Rugen Holdings (Cayman) Limited

Investigational Medicinal Product

B-124a

REVISED CLINICAL PROTOCOL

A Phase 1, Single-center, Randomized, Double-blind, Placebo-controlled Trial to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Single Ascending Oral Doses of B-124a in Healthy Subjects

A Study in Healthy Men and Women to Assess the Safety and Tolerability of Different Doses of B-124a and Their Uptake and Clearance From the Body

Protocol No. X06-201-00001
IND No. 153807

CONFIDENTIAL — PROPRIETARY INFORMATION

Drug Development Phase:	1
Sponsor:	Rugen Holdings (Cayman) Limited
Immediately Reportable Event	IQVIA Lifecycle Safety Phone: 855-638-2229 Fax: 855-638-1674 Email: QLS.OtsukaPKD@Quintiles.com
Amendment 4 Approval:	02 Sep 2022
Amendment 3 Approval:	10 Feb 2022
Amendment 2 Approval:	17 Dec 2021
Amendment 1 Approval:	05 Aug 2021
Approval:	25 May 2021

Table of Contents

List of In-text Tables

List of In-text Figures

List of Abbreviations

Abbreviation	Definition
ABPM	Ambulatory blood pressure monitoring
AE	Adverse event
AESI	Adverse event of special interest
API	Active pharmaceutical ingredient
AUC	Area under the concentration-time curve
AUC_{∞}	Area under the concentration-time curve from time zero to infinity
AUC_t	Area under the concentration-time curve calculated to the last observable concentration at time t
AUC_{∞}/dose	AUC_{∞} normalized to dose
AUC_t/dose	AUC_t normalized to dose
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
BPRS	Brief Psychiatric Rating Scale
CADSS	Clinician Administered Dissociative States Scale
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Science
CL/F	Apparent clearance of the drug normalized to body weight
C_{max}	Maximum (peak) plasma concentration of the drug
C_{max}/dose	C_{max} normalized to dose
CRO	Clinical Research Organization
C-SSRS	Columbia-Suicide Severity Rating Scale
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DRC	Dosing Review Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
eICF	Electronic informed consent form
ET	Early termination
FDA	Food and Drug Administration
FIH	First-in-human
FOCBP	Females of childbearing potential
FSH	Follicle-stimulating hormone
GCP	Good clinical practice
GPV	Global Pharmacovigilance
HIV	Human immunodeficiency virus
HR	Heart rate
IB	Investigator's Brochure
IC_{50}	Concentration of the drug producing 50% inhibition
ICF	Informed consent form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors

Abbreviation	Definition
ID	Identifier
IMP	Investigational medicinal product
IND	Investigational New Drug
IRB	Institutional review board
IRE	Immediately reportable event
MOAA/S	Modified Observer's Assessment of Alertness/Sedation Scale
MTD	Maximum tolerated dose
NAM	Negative allosteric modulator
NMDA	N-methyl-D-aspartate
NOAEL	No observed adverse effect level
OPDC	Otsuka Pharmaceutical Development & Commercialization, Inc
PD	Pharmacodynamic(s)
PGx	Pharmacogenomic(s)
PK	Pharmacokinetic(s)
PQC	Product quality complaint
qEEG	Quantitative electroencephalogram
QTc	Corrected QT interval
ΔQTcF	Change-from-baseline in QT interval corrected for heart rate using Fridericia's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
$t_{1/2,z}$	Terminal-phase elimination half-life
T_3	Triiodothyronine
T_4	Thyroxine
TEAE	Treatment-emergent adverse event
t_{max}	Time to maximum (peak) plasma concentration
TRD	Treatment-resistant depression
ULN	Upper limit of normal
US	United States
VAS	Visual analog scale
WBC	White blood cell

1 Protocol Summary

1.1 Synopsis

Name of Sponsor: Rugen Holdings (Cayman) Limited

Name of Investigational Medicinal Product: B-124a

Protocol No.: X06-201-00001

IND No.: 153807

Protocol Title: A Phase 1, Single-center, Randomized, Double-blind, Placebo-controlled Trial to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Single Ascending Oral Doses of B-124a in Healthy Subjects

Protocol Lay Person Short Title: A Study in Healthy Men and Women to Assess the Safety and Tolerability of Different Doses of B-124a and Their Uptake and Clearance From the Body

Clinical Phase: 1

Treatment/Indication: Treatment-resistant depression

Objectives and Endpoints:

Objectives	Endpoints
Primary: Arms 1 and 2: To determine the safety, tolerability, and PK of B-124a (API capsule and the liquid-filled capsule formulation) following ascending single oral doses in healthy subjects.	Safety and tolerability: Incidence of AEs, vital sign measurements, safety ECGs, clinical laboratory tests, physical/ neurological examinations, neurological scale scores, and the C-SSRS. PK: C_{max}, t_{max}, AUC_t, AUC_∞, CL/F, $t_{1/2,z}$, C_{max}/dose, AUC_t/dose, and AUC_∞/dose.
Secondary: Arms 1 and 2: To determine the effect of B-124a (API capsule and the liquid-filled capsule formulation) plasma concentrations on ECG parameters, including QTc, in healthy subjects.	PD: ΔQTcF; the relationship between B-124a plasma concentrations and QTc.

Objectives	Endpoints
Exploratory: Arm 1 and 2: To explore the relationship between B-124a (API capsule and the liquid-filled capsule formulation) plasma exposure and hemodynamic effect and other PD effects, if applicable.	Change in SBP and DBP (other endpoints, such as HR, may be explored), and qEEG measures (including, but not limited to, gamma oscillation activity).
Arms 1 and 2: To explore the relationship between B-124a plasma (API capsule and the liquid-filled capsule formulation) exposure and neurological scales.	Change in selected neurological scale scores (including, but not limited to, the CADSS and MOAA/S).

AE = adverse event; AUC_∞ = area under the concentration-time curve from time zero to infinity; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t; AUC_∞/dose = AUC_∞ normalized to dose; AUC_t/dose = AUC_t normalized to dose; CADSS = Clinician Administered Dissociative States Scale; CL/F = apparent clearance of the drug normalized to body weight; C_{max} = maximum (peak) plasma concentration of the drug; C_{max}/dose = C_{max} normalized to dose; C-SSRS = Columbia-Suicide Severity Rating Scale; DBP = diastolic blood pressure; ECG = electrocardiogram; HR = heart rate; MOAA/S = Modified Observer's Assessment of Alertness/Sedation Scale; PD = pharmacodynamics; PK = pharmacokinetics; qEEG = quantitative electroencephalogram; QTc = corrected QT interval; ΔQTcF = change-from-baseline in QT interval corrected for heart rate using Fridericia's formula; SBP = systolic blood pressure; $t_{1/2,z}$ = terminal-phase elimination half-life; t_{max} = time to maximum (peak) plasma concentration.

Trial Design:

This is a phase 1, single-center trial to assess the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single ascending oral doses of B-124a (Arm 1) and the liquid-filled capsule formulation B-124a (Arm 2) in healthy subjects.

In general, there will be 2 tested drug product presentations: a powder in capsule in Arm 1 and a liquid-filled capsule formulation in Arm 2.

The trial will consist of a screening period (Day −45 through Day −2), check-in (Day −1), in-clinic stay (minimum of 8 days), and a safety follow-up telephone call 30 (+ 2) days after the last dose of B-124a (the investigational medicinal product [IMP]) to assess any new or ongoing adverse events (AEs) and to record concomitant medications. The in-clinic stay will be 8 days.

Arm 1:

Arm 1 is a randomized, double-blind, placebo-controlled, single ascending oral dose administration of B-124a to healthy subjects in a fasted state.

Dosing is planned to be conducted in 7 cohorts of 8 subjects each. The proposed doses for Cohorts 1, 2, 3, 4, 5, 6, and 7 are 3, 6, 12, 25, 50, 100, and 200 mg of B-124a, respectively, or placebo. Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce systolic blood pressure [SBP] changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7.

For each cohort in Arm 1, subjects will be randomized on Day 1 to a single oral dose of B-124a or matching placebo in a 6:2 ratio (6 on B-124a subjects and 2 placebo subjects) and dosed in 2 groups (3 sentinel subjects [2 on B-124a and 1 on placebo] and the remaining subjects [4 on B-124a and 1 on placebo]). For each cohort, the second group will be dosed at least 24 hours after the sentinel group has been dosed and after AE data for the sentinel group has been reviewed and agreed by the principal investigator that it is safe and appropriate to dose the remaining subjects. The principal investigator will notify the Clinical Research Organization (CRO) medical monitor and the Otsuka Pharmaceutical Development & Commercialization, Inc (OPDC) Global Clinical Development (GCD) and Global Pharmacovigilance (GPV) representatives that dosing may continue in the cohort.

Once all the data for dose selection for the cohort are available, the unblinded safety, tolerability, PK (PK sampling data through 24 hours postdose), and/or PD data collected from each cohort will be provided to the Dosing Review Committee (DRC) for review. The investigator will remain blinded. The unblinded data from each cohort will be evaluated by the DRC to determine if the dose for the next cohort will be escalated as planned, if the dose from the previous cohort will be repeated, if the dose will be increased, or if the dose will be decreased. Dose escalation may be modified or stopped based upon the sponsor's and/or investigator's clinical judgment at any time. Dose escalation will continue until a non-tolerated dose is reached and the maximum tolerated dose (MTD) is established or the last cohort is completed. The DRC will consist of OPDC and Rugen trial personnel, including GPV, Clinical Management, Clinical Pharmacology, Medical, and Biostatistical representatives, CRO Medical representatives, as well as other staff, as needed.

The decision to proceed to the next cohort will be based on the observance of no serious treatment-emergent adverse events (TEAEs) and no more than 2 out of 6 subjects who received B-124a (after unblinding the cohort) experiencing toxicity ≥ Grade 3, as defined in the United States (US) Food and Drug Administration (FDA) Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials," with associated clinical signs and symptoms except blood pressure (BP). Grade 3 toxicities in vital signs and laboratory parameter assessments must be confirmed with a repeat measurement. If the dose for a cohort is determined not to be safe or well tolerated (ie, if, after unblinding the cohort, any subject who received B-124a experienced a serious TEAE with a reasonable possibility of a causal relationship to the IMP, or if ≥ 2 out of 6 subjects who received B-124a experienced toxicity ≥ Grade 3 with associated clinical signs and symptoms), the preceding dose level will then be considered as the MTD. The DRC will be provided with the blinded Council for International Organizations of Medical Science (CIOMS) form of any reported serious TEAE, when applicable, for additional information and details on the serious TEAE. The DRC will rely on the causality assessment (related, not related) as assessed by the independent sponsor medical reviewer and the investigator.

A score of 1 on the Modified Observer's Assessment of Alertness/Sedation Scale (MOAA/S) may be considered as not safe or well tolerated as well. Transient (ie, lasting for less than 2 hours) SBP up to 180 mmHg or a change (eg, increase) from baseline of up to 60 mmHg will be allowed for dose escalation.

Arm 2:

Arm 2 is a randomized, double-blind, placebo-controlled, single ascending oral dose administration of B-124a to healthy subjects in a fasted state.

Dosing is planned to be conducted in 3 or 4 cohorts of 8 subjects each. The starting dose of 72 mg was selected based on the preliminary physiologically-based pharmacokinetic modeling (PBPK) prediction of approximately 30% improvement of C_{max}, well within a 2-fold safety margin of the highest tested dose 200 mg in Arm 1. The dose escalation for the cohorts in Arm 2 will be 150 mg, 250 mg, and an optional cohort. The dose escalation for Arm 2 was based upon the available target engagement/PD data to match the comparable exposure from Arm 1 of approximately 1500 ng/mL (C_{max}) or until the maximum tolerated dose level was reached. The higher dose levels will be subject to change according to the data collected from earlier cohorts. Doses for latter cohorts may be repeated or adjusted based on PK, AEs, and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented.

For each cohort in Arm 2, subjects will be randomized on Day 1 to a single oral dose of B-124a or matching placebo in a 6:2 ratio (6 on B-124a subjects and 2 placebo subjects) and dosed in 2 groups (3 sentinel subjects [2 on B-124a and 1 on placebo] and the remaining subjects [4 on B-124a and 1 on placebo]). For each cohort, the second group will be dosed at least 24 hours after the sentinel group has been dosed and after AE data for the sentinel group has been reviewed and agreed by the principal investigator that it is safe and appropriate to dose the remaining subjects. The principal investigator will notify the CRO medical monitor and the OPDC GCD and GPV representatives that dosing may continue in the cohort.

The dose escalation process for Arm 2 will be the same as in Arm 1.

Trial Population:

Approximately 88 healthy subjects (56 subjects in Arm 1 and up to 32 subjects in Arm 2) are expected to be enrolled in the trial. Subjects will not be allowed to be enrolled and receive IMP in more than one cohort. Subjects who withdraw for reasons other than AEs may be replaced at the discretion of the sponsor.

Key Inclusion/Exclusion Criteria:

Male or female subjects between the ages of 18 and 55 years (inclusive), with a body mass index (BMI) between 18.0 and 32.0 kg/m^2 (inclusive), who are in good health as determined by medical history, physical and neurological examination, electrocardiogram (ECG), and serum/urine chemistry, hematology, and serology tests, and who are able to provide informed consent will be considered for inclusion in the trial.

