Title: A Randomized, Double-blind, Placebo-Controlled, 3-Period Crossover Study Followed by 1 Open-label Comparator Period to Evaluate Central Pharmacodynamic Activity of TAK-653 in Healthy Volunteers Using Transcranial Magnetic Stimulation

NCT Number: NCT03792672

Protocol Approve Date: 04 April 2019

Certain information within this protocol has been redacted (ie, specific content is masked irreversibly from view with a black/blue bar) to protect either personally identifiable information (PPD) or company confidential information (CCI).

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study.
- Proprietary information, such as scales or coding systems, which are considered confidential information under prior agreements with license holder.
- Other information as needed to protect confidentiality of Takeda or partners, personal information, or to otherwise protect the integrity of the clinical study.

TAKEDA PHARMACEUTICALS

PROTOCOL

A Randomized, Double-blind, Placebo-Controlled, 3-Period Crossover Study Followed by 1 Open-label Comparator Period to Evaluate Central Pharmacodynamic Activity of TAK-653 in Healthy Volunteers Using Transcranial Magnetic Stimulation

Sponsor: Takeda Pharmaceuticals

Study Identifier: TAK-653-1003

Compound: TAK-653

Date: 04 April 2019 **Amendment Number:** 01

Amendment History:

Date	Amendment Number	Amendment Type	Region
21 November 2018	Initial Protocol	Not applicable	Global
04 April 2019	Amendment 01	Substantial	Global

TABLE OF CONTENTS

1.0 STUDY SUMMARY 7
1.1 Protocol Amendment 01 Summary of Changes 7
1.2 Protocol Summary 8
2.0 STUDY SCHEMATIC 12
3.0 SCHEDULE OF STUDY PROCEDURES 13
4.0 INTRODUCTION 20
4.1 Background 20
4.2 Rationale for the Proposed Study 22
4.3 Benefit-Risk Profile 22
4.4 TAK-653 23
4.4.1 Ketamine 25
4.4.2 TMS 26
5.0 TRIAL OBJECTIVES AND ENDPOINTS 27
5.1 Trial Objectives 27
5.1.1 Trial Primary Objective 27
5.1.2 Trial Secondary Objective 27
5.1.3 Trial Exploratory Objectives 27
5.2 Endpoints 28
5.2.1 Primary Endpoints 28
5.2.2 Secondary Endpoints 28
5.2.3 Safety Endpoints 28
5.2.4 Exploratory Endpoints 28
6.0 TRIAL DESIGN AND DESCRIPTION 30
6.1 Trial Design 30
6.2 Rationale for Trial Design, Dose, and Endpoints 31
6.2.1 Rationale of Trial Design 31
6.2.2 Rationale for Dose 31
6.2.3 Rationale for Endpoints 32
6.2.4 Critical Procedures Based on Trial Objectives: Timing of Procedures 34
6.3 Trial Design/Dosing/Procedures Modifications Permitted Within Protocol Parameters 34
6.4 Trial Beginning and End/Completion 35
6.4.1 Definition of Beginning of the Trial 35
6.4.2 Definition of End of the Trial 35

6.4.3 Definition of Trial Discontinuation 35
6.4.4 Criteria for Premature Termination or Suspension of the Trial 36
7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS 37
7.1 Inclusion Criteria 37
7.2 Exclusion Criteria 38
7.3 Excluded Medications, Supplements, Dietary Products 39
7.3.1 Concomitant Medications 39
7.3.2 Fruit Juice 39
7.3.3 Alcohol 39
7.3.4 Caffeine 40
7.3.5 Smoking 40
7.4 Diet, Fluid, Activity 40
7.4.1 Diet and Fluid 40
7.4.2 Activity 40
7.5 Criteria for Discontinuation or Withdrawal of a Subject 40
7.6 Procedures for Discontinuation or Withdrawal of a Subject 41
7.7 Subject Replacement 42
8.0 CLINICAL STUDY MATERIAL MANAGAEMENT 43
8.1 Clinical Study Drug 43
8.1.1 Clinical Study Drug Labeling 43
8.1.2 Clinical Study Drug Inventory and Storage 43
8.1.3 Clinical Study Drug Blinding 43
8.1.4 Randomization Code Creation and Storage 43
8.1.5 Clinical Trial Blind Maintenance/Unblinding Procedure 43
8.1.6 Accountability and Destruction of sponsor-Supplied Drugs 44
8.2 Ancillary Supplies 44
9.0 STUDY PROCEDURES 45
9.1 Administrative Procedures 45
9.1.1 Informed Consent Procedure 45
9.1.2 Inclusion and Exclusion 45
9.1.3 Medical History/Demography 46
9.1.4 Concomitant Medications 46
9.2 Clinical Procedures and Assessments 46
9.2.1 Full Physical Exam 46
9.2.2 Height and Weight 46

9.2.3 BMI 46
9.2.4 Vital Signs 46
9.2.5 12-Lead ECG 47
9.2.6 Study Drug Administration 48
9.2.7 Ketamine Administration 48
9.2.8 AE Monitoring 48
9.3 Laboratory Procedures and Assessments 48
9.3.1 Clinical Laboratory Tests 49
9.3.2 Diagnostic Screening 50
9.4 PK and PD Evaluations 51
9.4.1 PK Samples 51
9.4.2 PK Measurements 51
9.4.3 PD Measurements 52
9.5 Confinement 54
9.6 Childbearing Status and Methods of Contraception 55
9.6.1 Women of Childbearing Potential 55
9.6.2 Women of Nonchildbearing Potential 55
10.0 ADVERSE EVENTS 56
10.1 Definitions and Elements of AEs 56
10.1.1 SAEs 58
10.1.2 Adverse Events of Special Interest (AESI) 59
10.2 AE Procedures 60
10.2.1 Assigning Severity/Intensity of AEs 60
10.2.2 Assigning Causality of AEs 60
10.2.3 Start Date 60
10.2.4 End Date 60
10.2.5 Pattern of Adverse Event (Frequency) 61
10.2.6 Action Taken With Study Treatment 61
10.2.7 Outcome 61
10.2.8 Collection and Reporting of AEs, SAEs, Special Interest AEs, and Abnormal Liver Function Tests (LFT)s 62
10.2.9 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities 64
11.0 STATISTICAL METHODS 65
11.1 Statistical and Analytical Plans 65
11.1.1 Analysis Sets 65
11.1.2 Analysis of Demography and Other Baseline Characteristics 65

11.1.3 PK Analysis 65
11.1.4 PD Analysis 66
11.1.5 Safety Analysis 67
11.2 Interim Analysis and Criteria for Early Termination 68
11.3 Determination of Sample Size 68
12.0 QUALITY CONTROL AND QUALITY ASSURANCE 69
12.1 Study-Site Monitoring Visits 69
12.2 Protocol Deviations 69
12.3 Quality Assurance Audits and Regulatory Agency Inspections 69
13.0 ETHICAL ASPECTS OF THE STUDY 70
13.1 IRB and/or IEC Approval 70
13.2 Subject Information, Informed Consent, and Subject Authorization 70
13.3 Subject Confidentiality 71
13.4 Publication, Disclosure, and Clinical Trial Registration Policy 72
13.4.1 Publication and Disclosure 72
13.4.2 Clinical Trial Registration 72
13.4.3 Clinical Trial Results Disclosure 73
13.5 Insurance and Compensation for Injury 73
14.0 ADMINISTRATIVE AND REFERENCE INFORMATION 74
14.1 Administrative Information 74
14.1.1 Study Contact Information 74
14.1.2 INVESTIGATOR AGREEMENT 75
14.1.3 Study-Related Responsibilities 76
14.1.4 List of Abbreviations 76
15.0 DATA HANDLING AND RECORDKEEPING 78
15.1 CRFs (Electronic and Paper) 78
15.2 Record Retention 78
16.0 REFERENCES 80
17.0 APPENDICES 83

LIST OF IN-TEXT TABLES

Table 4.a Mean Exposures to TAK-653 at the NOAELs in the 13-Week Repeat-Dose Toxicity and Neurotoxicity Studies and Clinical Margins 24
Table 6.a Study Drug and Doses 31
Table 9.a Primary Specimen Collections 51

Table 10.a Takeda Medically Significant AE List....59

LIST OF IN-TEXT FIGURES

Figure 4.a Schematic Representation of the Different Parameters Measured Using TMS 22

LIST OF APPENDICES

Appendix A Responsibilities of the Investigator....83
Appendix B Elements of the Subject Informed Consent....85
Appendix C Investigator Consent to the Use of Personal Information....88
Appendix D Pregnancy and Contraception....89
Appendix E Detailed Description of Amendments to Text....92

1.0 STUDY SUMMARY

1.1 Protocol Amendment 01 Summary of Changes

This document describes the changes in reference to the Protocol Incorporating Amendment No. 01.

Rationale for Amendment 01

The primary purpose of this amendment is to update the protocol regarding changing the primary and key secondary endpoints and changing the early data analysis from blinded to unblinded. Other minor changes in procedures are included, primarily to address inconsistencies in the text and schedule of events. Minor grammatical and editorial changes are included for clarification purposes only. Full details on changes of text are given in Appendix E, including detailed rationale.

Changes in Amendment 01

1. A second primary endpoint added and both endpoints are evaluated as changes from pre-dosing baseline, compared to placebo.
2. Modified secondary endpoints.
3. The primary endpoint analysis now includes both primary endpoints with an additional Hochberg step-up procedure. In addition, some details of the model are deferred to the SAP.
4. The early analysis, which may occur when at least 18 subjects have completed periods 1 through 3, will be unblinded (previously partially blinded).
5. The sample size justification is updated to include both primary endpoints.
6. The resting motor threshold (rMT) exclusion criterion is modified.
7. Three safety endpoints were deleted.
8. Subject instructions to stop concomitant medications before dosing are modified.
9. The window for predose ECG is widened to 3 hours.
10. Site clinic seizure precautions are updated for generalized use.
11. Pharmacokinetic (PK) blood sample collection instructions for ketamine analysis are added.
12. The time interval for routine collection of AEs after final dosing is clarified.
13. Deletion of the 25 hour visit.
14. Detail from study summary added to the trial design section.
15. Description of study blinding corrected.
16. Definition of safety analysis set revised.
17. Pregnancy and contraception language updated.
18. Revisions to the Schedule of Study Procedures table and footnotes.

1.2 Protocol Summary

Name of sponsor:	**Compound:**
Millenium Pharmaceuticals, Inc, a wholly owned subsidiary of Takeda Pharmaceutical Company, Ltd 40 Landsdowne Street Cambridge MA 02139 USA	TAK-653
Study Identifier: TAK-653-1003	**Phase:** 1
Protocol Title: A Randomized, Double-blind, Placebo-Controlled, 3-Period Crossover Study Followed by 1 Open-label Comparator Period to Evaluate Central Nervous System Pharmacodynamic Activity of TAK-653 in Healthy Subjects Using Transcranial Magnetic Stimulation	

Trial Design:

This randomized, double blind, placebo-controlled, 3-period cross-over study followed by one open label comparator period is designed to evaluate the central pharmacodynamic activity of TAK-653 in healthy subjects using transcranial magnetic stimulation (TMS). Approximately 24 healthy male and female subjects, aged 18 to 55 years, inclusive, will be enrolled.

The study will include 3 treatment periods, each 1 day in duration, followed by a 2-day comparator period, alternating with 3 washout periods of at least 10 days (not to exceed 15 days). There will be 5 clinic visits, including screening in which full medical, neurological, and psychiatric examinations will be conducted; Day 1 of each of the first 3 treatment periods, during which TMS testing will be conducted before and after subjects receive placebo or 1 of 2 doses of TAK-653; Day 1 of the fourth treatment period, during which TMS testing will be conducted before and after subjects receive the (NMDA) (N-methyl-D-aspartate) antagonist ketamine, which will be open label.

Before the start of each treatment period, the investigator will review the safety assessments for the subject, including vital signs, and electrocardiogram (ECG).

On the first day of each treatment period (Day 1), each subject will undergo a TMS session assessing baseline TMS-elicited motor-evoked potential (MEP) amplitude and threshold, followed by administration of TAK-653 or placebo (Treatment Periods 1-3), or ketamine (Treatment Period 4). For the first 3 treatment periods, TMS and MEP/electroencephalogram assessment sets, will be conducted along with blood sample collection for pharmacokinetic (PK) analyses at predose and starting at 30 and 150 minutes (2 ½ hours) after dosing. The 2 ½ hour time point is chosen to capture C_{max}, and the 30 minute time point to capture a lower exposure level in each subject. In Period 4, each subject will undergo a TMS session assessing baseline TMS-elicited MEP amplitude and threshold, and blood sample collection for PK analyses at predose, followed by administration of ketamine intravenously (0.5 mg/kg over 40 minutes). TMS assessments will be conducted at 150 minutes (2.5 hours) after ketamine initiation (when ketamine acute dissociative effects have subsided), and blood sample collection for PK analysis at selected timepoints. Subjects will undergo follow-up TMS session starting at 24 hours (Day 2 of Period 4) to capture ketamine modulation of TMS responses at a time in which antidepressant effects of ketamine are already present in a major depressive disorder population.

Subjects will remain in the clinic on treatment days until approximately 2 hours post-TMS procedures for safety observation. During the ketamine-testing period, subjects will remain overnight following the infusion, until the 24 hour follow-up TMS assessments have been completed. Before discharge, a neurological and mental state examination will be performed.

Trial Primary Objective:

1. To determine whether TAK-653 in comparison to placebo, increases central nervous system (CNS) excitability, assessed with TMS-evoked MEP in healthy subjects.

Trial Secondary Objectives:

1. To determine whether TAK-653, in comparison to placebo, modulates responses evoked with paired TMS pulses that capture intracortical circuitry modulation.
2. To determine whether ketamine increases CNS excitability assessed with TMS-evoked MEP in healthy subjects.

Trial Safety Objective

1. To determine the safety and tolerability of TAK-653 when administered as single dose in healthy subjects assessing responses evoked by TMS.

Trial Subject Population: Healthy adult subjects aged 18-55 years

Planned Number of Subjects:	**Planned Number of Sites:**
Up to approximately 24 subjects total enrolled for approximately 22 to complete at least Treatment Periods 1-3)	1 site in the Netherlands
Dose Levels: Study Drug TAK-653 0.5 mg TAK-653 6 mg Matching placebo Ketamine 0.5 mg/kg	**Route of Administration:** Study Drug Oral (Tablets) Ketamine Intravenous infusion
Duration of Treatment: 1 dose of study drug (TAK-653 0.5 or 6 mg or placebo) on Day 1 of each of 4 periods	**Planned Trial Duration:** Up to approximately 15 weeks

Main Criteria for Inclusion:

To be eligible for study participation, subjects must:

1. Understand the study procedures and agree to participate by providing written informed consent.
2. Be willing and able to comply with all study procedures and restrictions.
3. Be male or female (of nonchildbearing potential) aged 18 to 55 years, inclusive, at the Screening Visit.
4. Have a body mass index ≥18.5 and ≤30.0 kg/m^2 at the Screening Visit.
5. Be judged to be in good health by the investigator, based on clinical evaluations including laboratory safety tests, medical history, physical examination, 12-lead ECG, and vital sign measurements performed at the screening visit and before the first dose of study drug.
6. Meet the birth control requirements outlined in the protocol.

Main Criteria for Exclusion:

A subject must be excluded from participating in the study if the subjects:

1. Has a positive alcohol or drug screen.
2. Has a positive pregnancy test.
3. Is a lactating/nursing woman.
4. Has a clinically significant previous or current psychiatric disorder according to the (DSM5) Diagnostic and Statistical Manual of Mental Disorders, including substance use disorder.

5. Has a history of intracranial mass lesion, hydrocephalus and/or head injury or trauma.
6. Has metal objects in brain or skull
7. Has a cochlear implant or deep brain stimulation device.
8. Has a history of epilepsy, seizures, or convulsions.
9. Has a family history of epilepsy, seizures, or convulsions.
10. Has abnormal sleeping patterns (eg, working night shifts).
11. Has a resting motor threshold (rMT) of more than 75% of the maximum stimulator output, measured using TMS-electromyogram (EMG) during screening.

Main Criteria for Evaluation and Analyses:

The primary endpoints of the study are:

- The change of peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT) at 2½ hours after administration of TAK-653 from pre-dosing baseline, compared to placebo.
- The change of rMT obtained with single-pulse TMS at 2½ hours after administration of TAK-653 from pre-dosing baseline, compared to placebo.

The secondary endpoints are the change from baseline at 2½ hours for the following:

- Magnitude of long intracortical inhibition (LICI) obtained with paired-pulse TMS (stimulation intensity conditioning pulse and test pulse: 120% of baseline rMT).
- Magnitude of short intracortical inhibition (SICI) obtained with paired-pulse TMS (stimulation intensity: conditioning pulse 80% of baseline rMT; test pulse: 120% of baseline rMT).
- The rMT obtained with single-pulse TMS assessing ketamine effects, as well as at 24 hours.
- The peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT) assessing ketamine effects, as well as at 24 hours.

The safety endpoints include:

- Number/percentage of subjects with at least 1 AE.
- Number/percentage of subjects with at least 1 serious adverse event (SAE).
- Number/percentage of subjects with at least 1 clinically-defined abnormal laboratory value.
- Number/percentage of subjects with at least 1 clinically-defined abnormal vital sign value.

Statistical Considerations:

PK Analysis

Plasma concentrations of TAK-653 at each time point will be summarized from each treatment using descriptive statistics.

Individual plasma concentration data versus time will be presented in a data listing.

PD Analysis

Primary Endpoint Analysis

The primary endpoints are changes in rMT and changes in peak-to-peak amplitude of the MEP obtained with single-pulse TMS at 2 1/2 hours after administration of TAK-653 from their corresponding pre-dosing baseline levels compared to placebo. They will be analyzed using linear mixed effect models.

The primary endpoints will be analyzed using a linear mixed effects model appropriate for a three-period crossover design. Hochberg's step-up procedure will be used to adjust for multiple endpoints. One-sided t-tests comparing each of the primary endpoints for placebo versus treatment at each dose level will be conducted as secondary analyses,

adjusted for multiple testing to ensure type 1 error control at the 10% level.

Secondary Endpoint Analysis

The magnitude of LICI and SICI obtained with paired-pulse TMS will be analyzed using linear mixed effects models analogous to the model used to analyze the primary endpoint. One-sided t-tests (alpha = 0.05) will be used for the comparison of placebo versus one of the TAK-653 treatment groups.

The change of rMT at 24 hours post ketamine dosing and peak-to-peak amplitude of the MEP obtained with single-pulse TMS will be analyzed using linear mixed effect models appropriate for the design.

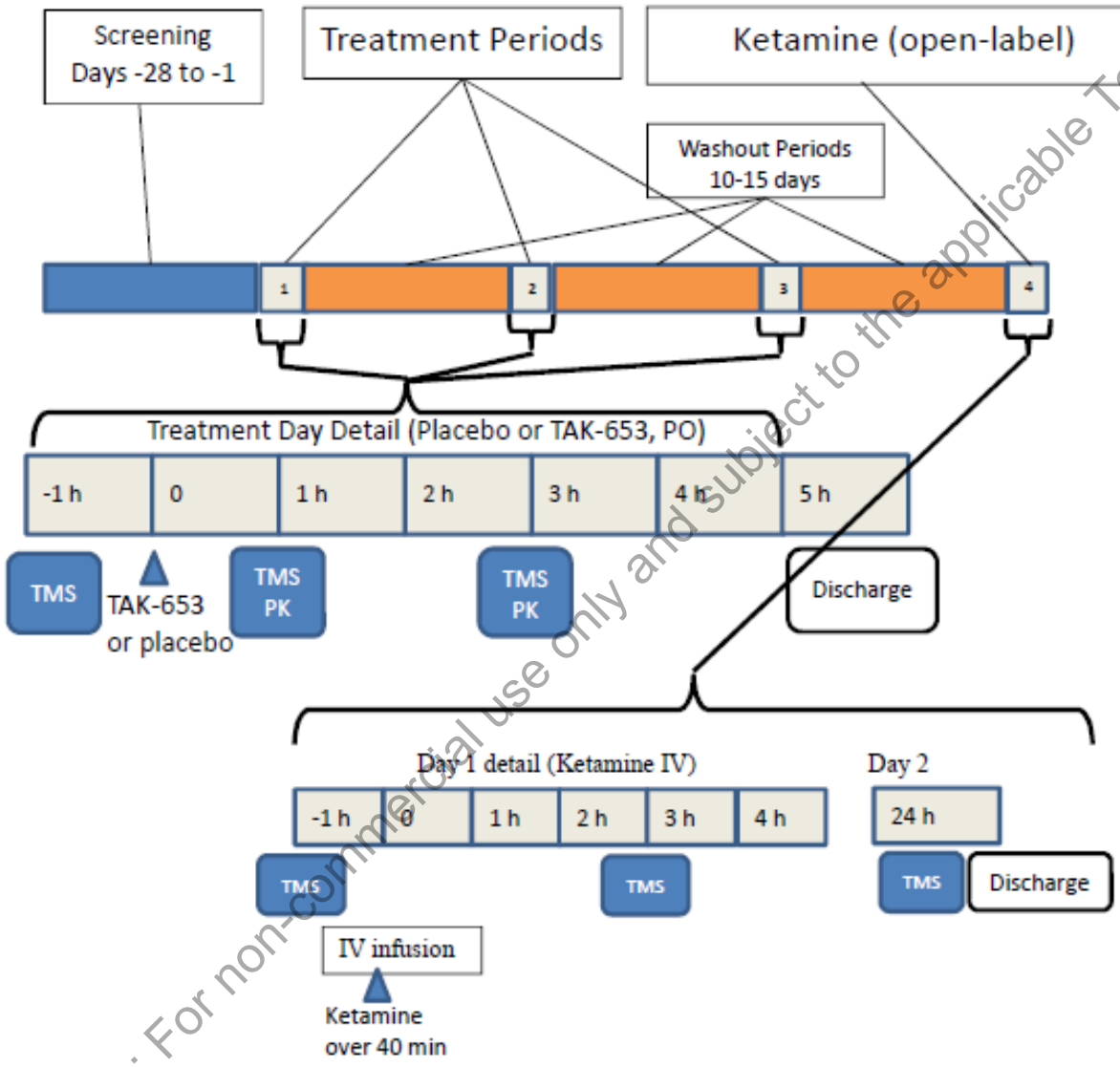
Safety Analysis

The safety of TAK-653 will be assessed through adverse events, clinical laboratory results, physical examinations, ECG findings, vital signs, suicidal assessments, and close monitoring of potential tremors, convulsions and seizures.

