subject

   MRI scanners have been in use for over three decades. Safety guidelines have been developed and updated allowing its dissemination to a wide range of clinical and non-clinical settings. As long as subjects are properly screened and sessions are run according to these safety guidelines, MRI scanners are generally considered NSR. While operated in research mode, the MRI will be under the International Electrotechnical Commission (IEC) 60601-2-33 First Level Controlled Operating Mode, which allows for research pulse sequences to be used within the FDA/IEC safety limits for MRI devices.

6.1.1.2 Research Pulse Sequences for 3T and 7T MRI

Research pulse sequences used during imaging procedures under this protocol are considered non-significant risk (NSR) devices.

Criteria establishing research pulse sequences as NSR devices:

a. is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject

   Research pulse sequences are not an implantable device.

b. is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject

   Research pulse sequences are not for use in supporting or sustaining human life. They do not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

c. is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject

   Research pulse sequences, as used under this protocol, are not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

d. otherwise presents a potential for serious risk to the health, safety or welfare of a subject

Research pulse sequences, as used under this protocol, do not present a potential for serious risk to the health, safety, or welfare of a subject. While operated in an investigational manner, research pulse sequences are used within the FDA/IEC safety limits for MRI devices.

6.1.1.3 Radiofrequency Research Coils for 3T and 7T MRI

Research coils used during MRI scans under this protocol are considered non-significant risk (NSR) devices.

Criteria establishing research coils as NSR devices:

a. is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject

   Research coils are not an implantable device.

b. is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject

   Research coils are not for use in supporting or sustaining human life. They do not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

c. is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject

   Research coils, as used under this protocol, are not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

d. otherwise presents a potential for serious risk to the health, safety or welfare of a subject

   Research coils, as used under this protocol, do not present a potential for serious risk to the health, safety, or welfare of a subject. The research coils are used within the FDA/IEC safety limits for MRI devices.

6.1.1.4 Image Reconstruction and Analysis Software for 3T and 7T MRI

Image reconstruction and analysis software used during MRI scans under this protocol are considered non-significant risk (NSR) devices.

Criteria establishing image reconstruction and analysis software as NSR devices:

a. is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject

   Image reconstruction and analysis software are not implantable devices.

b. is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject

Image reconstruction and analysis software are not for use in supporting or sustaining human life. They do not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

c. is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject

   Image reconstruction and analysis software, as used under this protocol, are not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

d. otherwise presents a potential for serious risk to the health, safety or welfare of a subject

   Image reconstruction and analysis software, as used under this protocol, do not present a potential for serious risk to the health, safety, or welfare of a subject. The software are used within the FDA/IEC safety limits for MRI devices.

6.1.2 Dosing and Administration

[redacted]

The starting dose will be 100 mg/day (given as a single AM dose approximately 30 minutes after start of breakfast) and the treatment target for all participants will be 100 mg/day unless tolerability issues ensue. The dose of TS-161/placebo will remain at 100 mg/day in subjects until the end of the study. If subjects are unable to tolerate the current dose, then the dose may be decreased down to 50 mg/day for one week at which time the dose may be increased back to 100 mg/day if response criteria are not met and side effects are tolerable. In case of continued side effects at 100 mg/day, the dose may be lowered to 50 mg/day until the end of the study. Subjects who are unable to tolerate the lowest allowable dose of study medication (50 mg/day) will be discontinued from the study. No dose increases will be allowed two weeks after the start of each research condition (Days 14 and 49) to allow ample time to evaluate the effect of the drug. Only licensed independent practitioners who are investigators on this protocol will be able to make dosing decisions. The maximum daily dose of TS-161 administered in this study will be 100 mg/day.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

Preparation, handling, storage, and accountability will be handled by the Clinical Center's Investigational Drug Management and Research Section (IDMRS) in accordance with the Investigational Brochure (IB).

6.3 ACQUISITION AND ACCOUNTABILITY

The study drug and placebo will be provided by the manufacturer, Taisho Pharmaceutical Co., Ltd, in accordance with a CRADA (see Collaborative Agreements). The study site pharmacist(s) responsible for study drug dispensation.

Study personnel will pick up the study intervention or the control product from the NIH pharmacy the day of the study visit. Any unused study intervention or control product will be returned to the NIH pharmacy for disposal.

6.4 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The name of the participant, name of the study, participant number, directions for administration, day of study visit, and the signature of the physician should appear on the study intervention and the control product.

6.4.1 Product Storage and Stability

[redacted]

6.4.2 Preparation

[redacted] Instructions for the preparation of study drugs are provided in a separate manual.

6.5 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

A randomization code will be generated by the unblinded statistician. The study site pharmacist(s) responsible for study drug dispensation will also be unblinded.

6.6 STUDY INTERVENTION COMPLIANCE

The study drug will be administered to participants on the inpatient unit to ensure adherence to the protocol.

6.7 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements. See Appendix B: Drugs Allowed and Not Allowed.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from TS-161 does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

To the extent possible, data to be collected at the time of study intervention discontinuation are those intended for the final day of the study (see Schedule of Events).

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Completion of study intervention
- Disease progression which requires discontinuation of the study intervention
- If any clinical AEs, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant. Examples include:
    - Extreme changes in vital signs
    - Persistent systolic blood pressure (SBP) > 165 mm Hg, diastolic blood pressure (DBP) > 100 mm Hg, SBP < 80 (symptomatic), pulse >130, pulse < 45 (symptomatic), SaO2 <90%, and T > 39 degrees C
- Investigator discretion
- Positive pregnancy test
- Participant unable to receive TS-161 for 3 weeks.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Prior to removal from study, effort must be made to have all subjects complete all end of study procedures listed on the Schedule of Events.

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject has completed the study follow-up period
- Death
- Screen Failure

The reason for participant discontinuation or withdrawal from the study will be recorded on the Enrollment log. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

This section is not applicable as this is an inpatient protocol.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

Prior to consenting to this study, subjects will have undergone screening under protocol 01-M-0254, "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers". Psychiatric history and diagnosis of MDE will be made using the SCID-P (First, Spitzer et al. 2001) as well as DSM-IV or DSM-5 diagnostic criteria. Subjects who meet DSM-IV or DSM-5 criteria for major depressive episode (without psychotic features) must have a score of ≥20 on the MADRS within one week of the start of Phase I and upon entry into Study Phase II. Subjects who previously enrolled in and completed studies at the ETPB may be enrolled in this protocol.

We will require that all subjects have physical examinations and specific laboratory tests (e.g. Acute Care Panel, CBC+Diff, Hepatic Panel, LFT, TFT, urinalysis, urine pregnancy, urine toxicology, HIV). Results of these tests will identify patients who should be excluded because of active medical problems or substance abuse that might affect clinical phenomenology or make participation in the protocol unsafe. Subjects are expected to meet all other eligibility criteria to be enrolled in the study. Protocol 01-M-0254 allows for the sharing of collected data, including history and physical exams, structural neuroimaging studies, and lab testing.

8.2 MEDICATION TAPERING

If participants with MDD are medicated at the time of consent, they will undergo tapering of all prohibited medications (see Appendix B, Drugs Allowed and Not Allowed for details). The medication taper will be followed with a subsequent washout of two weeks during which time patients will be medication-free. Subjects will be reminded to report all medications, OTC products, and other agents to the investigators so that they can screen to avoid interactions that might make participation unsafe or might confound the research results. The taper off of medications and drug-free period of the study may be done as an outpatient if clinically appropriate. Medications that are required for non-psychiatric medical reasons (e.g., antihistamines and topical antifungals) will not be discontinued for the purpose of the study.

8.3 TS-161 AND PLACEBO ADMINISTRATION

[redacted] All participants will use this dose range unless tolerability issues ensue. A randomization code will be generated by the unblinded statistician. The study site pharmacist(s) responsible for study drug dispensation will also be unblinded. [redacted] This study will use a double-dummy blind dosing design, all subjects will receive two capsules in the AM after start of breakfast, and dose adjustment, if required due to intolerance, will be done blindly. Instructions for the preparation of study drugs are provided in a separate manual. A flexible dose design (50 or 100 mg/day) will be used in this study since this type of dosing permits better assessment of safety and efficacy than does a fixed dose, especially when this compound has not been previously tested in subjects with major depression. All subjects except those who are responders at the end of the first Research Condition will cross over. To avoid carry-over effects between the different test sessions, there will be an interval of 14-21 days, pending response to Research Condition 1. Subjects will then be blindly crossed over to the second Research Condition (either TS-161 or placebo) for another three weeks.

No subject will receive more than 3 weeks of TS-161. All subjects who discontinue the study or who complete Study Phase II will then receive either short-term standard clinical treatment (for up to 2 months) or the opportunity to participate in another research protocol. Subjects maintaining response to Research Condition 1 after two weeks will receive an additional one-week washout before being crossed over to Research Condition 2. Subjects maintaining response (50% improved from baseline on the MADRS) after this additional week will be withdrawn from the study and will receive standard clinical treatment.

The starting dose will be 100 mg/day (given as a single AM dose approximately 30 minutes after start of breakfast) and the treatment target for all participants will be 100 mg/day unless tolerability issues ensue. The dose of TS-161/placebo will remain at 100 mg/day in subjects until the end of the study. If subjects are unable to tolerate the current dose, then the dose may be decreased down to 50 mg/day for one week at which time the dose may be increased back to 100 mg/day if response criteria are not met and side effects are tolerable. In case of continued side effects at 100 mg/day, the dose may be lowered to 50 mg/day until the end of the study. Subjects who are unable to tolerate the lowest allowable dose of study medication (50 mg/day) will be discontinued from the study. No dose increases will be allowed after Day 14 of each treatment period to allow ample time to evaluate the effect of the drug. Only licensed independent practitioners who are investigators on this protocol will be able to make dosing decisions.