Trial Site(s):

This will be a single-center trial conducted in the US.

Investigational Medicinal Product(s), Dose, Dosage Regimen, Treatment Duration, Formulation, Mode of Administration:

The IMP (B-124a) will be provided to the site as active pharmaceutical ingredient (API). The clinical pharmacy will compound capsules of the appropriate strengths using the API prepared at the site; the IMP should be prepared on the day of dosing no more than 6 hours prior to administration. Doses of IMP will be taken with 240 mL of still water or 240 mL of 100% cranberry juice.

Arm 1:

For Arm 1, up to 7 dose cohorts are planned. The proposed doses for Arm 1 are 3, 6, 12, 25, 50, 100, and 200 mg. Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce SBP changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7.

In Arm 1, a single oral dose of B-124a or placebo will be taken on Day 1. Treatments will be administered following an overnight fast of at least 10 hours and subjects will continue to fast for at least 4 hours after dosing. Water will be available ad libitum except for ± 1 hour around dosing.

Arm 2:

For Arm 2, 3 or 4 dose cohorts are planned. The proposed doses for Arm 2 are 72, 150, and 250 mg while Cohort 4 will be optional. Doses for cohorts may be repeated or adjusted based on PK, AEs, and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented.

In Arm 2, a single oral dose of B-124a or placebo will be taken on Day 1. Treatments will be administered following an overnight fast of at least 10 hours and subjects will continue to fast for at least 4 hours after dosing. Water will be available ad libitum except for ± 1 hour around dosing.

Trial Assessments:

Assessments for pharmacokinetics: blood sampling for B-124a plasma concentrations and PK parameters.

Assessments for pharmacodynamics: continuous (ie, Holter) 12-lead ECG extraction to assess the effect of B-124a on ECG parameters, including corrected QT interval (QTc); change from baseline in amplitude across quantitative electroencephalogram (qEEG) frequency bands and qEEG-derived measures (separately for oscillatory and fractal parts).

Assessments for pharmacogenomics: blood sampling for pharmacogenomic (PGx) testing.

Assessments for safety: AEs, clinical laboratory tests, vital signs (including ambulatory blood pressure monitoring [ABPM]), 12-lead ECGs, physical examinations, neurological examinations, the Columbia-Suicide Severity Rating Scale (C-SSRS), neurological scales (Clinician Administered Dissociative States Scale [CADSS], MOAA/S, Brief Psychiatric Rating Scale [BPRS], and the Cogstate Safety Battery + the One Back Test), and the Bond-Lader visual analog scale (VAS).

Screening/Other: demographics, medical and medication history, serum hepatitis and human immunodeficiency virus (HIV) screen, urine alcohol test and drug screen, urine (and confirmatory serum, if needed) pregnancy test (for females of childbearing potential [FOCBP]), follicle-stimulating hormone (FSH; for confirmation of postmenopausal females), and height/weight/BMI.

Dosing Review Committee:

Refer to the Trial Design section of the Synopsis for details.

Statistical Methods:

This trial is not powered for statistical comparisons of PK parameters and the sample size of 88 subjects was chosen from practical considerations. The number of subjects per cohort (6 active and 2 placebo) in Arms 1 and 2 is based on previous experience with other drugs for first-in-human trials. The number of subjects per dose level in Arms 1and 2 is generally considered to be adequate for determination of tolerability and estimation of the PK parameters.

Safety variables to be analyzed include AEs, clinical laboratory tests, vital sign measurements, safety ECGs, physical examinations, neurological examinations, neurological scale scores (CADSS, MOAA/S, BPRS, and the Cogstate Safety Battery + the One Back Test), and the C-SSRS. The safety analysis will be performed for each arm. Safety data will be summarized using descriptive statistics (as applicable).

In general, individual table and summary tables using descriptive statistics (N, median, mean, standard deviation, percent coefficient of variation, minimum, and maximum) will be presented for plasma concentrations and PK parameters. The individual, mean, and median plots of plasma concentrations will also be provided.

An exposure-response analysis will be attempted to examine the relationship between QTc and plasma concentrations of B-124a from the PK/QTc population using a linear mixed-effects modeling approach.

To explore the relationship between B-124a plasma exposure and change in BP, qEEG measures, and neurological scale scores (ie, CADSS and MOAA/S), exploratory PK/PD and/or exposure-response analyses will be attempted to understand the potential activity of B-124a and dissociation effect in healthy subjects.

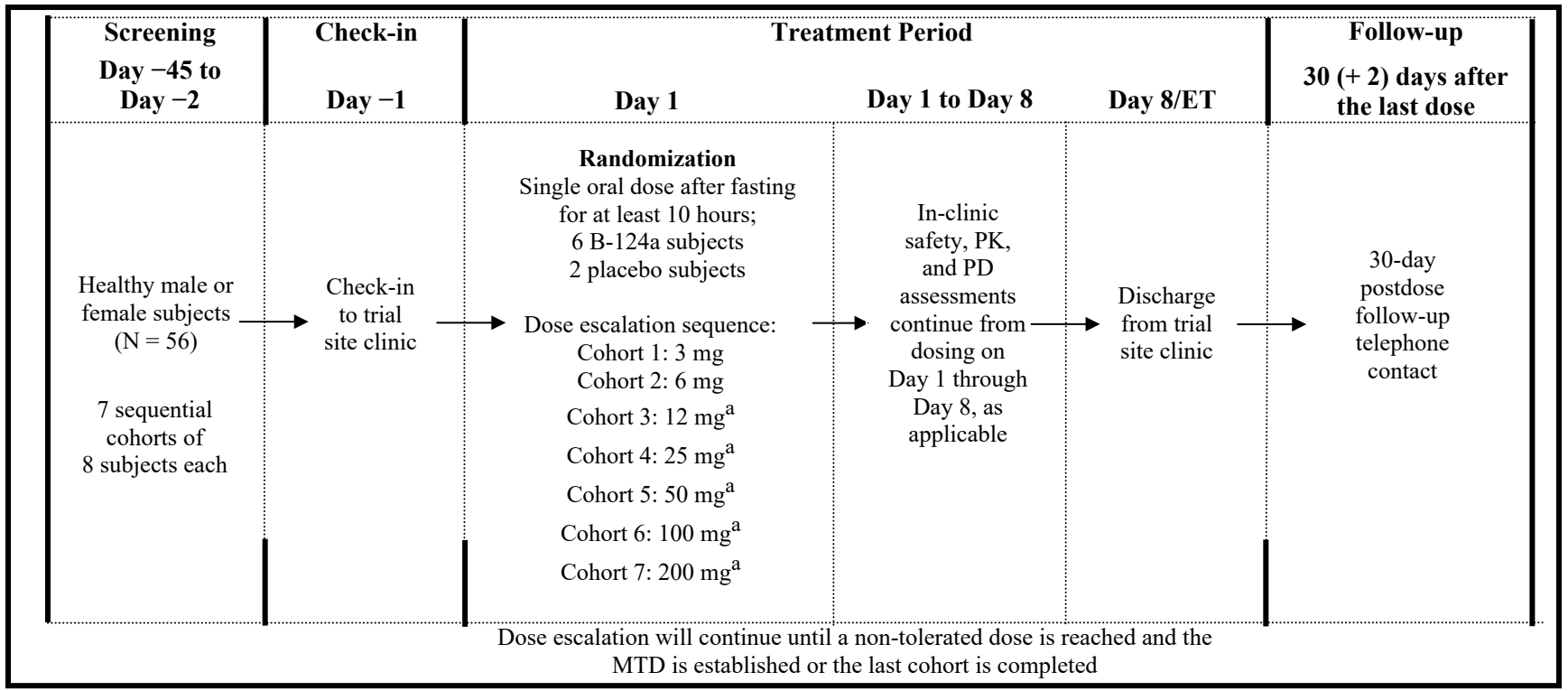
Trial Duration:

Each subject in this trial is expected to participate in the following periods of the trial (approximate durations listed), for up to 76 (+ 2) days:

- Eligibility screening period: 44 days (Day −45 to Day −2)
- Check-in: 1 day (Day −1)
- In-clinic treatment and assessment period: minimum of 8 days (Day 1 to discharge/ET)
- Post-treatment follow-up: 30 (+ 2) days after the last dose of IMP

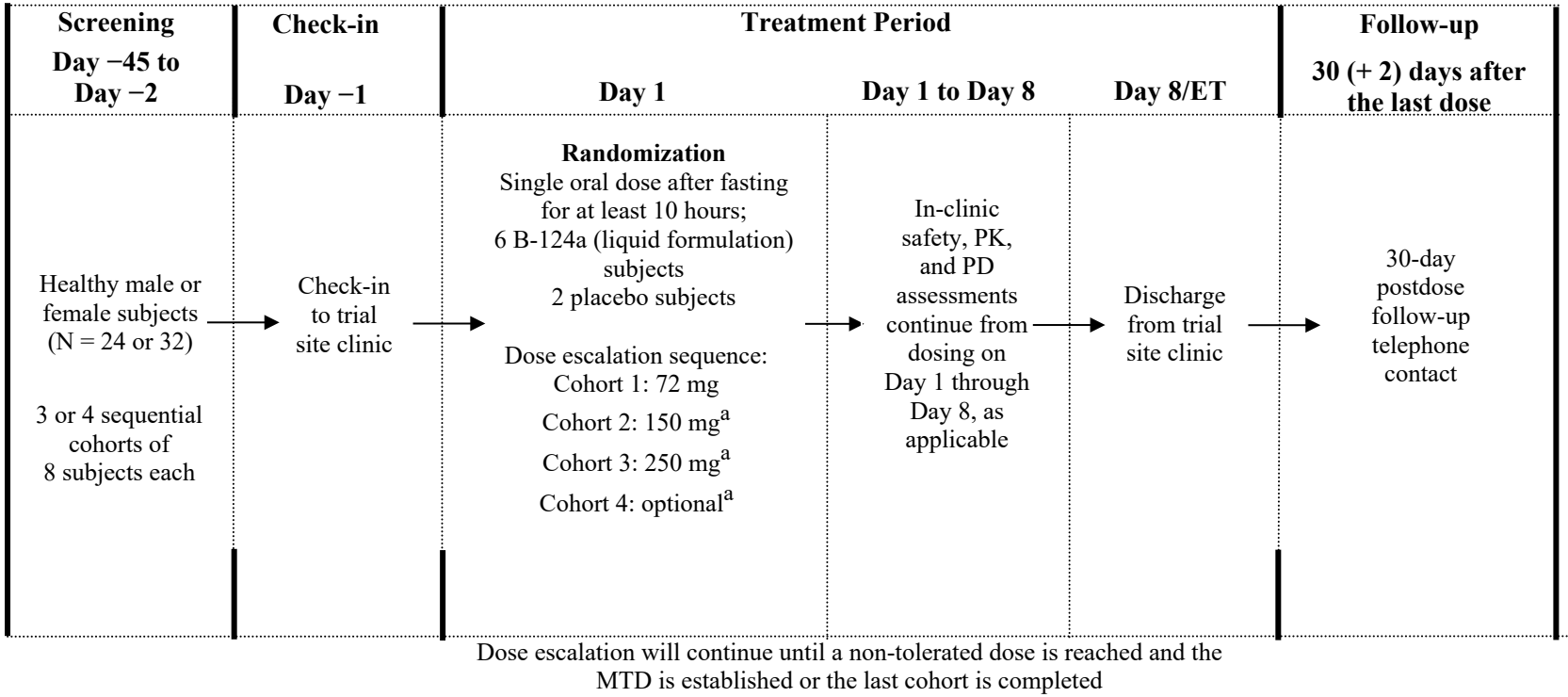
Overall, the trial duration from signing of the first informed consent form to the final subject assessment is expected to be approximately 7 months.

1.2 Schema



[a]Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce SBP changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7.

Figure 1.2-1 Trial Design Schematic for Arm 1



[a]Doses for later cohorts may be repeated or adjusted based upon the PK, AEs and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented. The dose escalation for Arm 2 was based upon the available target engagement/PD data to match the comparable exposure from Arm 1 of approximately 1500 ng/mL (C_{max}) or until the maximum tolerated dose level was reached.