Sample Size Justification:

The assumptions used in the sample size determination were based on consideration of a published study on Ketamine by di Lazzaro et al. (2003) and the preliminary results of TMS data in a CHDR study of healthy volunteers. Specifically, we assumed an intra-subject correlation of 0.9, a common standard deviation of 9% for rMT and 947 μV for the MEP peak-to-peak amplitude, a correlation coefficient of at least 0.5 between pre- and post-dosing outcome, and a familywise type I error rate of 10%. A sample size of 22 subjects, approximately 4 subjects for each of the 6 sequences of the first 3 treatment periods, will provide at least 80% power in detecting a reduction of 5.3 percentage point in rMT and/or an increase of 560 μV in the MEP peak-to-peak amplitude at the most effective dose level of TAK-653 versus placebo. Subjects who complete at least the first 3 treatment periods of the study are considered completers. Drop-out rate is expected to be low. Twenty-four subjects will be recruited (4 per sequence) to ensure at least 22 completers. If more than 2 subjects drop out before completing the first 3 treatment periods, up to 2 additional subjects may be enrolled.

2.0 STUDY SCHEMATIC



IV: intravenous; PK: pharmacokinetics; PO: orally; TMS: transcranial magnetic stimulation.

3.0 SCHEDULE OF STUDY PROCEDURES

	Screening Day -28 to -1	Treatment Period									
		TAK-653 or Placebo Single Dose						Ketamine			
Period		1		2		3		4			
Visit	1	2	Wash-out 10-15 Days	3	Wash-out 10-15 Days	4	Wash-out 10-15 Days	5		ET	Follow-up Phone Call (14 ± 2 Days After Last Dose)
								Day 1	Day 2		
Administrative Procedures											
Informed consent	X										
Inclusion/exclusion criteria	X	X		X		X		X			
Medical history/ demographics	X										
Prior and concomitant medication review	X	X		X		X		X	X	X	X
Clinical Procedures/ Assessments											
Full physical examination	X	X		X		X		X	X	X	
Neurological and psychiatric evaluation	X										
C-SSRS [a]	X	X		X		X		X	X	X	
Targeted neurological and mental state examination [b]		X		X		X		X	X		
Height and weight / BMI	X										
Semi-recumbent vital signs (HR, SBP, DBP) [c]	X	X		X		X		X	X	X	
Other vital signs (RR, tympanic temperature)	X	X		X		X		X	X	X	
Standard 12-lead ECG [e]	X [d]	X		X		X		X	X [e]	X	
Randomization		X									
AE monitoring	X	X		X		X		X	X	X	X
Seizure precautions [f]		X		X		X		X	X		
Study drug (TAK-653/placebo) administration		X		X		X					
Ketamine administration								X			

	Screening Day -28 to -1	Treatment Period									
		TAK-653 or Placebo Single Dose						Ketamine			
Period		1		2		3		4			
Visit	1	2	Wash-out 10-15 Days	3	Wash-out 10-15 Days	4	Wash-out 10-15 Days	5		ET	Follow-up Phone Call (14 ± 2 Days After Last Dose)
								Day 1	Day 2		
Laboratory Procedures/ Assessments											
Hematology	X	X		X		X		X		X	
Chemistry	X	X		X		X		X		X	
Urinalysis	X	X		X		X		X		X	
Urine pregnancy test	X										
Serum FSH (if applicable)	X										
Hepatitis/ HIV screen	X										
Alcohol screen	X	X		X		X		X			
Urine drug screen	X	X		X		X		X			
PK Evaluations [g]											
Plasma sample for TAK-653 PK		X		X		X				X	
Plasma sample for ketamine								X	X		
PD / Biomarker Evaluations											
TMS and MEP/EEG data collection [h]	X [i]	X		X		X		X	X		
CCI											
Overnight stay								X			

AE: adverse event; BMI: body mass index; C-SSRS: Columbia–Suicide Severity Rating Scale; CHDR: Centre for Human Drug Research; DBP: diastolic blood pressure; ECG: electrocardiogram; EEG: electroencephalogram; ET: early termination; FSH: follicle-stimulating hormone; HR: heart rate; MEP: motor evoked potential; PD: pharmacodynamic(s); PK: pharmacokinetic(s); RR: respiratory rate; SBP: systolic blood pressure; TMS: transcranial magnetic stimulation.

[a] C-SSRS will be administered at screening and at treatment visits.

[b] Targeted neurological and mental state examinations (Section 9.2.1) will be performed approximately 2 hours after TMS procedures and prior to discharging subjects from the clinic on treatment days.

[c] Vital signs and ECG measurements will be performed approximately 3 hours prior to dosing.

[d] The Screening (baseline) ECG will be repeated 3 times with at least a 1-minute interval between ECG measurements.

[e] A post-treatment ECG will be performed after TMS testing in all treatment periods before discharge.
[f] See Section 9.2.8 for seizure precautions as part of AE monitoring.
[g] See PK sampling times and windows in the tables below for Period 1 to 3 for TAK-653 and Period 4 for ketamine
[h] TMS procedures will include calculating threshold for MEP at baseline, and repeated testing of MEP amplitude at 120% of threshold at baseline and after dosing. The threshold after dosing will also be calculated to determine whether study drugs increased excitability. Paired pulse protocols (short intracortical inhibition, long intracortical inhibition) will be also tested at baseline and following dosing. In addition to the MEP assessments, TMS-evoked potentials will be collected with EEG every time TMS is assessed.
[i] CCI [redacted]
[j] For training purposes only.
[k] The treatment period time for all drug days is detailed in the tables for Treatment Periods 1 to 3 and Period 4 below.

TAK-653 Detailed Schedule of Assessments

Treatment Period 1 to 3

Time Point / Assessment	Predose	-40 min	0 min	30 min	70 min	150 min	185 min	240 min	300 min	360 min
Admission [a]	X									
Inclusion/exclusion criteria	X									
Concomitant medication review	Continuous									
C-SSRS	X									
Physical examination including neurological and mental state examination [b]	X								X	
Vital signs [c]	X				X		X		X	
Standard 12-lead ECG [d]	X							X		
AE monitoring	Continuous									
Study drug (TAK-653/placebo) administration			X							
Laboratory assessments [e]	X			X		X				
Urinalysis	X									
Urine drug screen	X									
TMS and MEP/EEG data collection [f, g]		X		X		X				
CCI										
PK sample TAK-653 [i]	X			X		X				
Discharge										X

AE: adverse event: C-SSRS: Columbia–Suicide Severity Rating Scale; CHDR: Centre for Human Drug Research; DBP: diastolic blood pressure; ECG: electrocardiogram; EEG: electroencephalogram; HR: heart rate; MEP: motor-evoked potential; PK: pharmacokinetic(s); RR: respiratory rate; SBP: systolic blood pressure; TMS: transcranial magnetic stimulation.

[a] Subjects should be in a fasted state from 5 hours before arrival; standardized meals will be provided after the first postdose TMS measurement has been performed.

[b] Targeted physical, neurological, and mental state examinations will be performed before dosing and before discharge.

[c] Vital signs will include HR, SBP, DBP, and RR. Temperature will be measured during the first predose vital signs measurement and during the last post dose measurement, but not

during all vital signs measurements. Vital signs and ECG measurements will be obtained about 3 hours prior to dosing after the subject has been in the supine semi-recumbent position for at least 5 minutes.

[d] ECGs will be made at least at 1 minute intervals. ECGs will be conducted after the subject has been in a semi-recumbent position for at least 5 minutes.

[e] Laboratory assessments consist of: hematology, chemistry. (Results do not have to be known before dosing).

[f] TMS procedures will include calculating threshold for MEP at baseline, and repeated testing of MEP amplitude at 120% of threshold at baseline and after dosing. The threshold after dosing will also be calculated to determine whether study drugs increased excitability. Paired pulse protocols (short-interval intracortical inhibition, long-interval cortical inhibition) will be also tested at baseline and following dosing. In addition to the MEP assessments, TMS-evoked potentials will be collected with EEG every time TMS is assessed.

[g] A time window of ±1 hour is allowed for the TMS and MEP/EEG data collection and for the CCI

[h] . CCI

A PK blood draw sampling time window of ±5 minutes is allowed.

Treatment Period 4

Time Point / Assessment	Predose	-40 min	0 min to 40 min	20 min	50 min	75 min	90 min	150 min	240 min	330 min	24 hours (Day 2)	26 hours
Admission [a]	X											
Inclusion/exclusion criteria	X											
Concomitant medication review	Continuous											
C-SSRS	X										X	
Physical examination including neurological and mental state examination [b]	X										X	
Vital signs [c]	X					X		X		X	X	
Standard 12-lead ECG [d]	X					X					X	
AE monitoring	Continuous											
Laboratory assessments [e]	X											
Urinalysis	X											
Urine drug screen	X											
Ketamine administration			X									
TMS and MEP/EEG data collection [f, g]		X						X			X [l]	
CCI												
PK sample ketamine [i]	X			X			X	X	X		X	
Discharge												X

AE: adverse event; C-SSRS: Columbia–Suicide Severity Rating Scale; CHDR: Centre for Human Drug Research; DBP: diastolic blood pressure; ECG: electrocardiogram; EEG: electroencephalogram; ET, early termination; HR: heart rate; MEP, motor-evoked potential; PK, pharmacokinetic(s); RR: respiratory rate; SBP: systolic blood pressure; TMS, transcranial magnetic stimulation.

[a] Subjects should be in a fasted state from 5 hours before arrival, standardized meals will be provided after the first postdose PD measurement has been performed.

[b] Targeted physical, neurological, and mental state examinations will be performed before dosing and before discharge.

[c] Vital signs will include HR, SBP, DBP, and RR., Temperature will be measured during the first predose vital signs measurement and last post dose vital sign measurement, but not during all vital sign measurements. Vital signs and ECG measurements will be obtained about 3 hours prior to dosing, after the subject has been in a supine semi-recumbent position for at least 5 minutes.

[d] Triplicate ECGs will be made with at least a 1 minute interval. ECGs will be made after the subject has been in a supine semi-recumbent position for at least 5 minutes.

[e] Laboratory assessments consist of: hematology, chemistry. (Results do not have to be known before dosing).
[f] TMS procedures will include calculating threshold for MEP at baseline, and repeated testing of MEP amplitude at 120% of threshold at baseline and after dosing. The threshold after dosing will also be calculated to determine whether study drugs increased excitability. Paired pulse protocols (short-interval intracortical inhibition, long-interval cortical inhibition) will be also tested at baseline and following dosing. In addition to the MEP assessments, TMS-evoked potentials will be collected with EEG every time TMS is assessed.
[g] A time window of ±1 hour is allowed for the TMS and MEP/EEG data collection and for the CCI .
[h] CCI

PK blood draw sampling time window of ±5 minutes is allowed.

4.0 INTRODUCTION

4.1 Background

TAK-653 is an alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) type glutamate receptor positive allosteric modulator in clinical development as a potential therapeutic for patients with treatment-resistant depression (TRD). The TRD field has recently been rejuvenated by the finding that ketamine, an N-methyl-d-aspartate (NMDA) glutamate receptor open channel blocker, has rapid-onset antidepressant properties in the TRD population and in rodent models of depression (eg, reduction of submissive behavior model). The rationale for an AMPA receptor potentiator to treat TRD follows from the observation that the antidepressant actions of ketamine in rodents are blocked with the co-administration of the AMPA receptor blocker NBQX. In addition, ketamine administration causes an increase in extracellular cortical glutamate, which can act on downstream AMPA receptors [1]. A current hypothesis on the mechanisms of the rapid-acting antidepressant effect of ketamine posits that increased AMPA receptor function is a key element in this mechanism. Taken together, these data suggest that by directly potentiating AMPA receptors, TAK-653 may act as a rapid onset antidepressant without the psychotomimetic side effects associated with ketamine administration.

To date, TAK-653 has shown no evidence of pharmacodynamic (PD) effects in humans, including no dose-related adverse events (AEs) suggestive of central nervous system (CNS) activity, such as dizziness or tremors. In a phase 1 single rising dose (SRD) and multiple rising dose (MRD) study, TAK-653 exhibited favorable safety/tolerability and pharmacokinetic (PK) profiles. In nonclinical studies, TAK-653 was not genotoxic but did show convulsions in rats and monkeys and neuropathology in rats at high doses. Accordingly, human exposures are limited by the maximum observed plasma concentration (C_{max}) no-observed-adverse-effect-level (NOAEL) for convulsions in monkeys (350 ng/mL) and the area under the plasma concentration-time curve (AUC) NOAEL for neuropathology in rats (17,400 h*ng/mL). Detecting CNS PD activity at sufficiently low doses is therefore critical for progression of TAK-653 into a clinical proof-of-concept (POC) study.

Nonclinical studies revealed effects of TAK-653 in rodent cognitive tasks and in a task relevant to depression (reversal of submissive behavior model in rat) at low doses; however, it's not clear if these paradigms are translatable to human depression studies. More recently, a transcranial magnetic stimulation (TMS) study in rats demonstrated that the same low doses of TAK-653 that elicited behavioral effects in nonclinical models can also modulate brain circuitry activity in a manner that can also be tested in humans. Therefore, to establish a therapeutic window and provide a firm basis for selecting a dose for a clinical dose-ranging trial in TRD, the present study is focused on determining if TAK-653 has measurable PD activity in healthy subjects at exposures being targeted for testing in the POC TRD study.

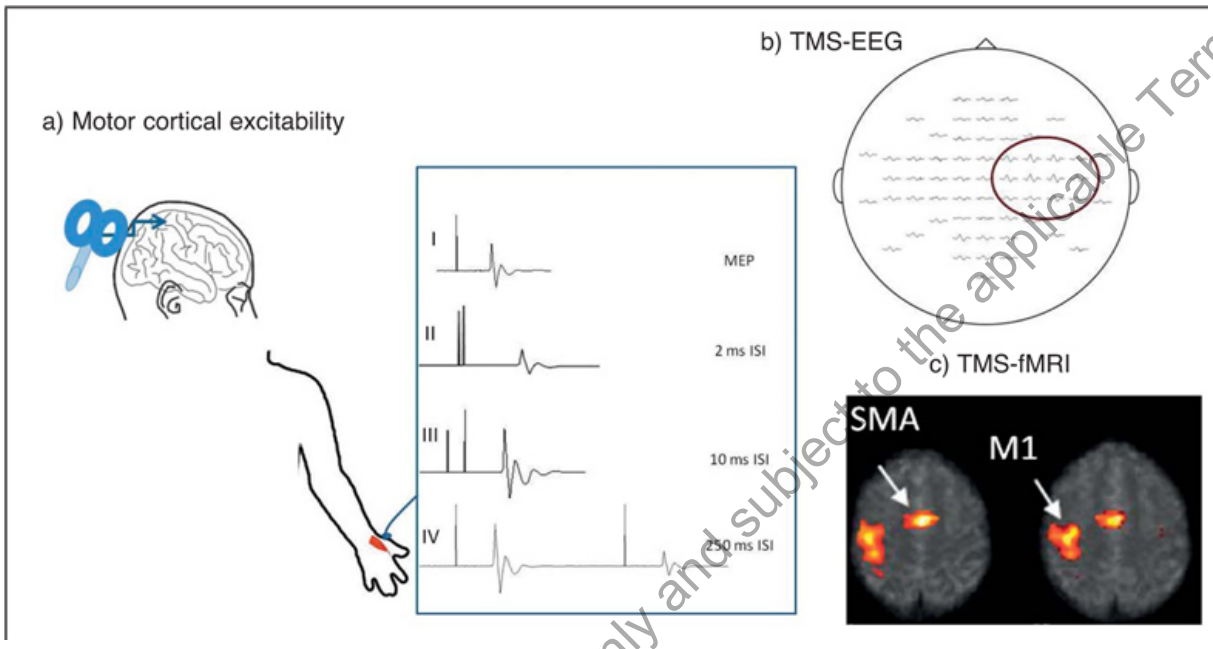
TMS is a noninvasive neurostimulation method involving the application of brief magnetic pulses to the skull that, based on the principles of electromagnetic induction, create an orthogonal electric current that can be sufficient to depolarize neurons and activate neuronal circuits. TMS has been used for many years to map epileptic foci and surrounding "eloquent" regions of cortex that must be avoided during neurosurgery. This diagnostic purpose uses single pulse TMS, in which TMS is

applied 1 stimulus at a time; other paradigms include paired-pulse TMS, in which pairs of stimuli are separated by a variable interval, and repetitive TMS (rTMS), in which TMS pulses are delivered in trains of various durations. rTMS use is rapidly expanding for a wide variety of therapeutic purposes, including psychiatric disorders (depression, acute mania, bipolar disorders, panic, hallucinations, obsessions/compulsions, schizophrenia, catatonia, posttraumatic stress disorder, and drug craving); neurologic diseases (Parkinson disease, dystonia, tics, stuttering, tinnitus, spasticity, epilepsy, and post-stroke rehabilitation); and pain syndromes, (neuropathic pain, visceral pain, and migraine).

Noninvasive brain stimulation techniques like TMS offer an opportunity to study mechanisms of cortical physiology at the systems level of the human brain [2]. The combination of brain stimulation with CNS-active drugs might help assessing the effects of these drugs on brain physiology. Combined with electroencephalography (EEG) and/or electromyography (EMG), both cortical excitability and the modulatory effects of CNS-penetrant drugs can be quantified. Although excitability of the cortex has been particularly relevant to the field of epilepsy and different anticonvulsants are known to affect different TMS measures of motor cortical excitability, it also holds potential for pathophysiological research in mood disorders and quantifying the effects novel glutamatergic drugs for the treatment of depression. However, although benzodiazepines have been shown to affect TMS-EEG and TMS-EMG in a few studies [3], the effects of excitatory glutamatergic compounds remain to be established.

The most extensively studied response using TMS is the motor-evoked potential (MEP) produced in the index finger after TMS stimulation of the motor cortex, Figure 4.a. Pharmacological modulation of MEP threshold and amplitude can be used to assess PD effects [4], and this endpoint is proposed to depend heavily on AMPA receptor function [4], and modulated by ketamine [5].

Figure 4.a Schematic Representation of the Different Parameters Measured Using TMS



Source: Badawy et al, 2012 [6].
EEG: electroencephalogram; EPI: echo planar image; fMRI: functional magnetic resonance imaging; ISI: interstimulus interval; M1: motor area; SMA: supplementary motor area; SPM: statistical parametric mapping; TMS: transcranial magnetic stimulation.
a) Motor cortical excitability showing (I) motor evoked potential recorded with a single pulse. Latency is measured from stimulus artefact to initial deflection in baseline; amplitude is measured peak to peak. Examples of (II) short-interval intracortical inhibition at the 2 ms ISI, (III) intracortical facilitation at the 10 ms ISI, and (IV) long-interval intracortical inhibition at the 250 ms ISI.
b) Averaged EEG responses evoked by TMS. The signals are arranged according to the layout of the electrodes. The amplitudes of the responses are highest in the vicinity of the stimulated site (highlighted) and attenuate with increasing distance from stimulation.
c) Sample of a connectivity map obtained after repetitive TMS of the right motor area on fMRI. SPMs are threshholded for corrected clusters ($p<0.05$ corrected for multiple comparisons) and are superimposed on the main EPI image.

4.2 Rationale for the Proposed Study

Given the absence of evidence for human target engagement or PD activity of TAK-653, the goal of this study is to leverage a translatable preclinical finding to assess PD activity in healthy subjects. Of specific interest is to determine whether low doses of TAK-653 modulate CNS activity. Based on these data, the study described herein will determine if doses and exposures of TAK-653 correlate with PD effects in TMS-evoked muscle evoked potentials and EEG signals.

4.3 Benefit-Risk Profile

This phase 1 trial has been designed to mitigate the known risks associated with AMPA receptor potentiators as a class and the potential risks based on the nonclinical toxicity data and preliminary

clinical data for TAK-653. In addition, this trial has been designed to mitigate the known clinical risks of ketamine. As this trial will be conducted in healthy subjects, there is no expected clinical benefit to trial participants.

The principal mitigations for these potential risks include the maintenance of an appropriate safety margin based on nonclinical study use of low doses that yield low drug exposure, appropriate selection of the trial population, the prespecified safety monitoring procedures, and the selection of the trial facility, where close monitoring can be performed and rapid institution of appropriate care can be given. The potential risks associated with AMPA receptor potentiators as a class, the potential risk based on nonclinical toxicity data, and the known clinical risks of ketamine can be monitored clinically and/or with laboratory tests and have been considered when determining the stopping rules for this clinical trial.

In addition to the potential risks associated with study drug administration, there is minimal risk associated with trial procedures including scheduled, periodic phlebotomy (limited to <500 mL) and noninvasive procedures including vital sign assessments, electrocardiograms (ECGs), and TMS assessment. Overall, the benefit-risk profile is considered appropriate for this trial.

4.4 TAK-653

TAK-653, like other AMPA receptor potentiators, may induce convulsions through the overactivation of AMPA receptors. Other potential risks of AMPA receptor potentiators include tremors. Based on nonclinical findings with TAK-653, the potential risks associated with the drug are seizures, convulsions and vomiting.