Concurrent with the first dose administration of study medication, participants will undergo MEG and MRS procedures (described below), unless technical or availability issues prevent their acquisition. Participants will also undergo MRI/MRS, MEG procedures on the final day of each Research Condition, unless technical or availability issues prevent acquisition.

8.4 EFFICACY ASSESSMENTS

8.4.1 Clinical Rating Scales

The following rating scales will be administered to participants at scheduled intervals during their participation (see the Schedule of Clinical Rating Scales).

Brief Psychiatric Rating Scale (BPRS, (Overall and Gorham 1962))

Symptoms and behaviors that are characteristic of schizophrenia will be assessed by using the BPRS. Four key BPRS items will be used as an index of positive symptoms of schizophrenia based on previous reports that indicated their utility and validity (Bowers, Heninger et al. 1980, Kane, Honigfeld et al. 1988) and inclusion within the empirically derived thought disorder factor of the BPRS. These four key positive symptoms are conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content. Three key BPRS items, blunted affect, emotional withdrawal, and motor retardation are selected as a measure of negative symptoms of schizophrenia based on a report of their reliability and validity and their inclusion within the empirically derived withdrawal-retardation factor of the BPRS.

Clinician Administered Dissociative States Scale (CADSS, (Bremner, Krystal et al. 1998))

The CADSSS is a 28-item scale for the assessment of dissociative states at discrete points in time. The instrument contains both subjective and objective items. This is intended to capture the fact that dissociation is both subjective experience as well as a set of behaviors which can be

observed by an outside observer. The CADSS can be used as a change measure to assess, for example, dissociative states before and after a course of treatment.

Columbia Suicide Severity Rating Scale (C-SSRS, (Posner, Brown et al. 2011))

The C-SSRS is a suicidal ideation rating scale created by researchers at Columbia University. It rates an individual's degree of suicidal ideation on a scale, ranging from "wish to be dead" to "active suicidal ideation with specific plan and intent. The scale identifies behaviors, which may be indicative of an individual's intent to commit suicide. An individual exhibiting even a single behavior identified by the scale was 8 to 10 times more likely to commit suicide.

Family History Screen (FHS, (Weissman, Wickramaratne et al. 2000))

The FHS is a clinician-administered structured interview designed to collect the mental health history of an informant and their first-degree relatives on 15 psychiatric disorders and suicidal behavior. A series of 31 questions are used to detect any evidence of psychiatric symptomatology in the participant's relatives. Its validity is best demonstrated for major depression, anxiety disorders, substance abuse, and suicide attempts. If the FHS was not conducted under 01-M-0254 it will be done under the current protocol.

Hamilton Depression Rating Scale (HDRS, (Hamilton 1960))

The HDRS is a widely used observational rating measure of depression severity. The estimated time to administer this scale is 30 minutes. It assesses both the presence and severity of individual signs and symptoms characterizing depression without psychotic features.

Hamilton Psychiatric Rating Scale for Anxiety (HAM-A, (Hamilton 1959))

The HAM-A is a widely used observational rating measure of anxiety severity. The scale consists of 14 items. Each item is rated on a scale of 0 to 4. This scale will be administered to assess the severity of anxiety and its improvement during the course of therapy. The HAM-A total score is the sum of the 14 items and the score ranges from 0 to 56.

Montgomery and Asberg Depression Rating Scale (MADRS; (Montgomery and Asberg 1979))

The MADRS is a 10-item instrument used for the evaluation of depressive symptoms in adults and for the assessment of any changes to those symptoms. The estimated time to administer this scale is 20 minutes. Inter-rater reliability of the scale is high and scores correlate significantly with those of the HAMD. Each of the 10 items are rated on a scale of 0 to 6, with differing descriptors for each item. These individual item scores are added together to form a total score, which can range between 0 and 60 points.

Positive and Negative Affect Schedule (PANAS, (Watson, Clark et al. 1988))

The PANAS is a questionnaire that consists of a number of words that describe different feelings and emotions.

Snaith–Hamilton Pleasure Scale (SHAPS, (Snaith, Hamilton et al. 1995))

The SHAPS is a self-reported *scale* evaluating *anhedonia* for neuropsychiatric disorders. This is a 14-item checklist covering four domains of hedonic experience, namely interest/pastimes, social interaction, sensory experience, and food/drink.

Scale for Suicidal Ideation (SSI, (Beck, Kovacs et al. 1979))

The SSI is a 19-item observer scale designed to quantify the intensity of current conscious suicidal ideation in various dimensions of self-destructive thoughts or wishes: the extent of the wish to die, the desire to make an actual suicide attempt, and details of any plans; also, internal deterrents to an active attempt, and subjective feelings of control and/or courage regarding a proposed attempt.

Temporal Experience of Pleasure Scale (TEPS, (Gard, Gard et al. 2006))

The TEPS is a self-report measure designed to distinguish between consummatory and anticipatory *anhedonia*.

Young Mania Rating Scale (YMRS; (Young, Biggs et al. 1978))

The YMRS consists of 11 items. Items 5, 6, 8, and 9 are rated on a scale from 0 (symptom not present) to 8 (symptom extremely severe). The remaining items are rated on a scale from 0 (symptom not present) to 4 (symptom extremely severe). Items 5, 6, 8, and 9 (irritability, speech, content and disruptive-aggressive behavior) are given twice the weight of the remaining 7 in order to compensate for the poor condition of severely ill patients. The YMRS total score ranges from 0 to 60. The time to administer this scale is 15-30 minutes. The YMRS scale is obtained should hypomanic/manic symptoms develop during the study protocol.

8.4.2 Magnetoencephalography (MEG) (Optional)

All subjects will have a total of six MEG scans: baselines preceding each treatment phase, 2 hours after the first dose administrations and at the end of each Research Condition (see the Schedule of Events for timing of procedures).

During the MEG scanning, participants will be in the shielded recording room with their heads inside the helmet. Brain magnetic fields will be recorded with the 275-channel OMEGA system. The 275 SQUID sensors are uniformly distributed, in a grid, over the inner surface of the helmet that covers the entire head with provisions for the eyes and ears. Visual and two-way audio communication with the participant will be maintained throughout the session. Head position within the magnetometer will be determined using three indicator coils that are attached to the preauricular and the nasion fiducial points. The positions define the coordinate system for the signals and allow for post-hoc registration. Digital photographs or 3d laser scans of the fiducial points on the participant's head may be used to facilitate registration with the anatomical MRI. A 10-minute resting state MEG scan will consist of continuous acquisition without presentation of a task, during which participants will be instructed to relax with their eyes closed. Tasks which measure visual, auditory, and somatosensory evoked potentials as well as simple cognitive tasks will also be performed (see below for a description of tasks). Physiological activity will be recorded via pulse oximetry and electrooculogram.

During MEG scanning, the following protocol of rest/task scans will be collected:

Auditory, Somatosensory, and Visual Evoked Fields Task

Subjects will be instructed to attend to a fixation while binaural auditory tones, left and right visual field static checkerboards, and left and right somatosensory “airpuff” stimulations are randomly presented during a 10-minute scan session. Subjects will be asked to maintain fixation on a centrally-presented dot during the scan session.

Monetary Incentive Delay Task

Subjects will be instructed to attend to stimuli (three different shapes: triangle, circle, square) that are presented briefly, followed by a variable delay, then a response window. During the response window, subjects will be instructed to button-press as quickly as possible. Some of the objects will allow participants to win money, some objects will allow participants to avoid losing money, and some will not be associated with reward/loss. This task will be collected during a 10-minute scan session.

Hariri Hamer Task

Subjects will be instructed to match faces and shapes during a 10-minute scan session. Subjects will first be presented with a target object (either face or shape), then after a brief delay, two simultaneous images (either 2 faces or 2 shapes). They will indicate via left or right button-press which of the two simultaneous images matches the target. For faces, subjects will be instructed to match the emotion displayed (either happy or sad). For shapes, subjects will be instructed to match the shape, even if the orientation changes.

8.4.3 MRI and MRS (Optional)

All subjects will have a total of six MRI/MRS scans: baselines preceding each treatment phase, 3 hours, and 4 hours after the first dose administrations and at the end of each Research Condition (see the Schedule of Events for the timing of procedures). Participants who have not received MRI scans at the NIH within the past 12 months will be scheduled for a battery of clinical scans. These scans take approximately 15 minutes to acquire. These clinical scans may be performed on either a 1.5, 3, or 7 Tesla system and may be acquired either during a separate scanning session before the baseline scanning session, or during the baseline scanning session. A structural MRI scan will be acquired for MEG data coregistration if one has not already been obtained.

MRI scans will be acquired using a multi-channel coil for increased signal-to-noise. The whole brain structural scan will be acquired with a gradient echo based T1 weighted whole brain sequence with a minimum of 1.2mm isotropic voxels.

Besides the clinical MRI, all subjects will have a total of six MRI/MRS scans: at baseline for both Research Conditions and at the c_{max} of the first and last dose administration for each treatment phase (placebo or active drug).