Figure 1.2-2 Trial Design Schematic for Arm 2

1.3 Schedule of Assessments

Table 1.3-1 Schedule of Assessments for Arms 1 and 2

Assessment	Screening	Check-in	Treatment Period[a]								Telephone Follow-up	Section (if applicable)
	Day −45 to Day −2	Day −1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8/ ET	30 (+ 2) days after the last dose	
Screening												
Informed consent	X											Section 5.4, Section 10.1.2
Inclusion/exclusion criteria	X	X										Section 5.2
Demographic information	X											Section 5.1
Medical history	X											
Height and weight[b]	X	X								X		Section 8.7.2
Urine (and confirmatory serum, if needed) pregnancy test (for FOCBP)	X	X								X		Section 10.3
FSH test (confirmation of postmenopausal female subjects)	X											
Urine alcohol and drug screen	X	X										Section 5.2.2
Hepatitis and HIV screen	X											Section 5.2.2
Trial Residency												
Check-in		X										
Randomization			X									Section 6.3
Discharge from clinic										X		
IMP Administration												
B-124a/placebo dosing			X									Section 6.1
Pharmacokinetics and Pharmacogenomics												
PK blood samples[c]			X	X	X	X	X	X	X	X		Section 8.2.1
PGx blood sample		X										Section 8.4.1
Safety and Tolerability												

Table 1.3-1 Schedule of Assessments for Arms 1 and 2

	Screening	Check-in	Treatment Period[a]								Telephone Follow-up	Section (if applicable)
Full physical/neurological examination	X	X								X		Section 8.7.2, Section 8.7.6.1
Targeted physical/ neurological examination[d]			X	X		X		X				Section 8.7.2, Section 8.7.6.1
Hematology, serum chemistry, coagulation, and urinalysis[e]	X	X	X	X	X					X		Section 8.7.1
Vital signs[f]	X	X	X	X	X	X	X	X	X	X		Section 8.7.3
ABPM/extraction[g]	X	X	X	X	X	X	X	X	X	X		Section 8.7.3
12-lead safety ECG[h]	X	X	X	X	X					X		Section 8.7.4
C-SSRS[i]	X	X	X			X				X		Section 8.7.5
CADSS[j]		X	X	X						X		Section 8.7.6.2.1
MOAA/S[j]		X	X	X						X		Section 8.7.6.2.2
BPRS[k]		X	X	X						X		Section 8.7.6.2.3
Cogstate Safety Battery + the One Back Test[l]		X	X	X						X		Section 8.7.6.2.4
Bond-Lader VAS[m]		X	X	X						X		Section 8.7.6.3
Record adverse events	<----------X---------->											Section 8.8
Record concomitant medications		<----------X---------->										Section 6.5
Pharmacodynamics												
Holter monitor/extraction			<----X[n]-->									Section 8.3
qEEG[o]		X	X	X								Section 8.3

Note: The sampling time points may be reduced in subsequent dosing cohorts based on data from earlier dosing cohorts for ABPM, CADSS, MOAA/S, BPRS, Cogstate Safety Battery + the One Back Test, and the Bond-Lader VAS.

[a]There will be 1 treatment period to assess 1 dose of IMP (6 active and 2 placebo).

[b]Height will be measured only at screening. The BMI will be calculated based on the weight and height information.

[c]The blood samples for PK analysis will be collected for all cohorts on Day 1: predose and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, and 16 hours postdose; Day 2: 24 and 36 hours postdose; Day 3: 48 hours postdose; Day 4: 72 hours postdose; and ET. Additional blood samples for PK analysis will also be collected for Cohorts 3 to 7 only on Day 5: 96 hours postdose, Day 6: 120 hours postdose, Day 7: 144 hours postdose, and Day 8: 168 hours postdose. The PK sampling time points may be changed in subsequent dosing cohorts in Arm 1 once PK data from earlier dose cohorts are available.

[d]A targeted physical and neurological examination will be performed on Day 1: 7 hours postdose, Day 2: 24 hours postdose, Day 4: 72 hours postdose, and Day 6: 120 hours postdose. The targeted neurological examination may trigger additional neurological scale administration (eg, BPRS, CADSS, and/or MOAA/S).

[e]Clinical laboratory samples will be collected on Day 1: 4 and 12 hours postdose, Day 2: 24 hours postdose, Day 3: 48 hours postdose, and Day 8/ET.

[f]Vital signs will be measured on Day 1: predose and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 16 hours postdose; Day 2: 24 hours postdose; Day 3: 48 hours postdose; Day 4: 72 hours postdose; and Day 8 (168 hours postdose)/ET. Additional vital signs will also measured for Cohorts 3 to 7 (Arm 1) and Cohorts 1 to 4 (Arm 2) only on Day 5: 96 hours postdose, Day 6: 120 hours postdose, and Day 7: 144 hours postdose.

[g]ABPM will occur for all cohorts on Day 1: predose, every 15 minutes during the 4-hour postdose period, and then hourly until 12 hours postdose. If BP does not stabilize (ie, return to baseline or clinically stable based on medical judgment) by 12 hours postdose, BP will be monitored and additional time points will be added until BP is clinically stable based on medical judgment. ABPM will occur for all cohorts in the supine position until 24 hours postdose, and in both the supine and standing positions for all cohorts on Day 2: 24 hours postdose; Day 3: 48 hours postdose; Day 4: 72 hours postdose; and Day 8 (168 hours postdose)/ET. Additional ABPM will also occur in both the supine and standing positions for Cohorts 3 to 7 (Arm 1) and Cohorts 1 to 4 (Arm 2) only on Day 5: 96 hours postdose, Day 6: 120 hours postdose, and Day 7: 144 hours postdose.

[h]ECGs will be performed on Day 1: predose and at 1, 2, 4, and 12 hours postdose; Day 2: 24 hours postdose; Day 3: 48 hours postdose; and Day 8/ET.

[i]The C-SSRS will be administered on Day 1: 8 hours postdose, Day 4: 72 hours postdose, and Day 8/ET.

[j]The CADSS and the MOAA/S will be administered on Day 1: 1, 2, and 3 hours postdose; Day 2: 24 hours postdose; and Day 8/ET. If symptoms persist beyond the 3-hour postdose assessments, the investigator may choose to collect additional measures until symptoms have resolved.

[k]The BPRS will be administered on Day 1: 8 hours postdose, Day 2: 24 hours postdose; and Day 8/ET.

[l]The Cogstate Safety Battery + the One Back Test will be administered on Day 1: 3 and 8 hours postdose, Day 2: 24 hours postdose; and Day 8/ET.

[m]The Bond-Lader VAS will be administered on Day 1: 3 and 8 hours postdose, Day 2: 24 hours postdose; and Day 8/ET.

[n]Holter extractions will be completed on Day 1: predose (at −45, −30, and −15 minutes) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 16 hours postdose; and Day 2: 24 hours postdose.

[o]A qEEG will be performed on Day 1: predose and at 0.75 hours postdose, and Day 2: 24 hours postdose. Additional time points may be added on Day 1 (eg, 1, 2, and 4 hours postdose) for Cohorts 2 through 7 (Arm 1) and Cohorts 2, and 3, and/or 4 (Arm 2) depending on analysis of the PK data from Cohort 1.

2 Introduction

B-124a is a new molecular entity with potential antidepressant activity for treatment-resistant depression (TRD). B-124a is a negative allosteric modulator (NAM) binding to the NR2B subunit of the N-methyl-D-aspartate (NMDA) glutamate receptor. Aberrant NMDA receptor-mediated glutamate transmission has been linked with depression.[1] The behavioral properties of B-124a have been shown in several relevant animal models, including a rat model of depression. The potential of TRD patients benefiting from B-124a will be further evaluated during drug development.

Trial X06-201-00001 is the first-in-human (FIH) trial for B-124a. This trial is designed to assess the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of B-124a following single ascending oral doses in healthy subjects. The effect of a high-fat meal will also be determined.

Please refer to the B-124a Investigator's Brochure (IB) for more detailed information.

2.1 Trial Rationale

B-124a was efficacious in preclinical models of anxiety, depression, and obsessive-compulsive disorder. This trial is the FIH trial for B-124a and is designed to determine the safety, tolerability, PK, and PD of B-124a following single ascending oral doses in healthy subjects. The effect of food on B-124a safety, tolerability, and PK will also be determined. In addition, the effect of B-124a on blood pressure (BP), quantitative electroencephalogram (qEEG) measures, and neurological scale scores will be explored. The data gathered from this trial will inform subsequent clinical trials for B-124a, including a multiple ascending dose trial and a phase 1b/proof-of-concept trial in subjects with TRD.

2.2 Background

2.2.1 Nonclinical Data

2.2.1.1 Pharmacology

B-124a is an orally bioavailable NAM of the NMDA receptor with conferred selectivity to the NR2B subunit. In vivo efficacy has been demonstrated after oral administration in preclinical models. Based on the results of the in vitro and in vivo data, B-124a is expected to produce sustained efficacy, analogous to the effects of ketamine.

2.2.1.2 Nonclinical Pharmacokinetics

The PK of B-124a has been studied in animal species. The oral bioavailability of B-124a was 22.3% in monkeys, 65.3% in mice, and 97.3% in rats. B-124a is highly brain permeable and is not a P-glycoprotein substrate. The brain to plasma concentration ratios were 0.498 at 8 hours postdose in rats and 0.980 at 0.25 hours postdose in mice after oral administration. B-124a is highly bound to plasma proteins as < 2% is found unbound in mouse, rat, monkey, and human plasma.

In vitro metabolism studies identified 2 metabolites, both of which were detected in rat, dog, monkey, and human hepatocytes. The more predominant metabolite in all species was generated by mono-oxygenation and glucuronide conjugation. Cytochrome P450 (CYP) 2C19, CYP2D6, and CYP3A4 are likely the main CYP enzymes involved in the metabolism of B-124a, with CYP1A2 and CYP2C9 as minor contributors. In human liver microsomes, B-124a caused moderate inhibition of CYP2C9 (concentration of the drug producing 50% inhibition [IC_{50}] = 9.83 μM); weak inhibition of CYP1A2 (IC_{50} = 24.7 μM), CYP2C19 (IC_{50} = 12.3 μM), and CYP2D6 (IC_{50} = 40.5 μM); and no inhibition of CYP3A4 (IC_{50} > 50.0 μM).

2.2.1.3 Safety Pharmacology and Toxicology

Safety pharmacology studies examined the effects of B-124a on neurobehavioral function and the cardiovascular and respiratory systems. At doses of 10, 30, and 100 mg/kg, B-124a has no effect on peripheral or central nervous system functions of male Sprague Dawley rats, as determined using the modified Irwin test. In male and female Sprague Dawley rats and female cynomolgus monkeys, increased arterial systolic blood pressure (SBP) and heart rate (HR) were observed, as expected for NMDA receptor antagonists. At 0.1 mg/kg the effects were minimal, although higher doses (0.2 - 3.0 mg/kg) increased arterial SBP and HR in a dose- and sex-dependent manner, with peak effects predominantly occurring at 1 hour postdose. The arterial SBP returned to baseline by 24 hours in both males and females, while the HR returned to baseline at 4 hours in males and 24 hours in females. In monkeys that were administered a single oral dose of 5.0, 15.0, or 45.0 mg/kg, there were modest, transient increases in systolic and mean arterial BP that subsided after the first 6 hours postdose, and HR at 18 to 24 hours postdose. The no observed adverse effect level (NOAEL) in the cardiovascular safety study was thus considered to be 45 mg/kg. B-124a inhibited the human ether-a-go-go-related gene (hERG) current at high concentrations (IC_{50} = 5.29 μM). There were no changes in electrocardiogram (ECG) morphology in monkeys at single doses of up to 45 mg/kg, the highest dose tested in the cardiovascular study. No ECG changes were observed in the

nonhuman primate 28-day daily repeat dose toxicology study at doses of up to 120 mg/kg/day.

B-124a was not mutagenic and did not cause chromosomal damage in in vitro and in vivo genotoxicity studies. A neurotoxicity study was conducted to provide an extensive review of the pathology of the brains of rats administered B-124a and there were no neurotoxicological findings related to B-124a.

Repeat-dose toxicity studies of up to 4 weeks have been conducted in Sprague Dawley rats and cynomolgus monkeys. The Sprague Dawley rats tolerated doses of up to 30 mg/kg of daily administration. At 100 mg/kg there were 2 early deaths, and at ≥ 30 mg/kg/day aggressiveness was observed. Aggressiveness was also seen in some females at 10 mg/kg/day, although at a reduced incidence. Mean body weights at the end of the treatment period were lower than the control at 3, 10, 30, and 100 mg/kg, but reached significance only in females. Microscopic findings were mainly limited to effects on reproductive and mammary tissues related to disturbances in the gonadotropin-releasing hormone-luteinizing hormone axis, consistent with NMDA antagonism. The lesions included minimal atrophy of the prostate at 100 mg/kg/day, hyperplasia of the mammary glands in males and females at 10, 30, and 100 mg/kg/day and in females only at 1 and 3 mg/kg/day, perturbations of the estrus cycle at 3, 10, 30, and 100 mg/kg/day, and decreased corpora lutea and follicular and/or luteal cysts at 0.3 to 100 mg/kg/day. Only reproductive findings at doses ≥ 30 mg/kg/day in males and 3 mg/kg/day in females were considered adverse. When the 10 mg/kg dose was administered biweekly, which is the expected dosing frequency in patients, the female rats developed minimal lobuloalveolar hyperplasia and minimal to mild decreased corpora lutea, but no follicular or luteal cysts were seen. Thus, based on the reproductive findings, the NOAEL in rats was determined to be 10 mg/kg biweekly in males and females, 10 mg/kg/day in males, and 1 mg/kg/day in females. Monkeys were administered B-124a at doses of 15, 45, and 120 mg/kg/day. In monkeys, postural instability was seen after a single dose of 300 mg/kg. At doses of 15 to 120 mg/kg/day in females and 45 to 120 mg/kg/day in males, transient decreased activity, tremors, and/or unsteady gait was observed, which disappeared on Day 4 of dosing. There were limited or no other in vivo or pathological findings and the NOAEL in monkeys was determined to be 120 mg/kg/day.