In the 2-part, first-in-human (FIH) trial (TAK-653-1001), 88 healthy subjects were enrolled and administered study drug (66 TAK-653 vs 22 placebo) in 6 SRD cohorts (0.3, 1, 3, 5, 9, and 18 mg) in Part 1 and 5 SRD/MRD cohorts (0.3, 1, 3, 6 and 9 mg once daily [QD] for 13 days) in Part 2. TAK-653 was generally well tolerated at single doses up to 18 mg and multiple doses up to 9 mg QD. No serious treatment-emergent adverse events (TEAEs), including serious CNS TEAEs or adverse events of special interest (AESIs) (seizures, convulsions), were reported. There were no concerning trends in clinical laboratory, vital sign, telemetry, ECG, or EEG data, and all AE were reported as mild in intensity and not related to the study drug.

PK data from Part 1 indicated that the mean C_{max} values increased slightly less than dose proportionally, ranging from 3.63 to 126 ng/mL, whereas the mean area under the plasma concentration-time curve from time 0 to infinity (AUC_{∞}) values increased dose proportionally, ranging from 148 to 8882 h*ng/mL, across the single-dose range (from 0.3 mg to 18 mg).

Mean exposures to TAK-653 in the 13-week toxicity studies and margins relative to exposures in human subjects administered a single oral dose of TAK-653 (6 and 18 mg) and an oral dose of TAK-653 (6 mg QD for 13 days) are presented in Table 4.a.

Table 4.a Mean Exposures to TAK-653 at the NOAELs in the 13-Week Repeat-Dose Toxicity and Neurotoxicity Studies and Clinical Margins

	C_{max} (ng/mL)	C_{max} Margin [a]	AUC_{24} (h*ng/mL)	AUC Margin [a]
Rat (B151026-Report) (13-week study)				
Male	1130 [b]	26.9/9.0/7.6	10,800 [c]	4.6/1.2/3.7
Female	2320	NA	28,900 [c]	12.4/3.3/9.9
Rat (B170759-Report) (Day 14)				
Male	1290	30.7/10.2/8.7	17,000	7.3/1.9/5.8
Female	1670	39.8/13.3/11.3	17,800	7.6/2.0/6.1
Monkey (1015-1193, 1015-1193 Amendment 1, 1015-1193 Amendment 2) (13-week study)				
Male	350 [d]	8.3/2.8/2.4	18,400 [e]	7.9/2.1/6.3
Female	362 [d]	8.6/2.9/2.4	18,900 [e]	8.1/2.1/6.4
Human (Study TAK-653-1001)				
6 mg single dose	42.0		2328 [f]	
18 mg single dose	126		8882 [f]	
6 mg multiple dose (QD for 13 days)	148		2933 [g]	

AUC: area under the plasma concentration-time curve; AUC_{24}: area under the plasma concentration-time curve from time 0 to 24 hours; C_{max}: maximum observed plasma concentration; NA: not applicable; NOAEL: no-observed-adverse-effect level; QD: once daily.
[a] 6 mg single dose/18 mg single dose/6 mg multiple dose (QD for 13 days).
[b] Day 1 at 50 mg/kg/day (males).
[c] Day 91 at 50 (males) and 100 (females) mg/kg/day.
[d] Day 2 at 10 (males) and 3 (females) mg/kg/day.
[e] Day 91 at 10 (males) and 6 (females) mg/kg/day.
[f] Area under the plasma concentration-time curve from time 0 to infinity.
[g] Area under the curve from 0 to time t.

Although data from the FIH trial suggest that the single doses (0.5 and 6 mg) proposed for the current trial were safe and well tolerated in healthy subjects, the potential risks described above, based on the mode of action of TAK-653, nonclinical findings, and human experience with other AMPA receptor potentiators, must still be considered.

As this protocol entails brain stimulation procedures using TMS, there is a risk for increasing the likelihood of convulsions and seizures. The TMS protocols used in this study have rarely resulted in seizures, but the risk should be considered especially in combination with TAK-653.

Given the above-mentioned potential risks, the following risk mitigation measures will be applied during the conduct of the current trial:

1. Subjects at risk of seizures will be excluded (see Section 10.1).
2. Convulsions and seizures will be monitored and reported as AESIs (see Section 10.1.2).
3. Subjects will be provided with emergency contact information to be carried on their person at all times.

4. The site will provide study physicians trained in basic life support and management of convulsions and seizures.

4.4.1 Ketamine

The administration of multiple subanesthetic doses of ketamine was generally well tolerated in previous studies performed at the Centre for Human Drug Research (CHDR) and in literature [7-11]. However, subjects who have no/limited experiences with the effects of recreational psychoactive substances such as alcohol may have a higher risk of intolerability to ketamine effects. Psychotomimetic reactions include anxiety, chest pain, palpitations, agitation, flashbacks, delirium, dystonia, psychosis and schizophrenia-like symptoms. The psychotomimetic reactions seem related to the infusion of ketamine and disappear soon after the discontinuation of the infusion [12]. Potential adverse effects of ketamine administration include hypersalivation, hyperreflexia, muscle hypertonicity, transient clonus, increased intraocular pressure, emesis, transient rash, agitation dizziness and seizures. Hypertension, tachycardia, increase pulmonary pressures, increased intraocular pressure and pulmonary edema can also be seen as an effect of sympathomimetic stimulation by ketamine. Laryngospasm (during infusion) is frequently cited as an adverse effect of ketamine, but it is rarely observed.

Ketamine undergoes extensive metabolism by hepatic cytochrome P450 (CYP). In humans, N-demethylation to norketamine is the major route of metabolism, which can undergo further metabolism into 2 diasteromeric HK (hydroxyketamine) and 6 diastereomeric HNK (hydroxynorketamine) metabolites and DHNK (dehydronorketamine) [13-15]. The major human hepatic CYPs that catalyze ketamine *N*-demethylation in vitro are CYP2B6 and CYP3A4, although there is ambiguity as to their comparative contributions to clinical ketamine metabolism [13,16,17]. The CYP enzymes responsible for the formation of norketamine metabolites include CYP2A6 and CYP2B6 [13]. Quantifiable concentrations of ketamine metabolites were found to be present up to 3 days after ketamine infusion [18]. Of the different active ketamine metabolites (2*R*,6*R*)-HNK induces an acute increase in glutamatergic signaling, followed by a long-term adaptation involving the upregulation of synaptic AMPA receptors, as evidenced by an increase in GluA1 and GluA2 in hippocampal synapses [19].

Ketamine has a wide therapeutic range and exhibits multiexponential compartmental PK after intravenous (IV) administration. The mean systemic clearance, steady-state distribution volume, and terminal disposition half-life ($t_{1/2z}$) values were 14.8 mL/min/kg, 2.2 L/kg, and 196 minutes respectively, following IV administration of ketamine to 10 healthy subjects [20]. The mean concentration was 187 ng/mL at 40-minutes post dose in 21 patients with depression who received 0.5 mg/kg of ketamine as a 40-minute infusion [21]. Ketamine will be administered once in the present study; IV at a dose level of 0.5 mg/kg over 40 minutes.

The use of ketamine in doses of 1 to 3 mg/kg has been associated with emergence reactions (~12% of subjects), which are unpleasant dreams or hallucinations when emerging from the dissociative state that ketamine induces. The psychological manifestations vary in severity between pleasant dream-like states, vivid imagery, hallucinations, and emergence delirium. In some cases, these states have been accompanied by confusion, excitement, and irrational behavior, which a few

patients recall as an unpleasant experience. The duration of these reactions ordinarily is no more than a few hours. In a few cases, however, recurrences have taken place up to 24 hours after ketamine administration.

Emergence phenomena are less frequent and less severe with the use of lower subanesthetic doses of ketamine (<1 mg/kg), as in the current trial. Emergence reactions may be reduced if verbal, tactile, and visual stimulation of the patient is minimized during the recovery period. If the subject experiences severe dissociative or hallucinatory experiences during the administration of ketamine, the infusion of ketamine should be immediately stopped. Supportive or other measures (ie, rescue medications) will be administered, if necessary.

Ketamine increases blood pressure and heart rate, and both parameters will be closely monitored during ketamine infusions. If the subject experiences an increase in systolic blood pressure to >180 mm Hg or an increase in diastolic blood pressure to >110 mm Hg during the ketamine infusion, the infusion rate will be reduced by half and blood pressure will continue to be carefully monitored. Supportive or other measures (ie, rescue medications) will be administered, if necessary.

During the ketamine infusion, all appropriate and necessary monitoring or other supportive measures will be followed in accordance with the current package labeling and medical use of ketamine. A qualified physician will always be present during ketamine administration and will examine subjects before they are discharged. Subjects will not be discharged until recovery from the effects of ketamine and/or other agents is complete. Recovery from the psychological manifestations of ketamine infusion will be confirmed with a mental state examination. Subjects will remain in the clinic overnight following the ketamine infusion.

Please refer to the current package labeling for ketamine for detailed information regarding the safety risks and risk mitigation measures for ketamine.

4.4.2 TMS

There are several side effects of TMS that have been reported, including scalp burns from electrodes as well as transient headache, local pain, neck pain, toothache, paresthesia, hearing changes, cognitive/neuropsychological changes, and EEG after effects. The most serious side effect reports have been neurocardiogenic syncope and epileptic seizures, which are sometimes clinically difficult to distinguish. The overwhelming majority of these events have occurred in the setting of rTMS, and the few that have been reported in single pulse TMS paradigms have occurred in patients with either epileptic risk factors (eg brain lesions in a patient with multiple sclerosis) or on seizure threshold-lowering medications such as many common anti-depressants [22].

Seizure risk is considered extremely low in the present study, as the TMS paradigms are limited to single and paired pulse protocols (vs. rTMS). Seizure risk will be mitigated by excluding subjects with a personal or family history of seizures; excluding subjects with histories of head trauma, psychiatric disorders, drug abuse, or abnormal sleep patterns; excluding use of all concomitant medications; and allowing only moderate alcohol consumption consistent with baseline use (ie instructing subjects not to change their normal drinking habits).

5.0 TRIAL OBJECTIVES AND ENDPOINTS

5.1 Trial Objectives

5.1.1 Trial Primary Objective

The primary objective of the study is to determine whether TAK-653, in comparison to placebo, increases CNS excitability, assessed with TMS-evoked MEP in healthy subjects.

5.1.2 Trial Secondary Objective

The secondary objective(s) of the study are:

1. To determine whether TAK-653, in comparison to placebo, modulates responses evoked with paired TMS pulses that capture intracortical circuitry modulation.
2. To determine whether ketamine increases CNS excitability assessed with TMS-evoked MEP in healthy subjects.
3. To determine the safety and tolerability of TAK-653 when administered as single dose in healthy subjects assessing responses evoked by TMS.

5.1.3 Trial Exploratory Objectives

Exploratory objectives of this study include:

CCI

[redacted]

5.2 Endpoints

5.2.1 Primary Endpoints

The primary endpoints of the study are:

- The change of peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline resting motor threshold [rMT]) at 2½ hours after administration of TAK-653 from pre-dosing baseline, compared to placebo.
- The change of rMT obtained with single-pulse TMS at 2 1/2 hours after administration of TAK-653 from pre-dosing baseline, compared to placebo.

5.2.2 Secondary Endpoints

Secondary endpoints include the change from baseline at 2 1/2 hours for the following:

1. Magnitude of long intracortical inhibition (LICI) obtained with paired-pulse TMS (stimulation intensity conditioning pulse and test pulse: 120% of baseline rMT).
3. Magnitude of short intracortical inhibition (SICI) obtained with paired-pulse TMS (stimulation intensity: conditioning pulse 80% of baseline rMT; test pulse: 120% of baseline rMT).
4. The rMT obtained with single-pulse TMS assessing ketamine effects, as well as at 24 hours.
5. The peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT) assessing ketamine effects, as well as at 24 hours.

5.2.3 Safety Endpoints

Safety endpoints include:

1. Number/percentage of subjects with at least 1 AE.
2. Number/percentage of subjects with at least 1 serious adverse event (SAE).
3. Number/percentage of subjects with at least 1 clinically-defined abnormal laboratory value.
4. Number/percentage of subjects with at least 1 clinically-defined abnormal vital sign value.

5.2.4 Exploratory Endpoints

CCI

[redacted]

CCI

[redacted]

6.0 TRIAL DESIGN AND DESCRIPTION

6.1 Trial Design

This randomized, double blind, placebo-controlled, 3-period cross-over study followed by one open label comparator period is designed to evaluate the central pharmacodynamic activity of TAK-653 in healthy subjects using transcranial magnetic stimulation (TMS). Approximately 24 healthy male and female subjects, aged 18 to 55 years, inclusive, will be enrolled.

The study will include 3 treatment periods, each 1 day in duration, followed by a 2-day comparator period, alternating with 3 washout periods of at least 10 days (not to exceed 15 days). There will be 5 clinic visits, including screening in which full medical, neurological, and psychiatric examinations will be conducted; Day 1 of each of the first 3 treatment periods, during which TMS testing will be conducted before and after subjects receive placebo or 1 of 2 doses of TAK-653; Day 1 of the fourth treatment period, during which TMS testing will be conducted before and after subjects receive the (NMDA) (N-methyl-D-aspartate) antagonist ketamine, which will be open label.

Before the start of each treatment period, the investigator will review the safety assessments for the subject, including vital signs, and electrocardiogram (ECG).

On the first day of each treatment period (Day 1), each subject will undergo a TMS session assessing baseline TMS-elicited motor-evoked potential (MEP) amplitude and threshold, followed by administration of TAK-653 or placebo (Treatment Periods 1-3), or ketamine (Treatment Period 4). For the first 3 treatment periods, TMS and MEP/electroencephalogram assessment sets, will be conducted along with blood sample collection for pharmacokinetic (PK) analyses at predose and starting at 30 and 150 minutes (2 ½ hours) after dosing. The 2 ½ hour time point is chosen to capture C_{max}, and the 30 minute time point to capture a lower exposure level in each subject. In Period 4, each subject will undergo a TMS session assessing baseline TMS-elicited MEP amplitude and threshold, and blood sample collection for PK analyses at predose, followed by administration of ketamine intravenously (0.5 mg/kg over 40 minutes). TMS assessments will be conducted at 150 minutes (2.5 hours) after ketamine initiation (when ketamine acute dissociative effects have subsided), and blood sample collection for PK analysis at selected timepoints. Subjects will undergo follow-up TMS session starting at 24 hours (Day 2 of Period 4) to capture ketamine modulation of TMS responses at a time in which antidepressant effects of ketamine are already present in a major depressive disorder population.

Subjects will remain in the clinic on treatment days until approximately 2 hours post-TMS procedures for safety observation. During the ketamine-testing period, subjects will remain overnight following the infusion, until the 24 hour follow-up TMS assessments have been completed. Before discharge, a neurological and mental state examination will be performed.

EEG and MEP signals evoked by TMS, as PD endpoints for assessing the effects of TAK-653 were selected on the basis of pre-clinical data showing that TAK-653 increases the amplitude of MEPs in rats. The goal of this study is to evaluate the PD effects of TAK-653 after single dose

treatment as measured by responses to TMS. Healthy adult (age 18 to 55 years) subjects are the intended population in this trial.

The planned dose levels of TAK-653 to be evaluated are outlined in Table 6.a. The study drug will be 6 mg TAK-653 for the high dose, 0.5 mg TAK-653 for the low dose, or placebo administered orally on Day 1 of Treatment Periods 1 to 3 at the clinical testing site. Both doses and placebo treatments will be delivered with 2 tablets, as indicated in Section 8.0. On Treatment Period 4, ketamine 0.5 mg/kg IV will be administered for 40 minutes.

Table 6.a Study Drug and Doses

Study Drug	Regimen	Comments
6 mg TAK-653 PO 0.5 mg TAK-653 PO Placebo	Single dose, PO administration on Day 1 of treatment periods 1-3	Double-blind, Placebo-controlled, Randomized, 3-period crossover (Treatment Periods 1-3)
Ketamine 0.5 mg/kg	Single dose, IV infusion over 40 minutes on Day 1	Open-label, Treatment Period 4

IV: intravenous; PO: orally.

6.2 Rationale for Trial Design, Dose, and Endpoints

6.2.1 Rationale of Trial Design

The crossover design has been selected because it is the most efficient design from the statistical perspective, as treatment comparisons are based on intra-subject differences which are associated with low variability. TAK-653 has been evaluated in a SRD and MRD study in healthy subjects at doses up to 18 mg in the SRD and 9 mg QD in the MRD. PK data indicate that TAK-653 has a mean $t_{1/2z}$ of approximately 40 hours, and longer in some subjects. Therefore, a >4 × $t_{1/2z}$ (~10 days) washout between the dose regimens of each treatment period is sufficient to ensure that there is no TAK-653 carryover effect. Ketamine was chosen as an active comparator, which will be administered to all subjects on Treatment Period 4 as open label. TMS assessments on the ketamine period will be 2½ hours after the start of infusion to capture acute effects and 24 hours after the infusion to capture a time point in which increased AMPA function is present, providing the basis for the antidepressant effect of ketamine.

6.2.2 Rationale for Dose

The dose of TAK-653 will be 6 mg for the high dose and 0.5 mg for the low dose, compared with placebo administered once orally on Day 1 of each treatment period according to the sequence group assigned. In the phase 1 SRD/MRD study, among 66 healthy subjects who took TAK-653, no SAEs or clinically meaningful changes in safety laboratory results, physical examinations, vital signs, ECGs or EEGs were observed. Given the ~40-hour $t_{1/2z}$ of TAK-653, the mean C_{max} associated with single 0.5 and 6 mg doses (estimated at 4.53 ng/mL for 0.5 mg and measured as 42.0 ng/mL for 6 mg) are similar to that with multiple daily dosing of 0.2 and 2 mg, which are the anticipated dose range to be used in a phase 2 study of TAK-653 for TRD to obtain maximal

treatment response while maintaining sufficient margins to potential class-based adverse effects (seizures). The lower dose of 0.5 mg will test whether plasma exposures that had activity in animal models have similar PD activity in healthy human subjects. Specifically, nonclinical studies revealed behavioral efficacy and PD effects with plasma concentrations ranging between 5 and 45 ng/mL. As multiple doses ranging between 0.5 and 2 mg provided plasma concentrations in that range in our safety studies, this will be the range we will explore for efficacy in subsequent studies, provided this study indicates modulation of CNS circuit activity at those exposures.

The NMDA antagonist, ketamine was chosen as an active comparator to be assessed during Treatment Period 4, open label. Ketamine will be delivered at subanesthetic doses, typically used for an antidepressant effect (0.5 mg/kg, IV, 40 minutes). Ketamine has been shown to increase excitability in a TMS study similar to what is proposed here [5].

6.2.3 Rationale for Endpoints

6.2.3.1 Safety Endpoints

Standard safety of TEAEs, vital signs, clinical laboratory test results, and 12-lead ECG parameters will be used to assess safety and tolerability of the investigational drug product. In addition, there are 2 compound-specific safety assessments in this study:

Seizures

Tremors, convulsions, and seizures are identified risks for TAK-653. Although TAK-653 was well tolerated at doses 3 times higher than the high dose in this study, adequate measures have been taken to minimize the occurrence of these risks in the study by limiting the top dose to below one-eighth the NOAEL exposure for convulsions observed in primates, and by excluding subjects who might be at increased risk for seizures. These events will be monitored during the study, as during all treatment periods subjects will don an EEG cap, and EEG signals will be recorded per exploratory objectives. In case of tremors, TMS procedures will be halted and the EEG signal will be collected to determine whether there was seizure like activity.

TMS also carries some risk for seizures, although this is extremely low and has been only seen with rTMS paradigms in healthy subjects; the risk is minimized in the present study by limiting TMS to single and paired-pulse TMS protocols, which carry a very low liability for seizures. EEGs will be recorded immediately if any of the events occur and steps outlined in Section 4.4 on whether to stop or suspend dosing in a cohort or the study will be followed. The standard query forms for the events in the electronic case report form (eCRF) will also be completed.

Columbia Suicide Symptoms Rating Scale

Suicidal ideation will be assessed using the Columbia Suicide Symptoms Rating Scale C-SSRS at the times stipulated in the Schedule of Study Procedures (Section 3.0). Two versions of the C SSRS will be used in this trial: The Screening C-SSRS Lifetime (Version 14Jan2009) and the Since-Last-Visit C-SSRS (Version 14Jan2009).

6.2.3.2 PK Endpoints

Blood samples (approximately 126.5 ml in total over the study) will be collected for the determination of the plasma concentrations of TAK-653 and ketamine. The sampling scheme is indicated in the Schedule of Events.

6.2.3.3 PD Endpoints

This study employs several functional tests to assess their utility as potential PD markers for the effects of TAK-653 and ketamine using EEG and electromyography (EMG) data driven by TMS in order to develop a toolbox to be used in future proof of activity studies as well as proof of concept studies as exploratory PD markers.

Primary PD Endpoints:

The peak-to-peak amplitude of MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT), assessing change from pre-dosing baseline for each treatment period. Enhancing MEP driven by TMS was selected as a primary PD endpoint on the basis of nonclinical data demonstrating that TAK-653 enhances TMS MEPs in rats.

The rMT of MEP obtained with single-pulse TMS assessing the change from pre-dosing baseline is the other primary endpoint.

A reduction in the threshold for TMS-evoked MEP was chosen as ketamine has been shown to increase TMS-driven excitability assessed with threshold to MEPs in healthy volunteers [5]. MEP amplitude and threshold capture modulation of excitatory synapses in motor cortex and spinal cord [4].

Secondary PD Endpoints:

LICI and SICI are TMS stimulation paradigms that capture modulation of cortical excitation-inhibition balance with pairs of TMS pulses. Because this is a local cortical circuitry driven phenomenon, it has the potential to expand the sensitivity of TMS as a biomarker of CNS activation beyond single pulse TMS for certain pharmacologies, including glutamate mediated neurotransmission.

Exploratory PD Endpoints:

CCI

[redacted]

6.2.3.4 Exploratory Biomarker Research

CCI

[redacted]

6.2.4 Critical Procedures Based on Trial Objectives: Timing of Procedures

For this trial, TMS-evoked MEP is the critical procedure.