The MRS scan will consist of the following elements and will last approximately 60-90 minutes. It is anticipated that these scans may not be collected in some subjects due to logistical issues (e.g., scheduling, or scanner not functioning).

- Whole brain structural image (15 minutes)
- Resting state functional MRI (10 min)
- Glutamate MRS (3 voxels, 10 minutes/voxel), pending sequence development
- Task MRI: motor/sensory/cognitive/emotional/working memory/ attention tasks (10 minutes each, time permitting)

MRIs are accomplished by having the subject lie down on a bed that is slid into the hollow, cylindrical “bore” of a magnet. Two persons with experience conducting MRIs will perform the scan from an adjacent room. Audible communication will occur by intercom. The NIH code team with full medical coverage will be available in case of emergency. Individuals will be removed

from the scanner immediately upon request or in case of emergency. During the scan, the subjects will hear a thumping or buzzing sound, but earplugs or MRI compatible headphones will be placed to minimize noise.

A customized, 3-D printed pillow may also be created to help stabilize the participant's head if movement is of particular concern in the study. If motion artifacts (e.g. ghosting, ringing) are present in the screening MRI, the optional custom pillow will be considered for use in subsequent research scans. In advance of imaging procedures, we will use a camera to take a series of pictures of the subject's head. These images will be used to map the participant's head shape and generate a pillow that is uniquely fitted to them.

Video monitoring may be used for motion correction and eye gaze tracking. These cameras are MR compatible and have been approved by the NMR safety committee. Video monitoring is optional.

8.4.4 Neurocognitive Battery (Optional)

The following optional neurocognitive tests will be administered as time/resources permit at each of the timepoints listed in the Schedule of Events. At each timepoint, a minimum of one test will be administered for each domain- verbal episodic memory, working memory/attention, and verbal fluency.

8.4.4.1 Verbal Episodic Memory

California Verbal Learning Test (CVLT; (Delis, Freeland et al. 1988))

The CVLT is a comprehensive battery used in memory research. For the purpose of this study, only one component, measuring immediate recall, will be employed. There are four standardized lists of 16 items to remember, allowing for repeated measurement. The test takes approximately 25 minutes to complete.

Rey Auditory Verbal Learning Test (RAVLT; (Ryan and Geisser 1986, Peaker and Stewart 1989))

The RAVLT is designed to assess verbal memory, including proactive inhibition, retroactive inhibition, retention, encoding versus retrieval, and subjective organization. The test also evaluates the severity of memory disjunction and changes that occur in memory function over a period of time. The test takes 10-15 minutes to complete.

Hopkins Verbal Learning Test – Revised (HVLT-R; (Benedict, Schretlen et al. 1998))

The HVLT-R is a test of verbal learning and delayed memory. The test includes a series trials. Each trial consists of a list of 12 nouns organized into semantic categories (e.g. precious gems, types of structures, animals). Participants are read the list and told to memorize the words. They are then asked to free-recall the items on the list. This same procedure is repeated during two additional trials. On the final trial, they are read 24 words and asked to respond with a "yes" if the word was included in the list or "no" if it was not. The test takes approximately 10 minutes to complete. There may be a 20- to 25-minute delay between hearing the list and recalling the words.

8.4.4.2 Working Memory/Attention

Digit Symbol Substitution Test (DSST; (Wechsler 1939))

The DSST, part of the original Wechsler Adult Intelligence Scale (WAIS), measures the presence of and change in cognitive dysfunction. The procedure involves matching symbols to corresponding Arabic number (e.g. ↔ is 1, ↕ is 2, ≡ is 3). The test is a series of boxes in two rows. The top row contains a number and the bottom row boxes are empty. Using the key, participants are asked to put the symbols in the boxes below their corresponding numbers. The score is the number of correct matches made within the specified length of time. The test takes approximately 5 minutes to complete.

Conners Continuous Performance Test 3rd Ed. (CPT 3; (Keith Conners, Sitarenios et al. 2018))

The CPT-3 is a revision of its predecessor, the CPT-II, and was designed to assess attention-related problems in four domains of attention. The test integrates the same key elements as its predecessor with a number of new features, including Validity scales, Screener items, Critical items, Impairment items, an assessment of Executive Functioning, and strengthened linkage to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The test takes approximately 15 minutes to administer.

8.4.4.3 Verbal Fluency

Controlled Oral Word Association Test (COWAT; (Benton and Hamsher 1983))

The COWAT is a measure used to assess verbal fluency. Patients are asked to produce words beginning with a letter as well as words that belong to a specific category. Patients have exactly one minute to produce as many words as possible per letter and category. The task takes approximately 5 minutes to complete.

Delis-Kaplan Executive Function System (D-KEFS; (Delis, Kaplan et al. 2001)), Verbal Fluency subtest

The D-KEFS evaluates the main components of executive function that are believed to be mediated by the frontal lobe. The assessment includes nine separate, stand-alone tests. The Verb al Fluency Test, one such component, measures letter fluency, category fluency, and category switching. The test takes approximately 10 minutes to complete.

8.5 BIOSPECIMEN EVALUATIONS

Blood will be collected according to site procedures through either an i.v. saline lock or directly by a needle. During the time that blood is obtained on multiple occasions, a saline lock will be used, thus the saline lock will be used via an antecubital iv line. If a single blood draw is done the sample will be obtained using a butterfly needle. Samples for safety and research purposes (samples for PK purposes are exclusionary) will be collected between the hours of 10 am and 2pm, to minimize potential diurnal variability (Begliuomini, Lenzi et al. 2008) at baseline prior to randomization and at the timepoints listed in the Schedule of Events. This will afford us the opportunity to explore the temporal relationship between changes in serum biomarkers and clinical improvement and changes in plasticity measures.

Blood samples will be drawn for safety, research, and PK purposes. No more than 370mL (25 tablespoons) of research blood will be drawn during the study.

8.5.1.1 Safety Labs

Samples will be collected for safety labs, including Acute Care Panel (sodium, potassium, chloride, total CO2, creatinine, eGFR, glucose, urea nitrogen anion gap), CBC+Diff (complete blood count with differential, including WBC, RBC, HGB, HCT, MCV, MCH, RDW, platelet count, MPY, nucleated RBC, absolute neutrophils, bands, immature granulocytes, lymphocytes, monocytes, eosinophils, basophils, neutrophil absolute, immature granulocytes absolute, lymphocyte absolute, monocyte absolute, eosinophil absolute, basophil absolute), hepatic panel (alkaline phosphatase, alanine aminotransferase, aspartame aminotransferase, bilirubin total, bilirubin direct), thyroid function tests (screening; thyroid stimulating hormone, free thyroxine). Safety labs will be collected at the timepoints listed in the table below, as well as in the Schedule of Events. Additional safety draws will be conducted as clinically indicated.

8.5.2 Correlative Studies for Research/Pharmacokinetic Studies

These draws will be conducted at the timepoints listed in the Schedule of Events. See Appendix C, for a detailed description of research-related blood draws.

We will not send PK samples for outside analysis taken from participants with COVID-19 or suspected COVID-19 (e.g., have respiratory symptoms or chest imaging findings).

8.5.3 Samples for Genetic/Genomic Analysis

8.5.3.1 Description of the scope of genetic/genomic analysis

See Appendix C for a description of genetic/genomic analyses.

Samples and data (including genomic data) will be shared with NIMH Data Archive, dbGaP, and the NIMH Repository and Genomics Resource.

8.5.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

All research activities will be conducted in as private a setting as possible. Clinical data will be managed in accordance with the NIH Clinical Center's privacy policy (http://www.cc.nih.gov/participate/patientinfo/legal.shtml). Research data and records will be coded, no individual will be identified by name, and the data will be stored in a locked filing cabinet stored in the principal investigator's locked offices to protect subject anonymity. Electronic data with identifiers (including neuroimaging) will be saved password-protected NIH-issued computers on secured servers. Neuroimaging data will be maintained on a secure internet-based server. Only study investigators will have access to the data. De-identified results from clinical trials will be posted on ClinicalTrials.gov. Clinical data will be managed according to NIH Clinical Center's standard policies.

Biological samples (i.e. blood) will be kept in freezers by the NIMH/ETPB with no participant identifiers. Information on processing and storage of samples is on record with the Clinical and Scientific Director's offices at the NIMH. Plasma samples will be collected, handled, stored, and shipped as outlined in the Data Handling and Record Keeping section. Results will be published as group data without the use of characteristics that would identify individual subjects. Samples and date will be stored using codes that we assign. Only study investigators will have access to the stored samples.

This study collects sensitive information, including personal medical and treatment history. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about NIH employees and staff through staff discussions and written branch/section procedures.

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data. De-identified results from clinical trials will be posted on cctrials.gov.

8.5.3.3 Management of results

Results from genetic testing will be obtained in a non-CLIA laboratory; therefore, results will not be provided back to the participant or health care provider for purposes of providing clinical diagnostic information.

8.5.3.4 Genetic counseling

Genetic counseling will not be provided under this protocol.

8.6 Safety and Other Assessments

8.6.1 Blood draw and laboratory screen

A blood draw and laboratory screen (e.g. Acute Care Panel, CBC+Diff, Hepatic Panel, LFT, TFT, HIV) will be conducted to screen for any medical condition that would increase risk for adverse events. Blood draw and laboratory screens will be conducted for screening and evaluation purposes.

8.6.2 Blood pressure

An assessment of blood pressure will be conducted as cardiovascular illness is an exclusion criterion for this study. Blood pressure will be collected for screening and evaluation purposes.