2.2.2 Clinical Data

No clinical trials with B-124a have been initiated; this will be the first clinical trial conducted in humans.

2.3 Known and Potential Risks and Benefits

The safety and efficacy of B-124a in human subjects has not been established. Possible effects in humans can only be predicted based on the results from safety pharmacology and toxicology studies, which are described in more detail in Section 2.2.1. Nonclinical toxicity studies have not identified any unique safety issues that will require additional monitoring in trial subjects.

The trial site will receive updated versions of the IB, when available, and the trial site should refer to the most current version as needed.

3 Objectives and Endpoints

Table 3-1 Trial Objectives and Endpoints

Objectives	Endpoints
Primary: Arms 1 and 2: To determine the safety, tolerability, and PK of B-124a (API capsule and the liquid-filled capsule formulation) following ascending single oral doses in healthy subjects.	Safety and tolerability: Incidence of AEs, vital sign measurements, safety ECGs, clinical laboratory tests, physical/ neurological examinations, neurological scale scores, and the C-SSRS. PK: C_{max}, t_{max}, AUC_t, AUC_∞, CL/F, $t_{1/2,z}$, C_{max}/dose, AUC_t/dose, and AUC_∞/dose.
Secondary: Arms 1 and 2: To determine the effect of B-124a (API capsule and the liquid-filled capsule formulation) plasma concentrations on ECG parameters, including QTc, in healthy subjects.	PD: ΔQTcF; the relationship between B-124a plasma concentrations and QTc.
Exploratory: Arms 1 and 2: To explore the relationship between B-124a (API capsule and the liquid-filled capsule formulation) plasma exposure and hemodynamic effect and other PD effects, if applicable. Arms 1 and 2: To explore the relationship between B-124a (API capsule and the liquid filled capsule formulation) plasma exposure and neurological scales.	Change in SBP and DBP (other endpoints, such as HR, may be explored), and qEEG measures (including, but not limited to, gamma oscillation activity). Change in selected neurological scale scores (including, but not limited to, the CADSS and MOAA/S).

AE = adverse event; AUC_∞ = area under the concentration-time curve from time zero to infinity; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t; AUC_∞/dose = AUC_∞ normalized to dose; AUC_t/dose = AUC_t normalized to dose; CADSS = Clinician Administered Dissociative States Scale; CL/F = apparent clearance of the drug

normalized to body weight; C_{max} = maximum (peak) plasma concentration of the drug; C_{max}/dose = C_{max} normalized to dose; DBP = diastolic blood pressure; MOAA/S = Modified Observer's Assessment of Alertness/Sedation Scale; PD = pharmacodynamics; QTc = corrected QT interval; ΔQTcF = change-from-baseline in QT interval corrected for heart rate using Fridericia's formula; $t_{1/2,z}$ = terminal-phase elimination half-life; t_{max} = time to maximum (peak) plasma concentration.

Section 9.4 describes the statistical analysis of the endpoints.

4 Trial Design

4.1 Type/Design of Trial

This is a phase 1, single-center trial to assess the safety, tolerability, PK, and PD of single ascending oral doses of B-124a (Arm 1) and the liquid-filled capsule formulation B-124a (Arm 2) in healthy subjects.

In general, there will be 2 tested drug product presentations: a powder in capsule in Arm 1 and a liquid-filled capsule formulation in Arm 2.

The trial will consist of a screening period (Day −45 through Day −2), check-in (Day −1), in-clinic stay (minimum of 8 days), and a safety follow-up telephone call 30 (+ 2) days after the last dose of B-124a (the investigational medicinal product [IMP]) to assess any new or ongoing adverse events (AEs) and to record concomitant medications. The in-clinic stay in Arms 1and 2 will be 8 days.

4.1.1 Arm 1

Arm 1 is a randomized, double-blind, placebo-controlled, single ascending oral dose administration of B-124a to healthy subjects in a fasted state (see Section 6.1.1 for details).

Dosing is planned to be conducted in 7 cohorts of 8 subjects each. The proposed doses for Cohorts 1, 2, 3, 4, 5, 6, and 7 are 3, 6, 12, 25, 50, 100, and 200 mg of B-124a, respectively, or placebo. Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce SBP changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7.

For each cohort in Arm 1, subjects will be randomized on Day 1, to a single oral dose of B-124a or matching placebo in a 6:2 ratio (6 B-124a subjects and 2 placebo subjects) and

dosed in 2 groups (3 sentinel subjects [2 on B-124a and 1 on placebo] and the remaining subjects [4 on B-124a and 1 on placebo]). For each cohort, the second group will be dosed at least 24 hours after the sentinel group has been dosed and after AE data for the sentinel group has been reviewed and agreed by the principal investigator that it is safe and appropriate to dose the remaining subjects. The principal investigator will notify the Clinical Research Organization (CRO) medical monitor and the Otsuka Pharmaceutical Development & Commercialization, Inc (OPDC) Global Clinical Development (GCD) and Global Pharmacovigilance (GPV) representatives that dosing may continue in the cohort.

Once all the data for dose selection for the cohort are available, the unblinded safety, tolerability, PK (PK sampling data through 24 hours postdose), and/or PD data collected from each cohort will be provided to the Dosing Review Committee (DRC) for review. The investigator will remain blinded. The unblinded data from each cohort will be evaluated by the DRC to determine if the dose for the next cohort will be escalated as planned, if the dose from the previous cohort will be repeated, if the dose will be increased, or if the dose will be decreased. Dose escalation may be modified or stopped based upon the sponsor's and/or investigator's clinical judgment at any time. Dose escalation will continue until a non-tolerated dose is reached and the maximum tolerated dose (MTD) is established or the last cohort is completed. The DRC will consist of OPDC and Rugen trial personnel, including GPV, Clinical Management, Clinical Pharmacology, Medical, and Biostatistical representatives, CRO Medical representatives, as well as other staff, as needed.

The decision to proceed to the next cohort will be based on the observance of no serious treatment-emergent adverse events (TEAEs) and no more than 2 out of 6 subjects who received B-124a (after unblinding the cohort) experiencing toxicity ≥ Grade 3, as defined in the United States (US) Food and Drug Administration (FDA) Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials," with associated clinical signs and symptoms except BP.[2] Grade 3 toxicities in vital signs and laboratory parameter assessments must be confirmed with a repeat measurement. If the dose for a cohort is determined not to be safe or well tolerated (ie, if, after unblinding the cohort, any subject who received B-124a experienced a serious TEAE with a reasonable possibility of a causal relationship to the IMP, or if ≥ 2 out of 6 subjects who received B-124a experienced toxicity ≥ Grade 3 with associated clinical signs and symptoms), the preceding dose level will then be considered as the MTD. The DRC will be provided with the blinded Council for International Organizations of Medical Science (CIOMS) form of any reported serious TEAE, when

applicable, for additional information and details on the serious TEAE. The DRC will rely on the causality assessment (related, not related) as assessed by the independent sponsor medical reviewer and the investigator.

A score of 1 on the Modified Observer's Assessment of Alertness/Sedation Scale (MOAA/S) may be considered as not safe or well tolerated as well. Transient (ie, lasting for less than 2 hours) SBP up to 180 mmHg or a change (eg, increase) from baseline of up to 60 mmHg will be allowed for dose escalation.

A schematic of the trial design for Arm 1 is provided in Figure 1.2-1.

4.1.2 Arm 2

Arm 2 will be a randomized, double-blind, placebo-controlled, single ascending oral dose administration of B 124a to healthy subjects in a fasted state. Dosing is planned to be conducted in 3 or 4 cohorts of 8 subjects each. The starting dose of 72 mg was selected based on the preliminary PBPK prediction of approximately 30% improvement of C_{max}, well within a 2-fold safety margin of the highest tested dose 200 mg in Arm 1. The dose escalation for the cohorts in Arm 2 will be 150 mg, 250 mg, and an optional cohort. The dose escalation for Arm 2 was based upon the available target engagement/PD data to match the comparable exposure from Arm 1 of approximately 1500 ng/mL (C_{max}) or until the maximum tolerated dose level was reached. The higher dose levels will be subject to change according to the data collected from earlier cohorts. Doses for latter cohorts may be repeated or adjusted based on PK, AEs, and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented.

For each cohort in Arm 2, subjects will be randomized on Day 1 to a single oral dose of B-124a or matching placebo in a 6:2 ratio (6 on B-124a subjects and 2 placebo subjects) and dosed in 2 groups (3 sentinel subjects [2 on B-124a and 1 on placebo] and the remaining subjects [4 on B-124a and 1 on placebo]). For each cohort, the second group will be dosed at least 24 hours after the sentinel group has been dosed and after AE data for the sentinel group has been reviewed and agreed by the principal investigator that it is safe and appropriate to dose the remaining subjects. The principal investigator will notify the CRO medical monitor and the OPDC GCD and GPV representatives that dosing may continue in the cohort.

The dose escalation process for Arm 2 will be the same as in Arm 1.

A schematic of the trial design for Arm 2 is provided in Figure 1.2-2.

4.2 Scientific Rationale for Trial Design

Given that this is the FIH trial, all doses of B-124a will be administered in the fasted state in healthy subjects. The safety, tolerability, and PK will be assessed in an expected relatively wide dose/exposure range, and the PK/PD analysis will be used to explore the correlation between drug exposure (ie, maximum [peak] plasma concentration of the drug [C_{max}]) and hemodynamic response (ie, BP effect), as well as the dissociation effect (ie, CADSS). In addition, an exploratory PD analysis will look at the effects of B-124a (dose and concentration) on qEEG measurements (ie, gamma power and oscillation). Assessment of the dose/exposure range in healthy subjects will inform safety and tolerability prior to exposing to TRD patients and will inform risk mitigation strategies for potential drug interactions. The exposure range in healthy subjects is clinically reasonable based upon the preclinical profile and the planned inpatient evaluation for treatment-emergent risks (ie, vital signs, ECG, and neuropsychiatric safety assessments), which will be frequently monitored.

4.3 Dosing Rationale

Nonclinical safety data were used to determine the FIH dose for this trial; repeat dose toxicity studies of up to 28 days were conducted in both cynomolgus monkeys and Sprague Dawley rats, as detailed in Section 2.2.1.3.

In monkeys, the NOAEL was determined to be 120 mg/kg. The NOAEL in rats was determined to be 10 mg/kg biweekly in males and females, 10 mg/kg/day in males, and 1 mg/kg/day in females due to the reproductive findings. Based on the NOAEL of 10 mg/kg in rats, a maximum starting dose of 10 mg was calculated in humans, using a safety factor of 10 for a 60-kg human subject.

Given that treatment with B-124a is expected to cause a transient increase in BP as an NMDA receptor on target mechanistic class effect,[3] an exploratory PK/PD analysis of the exposure of B-124a and SBP change was conducted using the data from both rats and monkeys. The plasma exposure (C_{max}) level in association with the average (≤ 6 hours) or peak blood pressure change from baseline (EC_{20} - EC_{25}) is 166 to 211 ng/mL corresponding to 7 to 8 mmHg in monkeys, and 39 to 52 ng/mL corresponding to 9 to 11 mmHg in rats. Predictive human PK models for B-124a were generated from cross-species allometric scaling. Based on the analysis and allometric scaling, a human dose of 3 mg is expected to produce a plasma concentration that would not result in a clinically significant increase (ie, > ~10 mmHg) in SBP. Therefore, the proposed starting dose is 3 mg.

For Arm 1, up to 7 dose cohorts are planned. The proposed doses for Arm 1 are 3, 6, 12, 25, 50, 100, and 200 mg. Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce SBP changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. For Cohorts 3 through 7, the dose may also be adjusted when administered with 100% cranberry juice. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7.

For Arm 2, the starting dose of 72 mg was selected based on the preliminary physiologically-based pharmacokinetic prediction of approximately 30% improvement of C_{max}, well within a 2-fold safety margin of the highest tested dose of 200 mg in Arm 1. The dose escalation for the cohorts in Arm 2 will be 150 mg, 250 mg, and an optional cohort. The higher dose levels will be subject to change according to the data collected from the earlier cohorts.

4.4 End of Trial Definition

The end of trial date is defined as the last date of contact or the date of final contact attempt from the post-treatment follow-up electronic case report form (eCRF) page for the last subject completing or withdrawing from the trial.

4.5 Definition of Completed Subjects

The treatment period is defined as the time period during which subjects are evaluated for primary and/or secondary objectives of the trial irrespective of whether or not the subject actually consumes all doses of the IMP. Subjects who are evaluated at the last scheduled visit during the treatment period will be defined as trial completers. For purposes of this trial, subjects who complete all PK and PD assessments all the way through discharge from the clinic, assuming PK washout and scheduled in-clinic AE assessment completion (ie, complete the Day 8 visit, will be defined as trial completers.

5 Trial Population

Approximately 88 subjects (56 subjects in Arm 1 and 32 subjects in Arm 2) are expected to be enrolled in the trial. The trial population will consist of healthy males and females, 18 to 55 years of age, inclusive. Subjects will not be allowed to be enrolled and receive IMP in more than one cohort. Subjects who withdraw for reasons other than AEs may be replaced at the discretion of the sponsor.