- TMS postdose assessments need to be performed as close to the exact nominal time point/scheduled time as possible.
- All other procedures should be performed as close as possible (either before or after the nominal time of the critical procedure).
- ECG and vital signs measurements should be performed before the PK blood draw, if scheduled together
- The order of priority can be changed during the study with joint agreement of the investigator and the sponsor.
- Any nonscheduled procedures required for urgent evaluation of safety concerns take precedence over all routine scheduled procedures.

6.3 Trial Design/Dosing/Procedures Modifications Permitted Within Protocol Parameters

This is a phase 1 assessment of TAK-653 in humans, and the PK, PD and safety profiles of the compound are still being elucidated. This protocol is written with some flexibility to accommodate the inherent dynamic nature of phase 1 clinical studies. Modifications to the dose, dosing regimen, and/or clinical or laboratory procedures, as outlined below, may be required to achieve the scientific goals of the study objectives and/or to ensure appropriate safety monitoring of the study subjects.

As such, the following alterations from the currently outlined dose and/or dosing regimen may be permitted on the basis of newly available data, but the maximum daily dose/exposure may not exceed that currently outlined in Section 6.1.

- The duration of the washout period between doses may be increased.
- The length of the washout period between doses may be decreased, if supported by safety and PK data.
- A PK data review may be added.
- The PK sampling scheme may be modified during the study. If indicated, these collected samples may also be assayed in an exploratory manner for metabolites.

- Up to an additional 25 mL of blood may be drawn for PK and/or PD analyses. This blood volume may include repeat samples or modified PK/PD time points on the basis of emerging data. The total blood volume withdrawn from any single subject will not exceed the maximum allowable volume during his/her participation in the entire study.
- The timing of planned procedures for assessment of safety procedures (eg, vital signs, ECGs, safety laboratory tests, etc) may be modified during the study based on newly available safety, tolerability, PK or PD data (eg, to obtain data closer to the [t_{max}] time of first occurrence of C_{max}). These changes will not increase the number of study procedures for a given subject during their participation in the entire study.
- Additional laboratory safety tests may be added to blood samples previously drawn to obtain additional safety information.

It is understood that the current study may employ some or none of the alterations described above. Any alteration made to this protocol to meet the study objectives must be detailed by the sponsor in a letter to the Trial File and forwarded to the investigator for retention. The letter may be forwarded to the institutional review board (IRB) /independent ethics committee (IEC) at the discretion of the investigator.

6.4 Trial Beginning and End/Completion

6.4.1 Definition of Beginning of the Trial

The overall study begins when the first subject signs the study informed consent form.

6.4.2 Definition of End of the Trial

The overall study ends when the last subject completes the last planned or follow-up visit/interaction associated with a planned visit (this can be a phone contact), discontinues from the study, or is lost to follow-up (ie, the investigator is unable to contact the subject).

6.4.3 Definition of Trial Discontinuation

Study discontinuation because of nonsafety reasons, such as the following:

- A finding (eg, PK, PD, efficacy, biologic targets) from another nonclinical or clinical study using the study treatment(s) results in the study being stopped for a nonsafety-related reason.
- Data from comparators, drugs of the same class, or methodologies used in this study become available and results in the study being stopped for a nonsafety-related reason.
- The study is stopped because of nonscientific and nonsafety reasons, such as slow enrollment.

Study discontinuation because of safety reasons:

- Early study termination because of unanticipated concerns of safety to the study subjects arising from clinical or nonclinical studies with the study treatments, comparators, drugs of the same class, or methodologies used in this study.

6.4.4 Criteria for Premature Termination or Suspension of the Trial

6.4.4.1 Criteria for Premature Termination or Suspension of Trial Sites

A study site may be terminated prematurely or suspended if the site (including the investigator) is found in significant violation of Good Clinical Practice, protocol, or contractual agreement, or is unable to ensure adequate performance of the study, or as otherwise permitted by the contractual agreement.

6.4.4.2 Procedures for Premature Termination or Suspension of the Trial or the Participation of Trial Sites

In the event that the sponsor, an IRB/IEC, or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the sponsor; the procedure will be followed by applicable investigational sites during the course of termination or study suspension.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria before the first dose of study drug:

1. The subject must understand the study procedures and agree to participate by providing written informed consent.
2. The subject must be willing and able to comply with all study procedures and restrictions.
3. The subject must be male or female (of nonchildbearing potential) aged 18 to 55 years, inclusive, at the screening visit.
4. The subject must have a body mass index (BMI) ≥18.5 and ≤30.0 kg/m2 at the screening visit.
5. The subject must be judged to be in good health by the investigator, based on clinical evaluations including laboratory safety tests, medical history, physical examination, 12-lead ECG, and vital sign measurements performed at the screening visit and before the first dose of study drug.
6. The subject must meet the following birth control requirements:
    - Is a male subject who is sterile or agrees to use an appropriate method of contraception, including a condom with or without spermicidal cream or jelly, from the first dose of study drug until 100 days after the last dose of study drug. No restrictions are required for a vasectomized male subject provided the subject is at least 1 year post-bilateral vasectomy procedure before the first dose of study drug. A male subject whose vasectomy procedure was performed less than 1 year before the first dose of study drug must follow the same restrictions as a non-vasectomized man. Appropriate documentation of surgical procedure should be provided.
    - Is a male subject who agrees to not donate sperm from the first dose of study drug until 100 days after the last dose of study drug.
    - Is a female subject of nonchildbearing potential, defined by at least 1 of the following criteria:
        a) Postmenopausal (defined as 12 months of spontaneous amenorrhea in women aged >45 years or 6 months of spontaneous amenorrhea in women aged >45 years with serum follicle-stimulating hormone levels [FSH] >40 mIU/mL). Appropriate documentation of FSH levels is required.
        b) Surgically sterile by hysterectomy and/or bilateral oophorectomy with appropriate documentation of surgical procedure.
        c) Had a tubal ligation with appropriate documentation of surgical procedure.
        d) Has a congenital condition resulting in no uterus.

7.2 Exclusion Criteria

1. The subject has participated in another investigational study within 4 weeks (or based on local regulations) before the screening visit. The 4-week window will be derived from the date of the last study procedure and/or AE related to the study procedure in the previous study to the screening visit of the current study.
2. The subject is an employee of the sponsor or study site or immediate family member (eg, spouse, parent, child, sibling) of the sponsor or study site.
3. The subject has a history of cancer (malignancy).
4. The subject has a history of significant multiple and/or severe allergies (eg, food, drug, latex allergy) or has had an anaphylactic reaction or significant intolerance to prescription or nonprescription drugs or food.
5. The subject has a known hypersensitivity to any component of the formulation of TAK-653 or related compounds.
6. The subject has a positive alcohol or drug screen.
7. The subject has a positive pregnancy test.
8. The subject is a lactating/nursing woman.
9. The subject has a positive test result for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency antibody/antigen, at the Screening Visit. Note: Subjects with positive hepatitis B virus or hepatitis C virus serology may be enrolled if quantitative polymerase chain reaction for hepatitis B virus or hepatitis C virus RNA is negative.
10. The subject had major surgery or donated or lost 1 unit of blood (approximately 500 mL) within 4 weeks before the screening visit.
11. The subject is unable to refrain from or anticipates using medications (see Section 7.3.1) beginning approximately 7 days before administration of the first dose of study drug, throughout the study (including washout intervals between treatment periods), until the follow-up phone call.
12. The subject has a history of alcohol consumption exceeding 2 standard drinks per day on average (1 glass is approximately equivalent to the following: beer [354 mL/12 oz], wine [118 mL/4 oz], or distilled spirits [29.5 mL/1 oz] per day).
13. Participants who regularly smoke more than 5 cigarettes daily or equivalent and unable or unwilling not to smoke during the in-house period.
14. The subject consumes excessive amounts, defined as greater than 6 servings (1 serving is approximately equivalent to 120 mg of caffeine) of coffee, tea, cola, energy drinks, or other caffeinated beverages per day.

15. The subject has a previous or current clinically significant psychiatric disorder according to (DSM-5) Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, including substance use disorder.
16. The subject has a history of intracranial mass lesion, hydrocephalus and/or head injury or trauma.
17. The subject has metal objects in brain or skull.
18. The subject has a cochlear implant or deep brain stimulation device.
19. The subject has a history of epilepsy, seizures, or convulsions.
20. The subject has a family history of epilepsy, seizures, or convulsions.
21. The subject has abnormal sleeping patterns (eg, working night shifts)
22. The subject has an rMT of more than 75% of the maximum stimulator output, measured using TMS-EMG during screening.

7.3 Excluded Medications, Supplements, Dietary Products

7.3.1 Concomitant Medications

Subjects are instructed to stop concomitant medications 7 days before administration of the first dose of study drug, throughout the study until the follow-up phone call. Subjects must be instructed not to take any medications without first consulting with the investigator. Any concomitant medication use must first be discussed with the sponsor, unless the investigator or designee considers immediate administration is necessitated. It is allowed to give medication to treat AEs, for example midazolam for a seizure.

The occasional use of acetaminophen (approximately <1 g/day) is allowed.

7.3.2 Fruit Juice

Subjects will refrain from consuming grapefruit juice, grapefruits, and products containing grapefruit beginning approximately 2 weeks before administration of the first dose of study drug, throughout the study (including the washout interval between treatment periods), and until the follow-up phone call.

Subjects also will refrain from consuming all juices 24 hours before and after administration of each dose of study drug. Consumption of all fruits other than grapefruit is allowed on all days of the study.

7.3.3 Alcohol

Subjects will refrain from consuming alcohol 7 days before the screening visit and from 7 days before dosing and until the last PK blood sample has been collected in each treatment period. At all other times, alcohol consumption is limited to no more than approximately 2 alcoholic beverages

or equivalent (1 alcoholic beverage is approximately equivalent to: beer [354 mL/12 oz], wine [118 mL/4 oz], or distilled spirits [29.5 mL/1 oz]) per day.

7.3.4 Caffeine

Subjects will refrain from consuming caffeinated beverages 24 hours before the Screening Visit and from 24 hours before dosing and until the last PK blood sample has been collected in each treatment period. At all other times, caffeinated beverages or xanthine-containing products will be limited to amounts of no more than 6 servings per day (1 serving=120 mg of caffeine).

7.3.5 Smoking

The use of tobacco will not be permitted from 48 hours before dosing until the end of the residential period and participants will be asked to limit tobacco use to a maximum of 5 cigarettes a day or equivalent amount of tobacco during the out-clinic period until follow-up.

7.4 Diet, Fluid, Activity

7.4.1 Diet and Fluid

Subjects will fast from all food and drink except water for at least 8 hours before screening as well as before study drug dosing in all 4 treatment periods. Subjects will receive a single oral dose of study drug (TAK-653 or placebo tablets in Treatment Periods 1 to 3) ketamine and TMS procedures, and a meal will be delivered after the second TMS assessment on dosing days, along with approximately 240 mL of water. Water will be restricted 1 hour before and 1 hour after study drug administration.

A standard breakfast, lunch, dinner, and snacks will be provided to subjects present during meal times on scheduled study visit days listed in the schedule of study procedures. Subjects will fast from all food and drink except water between meals and snacks. The caloric content and composition of meals will be the same for all subjects in each treatment period. For Period 4, after the 24-hour postdose procedures have been completed, subsequent meals and snacks will be unrestricted in caloric content, composition, and timing.

7.4.2 Activity

Subjects will avoid unaccustomed strenuous physical activity (ie, weight lifting, running, bicycling, etc) from the screening visit until administration of the initial dose of study drug, throughout the study (including washout intervals between treatment periods), and until the follow-up phone call.

7.5 Criteria for Discontinuation or Withdrawal of a Subject

The primary reason for discontinuation or withdrawal of the subject from the study or study drug should be recorded in the eCRF using the following categories.

1. Pretreatment event (PTE) or AE. The subject has experienced a PTE or AE that requires early termination because continued participation imposes an unacceptable risk to the subject's health or the subject is unwilling to continue because of the PTE or AE.
2. Liver Function Test (LFT) Abnormalities

   Study drug should be discontinued immediately with appropriate clinical follow-up (including repeat laboratory tests, until a subject's laboratory profile has returned to normal/baseline status), if the following circumstances occur at any time during study drug treatment:

   - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥8× upper limit of normal (ULN), or

ALT or AST >5 × and persists for more than 2 weeks, or

   - ALT or AST >3 × ULN in conjunction with elevated total bilirubin >2 × upper limit of normal or international normalized ratio >1.5, or
   - ALT or AST >3 × ULN with appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (>5%).

3. Significant protocol deviation. The discovery postrandomization that the subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unacceptable risk to the subject's health.
4. Lost to follow-up. The subject did not return to the clinic and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented in the subject's source documents.
5. Voluntary withdrawal. The subject wishes to withdraw from the study. The reason for withdrawal, if provided, should be recorded in the eCRF.

Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded (ie, withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category.

6. Study termination. The sponsor, IRB, IEC, or regulatory agency terminates the study.
7. Pregnancy. The subject is found to be pregnant.

Note: If the subject is found to be pregnant, the subject must be withdrawn immediately.

7.6 Procedures for Discontinuation or Withdrawal of a Subject

The investigator may discontinue a subject's study participation at any time during the study when the subject meets the study termination criteria described in Section 7.5. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Should a subject's participation be discontinued, the primary criterion for termination must be recorded by the investigator. In addition, efforts should be made to perform all procedures scheduled for the

early termination visit. For all endpoints except safety, only subjects that complete Treatment periods 1 to 3 will be included in the analyses.

7.7 Subject Replacement

If a subject discontinues from the study, a replacement subject may be enrolled, if deemed appropriate by the investigator and sponsor. The study site should contact the sponsor for the replacement of subject's treatment assignment and allocation number.

8.0 CLINICAL STUDY MATERIAL MANAGAEMENT

8.1 Clinical Study Drug

Details regarding the dosage form description and strengths, or composition for the extemporaneous preparation, of the active drug and placebo can be found in the pharmacy manual or in the referenced compounding manual when applicable. Study drug will be packaged to support enrollment and replacement of subjects as required.

8.1.1 Clinical Study Drug Labeling

A clinical label will be affixed to study drug containers in accordance with local regulatory requirements.

8.1.2 Clinical Study Drug Inventory and Storage

Study drug must be stored in a secure, limited-access location under the storage conditions specified on the label and must remain in the original container until dispensed. The temperature excursion information can be found in the pharmacy manual or in the referenced compounding manual when applicable. Receipt and dispensing of study drug must be recorded by authorized personnel at the study site.

8.1.3 Clinical Study Drug Blinding

This is a double-blind study; the investigator and subjects are blinded to sequence group assignment. An unblinded study drug supply will be provided to an unblinded pharmacist or other qualified personnel at the study site who will blind the study supplies. Treatment identity (name and strength or potency) will be included on the study drug container label. Randomization code/disclosure envelopes or lists will be provided per the standard operating procedures of the study site.

8.1.4 Randomization Code Creation and Storage

Randomization personnel of the sponsor or designee will generate the randomization schedule. All randomization information will be stored in a secured area, accessible only by authorized personnel.

8.1.5 Clinical Trial Blind Maintenance/Unblinding Procedure

The study drug blind will be maintained through a randomization schedule held by the unblinded pharmacist at the study site or by the sponsor or designee. The study drug blind shall not be broken by the investigator unless information concerning the study drug is necessary for the medical treatment of the subject. If possible, the medical monitor should be contacted before the blind is broken. Unblinding will be performed per the standard operating procedures of the study site.

8.1.6 Accountability and Destruction of sponsor-Supplied Drugs

The investigator is responsible for keeping accurate records of the study drug received from the sponsor or designee, the amount dispensed to and returned by the subjects, and the amount remaining at the end of the study. For all study sites, the local country sponsor personnel or designee will provide appropriate documentation that must be completed for study drug accountability, return, and destruction.

8.2 Ancillary Supplies

All ancillary supplies will be provided by either the study site or the sponsor or designee, depending upon availability. The list of ancillary supplies and source information can be found in the pharmacy manual or in the referenced compounding manual when applicable. If provided by the sponsor, unused ancillary supplies will be accounted for and disposed of as directed by the sponsor or designee.

9.0 STUDY PROCEDURES

The following sections describe the study procedures to be performed and data to be collected as indicated in the Schedule of Study Procedures (Section 3.0). For each procedure, subjects are to be assessed by the same investigator or site personnel whenever possible. Please note that it may become necessary to perform the following procedures at unscheduled time periods, per the discretion of the investigator.

9.1 Administrative Procedures

9.1.1 Informed Consent Procedure

Informed consent must be obtained before the subject enters into the study and before any protocol-directed procedures are performed. The requirements of informed consent are described in Appendix B.

9.1.1.1 Assignment of Screening and Randomization Numbers

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur before randomization or allocation. Each subject will be assigned only one screening number. Screening numbers must not be reused for different subjects. Any subject who is screened multiple times will be assigned a new screening number for each screening event.

All eligible subjects will be randomly allocated and will receive a randomization number. The randomization number identifies the subject for all procedures occurring after randomization. Once a randomization number is assigned to a subject, it can never be reassigned to another subject. A single subject cannot be assigned more than 1 randomization number.

9.1.1.2 Study Drug Assignment

On Day 1 of Treatment Period 1, subjects will be assigned a randomization number in ascending numerical order at the clinical site, and in Period 4 all subjects will receive ketamine. Therefore, the randomization number encodes the sequence in which each subject will receive the low and high doses of TAK-653 and placebo according to the randomization schedule generated before the study. Each subject will be dispensed blinded study drug, labeled with his/her unique randomization number, throughout the study.

9.1.2 Inclusion and Exclusion

Each subject will be assessed through randomization, according to the eligibility criteria provided in Section 7.0.

9.1.3 Medical History/Demography

Qualified site personnel will collect subject significant medical history (past and concurrent medical conditions), per the clinical site's standard of care and appropriate clinical judgment, and subject demographics.

9.1.4 Concomitant Medications

Qualified site personnel will review subject prior and concomitant medication use. Medications are defined as prescription and over-the-counter drugs, vaccines, supplements, nutraceuticals, and oral herbal preparations.

9.2 Clinical Procedures and Assessments

9.2.1 Full Physical Exam

Qualified site personnel will conduct full physical examinations. A baseline physical examination (defined as the assessment before first dose of study drug) will consist of the following body systems: eyes; ears, nose, throat; cardiovascular system; respiratory system; gastrointestinal system; dermatologic system; extremities; musculoskeletal system; nervous system; lymph nodes; targeted neurological and mental health; and other.

Any abnormal finding on a pretreatment physical examination assessment must be assessed as not clinically significant (NCS) or clinically significant (CS) by the investigator and recorded in the source document and eCRF.

On subsequent examinations, any abnormal change from the pretreatment physical examination assessment occurring immediately before the start of the study drug must be assessed as NCS or CS by the investigator and recorded in the source document and eCRF. Any CS change or new diagnosis as a result of a CS change, as determined by the investigator, will be recorded as an AE in source documentation and eCRF.

9.2.2 Height and Weight

Body weight and height will be obtained with the subject's shoes off, and in underwear.

9.2.3 BMI

BMI equals a subject's weight in kilograms divided by height in meters squared (BMI=kg/m^2).

9.2.4 Vital Signs

Body temperature will be measured with either an oral (temperature taken at floor of the mouth) or tympanic thermometer. The same method (ie, oral or tympanic) must be used for all measurements for each individual subject and should be the same for all subjects.

Subjects should rest in a semirecumbent position for at least 5 minutes before vital signs are measured. Vital signs will include pulse rate, respiratory rate, and systolic and diastolic blood

pressure. The same method (eg, same size cuff, manual or automated) must be used for all measurements for each individual subject and should be the same for all subjects.

Subjects should continue to rest in a semirecumbent position from the time of dosing until 4 hours postdose except to stand for other study-related procedure.

9.2.5 12-Lead ECG

Special care must be taken for proper lead placement by qualified personnel. Skin should be clean and dry before lead placement. Subjects may need to be shaved to ensure proper lead placement. Female subjects may need to remove their bra.

Subjects should be resting in a semirecumbent position for at least 5 minutes before each ECG measurement.

QT intervals with Fridericia correction method (QTcF intervals) will be calculated in this study.

For each treatment period, a predose ECG will be obtained within approximately 3 hours before study drug dosing. This measurement will be used as the baseline assessment. The principal investigator should arrange to have a study cardiologist available as needed to review ECG abnormalities.

During each treatment period, if a subject demonstrates an increase in QTcF interval ≥40 milliseconds compared with a predose baseline measurement, the ECG will be repeated within 5 minutes. The average value of the QTcF interval from the 2 ECGs will represent the value at that time point. If the average QTcF interval increase from baseline for any postdose time point is ≥40 milliseconds, the subject will continue to be monitored by repeat 12-lead ECGs every 60 minutes for at least 4 hours or until the QTcF interval is within 40 milliseconds of the baseline value. If prolongation of the QTcF interval ≥40 milliseconds persists, a consultation with a study cardiologist may be appropriate and the sponsor should be notified.

If the QTcF interval is ≥500 milliseconds, the sponsor should be notified and the ECGs should be reviewed by a cardiologist. The subject should be monitored by telemetry (until the QTcF interval is <500 milliseconds) or should be considered for transfer to a location where closer monitoring is available.

If the subject has unstable hemodynamics, or has any clinically significant dysrhythmias, the subject should be immediately transferred to an acute care setting for definitive therapy.

If repeat ECGs are required, the clinical site will decide whether to leave the electrodes in place or mark the position of the electrodes for subsequent ECGs. To mark the position of the electrodes, 12-lead electrode sites will be marked on the skin of each subject with an ECG skin marker pen to ensure reproducible electrode placement.

The following ECG parameters will be recorded: heart rate, PR interval, QRS interval, QT interval, QTcF interval, and the interpretation of the ECG profile by the principal investigator.

9.2.6 Study Drug Administration

On Day 1 of Treatment Periods 1, 2, and 3, study drug (TAK-653 or placebo) will be administered orally at the times and days detailed in Section 3.0.