8.6.3 Structural neuroimaging studies

Structural neuroimaging studies will be conducted within one year prior to study participation. In rare cases when an abnormality is discovered, participants are offered further assessment at the NIH via a neurology consult. Minor abnormalities with no clinical significance to participants may not be shared. Structural neuroimaging studies will be conducted for screening purposes.

8.6.4 Electrocardiogram

The assessment of cardiovascular function via electrocardiogram (ECG) will be conducted as cardiovascular illness is an exclusion criterion for this study. ECG will be collected for screening purposes.

8.6.5 Heart rate

The assessment of heart rate will be conducted as cardiovascular illness is an exclusion criterion for this study. Heart rate will be collected for screening and evaluation purposes.

Additionally, heart rate will be collected during the study visit to monitor for potential adverse events.

8.6.6 Height

The assessment of height will be conducted for screening and evaluation purposes.

8.6.7 Medical history

A review of the participants medical history will be conducted. The review will involve the assessment of current medications as the use of psychoactive medications is exclusion criteria for this study. The review of medical history will also involve screening for medical conditions that would increase risk for MRI or any component of study participation. A review of medical history will be conducted for screening purposes.

8.6.8 Monitoring of clinical symptoms

Clinical Rating Scales will be used to monitor mood and anxiety symptoms. Additionally, clinical personnel with monitor participants for adverse events and other clinical symptoms (e.g. headache, nausea, vomiting, abdominal pain, chest pain, light-headedness, dizziness, and blurry vision).

8.6.9 Physical exam

Participants will undergo a physical exam to screen for medical conditions that would increase risk for MRI, TS-161, or any component of study participation. Physical exams will be conducted for screening purposes and safety assessment at the end of study participation.

8.6.10 Respiration

The assessment of respiration rate will be conducted to monitor responses to the administration of the study intervention.

8.6.11 Review of changes to Medical Report Form and Medication Review

Changes to participant's medical report forms and medication histories will be conducted to screen for medical conditions that would increase risk for the study intervention or other components of study participation. Review of changes to their medical report form and medication history will be performed in the 24 hours prior to administration of the study intervention.

8.6.12 Temperature

The assessment of body temperature will be conducted to monitor responses to the administration of the study intervention.

8.6.13 Urine pregnancy test

Women of child-bearing potential will undergo urine pregnancy testing to rule out pregnancy. Urine pregnancy tests will be completed at the time of screening and no more than 24 hours prior to start of each research condition and imaging procedure.

8.6.14 Urine toxicology

Participants will undergo a urine toxicology screen as drugs that act on the central nervous system is exclusion criteria for this study. Urine toxicology will be conducted for screening purposes.

8.6.15 Weight

Participants will undergo the measurement of their weight for screening purposes and at time points indicated in the Schedule of Events.

8.7 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.7.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.7.2 Definition of Serious Adverse Events (SAE)

An AE or suspected AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.7.3 Classification of an Adverse Event

8.7.3.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

Mild

Events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate

Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe

Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

8.7.3.2 Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

Definitely Related

There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related

There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Potentially Related

There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely to be related

A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related

The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.7.3.3 Expectedness

The PI and LIPs on the study will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.7.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

LIPs on the study will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.7.5 Adverse Event Reporting

Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

8.7.6 Serious Adverse Event Reporting

All serious adverse event (SAE) reporting will adhere to the U.S. Code of Federal Regulations (21 CFR Part 312.32) for IND drugs. The PI will immediately report any SAEs to the sponsor, whether or not these are considered study intervention-related, including those listed in the protocol or investigator brochure; any such report must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. The PI or designee must send all SAEs on a MedWatch form to the Sponsor, copying the REGSupportORSC@nih.gov inbox at the time of awareness (within 24 hours for deaths, within 48 hours for all other SAEs). Additional information to include in the MedWatch Form 3500A is as follows:

- IND Number
- Protocol Number
- Principal Investigator's Name

- Event Attribution

The sponsor or designee will be responsible for determining if an SAE is reportable to the FDA as an IND Safety Report, i.e. the event is considered a serious unexpected suspected adverse reaction (SUSAR). The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) or other applicable regulatory agency, of any unexpected fatal or life-threatening suspected AE as soon as possible, but in no case later than seven calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify the FDA and all participating investigators of an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

All SAEs will be followed until satisfactory resolution. All AEs will be documented on appropriate study records, including case report forms and AE tracking logs. All AEs will be provided in aggregate to the sponsor upon request and no less than once a year.

8.7.7 Events of Special Interest

Events of Special Interest are not applicable to this study.

8.7.8 Reporting of Pregnancy

Pregnant or nursing women or women who plan to become pregnant are excluded from this study. Participants who tested positive for pregnancy or become pregnant while enrolled in the study will be discontinued from the protocol and followed through pregnancy outcome.

8.8 UNANTICIPATED PROBLEMS

8.8.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.8.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

8.8.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

9.1.1 Primary Endpoint

- Change from baseline in the MADRS total score
  - H_0: The 95% confidence interval for the effect of drug (TS-161 minus placebo) on MADRS total at 3 weeks, after controlling for baseline scores, will include zero.
  - H_A: The 95% confidence interval for the effect of drug (TS-161 minus placebo) on MADRS total at 3 weeks, after controlling for baseline scores, will be less than and exclude zero.

9.1.2 Secondary Endpoints

- Proportion of subjects in remission (defined as MADRS total score ≤10).
  - H_0: The 95% confidence interval for the odds ratio (TS-161 versus placebo) predicting MADRS total score ≤10 at 3 weeks will include one.
  - H_A: The 95% confidence interval for the odds ratio (TS-161 versus placebo) predicting MADRS total score ≤10 at 3 weeks will be greater than and exclude one.
- Proportion of subjects with response (defined as ≥50% reduction from baseline in MADRS total score).
  - H_0: The 95% confidence interval for the odds ratio (TS-161 versus placebo) predicting 50% change from baseline in MADRS total score at 3 weeks will include one.
  - H_A: The 95% confidence interval for the odds ratio (TS-161 versus placebo) predicting 50% change from baseline in MADRS total score at 3 weeks will be greater than and exclude one.
- Change from baseline in HDRS, BPRS, CADSS, C-SSRS, HAM-A, MADRS, PANAS, SHAPS, SSI, TEPS, YMRS total scores.
  - H_0: The 95% confidence interval for the effect of drug (TS-161 minus placebo) on [all listed outcomes] at the timepoints listed in the Schedule of Events after controlling for baseline scores, will include zero.
  - H_A: The 95% confidence interval for the effect of drug (TS-161 minus placebo) on [all listed outcomes] at the timepoints listed in the Schedule of Events after controlling for baseline scores, will be less than and exclude zero.

9.1.3 Surrogate Markers of Drug Effect, Target Engagement, and Antidepressant Response

- Change in MEG spectral power (gamma power).
  - H_0: Among TRD-MDD subjects, pre-treatment gamma power levels will predict the magnitude of clinical improvement to TS-161, but not to placebo.
- Change in resting and task based functional connectivity in fMRI.
  - H_0: Among TRD-MDD subjects, TS-161 administration will increase pgACC activity and decrease amygdala activity to emotional faces more than placebo. Similar effects will be observed in activity associated with the resting-state networks. TS-161 will also decrease pgACC activity during the tasks more than placebo.
- Change in glutamate levels in MRS.
  - H_0: Among TRD-MDD subjects, pre-treatment mPFC glutamate levels will predict the magnitude of clinical improvement to TS-161 in subjects with MDD, but not to placebo.
  - H_A: Among TRD-MDD subjects, post-treatment increases in glutamate levels will predict the magnitude of clinical improvement to TS-161 (but not placebo).
- Change in peripheral biomarkers (exploratory).

9.2 SAMPLE SIZE DETERMINATION

The estimated duration of this protocol is 36 months and we anticipate recruiting 1-2 subjects a month over this period.

The primary outcome measure is the MADRS total score. The null hypothesis is that the 95% confidence interval for the effect of drug (TS-161 minus placebo) on MADRS total at 3 weeks, after controlling for baseline scores, will include zero; the alternative hypothesis is that this 95% CI will be less than and exclude zero. We have observed in previous crossover studies of the rapid acting antidepressant ketamine an estimated difference of -6.7 (SE = 1.5) [95%CI: -9.6, -3.76]. This study will be powered to detect an effect within that confidence interval that translates to moderate effect size (difference = -7.5; d = 0.70) on the MADRS total score at end point. While we are primarily interested in identifying drugs with a large effect size, this effect size was selected to balance the risk of an underpowered study if the true effect size were not large and the cost of powering the study to detect a small effect size. Assuming α=.05, two-tailed, 20 patients with MDD would provide 80% power to detect a drug effect of this magnitude at end point. As discussed, we are interested only in a moderate effect size and have powered the study accordingly. However, if we were to power the study based on the reasonable range of treatment effect observed in the previous studies of rapid acting antidepressants, the sample size requirements would range from 13 subjects for the largest effect in the 95% CI to 67 subjects for the smallest effect. To account for a 25% rate of attrition, either from participants who do not complete the drug-free period or who drop out during the crossover double-blind portion of the study, we will recruit 25 participants. No interim analyses are planned. Secondary analyses with continuous outcomes will likely be sufficiently powered, as we anticipate that the effects on most secondary outcomes will be similar to the primary outcome. However, the power of the study to detect effects on the categorical secondary outcomes (response and remission) will be reduced as categorical outcomes de facto require a larger sample size. SAS 9.4 PROC POWER for paired mean designs was used for sample size calculations.