5.1 Subject Selection and Numbering

All subjects will be given a unique subject identifier (ID; site number [3 digits] + subject number ['S' + 5 digits]) upon providing consent. The site number will be designated by the sponsor. For each site, the subject number will be given sequentially from S00001.

Demographic information (collection date, date of birth, sex, childbearing potential, race, ethnicity) and medical history will be recorded in the eCRF at the screening visit.

Eligible subjects who are enrolled in the trial will be assigned a unique subject randomization number for treatment sequence assignment as noted below:

- Arm 1: the randomization number will be a 5-digit number (Cohort 1 will begin with 10001, Cohort 2 will begin with 20001, Cohort 3 will begin with 30001, with a similar pattern for Cohorts 4, 5, 6, and 7).
- Arm 2: the randomization number will be a 5-digit number (Cohort 1 will begin with 91001, Cohort 2 will begin with 92001, with a similar pattern for Cohorts 3 and 4).

Results of the eligibility assessment, date of randomization (or date of treatment assignment), and randomization number (or treatment group) will be recorded in the eCRF.

5.2 Eligibility Criteria

Exceptions for eligibility criteria will not be permitted during the trial, neither by the investigator nor by the medical monitor.

5.2.1 Inclusion Criteria

Subjects are required to meet the following inclusion criteria when assessed:

1) Male or female subjects between 18 and 55 years of age, inclusive.
2) Heterosexually active males or females of childbearing potential (FOCBP), or their partners, will commit to utilizing 2 different approved methods of birth control or remain fully abstinent (periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] or withdrawal are not acceptable methods of contraception) during the trial and for 90 days (for males) or 30 days

(for FOCBP) after the last dose of IMP. Abstinence will be permitted if it is confirmed and documented at every trial visit.

If employing birth control, 2 of the following methods must be used: vasectomy, tubal ligation, nonhormonal intrauterine device, condom, sponge, or occlusive cap (vaginal diaphragm or cervical/vault cap).
Female subjects of nonchildbearing potential (permanently sterilized [ie, hysterectomy, bilateral oophorectomy], postmenopausal for at least 12 months, or otherwise incapable of pregnancy) and male subjects who have had a bilateral orchiectomy are also eligible for enrollment.
Male subjects must also agree not to donate sperm from trial screening through 90 days after the last dose of IMP.

3) Body mass index (BMI) between 18.0 and 32.0 kg/m^2 (inclusive).
4) In good health as determined by:
   - Medical history
   - Physical and neurological examination
   - ECG
   - Serum/urine chemistry, hematology, and serology tests.
5) Ability to provide written, informed consent prior to the initiation of any trial-related procedures, and ability, in the opinion of the principal investigator, to comply with all the requirements of the trial.

A definition of childbearing potential can be found in Section 10.3.

5.2.2 Exclusion Criteria

Subjects will be excluded if they meet any of the following exclusion criteria when assessed:

1) Females who are breast-feeding and/or who have a positive pregnancy test result prior to receiving IMP.
2) Clinically significant abnormality in past medical history, or at the screening physical examination, that in the investigator's or sponsor's opinion may place the subject at risk or interfere with outcome variables including absorption, distribution, metabolism, and excretion of the IMP. This includes, but is not limited to, history of or concurrent cardiac, hepatic, renal, neurologic, endocrine, gastrointestinal, respiratory, hematologic, and immunologic disease.
3) Subjects who meet the Diagnostic and Statistical Manual of Mental Disorders (fifth edition) criteria for moderate or severe substance use within 2 years prior to screening.
4) History of or current hepatitis or acquired immunodeficiency syndrome or carriers of hepatitis B surface antigen, hepatitis C antibodies, and/or human immunodeficiency virus (HIV) antibodies.
5) History of any significant drug allergy.

6) A positive urine alcohol test and/or urine drug screen for substances of abuse at screening or upon check-in to the trial site.
7) Subject having taken an investigational drug within 30 days preceding screening.
8) Any history of significant bleeding or hemorrhagic tendencies.
9) Any history of difficulty in donating blood.
10) Subjects without a permanent physical residence.
11) Consumption of alcohol and/or food and beverages containing methylxanthines, grapefruit, grapefruit juice, Seville oranges, or Seville orange juice within 72 hours prior to dosing.
12) Use of prescription drugs, over-the-counter drugs, herbal medication, or vitamin supplements within 14 days prior to dosing and antibiotics within 30 days prior to dosing. The sponsor may allow exceptions only if the medication's administration is deemed unlikely to impact the PK results.
13) Exposure to any substances known to stimulate or induce hepatic microsomal enzymes within 30 days prior to screening (ie, occupational exposure to pesticides, organic solvents).
14) Use of tobacco products or daily exposure to second-hand smoke within 2 months prior to screening, or urine cotinine concentrations > 200 ng/mL or serum cotinine concentrations > 20 ng/mL at screening or at check-in to the trial site.
15) Supine BP, after resting for at least 3 minutes, higher than 130/80 mmHg or SBP lower than 100 mmHg at screening or at check-in to the trial site.
16) Supine pulse rate, after resting for at least 3 minutes, outside the range of 40 to 90 beats per minute (bpm).
17) Abnormal ECG findings at screening or check-in, as follows, that are considered to be clinically significant:
    - QT interval corrected for HR using Fridericia's formula (QTcF) > 450 msec in males or > 470 msec in females
    - QRS interval > 120 msec
    - PR interval > 220 msec
    - Abnormal U waves, or other minor ST or T wave changes.

    Note that ECGs should be performed in triplicate, with 5 minutes between each recording, to confirm any clinically significant abnormality.
18) History of serious mental disorders that, in the opinion of the investigator, would exclude the subject from participating in this trial.
19) Subjects should not be enrolled (ie, dosed) if any active suicidal ideation is present prior to dosing (as evidenced by clinical examination or an answer of "yes" on Questions 4 or 5 of the Columbia-Suicide Severity Rating Scale [C-SSRS] Since Last Visit version) or suicidal behavior is present in the C-SSRS Since Last Visit version.
20) Any subject who, in the opinion of the investigator, should not participate in the trial.

Subjects must agree to restrictions to medications described in Section 6.5.1 and other restrictions described in Section 5.3.1 through Section 5.3.3.

5.3 Lifestyle Considerations

Not applicable.

5.3.1 Meals and Dietary Restrictions

The consumption of food and beverages containing grapefruit, grapefruit juice, Seville oranges, or Seville orange juice is prohibited within 72 hours prior to dosing and during the trial.

Refer to Section 6.1 for fasting and fed requirements and the timing of meals relative to dosing.

5.3.2 Caffeine, Alcohol, and Tobacco

The consumption of alcohol and/or food and beverages containing methylxanthines is prohibited within 72 hours prior to dosing and during the trial. The use of tobacco products or daily exposure to second-hand smoke is prohibited within 2 months prior to screening and during the trial.

5.3.3 Activity

Subjects should not engage in strenuous physical exercise within 72 hours prior to dosing and during the trial.

5.4 Screen Failures

A screen failure is a subject from whom informed consent is obtained and is documented in writing (ie, subject signs an informed consent form [ICF]), but who is not randomized or assigned trial treatment. Subjects who sign an ICF but who are not started on treatment are permitted to be re-screened. Subjects with a positive drug or alcohol screen are not eligible to be re-screened for participation in the trial; however, subjects excluded for other reasons (including exclusionary cotinine concentrations) may be re-screened at any time, at the discretion of the medical monitor, if the exclusion characteristic has changed or resolved. In the event that the subject is re-screened for trial participation, and the re-screening was not completed within the original screening window, a new ICF must be signed and a new screening number assigned. All AEs must be reported after subject informed consent has been obtained, including screening failures due to AEs, irrespectively of IMP administration.

If the subject meets the definition of a screen failure in this trial, the following information will be recorded in the eCRF:

- Date of informed consent
- Visit date (screening visit)
- Demographics (collection date, birth date, sex, race, ethnicity)
- Result of eligibility assessment
- Screen failure date
- Reason for screen failure

6 Trial Treatments

6.1 Trial Treatments Administered

The IMP (B-124a) will be provided to the site as active pharmaceutical ingredient (API). The clinical pharmacy will compound capsules of the appropriate strengths using the API prepared at the site; the IMP should be prepared on the day of dosing no more than 6 hours prior to administration. Doses of IMP will be taken with 240 mL of still water or 240 mL of 100% cranberry juice. Additional details will be provided in the Pharmacy Manual.

6.1.1 Arm 1

For Arm 1, up to 7 dose cohorts are planned (Table 6.1.1-1). The proposed doses for Arm 1 are 3, 6, 12, 25, 50, 100, and 200 mg. Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce SBP changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7. For Cohorts 3 through 7, the dose may also be adjusted when administered with 100% cranberry juice.

In Arm 1, a single oral dose of B-124a or placebo will be taken on Day 1. Treatments will be administered following an overnight fast of at least 10 hours and subjects will continue to fast for at least 4 hours after dosing. Water will be available ad libitum except for ± 1 hour around dosing.

Table 6.1.1-1 Anticipated Number of Dose Cohorts and Dosage Levels in Arm 1

Cohort	B-124a Dose	Route of Administration	Number of Subjects
1	3 mg Placebo	Oral	6 2
2	6 mg Placebo	Oral	6 2
3	12 mg[a] Placebo	Oral	6 2
4	25 mg[a] Placebo	Oral	6 2
5	50 mg[a] Placebo	Oral	6 2
6	100 mg[a] Placebo	Oral	6 2
7	200 mg[a] Placebo	Oral	6 2

[a]Dose may be adjusted based on data from the earlier cohorts, as described above. For Cohorts 3 through 7, the dose may also be adjusted when administered with 100% cranberry juice.

6.1.2 Arm 2

For Arm 2, up to 4 dose cohorts are planned (Table 6.1.2-1). The proposed doses for Arm 2 are 72, 150, and 200 mg while Cohort 4 will be optional. Doses for latter cohorts may be repeated or adjusted based on PK, AEs, and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented.

In Arm 2, a single oral dose of B-124a or placebo will be taken on Day 1. Treatments will be administered following an overnight fast of at least 10 hours and subjects will continue to fast for at least 4 hours after dosing. Water will be available ad libitum except for ± 1 hour around dosing.

Table 6.1.2-1 Anticipated Number of Dose Cohorts and Dosage Levels in Arm 2

Cohort	B-124a Dose	Route of Administration	Number of Subjects
1	72 mg Placebo	Oral	6 2
2	150 mg Placebo	Oral	6 2
3	250 mg[a] Placebo	Oral	6 2
4	Optional	Oral	6 2

[a]Doses for later cohorts may be repeated or adjusted based upon the PK, AEs and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented.

For further information regarding the dose regimen and treatment period(s), including the follow-up period for each arm of the trial, see Section 4.1.

6.1.3 Medical Devices

Not applicable.

6.2 Management of Investigational Medicinal Product

For full details on IMP management, please refer to the B-124a IB.

6.2.1 Packaging and Labeling

The IMP will be provided by the sponsor or designated agent to the investigators and the persons designated by the investigator(s) or institution(s). The IMP will be supplied as API. Each package will be labeled to disclose the compound ID, batch number, sponsor's name and address, instructions for use, route of administration, and appropriate precautionary statements and other information required by local regulatory authorities.

6.2.2 Storage

The IMP will be stored in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees.

The IMP will be stored according to the conditions indicated on the IMP label. The trial site staff will maintain a temperature log in the IMP storage area to record the temperature.

6.2.3 Accountability

The investigator or designee must maintain an inventory record of IMP (including investigational or placebo) received, dispensed, administered, and destroyed. Neither the

investigator nor any designees may provide IMP to any subject not participating in this protocol.

6.2.4 Returns and Destruction

The IMP may only be destroyed by the trial site if approved by the sponsor and if the IMP destruction meets all local regulations. The IMP will be destroyed by the clinical trial site following completion and verification of accountability of the IMP by the assigned trial monitor. The trial site may utilize qualified third-party vendors for IMP destruction. A certificate of destruction should be filed within the IMP accountability records.

6.2.5 Reporting of Product Quality Complaints

A product quality complaint (PQC) is any written, electronic, or oral communication provided by a healthcare professional, consumer, clinical trial subject, medical representative, regulatory agency, partner, or other third party that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a medical device or medicinal product or a falsified, tampered, or diverted product after it is released for distribution.

Examples include, but are not limited to, communications involving:

- Failure of an API or product to meet any of its specifications
- Incorrect or missing labeling
- Packaging issues (eg, damaged, dirty, crushed, missing product)
- Bottle defects (eg, under-fill, over-fill, no safety seal)
- Product defect (eg, odor, chipped, broken, crushed, embossing illegible)
- Loss or theft of product

6.2.5.1 Eliciting and Reporting Product Quality Complaints

The investigator or designee must record each PQC identified through any means from the receipt of the IMP from the sponsor or sponsor's designee, through and including reconciliation and up to destruction, including subject dosing. The investigator or designee must notify the sponsor (or sponsor's designee) by email within 24 hours of becoming aware of the PQC according to the procedure outlined below.

Send PQC reporting information to the OPDC IMP complaints mailbox email: IMP-PQC@otsuka-us.com. Also indicate whether or not the complaint sample is available for return.

Identification of a PQC by the subject should be reported to the site/investigator, who should then follow the reporting mechanism above.