9.2.7 Ketamine Administration

On Day 1 of treatment period 4, Ketamine 0.5 mg/kg IV will be administered for 40 minutes.

9.2.8 AE Monitoring

AE monitoring begins after signing of the informed consent form. Changes in subject health status from the baseline assessment until study drug administration should be captured in the subject's medical history. A complete description of adverse event collections and procedures is provided in Section 10.0.

Seizure precautions will be taken at all clinic visits, consisting of suction set up and an ambu bag nearby. If there is evidence of seizure activity, subjects will be administered standard of care anticonvulsants as needed and remain in the clinic overnight for closer observation.

9.3 Laboratory Procedures and Assessments

Laboratory samples will be collected in accordance with acceptable laboratory procedures. Samples will be collected following a minimum 8-hour overnight fast at the time points stipulated in the Schedule of Study Procedures (Section 3.0).

9.3.1 Clinical Laboratory Tests

9.3.1.1 Hematology

Hematology will consist of the following tests:

Erythrocytes (red blood cells)	Hemoglobin
Hematocrit	Platelets
Leukocytes (white blood cells) with absolute differential	

9.3.1.2 Chemistry

Chemistry evaluations will consist of the following standard chemistry panel:

Albumin	Alkaline phosphatase
ALT	AST
Bicarbonate	Calcium
Blood urea nitrogen	Chloride
Creatinine	Glucose
γ-glutamyl transferase	Sodium
Potassium	Bilirubin (total), if above the ULN total bilirubin will be fractionated
Protein (total)	

If subjects experience ALT or AST >3× ULN, follow-up laboratory tests (at a minimum, serum alkaline phosphatase, ALT, aspartate aminotransferase, total bilirubin, γ-glutamyl transferase, and international normalized ratio) should be performed 48 to 72 hours after the abnormality was noted.

If alanine aminotransferase or aspartate aminotransferase remains elevated >3× the upper limit of normal on these 2 consecutive occasions, the investigator must contact the medical monitor for consideration of additional testing, close monitoring, possible discontinuation of study drug, and discussion of the relevant subject details and possible alternative etiologies. The abnormality should be recorded as an adverse event.

Please refer to Section 7.5 for subject discontinuation criteria regarding abnormal liver test results and Section 10.2.8.5 for guidance on reporting abnormal liver test results.

9.3.1.3 Urinalysis

Urinalysis will consist of the following tests:

Leucocytes	Nitrite
Protein	pH
Blood	Specific Gravity
Ketones	Bilirubin
Glucose	

Urine microscopy will be performed if urinalysis is abnormal. Microscopy consists of red blood cells (RBC)/high-power field, white blood cells (WBC)/high-power field, and casts.

9.3.2 Diagnostic Screening

9.3.2.1 Serum

The serum diagnostic screening assessment will include the following tests:

HIV (human immunodeficiency virus)	Hepatitis screen (hepatitis B surface antigen, hepatitis C virus antibody)

9.3.2.2 Alcohol Screen

Subjects will undergo an alcohol breath test. A urine alcohol test may be performed at the discretion of the investigator.

9.3.2.3 Urine

The urine drug screening assessment will include the following tests:

Amphetamines	3,4-methylenedioxy-methamphetamine
Barbiturates	Methadone/metabolite
Benzodiazepines	Opiates
Buprenorphine/metabolite	Oxycodone/oxymorphone
Cannabinoids	Phencyclidine
Cocaine/metabolites	

9.4 PK and PD Evaluations

9.4.1 PK Samples

Samples for plasma concentration analysis will be collected at the time points stipulated in the schedule of procedures (Section 3.0). Please refer to the laboratory manual for information on the collection, processing, and shipment of samples to the central laboratory.

It is anticipated that the total blood volume drawn in this study will be approximately 126.5 mL for each subject.

Primary specimen collection parameters are provided in Table 9.a

Table 9.a Primary Specimen Collections

Specimen Name	Primary Specimen	Primary Specimen Derivative	Description of Intended Use	Sample Collection
Plasma sample for PK	Plasma	Blood	Plasma sample for PK analysis	Mandatory

PK, pharmacokinetic

9.4.2 PK Measurements

The plasma concentrations of TAK-653 and ketamine in the PK sample will be determined for all evaluable subjects. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times.

Backup samples will be stored for analyses of ketamine metabolites (including but not limited to norketamine, and 2S, 6S; 2R, 6R hydroxynorketamine) when deemed necessary by the sponsor. Additional pharmacokinetic parameters of ketamine or metabolites may be calculated as deemed necessary.

9.4.2.1 Plasma for PK Measurements

Blood samples for plasma concentration analysis of TAK-653 will be collected into chilled blood collection tubes (vacutainer) containing the anticoagulant potassium ethylenediamine tetraacetic acid (K_2EDTA) at room temperature. After the blood draw, the tube will immediately be placed in a bucket with ice. The collected blood samples may be archived for additional analysis of potential metabolites.

Blood samples for plasma concentration analysis of ketamine will be collected into a tube containing the anticoagulant sodium heparin at room temperature and immediately after the sample is drawn placed in a tube in a bucket with ice.

Please refer to Section 3.0 SCHEDULE OF STUDY PROCEDURES for PK blood sampling visits, draw times and sampling windows. The actual time of sample collection will be recorded on the source document and electronic case report form. Sampling time points may be adjusted based

on the preliminary emerging concentration data collected from prior subjects, but the total number of samples collected per subject should not exceed the planned number.

9.4.2.2 PK Sample Analysis

Plasma concentrations of TAK-653 and ketamine will be measured by a validated high-performance liquid chromatography (HPLC) assay with tandem mass spectrometry assay.

9.4.3 PD Measurements

The pharmacodynamic assessments described in Section 6.2.3.3 will be performed at the time points stipulated in the schedule of procedures (Section 3.0). Additional details regarding these pharmacodynamic assessments are provided in the study manual.

9.4.3.1 Threshold and Amplitude of MEP driven by single-pulse TMS

Enhancing MEP driven by TMS was selected as the primary pharmacodynamic endpoint based on preclinical data demonstrating that TAK-653 enhances TMS-MEPs in rats. Furthermore, ketamine has been shown to increase TMS-driven excitability assessed with threshold to MEPs in healthy volunteers [5]. MEP amplitude and threshold capture modulation of excitatory synapses in motor cortex and spinal cord [4].

9.4.3.2 TMS stimulation paradigms

Long interval intracortical inhibition (LICI) and short interval intracortical stimulation (SICI) are TMS stimulation paradigms that capture modulation of cortical excitation-inhibition balance with pairs of TMS pulses. Because this is a local cortical circuitry driven phenomenon, it has the potential to expand the sensitivity of TMS as a biomarker of CNS activation beyond single pulse TMS for certain pharmacologies, including glutamate mediated neurotransmission.

9.4.3.3 CCI [redacted]

CCI [redacted]

9.4.3.4 CCI [redacted]

CCI [redacted]

CCI

[redacted]

CCI

[redacted]

9.5 Confinement

Subjects will report to the clinical site on Day 1 of all treatment periods, and will leave after completion of all study-related procedures on each day on Treatment Periods 1-3. At the discretion of the investigator, subjects may be requested to remain in the clinical site longer.

On Day 1 of Treatment Period 4, during the ketamine-testing period, subjects will remain overnight following the infusion, until the 24 hr follow-up TMS assessment has been completed. Before discharge, a Neurological and mental state examination will be performed.

9.6 Childbearing Status and Methods of Contraception

9.6.1 Women of Childbearing Potential

Women of child bearing potential are excluded from this study, and methods of contraception are not relevant.

9.6.1.1 Definition of Women of Childbearing Potential

A woman is considered of childbearing potential (ie, fertile) following menarche and until becoming postmenopausal, unless permanently sterile.

9.6.2 Women of Nonchildbearing Potential

9.6.2.1 Definition of Women of Nonchildbearing Potential

A female subject of nonchildbearing potential is defined as satisfying at least 1 of the following criteria:

1. Postmenopausal: At least 12 months of spontaneous amenorrhea and a follicle stimulating hormone (FSH concentration) >40 mIU/mL.
2. Surgically sterile by hysterectomy and/or bilateral oophorectomy with appropriate documentation of surgical procedure.
3. Has no uterus as a result of a congenital condition.

9.6.2.2 Contraception for Women of Nonchildbearing Potential

No contraception is required for women of nonchildbearing potential.

10.0 ADVERSE EVENTS

10.1 Definitions and Elements of AEs

A pre-treatment adverse event (PTE) is defined as any untoward medical occurrence in a clinical investigation subject who has signed informed consent to participate in a study but before administration of any study drug; it does not necessarily have to have a causal relationship with study participation.

An AE is defined as any untoward medical occurrence in a clinical investigation subject who has signed informed consent to participate in a study; it does not necessarily have to have a causal relationship with the treatment.

Treatment-emergent adverse event (TEAE): Any AE that starts or increases in severity during or after the first dose of study drug.

An AE can therefore be any unfavorable and unintended sign (eg, a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, whether or not it is considered related to the drug.

An untoward finding generally may:

- Indicate a new diagnosis or unexpected worsening of a preexisting condition. (Intermittent events for pre-existing conditions or underlying disease should not be considered AEs.)
- Necessitate therapeutic intervention.
- Require an invasive diagnostic procedure.
- Require discontinuation or a change in dose of study medication or a concomitant medication.
- Be considered unfavorable by the investigator for any reason.

Diagnoses versus signs and symptoms:

- Each event should be recorded to represent a single diagnosis according to the Medical Dictionary for Regulatory Activities (MedDRA) database. Accompanying signs (including abnormal laboratory values or ECG findings) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, sign(s) or symptom(s) should be recorded appropriately as an AE(s).

Laboratory values and ECG findings:

- Changes in laboratory values or ECG parameters maybe considered AEs if they are judged to be clinically significant (ie, if some action or intervention is required or if the investigator judges the change to be beyond the range of normal physiologic fluctuation). A laboratory re-test and/or continued monitoring of an abnormal value are not considered an intervention. In addition, repeated or additional noninvasive testing for verification, evaluation or monitoring of an abnormality is not considered an intervention.

- If abnormal laboratory values or ECG findings are the result of pathology for which there is an overall diagnosis (eg, increased creatinine in renal failure), the diagnosis only should be reported appropriately as an AE.

Pre-existing conditions:

- A pre-existing condition (present at the time of signing of informed consent) is considered a concurrent medical history condition and should NOT be recorded as an AE. A baseline evaluation (eg, laboratory test, ECG, X-ray, etc) should NOT be recorded as an AE unless related to a study procedure. However, if the subject experiences a worsening or complication of such a concurrent medical history condition, the worsening or complication should be recorded appropriately as an AE (worsening or complication occurs after informed consent is signed). Investigators should ensure that the event term recorded captures the change in the condition (eg, "worsening of…").
- If a subject has a pre-existing episodic condition (eg, asthma, epilepsy), any occurrence of an episode should only be captured as an AE if the episodes become more frequent, serious, or severe in nature, that is, investigators should ensure that the AE term recorded captures the change from Baseline in the condition (eg "worsening of…").
- If a subject has a degenerative concurrent condition (eg, cataracts, rheumatoid arthritis), worsening of the condition should only be captured as an AE if occurring to a greater extent to that which would be expected. Again, investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Worsening of AEs:

- If the subject experiences a worsening or complication of an AE after the first administration of study medication or after any change in study medication, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Changes in severity of AEs:

- If the subject experiences a change in the severity of an AE that is not associated with a change in study medication, the event should be captured once with the maximum severity recorded.

Preplanned surgeries or procedures:

- Preplanned procedures (surgeries or therapies) that were scheduled before signing of informed consent are not considered AEs. However, if a preplanned procedure is performed early (eg, as an emergency) due to a worsening of the pre-existing condition, the worsening of the condition should be captured appropriately as an AE. Complications resulting from any planned surgery should be reported as AEs.

Elective surgeries or procedures:

- Elective procedures performed where there is no change in the subject's medical condition should not be recorded as AEs but should be documented in the subject's source documents. Complications resulting from an elective surgery should be reported as AEs.

Overdose:

- An overdose is defined as a known deliberate or accidental administration of investigational drug, to or by a study subject, at a dose above that which is assigned to that individual subject according to the study protocol. It is up to the investigator or the reporting physician to decide whether a dose is to be considered an overdose, in consultation with the sponsor.
- All cases of overdose (with or without associated AEs) will be documented on an Overdose page of the (e)CRF, in order to capture this important safety information consistently in the database. AEs associated with an overdose will be documented on AE CRF(s) according to Section 9.5.
- Serious adverse events (SAEs) of overdose should be reported according to the procedure outlined in Section 10.2.8.
- In the event of drug overdose, the subject should be treated symptomatically.

10.1.1 SAEs

An SAE is defined as any untoward medical occurrence that at any dose:

1. Results in DEATH.
2. Is LIFE THREATENING.
    - The term "life threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
3. Requires inpatient HOSPITALIZATION or prolongation of existing hospitalization.
4. Results in persistent or significant DISABILITY/INCAPACITY.
5. Is a CONGENITAL ANOMALY/BIRTH DEFECT.
6. Is an IMPORTANT MEDICAL EVENT that satisfies any of the following:
    - May require intervention to prevent items 1 through 5 above.
    - May expose the subject to danger, even though the event is not immediately life threatening or fatal or does not result in hospitalization.
    - Includes any event or synonym described in the Takeda Medically Significant AE List (Table 10.a).

Table 10.a Takeda Medically Significant AE List

Term	
Acute respiratory failure/acute respiratory distress syndrome	Hepatic necrosis
	Acute liver failure
Torsade de pointes ventricular fibrillation / ventricular tachycardia	Anaphylactic shock
	Acute renal failure
Malignant hypertension	Pulmonary hypertension
Convulsive seizures	Pulmonary fibrosis
Agranulocytosis	Confirmed or suspected endotoxin shock
Aplastic anemia	Confirmed or suspected transmission of infectious agent by a medicinal product
Toxic epidermal necrolysis/ Stevens-Johnson syndrome	Neuroleptic malignant syndrome / malignant hyperthermia
	Spontaneous abortion / stillbirth and fetal death

AEs that fulfill 1 or more of the serious criteria above are to be considered SAEs and should be reported and followed up in the same manner (see Sections 10.0 and 10.1.1).

10.1.2 Adverse Events of Special Interest (AESI)

An adverse event of special interest (AESI) (serious or non-serious) is one of scientific and medical concern specific to the compound or program, for which ongoing monitoring and rapid communication by the investigator to Takeda may be appropriate. Such events may require further investigation in order to characterize and understand them and are described in Section 4.4 and instructions provided for investigators as to how and when they should be reported to Takeda.

Based on nonclinical data to date and AMPA receptor potentiator class effects, the AESIs for this study are:

Seizures

Convulsions

10.2 AE Procedures

10.2.1 Assigning Severity/Intensity of AEs

The different categories of severity/intensity are:

Mild: An adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: An adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: An adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

10.2.2 Assigning Causality of AEs

The relationship of each AE to study medication(s) will be assessed using the following categories:

Related: An AE that follows a reasonable temporal sequence from administration of a drug (including the course after withdrawal of the drug), or for which a causal relationship is at least a reasonable possibility, ie, the relationship cannot be ruled out, although factors other than the drug, such as underlying diseases, complications, concomitant drugs and concurrent treatments, may also be responsible.

Not Related: An AE that does not follow a reasonable temporal sequence from administration of a drug and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant medications and concurrent treatments.

10.2.3 Start Date

The start date of the AE is the date that the first signs/symptoms were noted by the subject and/or investigator.

10.2.4 End Date

The end date of the AE is the date at which the subject recovered, the event resolved but with sequelae or the subject died.

10.2.5 Pattern of Adverse Event (Frequency)

Episodic AEs (eg, headache) or those which occur repeatedly over a period of consecutive days are intermittent. All other events are continuous or once.

10.2.6 Action Taken With Study Treatment

- Drug withdrawn – a study medication is stopped due to the particular AE.
- Dose not changed – the particular AE did not require stopping a study medication.
- Unknown – only to be used if it has not been possible to determine what action has been taken.
- Not applicable – a study medication was stopped for a reason other than the particular AE eg, the study has been terminated, the subject died, dosing with study medication had not yet started or dosing with study medication was already stopped before the onset of the AE.
- Dose reduced – the dose was reduced due to the particular AE.
- Dose increased – the dose was increased due to the particular AE.
- Drug interrupted – the dose was interrupted due to the particular AE.

10.2.7 Outcome

- Recovered/resolved – subject returned to first assessment status with respect to the AE.
- Recovering/resolving – the intensity is lowered by one or more stages: the diagnosis has or signs/symptoms have almost disappeared; the abnormal laboratory value improved, but has not returned to the normal range or to the baseline value; the subject died from a cause other than the particular AE with the condition remaining "recovering/resolving."
- Not recovered/not resolved – there is no change in the diagnosis, signs or symptoms; the intensity of the diagnosis, signs/symptoms or laboratory value on the last day of the observed study period has become worse than when it started; is an irreversible congenital anomaly; the subject died from another cause with the particular AE state remaining "Not recovered/not resolved."
- Recovered/ Resolved with sequelae – the subject recovered from an acute AE but was left with permanent/significant impairment (eg, recovered from a cardiovascular accident but with some persisting paresis).
- Fatal – an AE that is considered as the cause of death.
- Unknown – the course of the AE cannot be followed up due to hospital change or residence change at the end of the subject's participation in the study.

10.2.8 Collection and Reporting of AEs, SAEs, Special Interest AEs, and Abnormal Liver Function Tests (LFT)s

10.2.8.1 Collection Period

Collection of AEs (ie, AEs, SAEs, Special Interest AEs, and abnormal liver function tests (LFT)s will commence at the time the subject signs the informed consent. Routine collection of AEs will continue until the follow-up phone call on Day 14 (±2 days). For subjects who discontinue before the administration of study medication, AEs will be followed until the subject discontinues study participation.

10.2.8.2 Reporting AEs

At each study visit, the investigator will assess whether any subjective AEs have occurred. A neutral question, such as "How have you been feeling since your last visit?" may be asked. Subjects may report AEs occurring at any other time during the study. Subjects experiencing an SAE before the first exposure to investigational product must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to Baseline or there is a satisfactory explanation for the change. Nonserious AEs that begin before the first exposure to investigational product, related or unrelated to the study procedure, need not be followed-up for the purposes of the protocol.

All subjects experiencing AEs, whether considered associated with the use of the study medication or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to Baseline or until there is a satisfactory explanation for the changes observed. All AEs will be documented in the AE page of the eCRF, whether or not the investigator concludes that the event is related to the drug treatment. The following information will be documented for each event:

- Event term.
- Start and end date and time.
- Pattern of AE (frequency).
- Severity/Intensity.
- Causality (Investigator's opinion of the causal relationship between the event and administration of study drug[s]).
- Action taken with trial drug.
- Outcome of event.
- Seriousness.

10.2.8.3 Reporting SAEs

When an SAE occurs through the AE collection period it should be reported according to the procedure outlined below:

A Takeda SAE form must be completed, in English and signed by the investigator immediately or within 24 hours of first onset or notification of the event. The information should be completed as fully as possible but contain, at a minimum:

- A short description of the event and the reason why the event is categorized as serious.
- Subject identification number.
- Investigator's name.
- Name of the study medication(s).
- Causality assessment.

The SAE form should be transmitted within 24 hours to the attention of the contact listed in Section 14.1.1.

Any SAE spontaneously reported to the investigator following the AE collection period should be reported to the sponsor if considered related to study participation.

Reporting of SAEs that begin before first administration of investigational product will follow the same procedure for SAEs occurring on treatment.

SAE Follow-Up

If information is not available at the time of the first report becomes available at a later date, the investigator should complete a follow-up SAE form or provide other written documentation and fax it immediately within 24 hours of receipt. Copies of any relevant data from the hospital notes (eg, ECGs, laboratory tests, discharge summary, postmortem results) should be sent to the addressee, if requested.

All SAEs should be followed up until resolution or permanent outcome of the event. The timelines and procedure for follow-up reports are the same as those for the initial report.

10.2.8.4 Reporting Adverse Events of Special Interest (AESI)

If an Adverse Event of Special Interest (AESI), seizures, or convulsion as in section 10.1.2, occurs during the treatment period or the follow-up period, and is considered to be clinically significant based on the investigators clinical judgment, the AESI should be recorded in an AESI eCRF or an SAE Form and identified by writing AESI on the form or circle AESI if possible. The Form should be completed and reported to the SAE reporting contact in Section 14.1.1, within 24 hours.

10.2.8.5 Reporting of Abnormal LFTs

If a subject is noted to have ALT or AST elevated >3 ×ULN on 2 consecutive occasions, the abnormality should be recorded as an AE. In addition, an LFT Increases eCRF must be completed providing additional information on relevant recent history, risk factors, clinical signs and symptoms and results of any additional diagnostic tests performed.

If a subject is noted to have ALT or AST >3 ×ULN and total bilirubin >2 ×ULN for which an alternative etiology has not been identified, the event should be recorded as an SAE and reported as per Section 10.2.8.3. The investigator must contact the Medical Monitor for discussion of the relevant subject details and possible alternative etiologies, such as acute viral hepatitis A or B or other acute liver disease. Follow-up laboratory tests as described in Section 9.3 must also be performed. In addition, an LFT Increases eCRF must be completed and transmitted with the Takeda SAE form (as per Section 10.2.9).

10.2.9 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities

The sponsor will be responsible for reporting all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, investigators and institutional review boards (IRB)s or institutional ethics committees (IEC)s, as applicable, in accordance with national regulations in the countries where the study is conducted. Relative to the first awareness of the event by/or further provision to the sponsor or sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other serious events, unless otherwise required by national regulations. The sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the trial. The investigational site also will forward a copy of all expedited reports to his or her IRB or IEC in accordance with national regulations.

11.0 STATISTICAL METHODS

11.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized before database lock. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives.