9.3 POPULATIONS FOR ANALYSES

There will be no interim analysis; the blind will be broken after the accrual goal has been met and all analyses will be based on the full dataset. 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 during period 1 will be included. The ITT dataset will be used for the primary and secondary analyses.

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

9.3.1 Evaluable for Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with TS-161.

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

The assumptions of all models will be checked. Specifically, given that the primary model of analysis is the generalized linear model, we will graphically assess the distribution and spread of residuals. We will perform transformation if exploratory data analysis suggests that it will improve adherence to model assumptions; we do not expect that this will be necessary for behavioral outcomes but a log transformation is commonly applied to biological outcomes. For the primary analysis no a priori covariates are specified, except for the baseline values of the outcome as described in this section. Significance will be evaluated at $p \leq .05$, two-tailed. The primary hypothesis has only one outcome, the treatment effect at week 3, and therefore no correction to alpha (or to p-values) will be made. Following the current guidelines of the American Statistical Association (Wasserstein, Schirm et al. 2019), no threshold will be applied to secondary outcomes of this trial. Instead, parameter estimates will be presented alongside both their 95%

9.4.2 Analysis of the Primary Endpoints

The primary aim will be accomplished by assessing the efficacy of three weeks of oral TS-161 (50 to 100 mg/day) compared with placebo in improving overall depressive symptomatology. The primary assessment of efficacy is a comparison of MADRS scores between treatment groups. The MADRS total score is measured at the interval level, and although repeated measurement of the outcome is obtained the primary hypothesis concerns the value at 3 weeks.

The difference between TS-161 and placebo will be examined using a mixed model for repeated measures with restricted maximum likelihood estimation where drug and time are within subjects factors. Fixed effects for drug, time period, and their interaction will be specified. Period-specific baseline scores and the subject-specific average baseline score will be entered as a covariate (Kenward and Roger 2010); no other covariates will be included *a priori*. The degrees of freedom will be corrected using the Kenward-Roger approach. The residual covariance matrix will account for nesting of observations within period for each participant; during the model fitting stage, fit indices (e.g., Schwarz's Bayesian criteria) will be used to determine the most appropriate structure for the residual covariance matrix, including the possibility that it will be specified by drug to allow for heteroscedasticity. Also, during the model fitting stage, a subject-specific (random) intercept will be tested and retained if indicated by model fit indices. During the model

fitting stage, the residuals will be graphically assessed for distribution and spread; if they violate the assumptions of the model then appropriate transformations will be applied before selecting the final model. The analysis will be performed in the ITT sample, and missing data may occur. Maximum likelihood estimation will be implemented, which allows for missingness without excluding the participant; no imputation will be performed. The effect of interest in this model is the effect of drug at the 3-week timepoint (with 95% CI), which will be obtained from the drug-by-time interaction using a contrast statement. Cohen's d, calculated using the model-estimated difference between groups, the standard error of that difference, and the degrees of freedom associated with that comparison, will be used to describe the effect size.

9.4.3 Analysis of the Secondary Endpoints

Secondary objectives are to evaluate the remission and response rates on the primary outcome measure (MADRS). A similar model to the one described above will be employed, with the difference that the outcome distribution will be specified as binary logistic. Because remission/response is determined based on change from baseline within a period, no baseline covariate will be included in the model. The parameter estimate for the effect of drug on remission/response rate at a given time point will be expressed as an odds ratio with a 95% CI. The remaining secondary analyses of clinical outcomes measured on the interval level (HAMD, BPRS, CADSS, C-SSRS, HAM-A, PANAS, SHAPS, SSI, TEPS, and YMRS) will be conducted in the manner described above for MADRS score. Following the current guidelines of the American Statistical Association (Wasserstein, Schirm et al. 2019), no threshold will be applied to secondary outcomes of this trial. Instead, parameter estimates will be presented alongside both their 95% confidence intervals and the raw p-value.

9.4.4 Safety Analyses

Safety will be evaluated throughout study participation in order to assess symptomology and the development of adverse events. AEs will be tracked in CRFs and reported in compliance with Policy 801.

9.4.5 Baseline Descriptive Statistics

The demographic composition of the sample, including sex assigned at birth, gender identity, race, ethnicity, age, markers of socioeconomic status, and BMI will be tabulated for the full sample in order to aid interpretation of the generalizability of the sample. Because the sequence groups are randomized, inferential tests of baseline differences are inappropriate.

9.4.6 Tabulation of individual Participant Data

Individual participant data will not be listed; only summary statistics will be presented.

9.4.7 Exploratory Analyses

We will conduct primary and secondary analyses as specified in Sections 9.4.2 and 9.4.3. We will not conduct exploratory analysis for confirmatory proof for registration trials.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. In-person consent will be obtained at the NIH CC (see below for telephone and telehealth consent processes). Participants will be provided as much time as is required to carefully review the written consent form and ask questions of consenting investigators regarding this study prior to signing.

Consent will be obtained by authorized licensed independent practitioners with patient care and clinical research privileges in the NIH CC. All those study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

An HSPU team member will monitor the informed consent process for patients, which may then, in certain circumstances, trigger the need for a formal independent capacity assessment. Subjects without consent capacity excluded from participation.

10.1.2 For assent of children, if applicable

This section is not applicable.

10.1.3 Consent for minors when they reach the age of majority

This section is not applicable.

10.1.4 Telephone and Telehealth Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was returned. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator. The investigator will confirm that, when required, written legally effective consent has been obtained prior to initiating any study interventions.

Sometimes it is necessary to obtain information about symptoms by electronic media, obtain measures of symptom severity, or conduct other eligible procedures (e.g. interviews, questionnaires, and psychological testing) by telehealth. Under such circumstances, this process will be utilized to obtain telehealth consent for this study using NIH-approved platforms.

10.1.5 Telephone Assent

This section is not applicable.

10.1.6 Consent of subjects who are or become, decisionally impaired

Adults unable to provide consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for

themselves during the course of this study. In the event this occurs, the subjects will be withdrawn from the study.

10.1.7 Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH staff will be obtained as detailed above with following additional protections:

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and, as applicable, the FDA.

10.3 Confidentiality and Privacy

Participant confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

This study collects sensitive information, including personal and family medical and treatment history. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about NIH employees and staff through staff discussions and written branch/section procedures.

Hard copy research data/records will be coded, no individual will be identified by name, and the data will be stored in a locked filing cabinet stored in locked offices maintained by the PI to

protect subject anonymity. Electronic data with identifiers will be saved on NIH secured servers. Data will be kept in password-protected computers. Samples will be kept in locked storage.

Samples and data will be stored using codes that we assign. Only study investigators and clinical monitors will have access to the samples and data with patient identifiable information (PII). Offsite associate investigators will not have access to PII.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Clinical Trials Database (CTDB) at NIH. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by ETPB/NIMH research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the CTDB.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 Future Use of Stored Specimens and Data

Biological samples will be kept in freezers by the NIMH/ETPB with no patient identifiers. Information on processing and storage of samples is on record with the Clinical and Scientific Director's offices at the NIMH. Samples will be collected, handled, stored, and shipped as outlined in the Data Handling and Record Keeping section. Results will be published as group data without the use of characteristics that would identify individual subjects. Samples and date will be stored using codes that we assign. Only study investigators will have access to the stored samples.

10.5 Safety Oversight

This protocol will be reviewed by the NIMH-IRP Data and Safety Monitoring Board. The DSMB meets two times per year. The board will review study accrual and progress, adverse events related to the study, and safety and outcome data. Specific data elements required by the DSMB will be established at the first meeting the protocol is reviewed. The DSMB will have the authority to require changes in the study design, or to stop all or part of any study based on accumulating safety data.

The medically responsible investigator has the authority to break the blind in the event of an emergency. Breaking of the blind, life threatening injury or death all require immediate reporting to the DSMB and the NIMH Clinical Director. A written notification to the DSMB chair must follow within 7 days.

10.6 CLINICAL MONITORING

Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored. Monitoring for the NIH site will be conducted according to the "NIMH Intramural Program Guidelines for Monitoring of Clinical Trials". Monitors under contract to the NIMH OCD ORO will visit the NIH site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information from clinical databases (e.g. CTDB) with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, clinical database records and pertinent hospital/sources or clinical records) readily available for inspection by the local IRB, FDA, the site monitors, and the NIMH staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference

on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including demographics information, clinical rating scales, and AEs) and clinical laboratory data will be recorded in paper form or entered into CTDB, a 21 CFR Part 11-compliant data capture system provided by the NICHD. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly or from the source documents.

Electronic data (including imaging data) and electronic personally-identifiable health information (ePHI) will be stored on secure servers within the NIH firewall with access for study personnel only.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to the NIMH Program Official and Office of the Clinical Director. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 6 years after the completion of the primary endpoint by contacting the ETPB.