6.2.5.2 Information Required for Reporting Purposes

- Description of complaint
- Reporter identification (eg, investigator, site, etc.)
- Reporter contact information (eg, name, address, phone number, e-mail address)
- Subject number
- Clinical site number
- ID of material (product/compound name, lot/batch number, kit number, shipment number, expiry date)
- Clinical protocol reference (number and/or trial name)
- Dosage form/strength (if known)
- Pictures of complaint sample (if available)
- Availability of complaint sample for return
- Was any subject at risk due to the identified issue?

6.2.5.3 Return Process

Indicate during the report of the PQC if the complaint sample is available for return.

If the complaint sample is available but not at the clinical site, please instruct the subject to bring the complaint sample to their next site visit.

If the complaint sample is available for return, the sponsor will provide return instructions, when applicable.

It must be documented in the site accountability record that the complaint sample has been forwarded to the sponsor for complaint investigation.

6.2.5.4 Assessment/Evaluation

Assessment and evaluation of PQCs will be handled by the sponsor.

6.3 Measures to Minimize/Avoid Bias

In Arms 1 and 2 of the trial, subjects will be randomly assigned to treatment (B-124a or placebo) in each of the cohorts. The randomization code will be created by the sponsor following its standard operating procedures. In order to avoid bias in the evaluation of safety parameters, the investigator and clinic personnel (with the exception of the personnel preparing the doses) will be blinded to the identity of the IMP that is given to each subject. Procedures for breaking the blind can be found in Section 8.8.7.

6.4 Subject Compliance

The time and dose of each IMP administration will be recorded in the eCRF. Information regarding any missed or inappropriately administered doses will also be documented in the eCRF. All doses of IMP will be administered while the subjects are in the clinic; compliance will be ensured by a mouth and/or hand check during the oral dosing administration of IMP. In addition, during the 4-hour postdose period on the first day of dosing, the subject's toilet use will be supervised to prevent self-induced emesis resulting in loss of the oral dose.

6.5 Concomitant Medications or Therapies

The investigator will record all medications (including prescription medications, over-the-counter medications, herbal remedies, etc) and therapies taken by the subject from 30 days prior to signing of informed consent through the end of the evaluation period (defined as the time period during which subjects are evaluated for primary and/or secondary objectives) in the eCRF. The investigator will also record all medications and therapies taken by the subject for treatment of an AE or which caused an AE until the end of the trial (defined as the last date of contact or date of final contact attempt) in the eCRF.

For concomitant medications, the following will be recorded in the eCRF: medication, indication, dose, frequency, route, start date, and end date. For concomitant therapy, the following will be recorded in the eCRF: therapy, indication, start date, and end date.

6.5.1 Prohibited Medications or Therapies

No medications other than the planned IMP may be taken during the trial. The use of prescription drugs, over-the-counter drugs, herbal medications, or vitamin supplements within 14 days prior to dosing and antibiotics within 30 days prior to dosing is prohibited. The sponsor may allow exceptions only if the medication is unlikely to affect the PK results.

6.5.2 Permitted Medications or Therapies

Not applicable.

6.5.3 Rescue Medications

Not applicable.

6.6 Intervention After the End of the Trial

Not applicable.

7 Stopping Rules, Withdrawal Criteria, and Procedures

7.1 Entire Trial or Treatment

If the sponsor terminates or suspends the trial for any reason, prompt notification will be given to investigators, the institutional review board (IRB), and regulatory authorities in accordance with regulatory requirements.

Dose escalation in Arm 1 may be modified or stopped based upon the sponsor's and/or investigator's clinical judgment at any time. The proposed doses for Cohorts 1, 2, 3, 4, 5, 6, and 7 are 3, 6, 12, 25, 50, 100, and 200 mg of B-124a, respectively, or placebo. Doses for later cohorts (Cohorts 3 through 7) may be adjusted based upon the PD response expected from the first 2 cohorts (ie, if the 6-mg dose does not produce SBP changes of ~16 mmHg or higher, the doses will be adjusted to 18, 36, 72, 150, and 200 mg for Cohorts 3, 4, 5, 6, and 7, respectively). Doses for Cohorts 3 through 7 may also be repeated or adjusted based on PK, AEs, and safety assessments. No more than a ~2-fold increase of the dose level in the immediately preceding dose cohort will be expected for Cohorts 4 through 7.

Dose escalation in Arm 2 may be modified or stopped based upon the sponsor's and/or investigator's clinical judgment at any time. The proposed doses for Cohorts 1, 2, and 3 are 72, 150, and 250 mg of B-124a respectively, or placebo while Cohort 4 will be optional. Doses for later cohorts may be repeated or adjusted based on PK, AEs, and safety assessments obtained from earlier cohort(s). No more than approximately 2-fold increase of the dose level in the immediately preceding dose cohort will be implemented.

It may be decided to repeat a dose level or decrease the dose. The criteria below may serve as a general guideline, and of note, these are guidelines only. Exceptions can be made and the reasons for exception should be clearly documented.

Dose escalation will be stopped when the dose for a cohort is determined not to be safe or well tolerated; for example, once the sponsor has been unblinded, one of the following is found in subjects who received B-124a without occurrence in the placebo group or the occurrence differs between B-124a and placebo treatment above the threshold numbers, as listed in the following:

1) One or more serious adverse events (SAEs).
2) Clinically significant (Grade ≥ 3 toxicity with associated clinical signs and symptoms) laboratory abnormalities of the same characteristics in 2 or more subjects.

3) Clinically significant (Grade ≥ 3 toxicity with associated clinical signs and symptoms) changes in safety ECG parameters of the same characteristics in 2 or more subjects.
4) Clinically significant (Grade ≥ 3 toxicity with associated clinical signs and symptoms) changes in vital signs of the same characteristics in 2 or more subjects (except BP; transient [ie, lasting for less than 2 hours] SBP up to 180 mmHg or a change (eg, increase) from baseline of up to 60 mmHg will be allowed for dose escalation).
5) Severe AEs of the same characteristics in 2 or more subjects or of a different characteristic in 3 or more subjects.
6) Three occurrences of any of the following: clinically significant (Grade ≥ 3 toxicity with associated clinical signs and symptoms) changes in laboratory values or vital signs (except BP; transient [ie, lasting for less than 2 hours] SBP up to 180 mmHg or a change (eg, increase) from baseline of up to 60 mmHg will be allowed for dose escalation).
7) A score of 1 on the MOAA/S.

Grade 3 toxicities in vital signs and laboratory parameter assessments must be confirmed with a repeat measurement.

7.2 Individual Site

Individual trial site participation may be discontinued by the sponsor, the investigator, or the IRB if judged to be necessary for medical, safety, regulatory, ethical, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP). The investigator will notify the sponsor promptly if the trial is terminated by the investigator or the IRB at the site.

7.3 Individual Subject Discontinuation

7.3.1 Treatment Interruption

Not applicable.

7.3.2 Treatment Discontinuation

After treatment assignment, a subject may stop treatment permanently for a variety of reasons. Treatment discontinuations may be initiated by a subject who is not satisfied with the treatment or may become medically necessary due to AEs, required treatment with a disallowed medication or therapy, or other issues, as determined by the investigator. If a subject discontinues treatment, their participation in the trial will be discontinued. Discontinued subjects should be encouraged to complete all ET and follow-up assessments with ET assessments conducted as soon as possible after the subject is withdrawn.

7.3.3 Documenting Reasons for Treatment Interruption or Discontinuation

A subject may discontinue IMP for the reasons listed below:

- Adverse event
  - Subject decides to discontinue because of annoyance or discomfort due to a nonserious AE which is not otherwise determined to be an undue hazard
  - Continuing IMP places the subject at undue risk as determined by the investigator (eg, a safety concern that is possibly, probably, or likely related to IMP)
    - SAE
    - Other potentially IMP-related safety concerns or AEs
- Death
- Lost to follow-up
- Physician decision
- Pregnancy (see Section 10.3)
- Protocol deviation
- Randomized by mistake
- Site terminated by sponsor
- Trial terminated by sponsor
- Technical problems
- Withdrawal by subject
- Other

If the subject discontinues IMP due to an AE, the investigator, or other trial personnel, will make every effort to follow the event until it has resolved or stabilized. Follow-up procedures in Section 7.3.2 must be followed.

7.3.4 Withdrawal of Consent or Assent

Each subject has the right to withdraw their consent from further participation in the trial at any time without prejudice. Subjects can withdraw consent for use of data that has not previously been anonymously transferred into trial data sets collected as part of the trial and can only withdraw consent for future participation. The investigator can also discontinue a subject's participation in the trial at any time if medically necessary. Unless the subject provides their written withdrawal of consent or there is other written documentation by the investigator confirming the subject's verbal intent to completely withdraw from the trial, subjects should be followed for all protocol-specified evaluations and assessments, if possible.

Complete withdrawal of consent requires a subject's refusal of ALL of the following methods of follow-up:

- Participation in all follow-up procedures specified in the protocol (whether in-clinic, by telephone, or by a home visit).
- Participation in a subset of protocol-specified follow-up procedures (by a frequency schedule and method, as agreed by subject and trial site staff).
- Contact of the subject by trial personnel, even if only by telephone, to assess current medical condition, and obtain necessary medical or laboratory reports relevant to the trial's objectives.
- Contact of alternative person(s) who have been designated in source records as being available to discuss the subject's medical condition, even if only by telephone, mail, or e-mail (eg, family, spouse, partner, legal representative, friend, neighbor, or physician).
- Access to medical information from alternative sources (eg, hospital/clinic medical records, referring doctor's notes, public records, dialysis, transplantation or vital registries, social media sources).

Withdrawal of consent is a critical trial event and, therefore, should be approached with the same degree of importance and care as is used in initially obtaining informed consent. The reasons for a subject's intended withdrawal need to be completely understood, documented, and managed to protect the rights of the subject and the integrity of the trial. A subject may initially express their desire to discontinue IMP administration, which is not equivalent to a complete withdrawal of consent for further participation (see Section 7.3.2). A subject may, however, indicate that further trial participation is creating a burden on their work, school, or social schedule. Therefore, the investigator should follow the procedures outlined in Section 7.3.3 to determine if the subject can continue participation in the trial if modifications to his/her treatment and/or schedule of assessments can be accommodated. Only subjects who withdraw their permission for all of the above methods of follow-up are considered to have completely withdrawn their consent to participate in the trial.

Details on the withdrawal of consent from the pharmacogenomic (PGx) assessment are provided in the ICF.

7.3.5 Procedures to Encourage Continued Trial Participation

In all cases of impending IMP discontinuation or consent withdrawal, investigators will be instructed to meet and discuss (without undue coercion) with the subject their options of continuing in the trial, preferably on therapy. The investigator should ensure understanding and documentation of the reasons for the subject's desire to withdraw consent.

7.4 Definition of Subjects Lost to Follow-up

Subjects who cannot be contacted on or before the follow-up telephone call (30 [+ 2] days after the last dose of IMP), who do not have a known reason for discontinuation (eg, withdrew consent or AE), and for whom a survival status at the end of the trial cannot be determined will be classified as "lost to follow-up". Survival status can be determined from a variety of sources, either by obtaining acceptable documentation for death (ie, death certificate, medical records, public records, statement by a family member or primary care physician) or acceptable documentation for life (ie, direct contact with the subject, medical records, successful telephone contact with the subject, statement by a family member or primary care physician, or public records).

The site will make 3 documented attempts to contact the subject by telephone and in the event the site is unable to reach the subject by telephone, the site will attempt to contact the subject via certified mail or an alternative similar method, where appropriate, before assigning a "lost to follow-up" status.

If the subject was classified as "lost to follow-up", "Were you able to contact the subject?", "Date of contact/Date of final contact attempt", and "Contact method" will be recorded in eSource.

8 Trial Procedures

The expected duration of trial participation for each subject will be approximately 76 (+ 2) days in Arm 1 and 80 (+ 2) days in Arm 2, consisting of a screening period from Day −45 through Day −2, check-in on Day −1, an in-clinic treatment and assessment period from Day 1 through discharge/ET, and a safety follow-up telephone call 30 (+ 2) days after the last dose of IMP. The in-clinic stay in Arm 1 will be 8 days; however, the in-clinic stay in Arm 2 may be adjusted if the washout period is extended beyond 4 days.

The total duration of this trial is expected to be approximately 7 months.

The assessments to be conducted during the trial are summarized in Table 1.3-1.

8.1 Efficacy Assessments

Not applicable.

8.2 Pharmacokinetic Assessments

For PK samples taken within the first 6 hours postdose, subjects will remain either in a seated or semi-recumbent position for the first 6 hours following dosing except during brief periods where protocol-related procedures need to be performed. Restroom visits must be supervised during the 6 hours postdose and should be brief (< 10 minutes). In addition, during the 6-hour period after oral dosing, the subject's toilet use must be supervised to prevent self-induced emesis resulting in loss of the oral dose. Following the 6-hour postdose period, the subjects will be allowed to ambulate, but should not exercise strenuously.