11.1.1 Analysis Sets

11.1.1.1 Safety Set

The Safety Analysis Set will consist of all subjects who are randomized and received 1 dose of study drug. Subjects in this analysis set will be used for demographics, baseline characteristics, and safety summaries.

11.1.1.2 PK Set

The PK Set will consist of all subjects who receive study drug and have at least 1 measurable plasma concentration.

If any subjects are found to be noncompliant in dosing schedule or with incomplete data, a decision will be made on a case-by-case basis as to their inclusion in the analysis, but the data will be presented in the subject listings.

11.1.1.3 PD Set

The PD Set will consist of all subjects who receive study drug and have at least 1 postdose PD measurement.

If any subjects are found to be noncompliant in dosing schedule or with incomplete data, a decision will be made on a case-by-case basis as to their inclusion in the analysis but will be presented in the subject listings.

11.1.2 Analysis of Demography and Other Baseline Characteristics

Demographic and Baseline characteristics will be summarized for all subjects in the Safety Set, by sequence groups as well as overall. For continuous variables (eg, age, height, weight, and BMI), the summary will consist of descriptive statistics (number of subjects, mean, standard deviation (SD), minimum, median, and maximum). For categorical variables (eg, ethnicity and race), the summary will consist of number and percentage of subjects in each category.

11.1.3 PK Analysis

Plasma concentrations of TAK-653 and ketamine at each time point will be summarized for each regimen using descriptive statistics.

Individual plasma concentration data versus time will be presented in a data listing.

11.1.4 PD Analysis

11.1.4.1 Primary Endpoint Analysis

The primary endpoints are changes in rMT and changes in peak-to-peak amplitude of the MEP obtained with single-pulse TMS at 2 1/2 hours after administration of TAK-653 from their corresponding pre-dosing baseline levels, compared to placebo. They will be analyzed using linear mixed effect models.

The primary endpoints will be analyzed using a linear mixed effects model appropriate for a three-period crossover design. Additional details of the model will be specified in the SAP. Hochberg's step-up procedure will be used to adjust for multiple endpoints. One-sided t-tests comparing each of the primary endpoints for placebo vs. treatment at each dose level will be conducted as secondary analyses, adjusted for multiple testing to ensure type 1 error control at the 10% level.

11.1.4.2 Secondary Endpoint Analysis

The magnitude of LICI and SICI obtained with paired-pulse TMS will be analyzed using linear mixed effect models analogous to the model used to analyze the primary endpoint. One-sided t-tests (alpha = 0.05) will be used for the comparison of placebo versus one of the TAK-653 treatment groups.

The change of rMT at 24 hours post ketamine dosing and peak-to-peak amplitude of the MEP obtained with single-pulse TMS will be analyzed using linear mixed effect models appropriate for the design. Additional details will be provided in the SAP.

11.1.4.3 Exploratory Endpoint Analysis

CCI

CCI

[redacted]

11.1.5 Safety Analysis

The safety of TAK-653 will be assessed through AEs, clinical laboratory results, physical examinations, ECG findings, vital signs, suicidal assessments, and monitoring of potential tremors, convulsions and seizures.

11.1.5.1 AEs and AESIs

Summary tables will include the number and percent of subjects in each treatment experiencing at least 1 TEAE by system organ class and preferred term. If a subject has more than 1 AE that codes to the same preferred term, the subject will be counted only once for that preferred term. Similarly, if a subject has more than 1 AE within a system organ class category, the subject will be counted only once for that system organ class.

TEAEs possibly or probably related to study medication will be tabulated in the same manner. TEAEs will also be summarized by maximum intensity. All AEs will be listed.

AEs will be summarized for each regimen using the safety analysis set. All AEs will be coded using MedDRA. Data will be summarized using preferred term and primary system organ class.

11.1.5.2 Clinical Laboratory Evaluation, Vital Signs and ECG

Clinical laboratory tests (hematology, chemistry, and urinalysis), vital signs, and 12-lead ECG parameters will be listed. The results that meet Takeda's criteria for markedly abnormal criteria will be flagged in the listing.

Summary statistics on observed values and changes from baseline will be provided by regimen and scheduled time point if appropriate.

The number and percentage of subjects who meet Takeda's criteria for markedly abnormal criteria at least once postdose will be summarized by dose.

11.1.5.3 Other Safety Parameters

Summary statistics of C-SSRS scores at each visit and changes from baseline during treatment and follow-up visits will be provided by dose and visit. Individual C-SSRS results will be listed.

The occurrence of Tremors, convulsions, or seizures will be presented in the data listings.

Physical examination findings will be presented in the data listings.

11.2 Interim Analysis and Criteria for Early Termination

After at least 18 subjects have completed the first three periods of the study, an unblinded data analysis may be provided on the primary endpoints. An unblinded team not involved in the study conduct or the program will perform the unblinded analysis. A firewall will be set up between the unblinded team and the study team. Details of the procedure will be described in a separate document. No decision on the trial or modification of sample size will be made based on the analysis. The investigator and study site will not have access to the analysis results until the final unblinding of the study. This early data review will be conducted only for the purpose of sponsor programmatic forward planning that is unrelated to the conduct of the present study.

11.3 Determination of Sample Size

The assumptions used in the sample size determination were based on consideration of a published study on ketamine by di Lazzaro et al. (2003) [5] and the preliminary results of TMS data in a CHDR study of healthy volunteers. Specifically, we assumed an intra-subject correlation of 0.9, a common standard deviation of 9% for rMT and 947 μV for the MEP peak-to-peak amplitude, a correlation coefficient of at least 0.5 between pre- and post-dosing outcome, and a familywise type I error rate of 10%. A sample size of 22 subjects, approximately 4 subjects for each of the 6 sequences of the first 3 treatment periods, will provide at least 80% power in detecting a reduction of 5.3 percentage points in rMT and/or an increase of 560 μV in the MEP peak-to-peak amplitude at the most effective dose level of TAK-653 versus placebo. Subjects who complete at least the first 3 treatment periods of the study are considered completers. Drop-out rate is expected to be low. Twenty-four subjects will be recruited (4 per sequence) to ensure at least 22 completers. If more than 2 subjects drop out before completing the first 3 treatment periods, up to 2 additional subjects may be enrolled.

12.0 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Study-Site Monitoring Visits

Monitoring visits to the study site will be made periodically during the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data recorded on the eCRFs. Source documents are defined as original documents, data, and records. The investigator and study site guarantee access to source documents by the sponsor or its designee contract research organisation (CRO) and by the IRB or IEC.

All aspects of the study and its documentation will be subject to review by the sponsor or the sponsor's designee (as long as blinding is not jeopardized), including but not limited to the Investigator's Binder, trial drug, subject medical records, informed consent documentation, and review of eCRFs and associated source documents. It is important that the investigator and other study personnel are available during the monitoring visits and that sufficient time is devoted to the process.

12.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to trial subjects. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the investigator should consult with the sponsor or designee (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospectively approved deviation) from the inclusion or exclusion criteria.

Significant deviations include, but are not limited to, those that involve fraud or misconduct, increase the health risk to the subject, or confound interpretation of primary study assessment.

12.3 Quality Assurance Audits and Regulatory Agency Inspections

The study site also may be subject to quality assurance audits by the sponsor or designees. In this circumstance, the sponsor-designated auditor will contact the site in advance to arrange an auditing visit. The auditor may ask to visit the facilities where laboratory samples are collected, where the medication is stored and prepared, and any other facility used during the study. In addition, there is the possibility that this study may be inspected by regulatory agencies, including those of foreign governments (eg, the Food and Drug Administration (FDA), the United Kingdom Medicines and Healthcare Products Regulatory Agency, the Pharmaceuticals and Medical Devices Agency of Japan). If the study site is contacted for an inspection by a regulatory body, the sponsor should be notified immediately. The investigator guarantees access for quality assurance auditors to all study documents as described in Section 12.1.

13.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (ie, subjects) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for good clinical practice (GCP). Each investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator" that are listed in Appendix A. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

13.1 IRB and/or IEC Approval

- This study will be conducted in accordance with the protocol and with the consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS), International Ethical Guidelines, International Conference on Harmonisation (ICH) GCP guidelines, and laws and regulations applicable to clinical studies (including US 21 Code of Federal Regulations [CFR] and European regulation 536/2014).
- The protocol, informed consent forms (ICF)s, Investigator's Brochure, and other relevant supporting documents (eg, advertisements, information on payments and compensation available to subjects) must be submitted to an IRB/IEC by the investigator. Written approval of the protocol from the IRB/IEC must be obtained before the study is initiated, ie, the first subject signs the study ICF.
- The study cannot be initiated until notification is received from the sponsor.
- Any amendment to the protocol, except when necessary to eliminate an immediate hazard to study participants, requires IRB/IEC written approval before implementation.
- If required by either country or regional regulations or procedures, approval from the Competent Regulatory Authority will be obtained before commencement of the study or implementation of an amendment.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.

13.2 Subject Information, Informed Consent, and Subject Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all

applicable laws and regulations. The informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for purposes of conducting the study. The informed consent form and the subject information sheet (if applicable) further explain the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is given. The informed consent form will detail the requirements of the participant and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

The investigator is responsible for the preparation, content, and IRB or IEC approval of the informed consent form and, if applicable, the subject authorization form. The informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) must be approved by both the IRB or IEC and the sponsor before use.

The informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) must be written in a language fully comprehensible to the prospective subject. It is the responsibility of the investigator to explain the detailed elements of the informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) to the subject. Information should be given in both oral and written form whenever possible and in the manner deemed appropriate by the IRB or IEC.

The subject, must be given ample opportunity to: (1) inquire about details of the study, and (2) decide whether or not to participate in the study. If the subject determines he or she will participate in the study, then the informed consent form and subject authorization form (if applicable) must be signed and dated by the subject at the time of consent and prior to the subject entering into the study. The subject should be instructed to sign using their legal names, not nicknames, using blue or black ballpoint ink. The investigator must also sign and date the informed consent form and subject authorization (if applicable) at the time of consent and before subject entering into the study; however, the sponsor may allow a designee of the investigator to sign to the extent permitted by applicable law.

Once signed, the original informed consent form, subject authorization form (if applicable), and subject information sheet (if applicable) will be stored in the investigator's site file. The investigator must document the date the subject signs the informed consent in the subject's medical record. Copies of the signed informed consent form, the signed subject authorization form (if applicable), and subject information sheet (if applicable) shall be given to the subject.

All revised informed consent forms must be reviewed and signed by relevant subjects in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the subject's medical record, and the subject should receive a copy of the revised informed consent form.

13.3 Subject Confidentiality

The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to

the sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires the investigator to permit its monitor or designee's monitor, representatives from any regulatory authority (eg, FDA, Medicines and Healthcare products Regulatory Agency, Pharmaceuticals and Medical Devices Agency), the sponsor's designated auditors, and the appropriate IRBs and IECs to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process (see Section 13.2).

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (ie, subject name, address, and other identifier fields not collected on the subject's eCRF).

13.4 Publication, Disclosure, and Clinical Trial Registration Policy

13.4.1 Publication and Disclosure

The investigator is obliged to provide the sponsor with complete test results and all data derived by the investigator from the study. During and after the study, only the sponsor may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the clinical study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the sponsor.

The sponsor may publish any data and information from the study (including data and information generated by the investigator) without the consent of the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

13.4.2 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations and guidance, Takeda will, at a minimum register all interventional clinical trials it sponsors anywhere in the world on ClinicalTrials.gov and/or other publicly accessible websites before start of study, as defined in Takeda Policy/Standard. Takeda contact information, along with investigator's city, state (for Americas investigators), country, and recruiting status will be registered and available for public viewing.

For some registries, Takeda will assist callers in locating study sites closest to their homes by providing the investigator name, address, and phone number to the callers requesting trial information. Once subjects receive investigator contact information, they may call the site requesting enrollment into the trial. The investigative sites are encouraged to handle the trial inquiries according to their established subject screening process. If the caller asks additional questions beyond the topic of trial enrollment, they should be referred to the sponsor.

Any investigator who objects to the sponsor providing this information to callers must provide the sponsor with a written notice requesting that their information not be listed on the registry site.

13.4.3 Clinical Trial Results Disclosure

Takeda will post the results of clinical trials on ClinicalTrials.gov or other publicly accessible websites, as required by Takeda Policy/Standard, applicable laws and/or regulations.

13.5 Insurance and Compensation for Injury

Each subject in the study must be insured in accordance with the regulations applicable to the site where the subject is participating. If a local underwriter is required, then the sponsor or sponsor's designee will obtain clinical study insurance against the risk of injury to study subjects. Refer to the study site agreement regarding the sponsor's policy on subject compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

14.0 ADMINISTRATIVE AND REFERENCE INFORMATION

14.1 Administrative Information

14.1.1 Study Contact Information

Contact Type / Role	Contact
Serious adverse event and pregnancy reporting	PPD

14.1.2 INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure, package insert and any other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also to protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonisation, E6 Good Clinical Practice: Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in Section 10.2.9 of this protocol.
- Terms outlined in the study site agreement.
- Responsibilities of the Investigator (Appendix A).

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Appendix C of this protocol.

Signature of Investigator Date

Investigator Name (print or type)

Investigator's Title

Location of Facility (City, State/Provence)

Location of Facility (Country)

14.1.3 Study-Related Responsibilities

The sponsor will perform all study-related activities with the exception of those identified in the Study-Related Responsibilities template. The vendors identified for specific study-related activities will perform these activities in full or in partnership with the sponsor.

14.1.4 List of Abbreviations

AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
ANOVA	analysis of variance
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
AUC_{∞}	AUC from time 0 to infinity
AUC_{24}	AUC from time 0 to 24 hours postdose
BMI	body mass index
CFR	code of federal regulations
CHDR	Centre Hospital for Drug Research
CIOMS	Council for Organizations of Medical Sciences
C_{max}	maximum observed plasma concentration
CNS	central nervous system
CRO	contract research organization
CS	clinically significant
C-SSRS	Columbia-Suicide Severity Rating Scale
CYP	cytochrome P-450
ECG	electrocardiogram
eCRF	electronic case report form
EEG	electroencephalograph
FIH	first-in-human
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
HPLC	high performance liquid performance
IRB	institutional review board
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	institutional ethics committee
IV	intravenous(ly)
K_2EDTA	potassium ethylenediamine tetraacetic acid
LFT	liver function test

LICI	long intracortical inhibition
LS	least squares
MDD	major depressive disorder
MedDRA	Medical Dictionary for Regulatory Activities
MEP	motor-evoked potential
MRD	multiple-rising dose
NCS	not clinically significant
NIMH	National Institute of Mental Health
NOAEL	no-observed-adverse-effect level
PD	pharmacodynamic(s)
PGx	pharmacogenomics
PK	pharmacokinetic(s)
PO	orally
PTE	pretreatment event
QD	once daily
QTcF	QT interval with Fridericia correction method
RBC	red blood cell
rMT	resting motor threshold
rTMS	resting TMS
SAE	serious adverse event
SAP	statistical analysis plan
SICI	short intracortical inhibition
SPM	statistical parametric mapping
SPV	saccidic peak velocity
SRD	single-rising dose
SD	standard deviation
SUSARs	suspected unexpected serious adverse reactions
$t_{1/2z}$	terminal disposition phase half-life
TEAE	treatment-emergent adverse event
TEP	TMS-evoked EEG potential
t_{max}	time of first occurrence of C_{max}
TRD	treatment-resistant depression
VAS	visual analog scales
WBC	white blood cell

15.0 DATA HANDLING AND RECORDKEEPING

The full details of procedures for data handling will be documented in the Data Management Plan. AEs, medical history, and concurrent conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs will be coded using the World Health Organization Drug Dictionary.

15.1 CRFs (Electronic and Paper)

Completed eCRFs are required for each subject who signs an informed consent.

The sponsor or its designee will supply investigative sites with access to eCRFs. The sponsor will make arrangements to train appropriate site staff in the use of the eCRF. These forms are used to transmit the information collected in the performance of this study to the sponsor and regulatory authorities. eCRFs must be completed in English. Data are transcribed directly onto eCRFs.

After completion of the entry process, computer logic checks will be run to identify items, such as inconsistent dates, missing data, and questionable values. Queries may be issued by Takeda personnel (or designees) and will be answered by the site.

Corrections are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for change. Reasons for significant corrections should additionally be included.

The principal investigator must review the eCRFs for completeness and accuracy and must sign and date the appropriate eCRFs as indicated. Furthermore, the investigator must retain full responsibility for the accuracy and authenticity of all data entered on the eCRFs.

After the lock of the clinical study database, any change of, modification of, or addition to the data on the eCRFs should be made by the investigator with use of change and modification records of the eCRFs. The principal investigator must review the data change for completeness and accuracy, and must sign and date.

eCRFs will be reviewed for completeness and acceptability at the study site during periodic visits by study monitors. The sponsor or its designee will be permitted to review the subject's medical and hospital records pertinent to the study to ensure accuracy of the eCRFs. The completed eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the sponsor.

15.2 Record Retention

The investigator agrees to keep the records stipulated in Section 15.1 and those documents that include (but are not limited to) the study-specific documents, the identification log of all participating subjects, medical records, temporary media such as thermal sensitive paper, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent forms), electronic copy of eCRFs, including the audit trail, and detailed records of drug disposition to

enable evaluations or audits from regulatory authorities, the sponsor or its designees. Any source documentation printed on degradable thermal sensitive paper should be photocopied by the site and filed with the original in the subject's chart to ensure long-term legibility. Furthermore, ICH E6 Section 4.9.5 requires the investigator to retain essential documents specified in ICH E6 (Section 8) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH E6 Section 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the Clinical Study Site Agreement between the investigator and sponsor.

Refer to the Clinical Study Site Agreement for the sponsor's requirements on record retention. The investigator and the head of the institution should contact and receive written approval from the sponsor before disposing of any such documents.

16.0 REFERENCES

1. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. Journal of Neuroscience 1997;17(8):8. 2921.
2. Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003;2(3):145-56.
3. Premoli I, Castellanos N, Rivolta D, Belardinelli P, Bajo R, Zipser C, et al. TMS-EEG signatures of GABAergic neurotransmission in the human cortex. J Neurosci 2014;34(16):5603-12.
4. Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol 2015;126(10):1847-68.
5. Di Lazzaro V, Oliviero A, Profice P, Pennisi MA, Pilato F, Zito G, et al. Ketamine increases human motor cortex excitability to transcranial magnetic stimulation. J Physiol 2003;547(Pt 2):485-96.
6. Badawy RA, Loetscher T, Macdonell RA, Brodtmann A. Cortical excitability and neurology: insights into the pathophysiology. Funct Neurol 2012;27(3):131-45.
7. Caddy C, Giaroli G, White TP, Shergill SS, Tracy DK. Ketamine as the prototype glutamatergic antidepressant: pharmacodynamic actions, and a systematic review and meta-analysis of efficacy. Ther Adv Psychopharmacol 2014;4(2):75-99.
8. DiazGranados N, Ibrahim LA, Brutsche NE, Ameli R, Henter ID, Luckenbaugh DA, et al. Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. J Clin Psychiatry 2010a;71(12):1605-11.
9. DiazGranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S, et al. A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. Arch Gen Psychiatry 2010b;67(8):793-802.
10. Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, Perez AM, et al. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. Am J Psychiatry 2013;170(10):1134-42.
11. Rasmussen KG, Lineberry TW, Galardy CW, Kung S, Lapid MI, Palmer BA, et al. Serial infusions of low-dose ketamine for major depression. J Psychopharmacol 2013;27(5):444-50.
12. Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 2006;63(8):856-64.
13. Portmann S, Kwan HY, Theurillat R, Schmitz A, Mevissen M, Thormann W. Enantioselective capillary electrophoresis for identification and characterization of human cytochrome P450

enzymes which metabolize ketamine and norketamine in vitro. J Chromatogr A 2010;1217(51):7942-8.

14. Trevor AJ, Woolf TF, Baillie TA, Adams JD, Castagnoli N. Stereoselective metabolism of ketamine enantiomers. In: Kamenka JM, Domino EF, Geneste P, editors. Phencyclidine and Related Arylcyclohexylamines, Present and Future Applications. Ann Arbor, MI: NPP Books; 1983, p. 279-89.
15. Woolf TF, Adams JD. Biotransformation of ketamine, (Z)-6-hydroxyketamine, and (E)-6-hydroxyketamine by rat, rabbit, and human liver microsomal preparations. Xenobiotica 1987;17(7):839-47.
16. Hijazi Y, Boulieu R. Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2002;30(7):853-8.
17. Yanagihara Y, Kariya S, Ohtani M, Uchino K, Aoyama T, Yamamura Y, et al. Involvement of CYP2B6 in n-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2001;29(6):887-90.
18. Zhao X, Venkata SL, Moaddel R, Luckenbaugh DA, Brutsche NE, Ibrahim L, et al. Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression. Br J Clin Pharmacol 2012;74(2):304-14.
19. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 2016;533(7604):481-6.
20. Ihmsen H, Geisslinger G, Schuttler J. Stereoselective pharmacokinetics of ketamine: R(-)-ketamine inhibits the elimination of S(+)-ketamine. Clin Pharmacol Ther 2001;70(5):431-8.
21. Zarate CA, Jr., Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, et al. Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol Psychiatry 2012;71(11):939-46.
22. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMSCG. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120(12):2008-39.
23. Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. Drug Discov Today Technol 2016;20:27-34.
24. Wright BM. A simple mechanical ataxia-meter. J Physiol 1971;218 Suppl:27P-8P.
25. van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis, and visual analog

lines to the effects of incremental doses of temazepam in healthy volunteers. Clinical Pharmacology & Therapeutics 1991;50(2):172-80.