10.10.2 Genomic Data Sharing Plan

This study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Agreement Type

A CRADA was established between the NIMH and Taisho Pharmaceutical Co., Ltd. Taisho Pharmaceutical Co., Ltd. will manufacture the study drug and placebo for this protocol. NIMH will be responsible for obtaining the IND, subject recruitment, clinical care, conduct of the research study and procedures, study monitoring, analyzing and writing, and publishing study results.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and

managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIMH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
ANCOVA	Analysis of Covariance
ATHF	Antidepressant Treatment History Form
BBB	Blood Brain Barrier
BCRP	Breast Cancer Resistance Protein
BDNF	Brain Derived Neurotrophic Factor
BLS	Basic Life Support
BMI	Body Mass Index
BOLD	Blood Oxygenation Level Dependent
BPRS	Brief Psychiatric Rating Scale
CADSS	Clinician-Administered Dissociative States Scale
CAMKIII	Calcium-Calmodulin Dependent Protein Kinase III
CBC	Complete Blood Count
CES	Carboxylesterase
CLIA	Clinical Laboratory Improvements Amendments
Cmax	Maximum plasma concentration
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
COVID-19	Coronavirus Disease 2019
COWAT	Controlled Oral Word Association Test
CPT 3	Conners Continuous Performance Test 3rd Edition
CRA	Clinical Research Advocate
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
CTDB	Clinical Trials Database
CVLT-II	California Verbal Learning Test - Second Edition
CYP	Cytochrome P-450

dbGap	database of Genotypes and Phenotypes
DSST	Digit Symbol Substitution Test
D-KEFS	Delis-Kaplan Executive Function System
DRTS	Designation of Reimbursement for Travel and Subsistence
ECG	Electrocardiogram
ECT	Electroconvulsive therapy
eEF2K	eukaryotic Elongation Factor-2 Kinase
ePHI	electronic Personally-Identifiable Health Information
FDA	Food and Drug Administration
fMRI	functional Magnetic Resonance Imaging
FST	Forced Swim Test
GDNF	Glial-Derived Neurotrophic Factor
HAM-A	Hamilton Anxiety Rating Scale
HCG	Human Chorionic Gonadotropin
HDRS	Hamilton Depression Rating Scale
HIV	Human Immunodeficiency Virus
HNK	Hydroxynorketamine
HSPU	Human Subjects Protection Unit
HVLT-R	Hopkins Verbal Learning Test - Revised
IDMRS	Investigational Drug Management and Research Section
IEC	International Electrotechnical Commission
IFN	Interferon
IGF-1	Insulin-like Growth Factor-1
IL	Interleukin
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intent-To-Treat
LPS	Lipopolysaccharide
MAD	Multiple Ascending Dose
MADRS	Montgomery-Asberg Depression Rating Scale
MAOI	Monoamine Oxidase Inhibitor
MDD	Major Depressive Disorder
MDEs	Major Depressive Episodes
MEG	Magnetoencephalography
mGlu	metabotropic Glutamate

mPFC	medial Prefrontal Cortex
mRNA	Messenger Ribonucleic Acid
MRS	Magnetic Resonance Spectroscopy
mTORC1	mammalian Target of Rapamycin
NIH	National Institutes of Health
NMDAR	N-Methyl-D-Aspartate Receptor
NO	Nitric Oxide
NOAEL	No Observed Adverse Effect Level
NSR	Non-Significant Risk
OAT3	Organic Anion Transporter 3
OATP1B1	Organic Anion Transporting Polypeptide 1B1
OHRP	Office for Human Research Protections
PANAS	Positive and Negative Affect Schedule
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PFC	Prefrontal Cortex
PI	Principal Investigator
PII	Personally Identifiable Information
POC	Proof-of-Concept
PRN	Pro Re Nata
RAVLT	Rey Auditory Verbal Learning Test
RDoC	Research Domain Criteria
RF	Radio Frequency
RH	Relative Humidity
SAD	Single-Ascending Dose
SCID-P	Structured Diagnostic Interview-Patient Version
SHAPS	Snaith Hamilton Pleasure Scale
SNP	Single Nucleotide Polymorphism
SOC	System Organ Class
SOP	Standard Operating Procedure
SSI	Scale for Suicidal Ideation
SWA	Slow-Wave sleep Activity
TEPS	Temporal Experience of Pleasure Scale
TMS	Transcranial Magnetic Stimulation
TRD	Treatment Resistant Depression

TrkB	Tropomyosin-Related Kinase B
TS-161	TP0473292
TSH	Thyroid Stimulating Hormone
TTX	Tetradotoxin
VEGF	Vascular Endothelial Growth Factor
YMRS	Young Mania Rating Scale

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13 APPENDICES

13.1 APPENDIX A. FIVE QUESTION CONSENT QUIZ

TS-161 is not an FDA-approved for major depressive disorder (MDD).	True	False
You will receive a placebo during this study.	True	False
If you are able to get pregnant, you will have a pregnancy test before taking TS-161. There may be birth defects with TS-161.	True	False
We will look at chemicals in the brain and how they respond to TS-161.	True	False
You may not get any benefit from participating in this study.	True	False

Note: Correct responses are highlighted for this document

13.2 APPENDIX B. DRUGS ALLOWED AND NOT ALLOWED

13.2.1 Medications Allowed (Y) and Not Allowed (N) as Concomitant Medications

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Exclusion: Safety or Study Confound	Restrictions/Comments
Analgesics	Y	N	—	Narcotic analgesics or long-acting nonsteroidal anti-inflammatory drugs are not allowed
Anesthetics, general	N	N	Safety	
Anorexics	N	N	Confound	—
Antacids	Y	N	—	—
Antiacne	N	N	—	
Antianginal agents	N	N	Safety	—
Antiarrhythmics	N	N	Safety	—
Antiasthma agents	N	N	—	—
Antibiotics	Y	N	Confound	—
Anticoagulants	N	N	Safety	Warfarin (Coumadin) and antiplatelet agents are not allowed
Anticholinergics	N	N	Confound	
Anticonvulsants	N	N	Confound	—
Antidepressants	N	N	Confound	—
Antidiarrheal preparations	Y	N	Safety	Only loperamide HCl (Imodium), bismuth subsalicylate (Pepto-Bismol), and kaolin preparations are allowed
Antifungals, systemic	N	N	Safety	—
Antifungals, topical	Y	Y	—	—

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Exclusion: Safety or Study Confound	Restrictions/Comments
Antihistamines	Y	Y	Confound	Sedating antihistamines are not allowed. Only, loratadine (Claritin), desloratadine (Clarinex), and cetirizine (Zyrtec) are allowed for episodic or chronic use. The use of combinations containing pseudoephedrine or phenylephrine is not allowed. Combination products containing the word nighttime or some synonym routinely include a sedating antihistamine are not allowed.
Antihypertensives	N	N	Both	
Anti-impotence medications	N	N	Safety	—
Anti-inflammatory drugs	Y	N	Confound	Long-acting nonsteroidal anti-inflammatory drugs are not allowed
Antimigraine	N	N	Both	
Antinauseants/ Antiemetics	Y	N	Confound	Only phosphoric acid preparations (Emetrol, Emecheck), bismuth subsalicylate (Pepto-Bismol), and cola syrup are allowed.
Antineoplastics/ Immunosuppressant agents	N	N	Safety	Use of chemotherapy agents or history of cancer, other than resolved skin cancer, within 5 years before the screening visit.
Antiobesity/Appetite suppressants	N	N	Confound	OTC Alli (Xenical), sibutramine (Meridia), and phentermine; (Adipex-P and others) are not allowed.
Antiplatelet agents	N	N	Safety	
Antipsoriatic treatments	N	N	—	Only topical agents and acitretin; (Soriatane) is not allowed.

Drug Name or Class	**Episodic Use (PRN)**	**Chronic Use**	**Exclusion: Safety or Study Confound**	**Restrictions/Comments**
Antipsychotics	N	N	Confound	—
Antismoking medications	N	N	Confound	Varenicline (Chantix) is not allowed.
Antiviral agents	N	N	—	
Anxiolytics	N	N	Confound	—
Benign prostatic hyperplasia treatments	N	N	Safety	
Buspirone	N	N	Confound	—
Cough/cold preparations	Y	N	Confound	Use of cough and cold preparations containing pseudoephedrine or phenylephrine are not allowed. Any sedative OTC (night) products are not allowed. Combination products containing the word nighttime or some synonym routinely include a sedating antihistamine and are not allowed.
Diuretics	N	N	—	
Dopaminergics	N	N	Confound	Dopamine agonists for restless leg syndrome are not allowed.
Gastrointestinal: H_2-blockers/ proton pump inhibitors/ prokinetic agents	N	N	—	
Hormonal (noncontraceptive) therapies	N	Y	—	see below
Hormone suppressants	N	N	Safety	

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Exclusion: Safety or Study Confound	Restrictions/Comments
Hormones: reproductive	N	Y	Safety	Systemic hormonal contraceptives (oral contraceptives of estrogen and progestin combinations, depot injections such as Depo-Provera, the contraceptive implant Implanon, or transdermally delivered contraceptives such as Ortho Evra) are allowed.
Hormones: thyroid	N	Y	Confound	Thyroid hormone replacement is allowed (dosage of thyroid medication should be stable for 2 months before screening). Therapeutic use for psychiatric disorders (e.g., T3 augmentation) is not allowed
Hypoglycemic agents	N	N	Safety	Oral hypoglycemic agents or insulin are not allowed.
Hypolipidemics	N	N	Safety	
Hypolipidemics: bile acid sequestrants	N	N	Confound	—
Hypolipidemics: fibrates	N	N	Confound	
Hypolipidemics: niacin	N	N	Confound	
Hypolipidemics: statins	N	N	Safety	
Investigational drug	N	N	Safety	No investigation drug within 30 days of starting the study.
Laxatives	Y	N	—	Only fiber-based products and docusate sodium (Colace) are allowed.
Lithium	N	N	Confound	—
Muscle relaxants	N	N	Confound	—