8.2.1 Pharmacokinetic Blood Samples

Pharmacokinetic samples will be collected at the time points described in the schedule of assessments (Table 1.3-1). The PK sampling time points may be changed in subsequent dosing cohorts once PK data from earlier dose cohorts are available. Predose samples should be collected within 60 minutes before administration of the IMP. Pharmacokinetic sampling windows for other time points will be provided separately. When vital signs or ECGs are scheduled at the same nominal time as PK sample collections, ECGs should be performed before PK samples are collected and blood draws for PK samples should take priority over vital signs. The actual date and time of the PK sample collection will be recorded in the eCRF.

Serial blood samples (4 mL, dipotassium ethylenediaminetetraacetic acid [K_2EDTA]) will be collected by catheter or venipuncture, as necessary, and processed into plasma to determine the concentrations of B-124a. After processing into plasma, 2 approximately equally sized aliquots will be placed into appropriately labeled tubes and will be placed in a freezer set at −70°C, unless otherwise instructed in the Operations/Laboratory Manual.

All plasma samples will be shipped to the bioanalytical laboratory for analysis of B-124a. A second aliquot will be sent to an exploratory analysis laboratory for identification and analysis of B-124a and additional metabolites. Additional information will be provided in the Operations/Laboratory Manual.

8.3 Pharmacodynamic Assessments

This trial will assess the effect of B-124a plasma concentrations on ECG parameters, including corrected QT interval (QTc), in healthy subjects. As such, Holter extractions will be completed for Arm 1 at the time points described in the schedule of assessments (Table 1.3-1).

At each protocol-specified time point, ten 14-second digital 12-lead ECG tracing replicates will be extracted from a 5-minute "ECG window" (typically, the last 5 minutes of the 15-minute period when the subject is maintained in a supine or semi-recumbent quiet position). Subjects need to be in the supine position for at least 10 minutes prior to the measurement and for 5 minutes after each sampling time point. When ECG extractions coincide with safety ECGs, vital sign measurements, and blood draws, procedures will be carried out in the said order. Once human PK data (ie, C_{max}) are available, the number of ECG time points may be reduced in subsequent dosing cohorts in Arm 1.

An exposure-response analysis will be conducted; refer to Section 9.4.3.4 for details.

A qEEG will be performed for Arm 1 at the time points described in the schedule of assessments (Table 1.3-1). Additional time points may be added on Day 1 (eg, 1, 2, and 4 hours postdose) for Cohorts 2 through 7 depending on analysis of the PK data from Cohort 1.

A qEEG will be used to assess the frequency bands of the electroencephalogram, before and after receiving the IMP (B-124a or placebo). A qEEG will be conducted for prespecified intermittent periods of approximately 10 minutes, during which the subject will have their eyes closed for 5 minutes and open for 5 minutes. A qEEG technologist will monitor the subjects during the assessment to ensure minimization of any type of artifact (biological or external).

A minimum of 30 (artifact-free EEG segments) will be analyzed. The qEEG segments will be 2 seconds long. Selection of the segments will involve detection and rejection, or reduction by algorithm, of various extracerebral artifacts, including removal of eye blink, eye movement, head movement, electromyographic, salt bridge (sweat), and cardioballistic artifacts, electrode disconnection, and any other detectable contamination. Once the analysis is performed, there will be a secondary quality check in which extreme results will be evaluated for possible contamination. To further reduce the influence of extreme data, endpoints from subjects with a mean value (change from baseline) more than 3 standard deviations from the group mean value will be marked as outliers and excluded from further analysis. Additional data manipulations may be utilized to enhance

signal detection; eg, current source density or Laplacian transform, especially for estimation of gamma-band EEG amplitude. Additional details will be provided in the EEG manual.

In addition, the relationship between B-124a plasma exposure and change in BP will be explored in Arms 1 and 2 respectively (see Section 9.4.3.6 for additional details).

8.4 Pharmacogenomic Assessments

A blood sample for PGx analysis will be collected on Day −1 (check-in) from all subjects in both arms.

8.4.1 Pharmacogenomic Samples

Blood samples (8.5 mL) will be collected in PAXGene™ blood collection tubes for PGx testing. Genomic deoxyribonucleic acid (DNA) will be extracted from a whole blood sample and used to determine genotypes and related phenotypes for genes related to absorption, distribution, metabolism, and excretion. The genotyping data may be included as part of a covariate analysis in a population PK analysis to be reported separately.

The date and time of each sample collected for PGx analysis will be recorded in the eCRF. All PGx samples will be shipped to the PGx laboratory for analysis. Additional information will be provided in the Operations/Laboratory Manual.

8.5 Biomarker Assessments

Not applicable.

8.6 Future Biospecimen Research Samples

Not applicable.

8.7 Safety Assessments

Safety assessments in this trial include AEs, clinical laboratory tests, vital signs (including ambulatory blood pressure monitoring [ABPM]), 12-lead ECGs, physical examinations, neurological examinations, the C-SSRS, neurological scales (Clinician Administered Dissociative States Scale [CADSS], MOAA/S, Brief Psychiatric Rating Scale [BPRS], and the Cogstate Safety Battery + the One Back Test).

Details pertaining to the definitions, collection, reporting, and follow-up of AEs are described in Section 8.8.

8.7.1 Clinical Laboratory Assessments

Clinical laboratory samples will be collected at the time points described in the schedule of assessments (Table 1.3-1) to perform the clinical laboratory assessments described in Section 10.2. The total volume of blood to be collected during the trial will be documented in the ICF.

Laboratory data will be captured manually in the eCRF; a local laboratory will be used for all laboratory testing required during the trial. Electrocardiograms should be performed before any blood samples are collected and blood draws for clinical laboratory assessments should take priority over vital signs.

8.7.2 Physical Examination

Physical examinations will be performed at the time points described in the schedule of assessments (Table 1.3-1). The complete physical examination will include height (screening only), weight, and calculation of BMI (screening only) as well as an assessment of the head, eyes, ears, nose, and throat; thorax; abdomen; urogenital; skin and mucosae; and extremities. The urogenital portion of the physical examination will be performed at screening and discharge only (and not at check-in) and should be a standard urogenital examination that in the investigator's judgment is appropriate given the subject's history and current presentation. The targeted physical examination will focus on subject-driven issues based on investigator judgment (eg, the investigator enquires if the subject has any complaints, pains, or disturbances and this would lead to further evaluation of the problematic area).

Whenever possible, the same individual should perform all physical examinations for any individual subject throughout the course of the trial. Individuals performing the physical examination must be permitted to do so by local regulations, must be listed on the FDA Form 1572 as principal investigator or subinvestigator, and must be listed on the trial site delegation of authority form as performing this function.

Any physical examination findings at screening and after screening that are considered by the investigator to be clinically significant are to be recorded as AEs.

8.7.3 Vital Signs

Vital signs (temperature and respiratory rate) will be measured at the time points described in the schedule of assessments (Table 1.3-1). Temperature and respiratory rate will be taken after the subject has been in the supine position for at least 3 minutes, and BP and HR will be taken after the subject has been in the supine (performed first) and standing positions after remaining in each position for at least 3 minutes.

In Arms 1 and 2, ABPM will be used to measure BP and HR at the time points described in the schedule of assessments (Table 1.3-1). The subject's BP and HR will be measured in the supine position until 24 hours postdose (≥ 3 minutes in that position), and in both the supine and standing positions on Day 2 (24 hours postdose) and at all later time points (≥ 3 minutes in each position). The number of ABPM time points may be reduced in subsequent dosing cohorts based on data from previous cohorts.

All blood draws should take priority over vital signs.

8.7.4 Electrocardiogram

Electrocardiograms will be performed at the time points described in the schedule of assessments (Table 1.3-1). At screening, ECGs should be performed in triplicate, with 5 minutes between each recording, to confirm any clinically significant abnormality. The predose ECGs should be performed within 2 hours before the administration of IMP. Safety ECGs should be performed before any blood samples are collected. The 12-lead ECGs will be recorded with the subject supine and at rest (for at least 10 minutes). The HR, PR interval, QRS duration, QT intervals, and QTc will be recorded. The ECG data will be read by a central vendor.

Details regarding the continuous (ie, Holter) ECG assessments are in Section 8.3.

8.7.5 Suicidality Monitoring

Suicidality monitoring will occur at the time points described in the schedule of assessments (Table 1.3-1). The "Baseline/Screening" version of the C-SSRS will be completed at screening and the "Since Last Visit" version will be completed at all other defined time points (including ET, if applicable).

The C-SSRS is a semi-structured interview that captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period. The interview includes definitions and suggested questions to solicit the type of information needed to determine if a suicide-related thought or behavior has occurred. The interview and rating for the C-SSRS must be completed by a licensed clinician or designee who has completed the required rater training to complete this assessment and who has been approved by the sponsor to administer this assessment. Documentation of trial training should be maintained in the investigational site's files. Whenever possible, the same individual should perform all C-SSRS assessments for any individual subject throughout the course of the trial.

Subjects should not be enrolled (ie, dosed) if any active suicidal ideation is present prior to dosing (as evidenced by clinical examination or an answer of "yes" on Questions 4 or 5 of the C SSRS Since Last Visit version) or suicidal behavior is present in the C-SSRS Since Last Visit version.

8.7.6 Other Safety Variables

8.7.6.1 Neurological Examination

Neurological examinations will be performed at the time points described in the schedule of assessments (Table 1.3-1). The complete neurological examination will include an evaluation of mental status, cranial nerves, motor system, reflexes, sensory system, coordination, and station and gait. The targeted neurological examination will focus on subject-driven issues based on investigator judgment (eg, the investigator enquires if the subject has any complaints, pains, or disturbances and this would lead to further evaluation of the problematic area). The targeted neurological examination may trigger additional neurological scale administration (eg, BPRS, CADSS, and/or MOAA/S).

8.7.6.2 Neurological Scales

8.7.6.2.1 Clinician Administered Dissociative States Scale

The CADSS will be performed at the time points described in the schedule of assessments (Table 1.3-1). If symptoms persist beyond the 3-hour postdose assessments, the investigator may choose to collect additional measures until symptoms have resolved. The sampling time points may be reduced in subsequent dosing cohorts based on data from earlier dosing cohorts.

The CADSS is a 28-item scale with 23 subject-rated items and 5 items scored by an observer. Only the subjective items will be utilized in this trial. The subjective component consists of 23 items that are administered by a clinician who begins each question with the phrase “at this time” and then reads the item to the subject. The subject then endorses one of a range of possible responses: 0 = not at all, 1 = slightly, 2 = moderately, 3 = considerably, 4 = extremely. The subject’s response on this 0 to 4 scale is recorded and the clinician moves on to the next item.[5]

8.7.6.2.2 Modified Observer's Assessment of Alertness/Sedation Scale

The MOAA/S will be performed at the time points described in the schedule of assessments (Table 1.3-1). If symptoms persist beyond the 3-hour postdose assessments, the investigator may choose to collect additional measures until symptoms have resolved. The sampling time points may be reduced in subsequent dosing cohorts based on data from earlier dosing cohorts.

The MOAA/S scale is a measure of alertness/sedation and is used widely in clinical research. It is derived from the original Observer's Assessment of Alertness/Sedation scale. This scale was originally validated for use with midazolam. The observer rates the subject’s responsiveness, speech, and facial expression/eye movements. The modified form uses only the responsiveness component of the original scale: 1 (deep sleep) = does not respond to mild prodding or shaking, 2 = responds only after mild prodding or shaking, 3 = responds only after name is called loudly and/or repeatedly, 4 = lethargic response to name spoken in normal tone, 5 (alert) = responds readily to name spoken in normal tone.[6,7]

8.7.6.2.3 Brief Psychiatric Rating Scale

The BPRS will be performed at the time points described in the schedule of assessments (Table 1.3-1). The sampling time points may be reduced in subsequent dosing cohorts based on data from earlier dosing cohorts.

The BPRS is a rating scale that a clinician or researcher may use to measure psychiatric symptoms such as depression, anxiety, hallucinations, and unusual behavior. Each symptom is rated on a scale from 1 (not present) to 7 (extremely severe) and a total of 24 symptoms are scored.[8]

8.7.6.2.4 Cogstate Safety Battery Plus the One Back Test

The Cogstate Safety Battery + the One Back Test will be performed at the time points described in the schedule of assessments (Table 1.3-1). The sampling time points may be reduced in subsequent dosing cohorts based on data from earlier dosing cohorts.

The Cogstate early phase battery has been used extensively in phase 1 research to determine the extent to which treatment with different doses of experimental compounds is associated with changes in cognitive function. Cogstate's computerized early phase battery measures the cognitive domains of processing speed, attention, visual learning, and executive function.[9] The Cogstate Safety Battery + the One Back Test will be used in this trial.

8.7.6.3 Bond-Lader Visual Analog Scale

The Bond-Lader visual analog scale (VAS) will be administered at the time points specified in the schedule of assessments (Table 1.3-1). The sampling time points may be reduced in subsequent dosing cohorts in Arm 1 based on data from earlier dosing cohorts. The Bond-Lader VAS will be used to assess central nervous system function and is a questionnaire in which subjects rate the way they feel regarding 16 separate items related to mood using a VAS.[10]

8.8 Adverse Events

8.8.1 Definitions

An AE is defined as any untoward medical occurrence in a clinical trial subject administered an IMP and which does not necessarily have a causal relationship with this treatment. Adverse events would not include information recorded as medical history at screening for pre-planned procedures for which the underlying condition was known and no worsening occurred. An adverse reaction is any untoward and unintended response to an IMP related to any dose administered.