26. van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999;13(1):10-7.
27. Borland R, Nicholson A. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. British journal of clinical pharmacology 1975;2(1):9-17.
28. Borland R, Nicholson A. Visual motor co-ordination and dynamic visual acuity. British journal of clinical pharmacology 1984;18(S1):69S-72S.
29. Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971;10(2):181-91.
30. De Visser S, Van Der Post J, Pieters M, Cohen A, Van Gerven J. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. British journal of clinical pharmacology 2001;51(2):119-32.
31. Bond A, Lader M. The use of analogue scales in rating subjective feelings. British Journal of Medical Psychology 1974;47(3):211-8.
32. Bowdle AT, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers relationship to steady-state plasma concentrations. Anesthesiology: The Journal of the American Society of Anesthesiologists 1998;88(1):82-8.

17.0 APPENDICES

Appendix A Responsibilities of the Investigator

Clinical research studies sponsored by the sponsor are subject to ICH GCP and all the applicable local laws and regulations. The responsibilities imposed on investigators by the FDA are summarized in the "Statement of Investigator" (Form FDA 1572), which must be completed and signed before the investigator may participate in this study.

The investigator agrees to assume the following responsibilities by signing a Form FDA 1572:

1. Conduct the study in accordance with the protocol.
2. Personally conduct or supervise the staff that will assist in the protocol.
3. If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution should ensure that this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.
4. Ensure that study related procedures, including study specific (nonroutine/nonstandard panel) screening assessments are NOT performed on potential subjects, before the receipt of written approval from relevant governing bodies/authorities.
5. Ensure that all colleagues and employees assisting in the conduct of the study are informed of these obligations.
6. Secure prior approval of the study and any changes by an appropriate IRB/IEC that conform to 21 CFR Part 56, ICH, and local regulatory requirements.
7. Ensure that the IRB/IEC will be responsible for initial review, continuing review, and approval of the protocol. Promptly report to the IRB/IEC all changes in research activity and all anticipated risks to subjects. Make at least yearly reports on the progress of the study to the IRB/IEC, and issue a final report within 3 months of study completion.
8. Ensure that requirements for informed consent, as outlined in 21 CFR Part 50, ICH and local regulations, are met.
9. Obtain valid informed consent from each subject who participates in the study, and document the date of consent in the subject's medical chart. Valid informed consent is the most current version approved by the IRB/IEC. Each informed consent form should contain a subject authorization section that describes the uses and disclosures of a subject's personal information (including personal health information) that will take place in connection with the study. If an informed consent form does not include such a subject authorization, then the investigator must obtain a separate subject authorization form from each subject.
10. Prepare and maintain adequate case histories of all persons entered into the study, including eCRFs, hospital records, laboratory results, etc, and maintain these data for a minimum of 2 years following notification by the sponsor that all investigations have been discontinued or

that the regulatory authority has approved the marketing application. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

11. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.
12. Maintain current records of the receipt, administration, and disposition of sponsor-supplied drugs, and return all unused sponsor-supplied drugs to the sponsor.
13. Report adverse reactions to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.

Appendix B Elements of the Subject Informed Consent

In seeking informed consent, the following information shall be provided to each subject:

1. A statement that the study involves research.
2. An explanation of the purposes of the research.
3. The expected duration of the subject's participation.
4. A description of the procedures to be followed, including invasive procedures.
5. The identification of any procedures that are experimental.
6. The estimated number of subjects involved in the study.
7. A description of the subject's responsibilities.
8. A description of the conduct of the study.
9. A statement describing the treatment(s) and the probability for random assignment to each treatment.
10. A description of the possible side effects of the treatment that the subject may receive.
11. A description of any reasonably foreseeable risks or discomforts to the subject and, when applicable, to an embryo, fetus, or nursing infant.
12. A description of any benefits to the subject or to others that reasonably may be expected from the research. When there is no intended clinical benefit to the subject, the subject should be made aware of this.
13. Disclosures of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject and their important potential risks and benefits.
14. A statement describing the extent to which confidentiality of records identifying the subject will be maintained, and a note of the possibility that regulatory agencies, auditor(s), IRB/IEC, and the monitor may inspect the records. By signing a written informed consent form, the subject is authorizing such access.
15. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of or where further information may be obtained.
16. The anticipated prorated payment(s), if any, to the subject for participating in the study.
17. The anticipated expenses, if any, to the subject for participating in the study.
18. An explanation of whom to contact for answers to pertinent questions about the research (investigator), subject's rights, and IRB/IEC and whom to contact in the event of a research-related injury to the subject.

19. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject otherwise is entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.
20. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.
21. A statement that the subject will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the study.
22. The foreseeable circumstances or reasons under which the subject's participation in the study may be terminated.
23. A written subject authorization (either contained within the informed consent form or provided as a separate document) describing to the subject the contemplated and permissible uses and disclosures of the subject's personal information (including personal health information) for purposes of conducting the study. The subject authorization must contain the following statements regarding the uses and disclosures of the subject's personal information:
    a) that personal information (including personal health information) may be processed by or transferred to other parties in other countries for clinical research and safety reporting purposes, including, without limitation, to the following: (1) Takeda, its affiliates, and licensing partners; (2) business partners assisting Takeda, its affiliates, and licensing partners; (3) regulatory agencies and other health authorities; and (4) IRBs/IECs;
    b) it is possible that personal information (including personal health information) may be processed and transferred to countries that do not have data protection laws that offer subjects the same level of protection as the data protection laws within this country; however, Takeda will make every effort to keep your personal information confidential, and your name will not be disclosed outside the clinic unless required by law;
    c) that personal information (including personal health information) may be added to Takeda's research databases for purposes of developing a better understanding of the safety and effectiveness of the study medication(s), studying other therapies for patients, developing a better understanding of disease, and improving the efficiency of future clinical studies;
    d) that subjects agree not to restrict the use and disclosure of their personal information (including personal health information) upon withdrawal from the study to the extent that the restricted use or disclosure of such information may impact the scientific integrity of the research; and
    e) that the subject's identity will remain confidential in the event that study results are published.

24. Male subjects must use highly effective contraception (as defined in the informed consent) from signing the informed consent throughout the duration of the study and for 5 half lives PLUS 90 days after the last dose of study drug. If the partner or wife of the subject is found to be pregnant during the study, the investigator will offer the subject the choice to receive unblinded treatment information.
25. A statement that clinical trial information from this trial will be publicly disclosed in a publicly accessible website, such as ClinicalTrials.gov.

Appendix C Investigator Consent to the Use of Personal Information

Takeda will collect and retain personal information of investigator, including his or her name, address, and other personally identifiable information. In addition, investigator's personal information may be transferred to other parties located in countries throughout the world (eg, the United Kingdom, United States, and Japan), including the following:

- Takeda, its affiliates, and licensing partners.
- Business partners assisting Takeda, its affiliates, and licensing partners.
- Regulatory agencies and other health authorities.
- IRBs and IECs.

Investigator's personal information may be retained, processed, and transferred by Takeda and these other parties for research purposes including the following:

- Assessment of the suitability of investigator for the study and/or other clinical studies.
- Management, monitoring, inspection, and audit of the study.
- Analysis, review, and verification of the study results.
- Safety reporting and pharmacovigilance relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to other medications used in other clinical studies that may contain the same chemical compound present in the study medication.
- Inspections and investigations by regulatory authorities relating to the study.
- Self-inspection and internal audit within Takeda, its affiliates, and licensing partners.
- Archiving and audit of study records.
- Posting investigator site contact information, study details and results on publicly accessible clinical trial registries, databases, and websites.

Investigator's personal information may be transferred to other countries that do not have data protection laws that offer the same level of protection as data protection laws in investigator's own country.

Investigator acknowledges and consents to the use of his or her personal information by Takeda and other parties for the purposes described above.

Appendix D Pregnancy and Contraception

Contraception and Pregnancy Avoidance Procedure

Male Subjects and Their Female Partners

From signing of informed consent, throughout the duration of the study, and for 5 half-lives PLUS 90 days after last dose of study drug, nonsterilized** male subjects who are sexually active with a female partner of childbearing potential* must use barrier contraception (eg, condom with or without spermicidal cream or jelly). In addition, they must be advised not to donate sperm during this period.

Female Subjects and Their Male Partners

Women of childbearing potential (WOCBP) will be excluded from the study.

Definitions and Procedures for Contraception and Pregnancy Avoidance

The following definitions apply for contraception and pregnancy avoidance procedures.

* A woman is considered a WOCBP, ie fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, and bilateral oophorectomy. 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 (FSH >40 IU/L) may be used to confirm a post-menopausal state in younger women (eg, those <45 years old) or women who are not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Sterilized males should be at least 1 year post-bilateral vasectomy and have confirmed that they have obtained documentation of the absence of sperm in the ejaculate or have had bilateral orchidectomy.

The following procedures apply for contraception and pregnancy avoidance.

1. Highly effective methods of contraception are defined as "those, alone or in combination, that result in a low failure rate (ie, less than 1% failure rate per year when used consistently and correctly). In this study, where medications and devices containing hormones are included/excluded, the only acceptable methods of contraception are:
   - Non-Hormonal Methods:
     - Vasectomised partner (provided that partner is the sole sexual partner of the trial participant and that the vasectomised partner has received medical assessment of the surgical success.
     - True sexual abstinence, only if this is in line with the preferred and usual lifestyle of the subject. True abstinence is defined as refraining from heterosexual intercourse during the entire period of the study, from 1 month before the first dose until 5 half lives PLUS 30 days after last dose.

6. Effective method of contraception (there may be a higher than 1% failure rate) are:
    - Double-barrier method (contraceptive sponge, diaphragm or cervical cap with spermicidal jellies or creams PLUS male condom).
7. Unacceptable methods of contraception are:
    - Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods).
    - Spermicides only.
    - Withdrawal.
    - No method at all.
    - Use of female and male condoms together.
    - Cap/diaphragm/sponge without spermicide and without condom.
    - Sexual abstinence is NOT an acceptable method of contraception.
8. Subjects will be provided with information on highly effective/effective methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy, donation of ova, and sperm donation during the course of the study.
9. During the course of the study, all subjects (male and female) will receive continued guidance with respect to the avoidance of pregnancy and sperm donation as part of the study procedures. Such guidance should include a reminder of the following:
    a) contraceptive requirements of the study
    b) reasons for use of barrier methods (ie, condom) in males with pregnant partners
    c) assessment of subject compliance through questions such as
        i. Have you used the contraception consistently and correctly since the last visit?
        ii. Have you forgotten to use contraception since the last visit?
        iii. Are your menses late (even in women with irregular or infrequent menstrual cycles a pregnancy test must be performed if the answer is "yes")
        iv. Is there a chance you could be pregnant?

General Guidance With Respect to the Avoidance of Pregnancy

Such guidance should include a reminder of the following:

- contraceptive requirements of the study.
- reasons for use of barrier methods (ie, condom) in males with pregnant partners.
- assessment of subject compliance through questions such as:

- Have you used the contraception consistently and correctly since the last visit?
- Have you forgotten to use contraception since the last visit?

Pregnancy

Women of childbearing potential will not be included in this study.

If any subject is found to be pregnant during the study she should be withdrawn and any sponsor-supplied drug [list all that apply] should be immediately discontinued. In addition, any pregnancies in the partner of a male subject during the study or for 5 half lives PLUS 90 days after the last dose, should also be recorded following authorization from the subject's partner.

Should the pregnancy occur during or after administration of blinded drug, the investigator must inform the subject of their right to receive treatment information. If the subject chooses to receive unblinded treatment information, the individual blind should be broken by the investigator. Subjects randomized to placebo need not be followed.

If the female subject and/or female partner of a male subject agrees to the primary care physician being informed, the investigator should notify the primary care physician that the female subject/female partner of the subject was participating in a clinical study at the time she became pregnant and provide details of the study drug the subject received (blinded or unblinded, as applicable).

All pregnancies, including female partners of male subjects, in subjects on active study drug (including comparator, if applicable) will be followed up to final outcome, using the pregnancy form. Pregnancies will remain blinded to the study team. The outcome, including any premature termination, must be reported to the sponsor. An evaluation after the birth of the child will also be conducted.

Appendix E Detailed Description of Amendments to Text

The primary sections of the protocol affected by the changes in Amendment No. 01 are indicated. The corresponding text has been revised throughout the protocol.

Change 1: A second primary endpoint added and both endpoints are evaluated as changes from pre-dosing baseline, compared to placebo.

The primary changes occur in Section 5.2.1 Primary Endpoints

Initial wording: **5.2.1 Primary Endpoint**

The primary endpoint of the study is:

- The resting motor threshold (rMT) obtained with single-pulse TMS assessing the effect of TAK-653 compared with placebo.

Amended or new wording: **5.2.1 Primary ~~Endpoint~~Endpoints**

The primary ~~endpoint~~**endpoints** of the study ~~is~~**are**:

- The **change of peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline** resting motor threshold ~~(rMT)~~**[rMT]) at 2½ hours after administration of TAK-653 from pre-dosing baseline, compared to placebo.**
- **The change of rMT** obtained with single-pulse TMS ~~assessing the effect of TAK-653 compared with placebo~~**at 2 1/2 hours after administration of TAK-653 from pre-dosing baseline, compared to placebo**.

Rationale for Change: One of the original secondary endpoints has been changed to a primary endpoint so that either endpoint may be used to assess excitability.

The following sections also contain this change:

- Section 1.0 STUDY SUMMARY

Change 2: Modified secondary endpoints.

The primary changes occur in Section 5.2.2 Secondary Endpoints

Initial Wording: Secondary endpoints include:

1. The peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT), assessing TAK-653 effects over pre-dosing baseline for each treatment period (key secondary endpoint).
2. Magnitude of long intracortical inhibition (LICI) obtained with paired-pulse TMS (stimulation intensity conditioning pulse and test pulse: 120% of baseline rMT).
3. Magnitude of Short intracortical inhibition (SICI) obtained with paired-pulse TMS (stimulation intensity: conditioning pulse 80% of baseline rMT; test pulse: 120% of baseline rMT).
4. The resting motor threshold (rMT) obtained with single-pulse TMS assessing ketamine effects.

The peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT) assessing ketamine effects.

Amended or new wording: Secondary endpoints include **the change from baseline at 2 1/2 hours for the following:**

~~1. The peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT), assessing TAK-653 effects over predosing baseline for each treatment period (key secondary endpoint).~~

1. Magnitude of long intracortical inhibition (LICI) obtained with paired-pulse TMS (stimulation intensity conditioning pulse and test pulse: 120% of baseline rMT).

2. Magnitude of short intracortical inhibition (SICI) obtained with paired-pulse TMS (stimulation intensity: conditioning pulse 80% of baseline rMT; test pulse: 120% of baseline rMT).

3. The rMT obtained with single-pulse TMS assessing ketamine effects, **as well as at 24 hours**.

4. The peak-to-peak amplitude of the MEP obtained with single-pulse TMS (stimulation intensity: 120% of baseline rMT) assessing ketamine effects, **as well as at 24 hours**.

Rationale for Change: To specify the 2 ½ hour change from baseline for the assessments

Change 3: The primary endpoint analysis now includes both primary endpoints with an additional Hochberg step-up procedure. In addition, some details of the model are deferred to the SAP. In addition, some details of the model are deferred to the SAP.

The primary changes occur in Section 11.1.4.1 Primary Endpoint Analysis

Initial wording: *11.1.4.1 Primary Endpoint Analysis*

The change of primary endpoint (rMT at 2 ½ hours post TAK-653 dosing obtained with single-pulse TMS) from pre-dosing baseline level will be analyzed using mixed analysis of variance (ANOVA) model appropriate for a three-period crossover with fixed factors for treatment, sequence, period, time, and treatment-by-time interaction and a random factor for subject nested in sequence. One-sided t-tests comparing the primary endpoint results for placebo vs. treatment at each dose level will be conducted as secondary analyses, adjusted for multiple testing to ensure type 1 error control at the 10% level.

Amended or new wording: *11.1.4.1 Primary Endpoint Analysis*

The ~~change of primary endpoint (rMT at 2 ½ hours post TAK-653 dosing~~**primary endpoints are changes in rMT and changes in peak-to-peak amplitude of the MEP** obtained with single-pulse TMS~~) from~~ **at 2 1/2 hours after administration of TAK-653 from their corresponding** pre-dosing baseline~~ level~~**levels, compared to placebo. They** will be analyzed using **linear** mixed ~~analysis of variance (ANOVA)~~**effect models.**

The primary endpoints will be analyzed using a linear mixed effects model appropriate for a three-period crossover ~~with fixed factors for treatment, sequence, period, time, and treatment-by-time interaction and a random factor forsubject nested in sequence~~**design. Additional details of the model will be specified in the SAP. Hochberg's step-up procedure will be used to adjust for multiple endpoints**. One-sided t-tests comparing **each of** the primary ~~endpoint results~~**endpoints** for placebo vs. treatment at each dose level will be conducted as secondary analyses, adjusted for multiple testing to ensure type 1 error control at the 10% level.

Rationale for Change: Hochberg's step-up procedure is used to adjust for multiple testing. Deferring details to the SAP allows for refinement of the statistical analysis whilst assuring that the model used will be pre-specified.

The following sections also contain this change:

- Section 1.0 STUDY SUMMARY

Change 4: The early analysis, which may occur when at least 18 subjects have completed periods 1 through 3, will be unblinded (previously partially blinded).

The primary changes occur in Section 11.2 Interim Analysis and Criteria for Early Termination

Initial wording:

11.2 Interim Analysis and Criteria for Early Termination

No interim analysis is planned. Blinded summary statistics will be provided for safety and primary endpoints without statistical testing. No decision on the study will be made based on the results.

11.3 Early Blinded Analysis of Data

After at least 18 subjects have completed the first three periods of the study, blinded summary statistics (number of subjects, mean, SD, minimum, median, and maximum) may be provided on the change of primary endpoint (rMT at 2 ½ hours post TAK-653 dosing obtained with single-pulse TMS) from pre-dosing baseline level and the peak-to-peak amplitude of the MEP obtained with single-pulse TMS. Only summary statistics with dummy treatment code will be provided. No decision on the trial or modification of sample size will be made based on the analysis. The investigator and study site will not have access to the summary statistics until the study has ended.

Amended or new wording:

~~No interim analysis is planned. Blinded summary statistics will be provided for safety and primary endpoints without statistical testing. No decision on the study will be made based on the results.~~

~~**11.3 Early Blinded Analysis of Data**~~

~~After at least 18 subjects have completed~~ the first three periods of the study, ~~blinded summary statistics (number of subjects, mean, SD, minimum, median, and maximum)~~an unblinded data analysis may be provided on the change of primary ~~endpoint (rMT at 2 ½ hours post TAK-653 dosing obtained with single-pulse TMS) from pre-dosing baseline level and the peak-to-peak amplitude of the MEP obtained with single-pulse TMS. Only summary statistics with dummy treatment code will be provided~~endpoints. An unblinded team not involved in the study conduct or the program will perform the unblinded analysis. A firewall will be set up between the unblinded team and the study team. Details of the procedure will be described in a separate document. No decision on the trial or modification of sample size will be made based on the analysis. The investigator and study site will not have access to the ~~summary statistics~~analysis results until the final unblinding of the study ~~has ended~~. This early data review will be conducted only for the purpose of sponsor programmatic forward planning that is unrelated to the conduct of the present study.

Rationale for Change: Procedures adjusted to conduct an unblinded rather than partially blinded analysis.

The following sections also contain this change:

No other section

Change 5: The sample size justification is updated to include both primary endpoints.

The primary changes occur in Section 11.3 Determination of Sample Size

Initial wording:	A sample size of 22 subjects, approximately four subjects for each of the six sequences of the first three treatment periods, will be considered sufficient for evaluation of the primary endpoint for this study based on consideration of a published study on Ketamine by di Lazzaro et al. (2003) and preliminary results of baseline TMS data in a CHDR study of healthy volunteers. Subjects who complete at least the first three treatment periods of the study are considered completers. Drop-out rate is expected to be low. Twenty-four subjects will be recruited (4 per sequence) to ensure at least 22 completers. If more than two subjects drop out before completing the first treatment three periods, up to two additional subjects may be enrolled. Specifically, this sample-size calculation was based on a consideration of effect sizes of Ketamine on rMT at 0.01 mg/kg and 0.02 mg/kg, as reported in the study by di Lazzaro et al (2003). Based on a type-I error rate of 0.10, a common standard deviation of 9% and a within-subject correlation of 0.9, this study has at least 80% power to detect a reduction of 2.7 and 4.6 percentage point in rMT at the two dose levels of TAK-653 vs placebo, assuming a total of 22 completers.
Amended or new wording:	**Determination of Sample Size** **The assumptions used in the sample size determination were** based on consideration of a published study on **ketamine** by di Lazzaro et al. (2003) **[5] and the preliminary results of TMS data in a CHDR study of healthy volunteers. Specifically, we assumed an intra**-subject correlation of 0.9, **a common standard deviation of 9% for rMT and 947 µV for the MEP peak-to-peak amplitude, a correlation coefficient of at least 0.5 between pre- and post-dosing outcome, and a familywise type I error rate of 10%.** A sample size of 22 subjects, approximately ~~four~~**4** subjects for each of the ~~six~~**6** sequences of the first ~~three~~**3** treatment periods, will ~~be considered sufficient for evaluation of the primary endpoint for this study based on consideration of a published study on Ketamine by di Lazzaro et al. (2003)[5] and preliminary results of baseline TMS data in a CHDR study of healthy volunteers~~**provide at least 80% power in detecting a reduction of 5.3 percentage points in rMT and/or an increase of 560 µV in the MEP peak-to-peak amplitude at the**

most effective dose level of TAK-653 versus placebo. Subjects who complete at least the first ~~three~~**3** treatment periods of the study are considered completers. Drop-out rate is expected to be low. Twenty-four subjects will be recruited (4 per sequence) to ensure at least 22 completers. If more than ~~two~~**2** subjects drop out before completing the first **3** treatment ~~three~~ periods, up to ~~two~~**2** additional subjects may be enrolled.