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Exclusion: Safety or Study Confound	Restrictions/Comments
Psychotropic drugs not otherwise specified (including herbal products)	N	N	Confound	No drugs with psychomotor effects or with anxiolytic, stimulant, antipsychotic, or sedative properties are allowed. Herbal/dietary products and supplements with potential psychoactive actions including St. John's wort, kava kava, SAMe, valerian root, DHEA, tyrosine, methylfolate. and 5-HTP are not allowed.
Sedatives/hypnotics	N	N	Confound	—
Steroids/systemic	N	N	Confound	
Steroids/topical and inhalant	N	N	—	—
Steroids/intra-articular	Y	NA	—	—
Stimulants	N	N	Confound	Oral or transdermal methylphenidate, amphetamine products or prodrugs, pseudoephedrine, modafinil (Provigil), and other medications of same category are not allowed.
Vaccines	Y	NA	—	—
Elagolix	N	N	Safety	Elagolix, for endometriosis treatment, is not allowed. This compound is a substrates of a drug transporter (OATP1B1) which might be inhibited by TP0473292 (prodrug).
Eluxadoline	N	N	Safety	Eluxadoline, for irritable bowel syndrome with diarrhea treatment, is not allowed. This compound is a substrates of a drug transporter (OATP1B1) which might be inhibited by TP0473292 (prodrug).

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Exclusion: Safety or Study Confound	Restrictions/Comments
Pulmonary arterial hypertension treatment	N	N	Safety	Ambrisentan, bosentan, and selexipag are not allowed. These compounds are substrates of drug transporter (OATP1B1 and/or OATP1B3) which might be inhibited by TP0473292 (prodrug).

5-HTP = 5-hydroxytryptophan; DHEA = dehydroepiandrosterone; N = no; NA = not applicable; OATP = organic anion transporting polypeptide; OTC = over the counter; PRN = as needed (pro re nata); SAMe = S-adenosylmethionine; T3 = triodothyronine; Y = yes.

13.2.2 List of Psychiatric Medications Allowed and Not Allowed During the Study*

Drug Class	Episodic Use (as needed)	Chronic Use	Restrictions
Antidepressants	No	No	Treatment with fluoxetine or aripiprazole is an exclusion because of their long half-life.
Antipsychotics	No	No	
Anxiolytics	No	No	
Mood Stabilizers	No	No	
Psychotropic drugs not otherwise specified (including herbal products)	No	No	No drugs with psychomotor effects or with anxiolytic, stimulant, antipsychotic, or sedative properties are allowed.
Sedatives/Hypnotics	No	No	

* Some medications in this table may be indicated for exclusionary conditions; therefore, it would be unlikely that participants meeting inclusion will be taking them.

13.3 APPENDIX C. PERIPHERAL (PLASMA/SERUM) MEASURES OF INTEREST AS BIOMARKERS IN MAJOR DEPRESSIVE DISORDERS

13.3.1 General Background

Our conceptualization of the pathophysiology of major depressive disorders has evolved significantly over the past decade from a simple chemical imbalance model to a model of impaired neural plasticity and resilience (Zarate, Singh et al. 2006). This new conceptual framework affords us the opportunity to specifically examine the clinical utility of novel drugs affecting neuroplasticity (Manji, Quiroz et al. 2003, Zarate, Du et al. 2003). Moreover, this conceptual approach facilitates exploration of drug effects and several potential predictors, moderators/mediators and the surrogate endpoint response to treatment. Here, we provide a brief discussion of the rationale and goals of our group's concerted effort to identify biomarkers of TS-161 antidepressant effects.

13.3.2 Plasma/Serum Biomarkers: Neurotrophic Factors, Cell Cycle/Signal Transduction Regulators, Neuroinflammatory, Neuroendocrinological Measures, PBMC, Metabolomic and Proteomic Measures

Neurotrophic Factors

Animal studies clearly demonstrate that neurotrophins like brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF/FGF-2), vascular endothelial growth factor (VEGF), glial-derived neurotrophic factor (GDNF) and insulin-like growth factor (IGF-1) mediate some of the beneficial neuromodulatory effects of antidepressant medications.

S-100B protein is highly specific for neural tissue, where it is predominantly synthesized and secreted by glial cells; yet, its role is not yet fully understood. It may have intracellular and extracellular neurotrophic as well as neurotoxic effects. Structural damage to glial cells causes leakage of S-100B protein into the extracellular milieu and CSF, which then enters the blood. Recently, serum S-100B protein has emerged as an attractive surrogate marker of CNS injury. It can be measured in arterial and venous serum, is not affected by hemolysis and remains stable for several hours (Beaudeux, Dequen et al. 1999, Beaudeux 2009). Several studies have demonstrated elevated levels of S-100B in the CSF (Grabe, Ahrens et al. 2001) and serum in MDD, especially melancholia (Rothermundt, Arolt et al. 2001). A recent meta-analysis of nine studies with 198 depressed and 209 healthy subjects revealed very strong evidence of elevated S-100B levels in MDD (Cohen's d=2.57, 95% CI: 1.87 to 3.27) (Schroeter, Abdul-Khaliq et al. 2008). S-100B levels are also positively correlated with the numbers of depressive episodes, family history of major depression and cognitive disruption (Yang, Xie et al. 2008), which can be normalized by effective antidepressant treatment (Schroeter, Abdul-Khaliq et al. 2002, Schroeter, Abdul-Khaliq et al. 2008). An association between S-100B levels and memory processes in patients with recurrent depression has been hypothesized, which suggests a neuroprotective role for moderately-increased S-100B serum levels in the course of affective disorders (Zhang, Rothermundt et al. 2009). In sum, although still preliminary, there is now mounting evidence to suggest serum S-100B levels may serve as a useful biomarker in MDD. Considering its proposed function in regulating cell survival and its putative origin from damaged glial cells, it is a likely candidate surrogate endpoint of ketamine's antidepressant response and thus a promising area of study with TS-161.

Samples will be collected at the timepoints listed in the Schedule of Events at the timepoints listed in the to minimize potential diurnal variability (Begliuomini, Lenzi et al. 2008) at baseline prior to randomization and at the timepoints listed in the Schedule of Events. This will afford us the opportunity to explore the temporal relationship between levels, drug effects, and clinical improvement.

Objective

To determine if there are baseline differences in peripheral neurotrophins in MDD/current MDE and whether changes in serum concentrations are correlated with neurophysiological parameters and the antidepressant effects and response to TS-161.

13.3.3 Cell Cycle/Signal Transduction Regulators

The importance of intracellular signal transduction cascades in mood disorders may be inferred from the activation of synaptic membrane receptors leading to antidepressant effects. Preclinical models of despair have demonstrated that the modulation of signal transduction pathways, *e.g.* activation of mammalian target of rapamycin (mTORC1) (Li, Lee et al. 2010) and release of inhibition on eukaryotic elongation factor-2 kinase (eEF2K)/calcium-calmodulin dependent protein kinase III (CAMKIII) (Autry, Adachi et al. 2011), is necessary (as inhibition abrogates antidepressant-like effects) and sufficient (as activation induces antidepressant-like effects) for ketamine's rapid antidepressant effects. To date, there have been few clinical studies to analyze signal transduction mediators and their response to antidepressants. A significant reduction in mTORC1 and other downstream signal transduction mediators was observed in postmortem MDD PFC relative healthy control brains (Jernigan, Goswami et al. 2011). In a single treatment-resistant depressed subject, ketamine's antidepressant time course correlated with increasing mTORC1 expression in peripheral leukocytes (Denk, Rewerts et al. 2011), but, to date, this response has yet to be replicated in a larger sample.

Other cell cycle regulators have been implicated in the pathogenesis of depression, *i.e.* p11 (Snyder 2011). p11 is a member of the S-100 family of proteins (discussed above) that interacts with both the 5-HT_{1B} (Svenningsson, Chergui et al. 2006) and 5-HT_4 (Warner-Schmidt, Flajolet et al. 2009) receptors. p11 mutant mice display despair-like phenotypes, and overexpressing p11 mice are more resilient to stress than their wild-type littermates ((Svenningsson, Chergui et al. 2006)). In clinical studies, p11 levels are decreased in depressed patients (including suicide completers) (Zhang, Su et al. 2011).

13.3.4 Inflammatory Mediators

Proinflammatory cytokines have also been implicated in the underlying etiopathogenesis of mood disorders. TNFα, IL-1, IL-6 administration (either centrally or peripherally) has been linked to "sickness behavior", which mimics several characteristic signs and symptoms of major depression including anhedonia, decreased social exploration, psychomotor disturbances and decreased libido (Dantzer 2001, Dantzer 2001). A recent PET study in non-human primates with a ligand binding to activated microglia revealed lipopolysaccharide (LPS)-induced activation within hours, which is likely mediated by alterations in cytokine release (Hannestad, Gallezot et al. 2012). Likewise, peripheral cytokine levels are elevated in isolated and co-occurring depressive disorders (Penninx, Kritchevsky et al. 2003, Fitzgerald, O'Brien et al. 2006, Kahl, Bens et al. 2006, O'Brien, Scully et al. 2007), and proinflammatory cytokines decrease in response to effective

antidepressant therapy (Lanquillon, Krieg et al. 2000, Narita, Murata et al. 2006, O'Brien, Scully et al. 2007). Microglial NMDAR upregulation (as detected *in vitro* with the agonist quinolinic acid) was detected in several brain areas in MDD but not BD (Steiner, Walter et al. 2011).

Similar neuroinflammatory effects have also been observed in preclinical models of depression. Repeated restraint stress in rodents induced microglial activation in the medial PFC, which could be reversed by tetracycline antibiotic and microglial inhibitor minocycline (Hinwood, Morandini et al. 2012). TNF receptor ($TNFR1^{-/-}$ and $TNFR2^{-/-}$) knockout mice display increased resilience in the FST relative to their wild-type littermates (Simen, Duman et al. 2006). Additionally, TNFR1 knockout mice exhibited reduced fear conditioning while TNFR2 nulls displayed increased sucrose drinking after water deprivation stress; however, no difference was observed between wild-type and knockout littermates in anxiety-related tests, *e.g.* open field test and elevated plus maze (Simen, Duman et al. 2006). Treatment with kinin B1 receptor antagonists (an important modulator of TNFα secretion from activated microglia) reduced depressive-like behaviors in rodents (Viana, Maciel et al. 2010).

Several studies have reported physiological effects of antidepressants on microglial cells. SSRIs and the SNRI venlafaxine are anti-inflammatory *in vitro* as demonstrated by decreased production of several pro-inflammatory cytokines from microglia (Liu, Wang et al. 2011, Tynan, Weidenhofer et al. 2012). Additionally, paroxetine and sertraline significantly inhibited the generation of nitric oxide (NO) and TNFα from interferon (IFN)-γ-activated microglia in an intracellular Ca^{2+}-dependent manner. However, the monoamine oxidase inhibitor (MAOI) phenelzine *increased* the release of TNFα and IL-6 in a NF-kB-dependent manner in cultured microglia, which suggests differential effects among different antidepressant classes. It should be noted that the mGlu3 receptor on astrocytes mediates IL-6 secretion in the presence of IL-1β (Aronica, Gorter et al. 2005), while the mGlu2 receptor on microglia mediates TNFα secretion (Taylor, Jones et al. 2005).

Several groups have investigated immunomodulatory therapy as treatment alternatives in MDD due to the retrospective antidepressant effects of novel immunomodulators in autoimmune disease. For example, 618 subjects with moderate-to-severe psoriasis were randomized to receive the TNFα monoclonal antibody etanercept or placebo. Although not the primary outcome of the study, etanercept treatment resulted in a ~50% reduction in depression scores; additionally, unlike fatigue, which was highly correlated with psoriatic symptomatology, the antidepressant effects did not strongly correlate with improvement in the dermatological and/or musculoskeletal signs/symptoms of psoriasis (Tyring, Gottlieb et al. 2006). Although a recent placebo controlled trial of the TNFα neutralizing antibody infliximab did not reveal global efficacy in TRD, subjects with a baseline level of the acute phase reactant C-reactive protein (CRP) >5 mg/L had a positive antidepressant response (Raison, Rutherford et al. 2012). Targets of other novel immunomodulators, *e.g.* IL-1 and IFN inhibitors, are also rational drug approaches in depressive disorders.

Objective

To assess baseline differences in cell cycle/signal transduction and inflammatory mediators in MDD/current MDE and whether changes in serum concentrations are correlated with neurophysiological responses of drug effects, and the antidepressant response to TS-161.

13.3.5 Neuroendocrinological Mediators

There has been extensive research to date on putative neuroendocrinological measures in depressive disorders. These findings, *e.g.* impaired dexamethasone suppression and aberrant thyroid functioning, have been observed in some but not all depressed patients, which may assist in identifying biologically relevant subtypes among the clinical heterogeneity of MDD. Many preclinical studies (with rodents and non-human primates) have implicated the hypothalamic-pituitary-adrenal axis in stress-induced relapse and craving, and these findings are presently being translated into humans. Neuropeptides and stress-related hormones may be altered acutely and/or chronically in currently depressed subjects. As such, we will investigate stress-related neuropeptide and hormone levels at baseline and after TS-161 treatment.

Objective

To determine if there are baseline differences in neuroendocrinological markers including but not limited to cortisol, ACTH, corticotropin-releasing hormone in MDD/current MDE and whether changes in these markers are correlated with neurophysiological parameters during TS-161 and the antidepressant effects of TS-161.

13.3.6 Peripheral Blood Mononuclear Cells (PBMCs)

Objective

To determine if there are baseline differences in PBMC (lymphocyte) NMDARs gene expression levels in MDD/current MDE and whether changes in PBMC are correlated with neurophysiological parameters during TS-161 administration and the antidepressant response to TS-161. Real-time PCR will be used to investigate the mRNA expression level of NMDAR/AMPA subunits in peripheral blood lymphocytes. Metabolomic and Proteomic Measures

Objective

The objective of this part of study is to determine whether there are specific metabolomic signatures in patients with MDD receiving TS-161 and whether these signatures or changes in these signatures with treatment are correlated with antidepressant change scores following treatment within different subgroups.

13.3.7 Transcriptional Profiling

Objective

Gene expression changes in neuropsychiatric and neurodegenerative disorders, and gene responses to therapeutic drugs, provide new ways to identify CNS targets for drug discovery. In the absence of a deeper understanding of disease pathology and mechanisms of side effects, CNS drug discovery would remain dominated by the redesign of drugs for familiar targets and reduced approval rates for ‘me too’ drugs. The ability to evaluate changes in the expression of the entire genome in brain areas affected by CNS disease and drug effects on multiple pathways is an alternative, ‘bottom–up’ approach to drug discovery (Altar, Vawter et al. 2009).

13.4 APPENDIX D: CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies. In this section, the terms "male", "female", or "woman" refer to biological sex.

13.4.1 Male participants

Male participants are eligible to participate if they agree to the following during the intervention period and for at least 90 days after the last dose of study intervention:

- Refrain from donating sperm
- PLUS either a) OR b):
    a. Be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent;
    b. Must agree to use contraception/barrier as detailed below
        - Agree to use a male condom and female partner use of an additional highly effective contraceptive method with a failure rate of <1% per year as described in this appendix, when having sexual intercourse with a woman of childbearing potential (WOCBP) who is not currently pregnant
        - Agree to use male condom when engaging in any activity that allows for passage of ejaculate to another person

13.4.2 Female participants

A female participant is eligible to participate if they are not pregnant or breastfeeding, and at least

- One of the following conditions, c) or d) applies:
    a. Is not a WOCBP
    b. Is a WOCBP and using a contraceptive method that is highly effective (with a failure rate of <1% per year), preferably with low user dependency, as described in this appendix, during the trial and for at least 30 days after the last dose of study intervention. The investigator should evaluate the effectiveness of the contraceptive method in relationship to the first dose of study intervention.
- A WOCBP must have a negative serum pregnancy test on Screening Visit and Day -1.
    - The investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy

WOCBP

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal female with one of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's: review of the participant's medical records, medical examination, or medical history interview.

- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause (A high follicle stimulating hormone [FSH] level in the postmenopausal range may be used to confirm a postmenopausal state in women not, using hormonal contraception or hormonal replacement therapy [HRT]).
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

13.4.3 Contraception Guidance

Contraception for Female (WOCBP) Participants

Female participants of child-bearing potential must agree to use a contraceptive method that is highly effective, preferably with low user dependency, as listed below during the trial and at least 30 days following the last dose of TS-161.

Contraceptives[1] Allowed During the Study Include:

Highly Effective Methods[2] That Have Low User Dependency

Failure rate of <1% per year when used consistently and correctly.

- Implantable progestogen-only hormone contraception associated with inhibition of ovulation[3]
- Intrauterine device (IUD)

[1] Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies.

[2] Failure rate of <1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly.

[3] If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action.

- Intrauterine hormone-releasing system (IUS)[3]
- Bilateral tubal occlusion
- Vasectomized partner
  - Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.

Highly Effective Methods[3] That Are User Dependent Failure rate of <1% per year when used consistently and correctly.

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[3]
  - Oral
  - Intravaginal
  - Transdermal
  - injectable
- Progestogen-only hormone contraception associated with inhibition of ovulation[3]
  - Oral
  - Injectable
- Sexual abstinence

  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

  Note: Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Male condom and female condom should not be used together (due to risk of failure with friction)

Contraception for Male Participants

Male participants with a partner of child-bearing potential must agree to use following methods of contraception listed below from the initial administration of study intervention until at least 90 days following the last dose of TS-161.

Contraceptives Allowed During the Study Include:

- Condom for male participant AND his partner of child-bearing potential must use one of the methods of contraception for female (WOCBP) participants (listed above)
- Sexual abstinence

  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study

intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

Note: Male participants should refrain from fathering a child or donating sperm from the initial administration of study intervention until at least 90 days following the last dose of TS-161.

13.4.4 Collection of Pregnancy Information

Male participants with partners who become pregnant

- The investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study or prior to 90 days following his last dose of study intervention. This applies only to male participants who receive TS-161 (excluding placebo).
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. Pregnancy of the participant's partner is not considered to be an AE, however, the female partner will be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure. Any congenital anomaly must be reported as an SAE.

Female participants who become pregnant

- The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the sponsor within 24 hours of learning of a participant's pregnancy.
- The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion or congenital anomaly is always considered to be an SAE and will be reported as such. Any post-study pregnancy related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in this appendix. While the investigator is not obligated to actively seek this information in former study participants, they may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention or be withdrawn from the study.