~~Specifically, this sample-size calculation was based on a consideration of effect sizes of Ketamine on rMT at 0.01 mg/kg and 0.02 mg/kg, as reported in the study by di Lazzaro et al (2003). Based on a type-I error rate of 0.10, a common standard deviation of 9% and a within-subject correlation of 0.9, this study has at least 80% power to detect a reduction of 2.7 and 4.6 percentage point in rMT at the two dose levels of TAK-653 vs placebo, assuming a total of 22 completers.~~

Rationale for Change: Needed to justify the power for each primary endpoint when the Hochberg procedure is applied.

The following sections also contain this change:

- Section 1.0 STUDY SUMMARY

Change 6: The resting motor threshold (rMT) exclusion criterion is modified.

The primary changes occur in Section 7.2 Exclusion Criteria

Initial wording:	22. The subject has an rMT of more than 83**%** of the maximum stimulator output, measured using TMS-EMG during screening.
Amended or new wording:	22. The subject has an rMT of more than ~~83~~**75**% of the maximum stimulator output, measured using TMS-EMG during screening.

Rationale for Change: The rMT exclusion threshold was lowered to avoid a ceiling in stimulator output in subjects for which the rMT is at or near 83% of maximum stimulator output. One of the primary endpoints will be tested at 120% of the baseline rMT, so if subjects are at 83% of the maximum, 120% of that will be at maximum. A 75% of maximum stimulator output will enable a stimulation range that does not reach the apparatus limit.

The following sections also contain this change:

- Section 1.0 STUDY SUMMARY

Change 7: Three safety endpoints were deleted.

The primary changes occur in Section 5.2.3 Safety Endpoints

Initial wording: **5.2.3 Safety Endpoints**

Safety endpoints include:

1. Number/percentage of subjects with at least 1 AE.
2. Number/percentage of subjects with at least 1 SAE.
3. Number/percentage of subjects with at least 1 clinically-defined abnormal laboratory value.
4. Number/percentage of subjects with at least 1 clinically-defined abnormal vital sign value.
5. Number/percentage of subjects with at least 1 dose-limiting toxicity.
6. Number/percentage of subjects with at least 1 AE.
7. Number/percentage of subjects with at least 1 AE Grade 3 or higher.

Amended or new wording:

Safety endpoints include:

1. Number/percentage of subjects with at least 1 AE.
2. Number/percentage of subjects with at least 1 serious adverse event (SAE).
3. Number/percentage of subjects with at least 1 clinically-defined abnormal laboratory value.
4. Number/percentage of subjects with at least 1 clinically-defined abnormal vital sign value.
5. ~~Number/percentage of subjects with at least 1 dose-limiting toxicity.~~
6. ~~Number/percentage of subjects with at least 1 AE.~~
7. ~~Number/percentage of subjects with at least 1 AE Grade 3 or higher~~.

Rationale for Change: The deleted endpoints are already included in the first 4 points, or are not applicable.

The following sections also contain this change:

- Section 1.0 STUDY SUMMARY

Change 8: Subject instructions to stop concomitant medications before dosing are modified.

The primary changes occur in Section 7.3.1 Concomitant Medications

Initial wording: **Concomitant Medications**

The use of concomitant medications after randomization (ie, Day 1 of Period 1) until the follow-up phone call is not permitted. Subjects must be instructed not to take any medications without first consulting with the investigator. Any concomitant medication use must first be discussed with the sponsor, unless the investigator or designee considers immediate administration is necessitated. It is allowed to give medication to treat AEs, for example midazolam for a seizure.

The occasional use of acetaminophen (approximately <1 g/day) is allowed.

Amended or new wording: **Concomitant Medications**

~~The use of~~**Subjects are instructed to stop** concomitant medications ~~after randomization (ie, Day 1 of Period 1)~~**7 days before administration of the first dose of study drug and throughout the study**, until the follow-up phone cal~~l is not permitted~~. Subjects must be instructed not to take any medications without first consulting with the investigator. Any concomitant medication use must first be discussed with the sponsor, unless the investigator or designee considers immediate administration is necessitated. It is allowed to give medication to treat AEs, for example midazolam for a seizure.

The occasional use of acetaminophen (approximately <1 g/day) is allowed.

Rationale for Change: To ensure subjects are off concomitant medications one week before dosing and to also mirror Exclusion Criteria #11.

The following sections also contain this change:

No other section

Change 9: The window for predose ECG is widened to 3 hours.

The primary changes occur in Section 9.2.5 12-Lead ECG

Initial wording: **9.2.5 12-Lead ECG**

For each treatment period, a predose ECG will be obtained within approximately 1 hour before study drug dosing. This measurement will be used as the baseline assessment. The principal investigator should arrange to have a study cardiologist available as needed to review ECG abnormalities.

During each treatment period, if a subject demonstrates an increase in QTcF interval ≥40 milliseconds compared with a predose baseline measurement, the ECG will be repeated within 5 minutes. The average value of the QTcF interval from the 2 ECGs will represent the value at that time point. If the average QTcF interval increase from baseline for any postdose time point is ≥40 milliseconds, the subject will continue to be monitored by repeat 12-lead ECGs every 60 minutes for at least 4 hours or until the QTcF interval is within 40 milliseconds of the baseline value. If prolongation of the QTcF interval ≥40 milliseconds persists, a consultation with a study cardiologist may be appropriate and the sponsor should be notified

Amended or new wording: For each treatment period, a predose ECG will be obtained within approximately ~~1~~3 hour before study drug dosing. This measurement will be used as the baseline assessment. The principal investigator should arrange to have a study cardiologist available as needed to review ECG abnormalities.

During each treatment period, if a subject demonstrates an increase in QTcF interval ≥40 milliseconds compared with a predose baseline measurement, the ECG will be repeated within 5 minutes. The average value of the QTcF interval from the 2 ECGs will represent the value at that time point. If the average QTcF interval increase from baseline for any postdose time point is ≥40 milliseconds, the subject will continue to be monitored by repeat 12-lead ECGs every 60 minutes for at least 4 hours or until the QTcF interval is within 40 milliseconds of the baseline value. If prolongation of the QTcF interval ≥40 milliseconds persists, a consultation with a study cardiologist may be appropriate and the sponsor should be notified.

Rationale for Change: To permit scheduling of baseline ECG in a busy clinic and clarify timing.

The following sections also contain this change:

- Section 3.0 Schedule of Study Procedures

Change 10: Site clinic seizure precautions are updated for generalized use.

The primary changes occur in Section 9.2.8 AE Monitoring

Initial wording: **9.2.8 AE Monitoring**

Seizure precautions will be taken at all clinic visits, consisting of availability of a hospital bed with padded side rails, suction set up and appropriately tested, and an ambu bag nearby. If there is evidence of seizure activity, subjects will be administered standard of care anticonvulsants as needed and remain in the clinic overnight for closer observation.

Amended wording: Seizure precautions will be taken at all clinic visits, consisting of ~~availability of a hospital bed with padded side rails~~, suction set up ~~and appropriately tested,~~ and an ambu bag nearby. If there is evidence of seizure activity, subjects will be administered standard of care anticonvulsants as needed and remain in the clinic overnight for closer observation.

Rationale for Change: To remove restrictive precautions which are already addressed in the clinic setting.

The following sections also contain this change:

- No other section

Change 11: Pharmacokinetic (PK) blood sample collection instructions for ketamine analysis are added.

The primary changes occur in Section 9.4.2.1 Plasma for PK Measurements.

Initial wording: Blood samples for plasma concentration analysis of TAK-653 and ketamine will be collected into chilled blood collection tubes (vacutainer) containing the anticoagulant K_2EDTA. The collected blood samples may be archived for additional analysis of potential metabolites.

The actual time of sample collection will be recorded on the source document and electronic case report form. Sampling time points may be adjusted based on the preliminary emerging concentration data collected from prior subjects, but the total number of samples collected per subject should not exceed the planned number.

Amended or new wording: Blood samples for plasma concentration analysis of TAK-653 ~~and ketamine~~ will be collected into chilled blood collection tubes (vacutainer) containing the anticoagulant ~~K_2EDTA~~potassium ethylenediamine tetraacetic acid **(K_2EDTA) at room temperature. After the blood draw, the tube will immediately be placed in a bucket with ice.** The collected blood samples may be archived for additional analysis of potential metabolites.

Blood samples for plasma concentration analysis of ketamine will be collected into a tube containing the anticoagulant sodium heparin at room temperature and immediately after the sample is drawn placed in a tube in a bucket with ice.

Please refer to Section 3.0 SCHEDULE OF STUDY PROCEDURES for PK blood sampling visits, draw times and sampling windows. The actual time of sample collection will be recorded on the source document and electronic case report form. Sampling time points may be adjusted based on the preliminary emerging concentration data collected from prior subjects, but the total number of samples collected per subject should not exceed the planned number.

Rationale for Change: Specific details on sample collection for ketamine analysis were omitted in the initial version.

The following sections also contain this change:

- No other section.

Change 12: The time interval for routine collection of AEs after final dosing is clarified.

The primary changes occur in Section 10.2.8 Collection and Reporting of AEs, SAEs, Special Interest AEs, and Abnormal Liver Function Tests (LFT)s

Initial wording: *10.2.8.1 Collection Period*

Collection of AEs (ie, AEs, SAEs, Special Interest AEs, and abnormal liver function tests (LFT)s will commence at the time the subject signs the informed consent. Routine collection of AEs will continue until the follow-up phone call on Day 14 (± 2 days), approximately 30 days after the last dose of investigational product. For subjects who discontinue before the administration of study medication, AEs will be followed until the subject discontinues study participation.

Amended wording: *10.2.8.1 Collection Period*

Collection of AEs (ie, AEs, SAEs, Special Interest AEs, and abnormal liver function tests (LFT)s will commence at the time the subject signs the informed consent. Routine collection of AEs will continue until the follow-up phone call on Day 14 (± 2 days)~~, approximately 30 days after the last dose of investigational product~~. For subjects who discontinue before the administration of study medication, AEs will be followed until the subject discontinues study participation.

Rationale for Change: Collection period clarified, based on elimination half-life of TAK-653.

The following sections also contain this change:

- No other section

Change 13: Deletion of the 25 hour visit.

The primary changes occur in Section 5.1.3 Trial Exploratory Objectives

Initial wording: CCI [redacted]

Amended wording: CCI [redacted]

Rationale for Change: 25 hour assessment not needed

The following sections also contain this change:

Section 1.0 STUDY SUMMARY and Section 3.0 Schedule of Study Procedures

Change 14: Detail from study summary added to the trial design section.

The primary changes occur in Section 6.1 Trial Design

Initial wording: EEG and MEP signals evoked by TMS, as PD endpoints for assessing the effects of TAK-653 were selected on the basis of pre-clinical data showing that TAK-653 increases the amplitude of MEPs in rats. The goal of this study is to evaluate the PD effects of TAK-653 after single dose treatment as measured by responses to TMS. Healthy adult (age 18 to 55 years) subjects are the intended population in this trial.

The planned dose levels of TAK-653 to be evaluated are outlined in Table 6.a. The study drug will be 6 mg TAK-653 for the high dose, 0.5 mg TAK-653 for the low dose, or placebo administered orally on Day 1 of Treatment Periods 1 to 3 at the clinical testing site. Both doses and placebo treatments will be delivered with 2 tablets, as indicated in Section 8.0. On Treatment Period 4, ketamine 0.5 mg/kg IV will be administered for 40 minutes.

Amended wording:

This randomized, double blind, placebo-controlled, 3-period cross-over study followed by one open label comparator period is designed to evaluate the central pharmacodynamic activity of TAK-653 in healthy subjects using transcranial magnetic stimulation (TMS). Approximately 24 healthy male and female subjects, aged 18 to 55 years, inclusive, will be enrolled.

The study will include 3 treatment periods, each 1 day in duration, followed by a 2-day comparator period, alternating with 3 washout periods of at least 10 days (not to exceed 15 days). There will be 5 clinic visits, including screening in which full medical, neurological, and psychiatric examinations will be conducted; Day 1 of each of the first 3 treatment periods, during which TMS testing will be conducted before and after subjects receive placebo or 1 of 2 doses of TAK-653; Day 1 of the fourth treatment period, during which TMS testing will be conducted before and after subjects receive the (NMDA) (N-methyl-D-aspartate) antagonist ketamine, which will be open label.

Before the start of each treatment period, the investigator will review the safety assessments for the subject, including vital signs, and electrocardiogram (ECG).

On the first day of each treatment period (Day 1), each subject will undergo a TMS session assessing baseline TMS-elicited motor-evoked potential (MEP) amplitude and threshold, followed by administration of TAK-653 or placebo (Treatment Periods 1-3), or ketamine (Treatment Period 4). For the first 3 treatment periods, TMS and MEP/electroencephalogram assessment sets, will be conducted along with blood sample collection for pharmacokinetic (PK) analyses at predose and starting at 30 and 150

minutes (2 ½ hours) after dosing. The 2 ½ hour time point is chosen to capture C_{max}, and the 30 minute time point to capture a lower exposure level in each subject. In Period 4, each subject will undergo a TMS session assessing baseline TMS-elicited MEP amplitude and threshold, and blood sample collection for PK analyses at predose, followed by administration of ketamine intravenously (0.5 mg/kg over 40 minutes). TMS assessments will be conducted at 150 minutes (2.5 hours) after ketamine initiation (when ketamine acute dissociative effects have subsided), and blood sample collection for PK analysis at selected timepoints. Subjects will undergo follow-up TMS session starting at 24 hours (Day 2 of Period 4) to capture ketamine modulation of TMS responses at a time in which antidepressant effects of ketamine are already present in a major depressive disorder population.

Subjects will remain in the clinic on treatment days until approximately 2 hours post-TMS procedures for safety observation. During the ketamine-testing period, subjects will remain overnight following the infusion, until the 24 hour follow-up TMS assessments have been completed. Before discharge, a neurological and mental state examination will be performed.

EEG and MEP signals evoked by TMS, as PD endpoints for assessing the effects of TAK-653 were selected on the basis of pre-clinical data showing that TAK-653 increases the amplitude of MEPs in rats. The goal of this study is to evaluate the PD effects of TAK-653 after single dose treatment as measured by responses to TMS. Healthy adult (age 18 to 55 years) subjects are the intended population in this trial.

The planned dose levels of TAK-653 to be evaluated are outlined in Table 6.a. The study drug will be 6 mg TAK-653 for the high dose, 0.5 mg TAK-653 for the low dose, or placebo administered orally on Day 1 of Treatment Periods 1 to 3 at the clinical testing site. Both doses and placebo treatments will be delivered with 2 tablets, as indicated in Section 8.0. On Treatment Period 4, ketamine 0.5 mg/kg IV will be administered for 40 minutes.

Rationale for Change: Text from study summary replicated in study design section for clarity

The following sections also contain this change:

No other sections.

Change 15: Description of study blinding corrected.

The primary changes occur in Section 8.1.3 Clinical Study Drug Blinding

Initial wording: **Clinical Study Drug Blinding**

This is a double-blind study; the investigator and subjects are blinded to treatment assignment. An unblinded study drug supply will be provided to an unblinded pharmacist or other qualified personnel at the study site who will blind the study supplies. Treatment identity (name and strength or potency) will be included on the study drug container label. Randomization code/disclosure envelopes or lists will be provided per the standard operating procedures of the study site.

Amended wording: **Clinical Study Drug Blinding**

This is a double-blind study; the investigator and subjects are blinded to ~~treatment~~**sequence group** assignment. An unblinded study drug supply will be provided to an unblinded pharmacist or other qualified personnel at the study site who will blind the study supplies. Treatment identity (name and strength or potency) will be included on the study drug container label. Randomization code/disclosure envelopes or lists will be provided per the standard operating procedures of the study site.

Rationale for Change: To correctly state blinding

The following sections also contain this change:

- No other section

Change 16: Definition of safety analysis set revised.	
The primary changes occur in Section 11.1.1.1 Safety Set	
Initial wording:	*Safety Set* The Safety Analysis Set will consist of all subjects who are enrolled and received 1 dose of study drug. Subjects in this analysis set will be used for demographics, baseline characteristics, and safety summaries.
Amended wording:	*Safety Set* The Safety Analysis Set will consist of all subjects who are ~~enrolled~~**randomized** and received 1 dose of study drug. Subjects in this analysis set will be used for demographics, baseline characteristics, and safety summaries.
Rationale for Change: To correct the definition of the safety set.	
The following sections also contain this change: • No other section	

Change 17: Pregnancy and contraception language updated.

The primary changes occur in Appendix D Pregnancy and Contraception

Initial wording: *The following procedures apply for contraception and pregnancy avoidance.*

1. Highly effective methods of contraception are defined as "those, alone or in combination, that result in a low failure rate (ie, less than 1% failure rate per year when used consistently and correctly). In this study, where medications and devices containing hormones are included/excluded, the only acceptable methods of contraception are:

   - Non-Hormonal Methods:
     - Intrauterine device (IUD).
     - Bilateral tubal occlusion.
     - Vasectomised partner (provided that partner is the sole sexual partner of the trial participant and that the vasectomised partner has received medical assessment of the surgical success.
     - True sexual abstinence, only if this is in line with the preferred and usual lifestyle of the subject. True abstinence is defined as refraining from heterosexual intercourse during the entire period of the study, from 1 month before the first dose until 5 half lives PLUS 30 days after last dose.
   - Hormonal Methods: Hormonal contraception may be susceptible to interaction with the investigative compound, comparator, concomitant medications, which may reduce the efficacy of the contraception method (Evaluate on compound-by-compound and protocol –by-protocol basis and obtain clinical pharmacology justification).
     - Combined (estrogen and progestogen) hormonal contraception associated with inhibition of ovulation initiated at least 3 months before the first dose of study drug OR combined with a barrier method (male condom, female condom or diaphragm) if for shorter duration until she has been on contraceptive for 3 months;
       - Oral †.
       - Intravaginal † (eg, ring).
       - transdermal †.
     - Progestogen-only hormonal contraception associated with inhibition of ovulation1 initiated at least 3 months before the first dose of study drug OR combined with a barrier method (male condom, female condom or diaphragm) if shorter till she has been on contraceptive for

3 months;

- oral †.
- Injectable.
- Implantable.

2. If genotoxicity/teratogenicity/embryotoxicity is unlikely to be caused by the investigational drug, comparator, background therapy or standard of care medications effective methods of contraception (there may be a higher than 1% failure rate) are:
   - Double-barrier method (contraceptive sponge, diaphragm or cervical cap with spermicidal jellies or creams PLUS male condom).
   - Progestogen only hormonal contraception, where inhibition of ovulation is not the primary mode of action PLUS condom with or without spermicide.

Amended wording:

The following procedures apply for contraception and pregnancy avoidance.

1. Highly effective methods of contraception are defined as "those, alone or in combination, that result in a low failure rate (ie, less than 1% failure rate per year when used consistently and correctly). In this study, where medications and devices containing hormones are included/excluded, the only acceptable methods of contraception are:
   - Non-Hormonal Methods:
     - ~~Intrauterine device (IUD).~~
     - ~~Bilateral tubal occlusion.~~
     - Vasectomised partner (provided that partner is the sole sexual partner of the trial participant and that the vasectomised partner has received medical assessment of the surgical success.
     - True sexual abstinence, only if this is in line with the preferred and usual lifestyle of the subject. True abstinence is defined as refraining from heterosexual intercourse during the entire period of the study, from 1 month before the first dose until 5 half lives PLUS 30 days after last dose.
   - ~~Hormonal Methods: Hormonal contraception may be susceptible to interaction with the investigative compound, comparator, concomitant medications, which may reduce the efficacy of the contraception method (Evaluate on compound-by-compound and protocol - by-protocol basis and obtain clinical pharmacology justification).~~
     - ~~Combined (estrogen and progestogen) hormonal contraception~~

~~associated with inhibition of ovulation initiated at least 3 months before the first dose of study drug OR combined with a barrier method (male condom, female condom or diaphragm) if for shorter duration until she has been on contraceptive for 3 months;~~

- ~~Oral †.~~
- ~~Intravaginal † (eg, ring).~~
- ~~transdermal †.~~

~~Progestogen-only hormonal contraception associated with inhibition of ovulation1 initiated at least 3 months before the first dose of study drug OR combined with a barrier method (male condom, female condom or diaphragm) if shorter till she has been on contraceptive for 3 months;~~

- ~~oral †.~~
- ~~Injectable.~~
- ~~Implantable.~~

2. ~~If genotoxicity/teratogenicity/embryotoxicity is unlikely to be caused by the investigational drug, comparator, background therapy or standard of care medications effective methods~~**Effective method** of contraception (there may be a higher than 1% failure rate) are:
   - Double-barrier method (contraceptive sponge, diaphragm or cervical cap with spermicidal jellies or creams PLUS male condom).
   - ~~Progestogen only hormonal contraception, where inhibition of ovulation is not the primary mode of action PLUS condom with or without spermicide.~~

Rationale for Change: To update template language to correctly match required conditions for the study

The following sections also contain this change:

- No other section

Change 18: Revisions to the Schedule of Study Procedures table and footnotes.
The primary changes occur in Section 3.0 Schedule of Study Procedures
Full Physical Exam Assessment added at visits 3,4,5 and ET
Vital signs Other vital signs (RR, tympanic temperature) added on Day 2 of Period 4 (Ketamine)
12 lead ECG: Assessments added: Day 1 Period 4 (Ketamine) and ET visit
Urine pregnancy test added: Screening
Plasma sample for TAK-653 PK added: ET visit
Footnote to ECG: Post treatment ECG in all treatment periods before discharge
Treatment Period 1 to 3: 90 minute assessment column deleted as no assessments occur
CCI [redacted]
Treatment period 4: • 100 and 480, and 25 hour columns deleted as no assessments assigned. • The 70 minute column for ECG and vital signs adjusted to 75 minutes. • Ketamine administration shown as 0 to 40 minute infusion column. • CCI [redacted]
C-SSRS 24 hr assessment added; **ECG** 24 hour assessment added

ELECTRONIC SIGNATURES

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy HH:mm 'UTC')
PPD	Clinical Approval	08-Apr-2019 20:13 UTC
	Biostatistics Approval	08-Apr-2019 21:51 UTC