A suspected adverse reaction is any AE for which there is a reasonable possibility that the IMP caused the AE. For the purpose of Investigational New Drug (IND) safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the IMP and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality.

Treatment-emergent AEs are defined as AEs with an onset date on or after the start of double-blind treatment. In more detail, TEAEs are all AEs which started after the start of double-blind IMP treatment; or if the event was continuous from baseline and was worsening, serious, IMP related, or resulted in death, discontinuation, interruption, or reduction of IMP.

An SAE includes any event that results in any of the following outcomes:

- Death
- Life-threatening; ie, the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred. It does not include an event that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity/disability or substantial disruption of the ability to conduct normal life functions.
- Requires inpatient hospitalization or prolongs hospitalization.
  - Hospitalization itself should not be reported as an SAE; whenever possible the reason for the hospitalization should be reported.
  - Hospitalizations or prolonged hospitalizations for social admissions (ie, those required for reasons of convenience or other nonmedical need) are not considered SAEs.
  - Prescheduled hospitalization to address a condition that has existed prior to the signing of the ICF should not be considered an SAE.
- Congenital anomaly/birth defect.
- Other medically significant events that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to

prevent one of the outcomes listed above; eg, allergic bronchospasm requiring intensive treatment in an emergency room or home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

Nonserious AEs are all AEs that do not meet the criteria for a "serious" AE.

Adverse Events of Special Interest (AESIs): A noteworthy event for the particular product/IMP or class of products that a sponsor may wish to monitor carefully. No AESIs have been identified for the IMP to be administered during this trial.

Immediately Reportable Event (IRE):

- Any SAE.
- Any AE related to occupational exposure.
- Potential serious hepatotoxicity (see Section 8.8.6).
- Pregnancies are also defined as IREs. Although normal pregnancy is not an AE, it will mandate IMP discontinuation and must be reported on an IRE form and the Pregnancy Surveillance Form(s) to the sponsor. This includes pregnancy of the subject or the partner of the subject. Pregnancy will only be documented on the AE eCRF if the pregnancy occurs in a female subject and there is an abnormality or complication.

Clinical Laboratory Test Value Changes: It is the investigator's responsibility to review the results of laboratory tests for each individual subject as they become available. This review will be documented by the investigator's dated signature on the laboratory report. The investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests. If this laboratory value is considered medically relevant (ie, clinically significant) by the investigator (subject is symptomatic, requiring corrective treatment or further evaluation), or if the laboratory value leads to discontinuation, and/or fulfills a seriousness criterion, this is considered an AE.

Severity: Adverse events will be graded on a 3-point scale and reported as indicated in the eCRF. The severity of an adverse experience is defined as follows:

1 = Mild: Discomfort noticed, but no disruption to daily activity.

2 = Moderate: Discomfort sufficient to reduce or affect normal daily activity.

3 = Severe: Inability to work or perform normal daily activity.

IMP Causality: Assessment of causal relationship of an AE to the use of the IMP is defined as follows:

Related:	There is a reasonable possibility of a temporal and causal relationship between the IMP and the AE.
Not Related:	There is no temporal or causal relationship between the IMP and the AE.

8.8.2 Eliciting and Reporting Adverse Events

The investigator will regularly assess subjects for the occurrence of AEs. To avoid bias in eliciting AEs, subjects should be asked the nonleading question: "How have you felt since your last visit?" All AEs (serious and nonserious) reported by the subject must be recorded on the source documents and eCRF provided by the sponsor. Adverse event collection will begin after a subject signs the ICF and will continue until 30 (+ 2) days after the last dose of IMP. All AEs must be reported after subject informed consent has been obtained, including screening failures due to AEs, irrespective of IMP administration.

Medical terminology should be used for AE reporting. Adverse events should be reported as a single unifying diagnosis whenever possible or, in the absence of a unifying diagnosis, as individual signs or symptoms.

Exacerbation of conditions reported in the medical history should be reported as an AE only if there are unusual or severe clinical features that were not present, or experienced earlier, or not expected based on the course of the condition.

An AE that undergoes a change in severity, seriousness, or toxicity should be reported as a new AE in the eCRF.

In addition, the sponsor must be notified immediately by telephone, fax, or e-mail of any IREs according to the procedure outlined below, in Section 8.8.3. Special attention should be paid to recording hospitalization and concomitant medications.

The AE, start date (and start time, if possible), end date (and end time, if possible), seriousness, severity, relationship to trial treatment (IMP causality), action taken with trial treatment, and outcome will be recorded on the source documents and in the eCRF.

8.8.3 Immediately Reportable Events

The investigator must immediately report (within 24 hours), using an IRE form, after he/she or site personnel become aware of any IRE (SAE, AE related to occupational exposure, potential serious hepatotoxicity, or confirmed pregnancy), by telephone, fax, or e-mail to the sponsor or designee using the contact information on the cover page of this protocol (please note that the IRE form is NOT the AE eCRF). Subject confidentiality must be protected and contact information such as name, address, phone number, or any other protected health information as determined by applicable local regulation must be redacted when forwarding safety information and supporting documentation. Details regarding the follow-up of IREs are included in Section 8.8.8.2.

8.8.4 Medical Device Incidents (Including Malfunctions)

Not applicable.

8.8.5 Adverse Events of Special Interest

Not applicable.

8.8.6 Potential Serious Hepatotoxicity

For a subject who experiences an elevation in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) that is ≥ 3 times the upper limit of normal (ULN), a total bilirubin level should also be evaluated. If the total bilirubin is ≥ 2 times the ULN, complete an IRE form with all values listed and also report as an AE in the eCRF.

8.8.7 Procedure for Breaking the Blind

The investigator is encouraged to contact the sponsor/CRO medical advisor to discuss their rationale for unblinding. However, to prevent delays to the investigator or medical personnel responding to a potentially emergent situation, unblinding of the IMP will not be dependent upon the investigator receiving approval from the sponsor/CRO medical advisor (ie, the investigator will be able to obtain the code break information independent of the sponsor/CRO medical advisor). The investigator must contact the sponsor/CRO medical advisor by telephone or e-mail with an explanation of the need for opening the treatment assignment code within 24 hours of opening the code. If the blind is broken, the GPV department must be notified immediately (see the cover page of this protocol for contact information). Documentation of breaking the blind should be recorded in the subject's medical record with the date and time the blind was broken and the names of the personnel involved. Once the blind is broken for a subject, that subject may not reinitiate treatment with the IMP.

For the DRC meetings, the unblinded safety, tolerability, PK, and/or PD data collected from each cohort will be provided to the DRC for review.

8.8.8 Follow-up of Adverse Events

8.8.8.1 Follow-up of Nonserious Adverse Events

Nonserious AEs that are identified at any time during the trial must be recorded on the AE eCRF with the current status (ongoing or resolved/recovered) noted. All nonserious events (that are not IREs) that are ongoing at the last scheduled contact will be recorded as ongoing in the eCRF. For any AE having been identified throughout the trial, during analysis, additional relevant medical history information may be requested by the sponsor to further ascertain causality (including, but not limited to, information such as risk-related behavior, family history, and occupation).

8.8.8.2 Follow-up of Immediately Reportable Events

This trial requires that subjects be actively monitored for IREs up to 30 (+ 2) days after the last dose of IMP is administered.

Immediately reportable events that are **identified or ongoing at the last scheduled contact** must be recorded as such on the AE eCRF page and the IRE form. If updated information (eg, resolved status) on IRE status becomes available after a subject's last scheduled contact (up to last in-clinic visit for the entire trial), this must be reported to the sponsor and recorded on the AE eCRF page and the IRE form, according to the appropriate reporting procedures described in Section 8.8.3.

It is expected that the investigator will provide or arrange appropriate supportive care for the subject and will provide prompt updates on the subject's status to the sponsor. The investigator will follow IREs until the events are:

- Resolved,
- Stabilized,
- The subject is lost to follow-up, or
- Has died.

Resolution means that the subject has returned to the baseline state of health and stabilized means that the investigator does not expect any further improvement or worsening of the subject's condition. The investigator will continue to report any significant follow-up information to the sponsor up to the point the event has resolved or stabilized, or the subject is lost to follow-up or has died.

Refer to Section 10.3 for additional information regarding the follow-up period for subjects that become pregnant or for pregnant partners of male subjects.

8.8.8.3 Follow-up and Reporting of Immediately Reportable Events Occurring After Last Scheduled Contact

Any new IREs reported to the investigator which occur after the last scheduled contact and are determined by the investigator to be reasonably associated with the use of the IMP should be reported to the sponsor according to the procedures outlined in Section 8.8.3. This may include IREs that are captured on follow-up telephone contact or at any other time point after the defined trial period and continue to report any significant follow-up information to the sponsor until the events are resolved or stabilized, or the subject is lost to follow-up or has died.

8.9 Treatment of Overdose

For this trial, any dose of B-124a greater than a planned dose level will be considered an overdose. There is no specific antidote to B-124a. In the event of an overdose, the medical monitor will be notified immediately. The investigator will use clinical judgment to treat any overdose and the subject should receive appropriate supportive care. Any AEs, as well as the quantity of the excess dose amount and duration of the overdose, should be documented.

8.10 Subject Assessment Recording

Not applicable.

8.11 Other Assessments

Not applicable.

9 Statistical Considerations

9.1 Sample Size

This trial is not powered for statistical comparisons of PK parameters and the sample size of 88 subjects was chosen from practical considerations. The number of subjects per cohort (6 active and 2 placebo) in Arms 1 and 2 is based on previous experience with other drugs for FIH trials. The number of subjects per dose level in Arms 1and 2 is generally considered to be adequate for determination of tolerability and estimation of the PK parameters.

9.2 Datasets for Analysis

The analysis datasets are defined as follows:

- Safety dataset: includes all subjects who receive at least 1 dose of IMP.
- PK dataset: includes all subjects who receive at least 1 dose of IMP and have at least 1 postdose evaluable plasma concentration.
- PD dataset: includes all subjects who receive at least 1 dose of IMP and have baseline and at least 1 postdose evaluable PD measure.
- PK/QTc dataset: includes all subjects who are in the PK dataset and have at least 1 paired postdose PK and QTc value from the same time point.
- PK/PD (no QTc) dataset: includes all subjects who are in the PK dataset and have at least 1 postdose PK/PD value.

9.3 Handling of Missing Data for Primary and Secondary Endpoint Analysis

Missing safety data, and PK and PD parameters, will not be imputed.

9.4 Statistical Analyses

9.4.1 Efficacy Analyses

Not applicable.

9.4.2 Safety Analysis

Safety variables to be analyzed include AEs, clinical laboratory tests, vital sign measurements, safety ECGs, physical examinations, neurological examinations, neurological scale scores (CADSS, MOAA/S, BPRS, and the Cogstate Safety Battery + the One Back Test), and the C-SSRS.

The safety analysis will be performed for each arm. Safety data will be summarized using descriptive statistics (as applicable); data from subjects administered placebo will be pooled together for data summaries. Changes from baseline in safety variables will be summarized using descriptive statistics as applicable; baseline will be the last predose value at the start of the treatment period.

9.4.2.1 Adverse Events

All AEs will be coded by system organ class and Medical Dictionary for Regulatory Activities (MedDRA) preferred term. The incidence of the following events will be summarized by treatment group and dose cohort:

- TEAEs
- TEAEs by severity
- TEAEs potentially causally related to the IMP
- TEAEs with an outcome of death
- Serious TEAEs
- TEAEs leading to discontinuation of the IMP

Listings of the above TEAEs will also be provided.

9.4.2.2 Clinical Laboratory Data

Actual values for clinical laboratory parameters and changes from baseline will be summarized by treatment and visit using descriptive statistics. The incidence of potentially clinically relevant clinical laboratory tests will be summarized by treatment. Clinical laboratory test data will also be presented in listings.

9.4.2.3 Physical Examination and Vital Signs Data

Physical examination data will be provided in a data listing.

Actual values for vital sign parameters and changes from baseline will be summarized by treatment and visit using descriptive statistics. The incidence of potentially clinically relevant vital sign values will be summarized by treatment. Vital signs data will also be presented in listings.

9.4.2.4 Electrocardiogram Data

Actual values for ECG intervals and changes from baseline will be summarized by treatment and visit using descriptive statistics. Clinical interpretation of ECG results will be listed by subject and dose cohort. The incidence of potentially clinically relevant ECG values will be summarized by treatment.

9.4.2.5 Other Safety Data

The C-SSRS data will be analyzed for evidence of any treatment-emergent issues related to suicidal ideation or behavior. The incidence of suicidality, suicidal behavior, and suicidal ideation will be summarized by treatment and visit.

Neurological scales (CADSS, MOAA/S, BPRS, and the Cogstate Safety Battery + the One Back Test) will be summarized using descriptive statistics by treatment and visit and also provided in data listings.

The Bond-Lader visual analog scale will be analyzed in an exploratory analysis. Details will be included in the statistical analysis plan (SAP).

9.4.3 Other Analyses

9.4.3.1 Analysis of Demographic and Baseline Characteristics

Demographic and baseline characteristics (age, sex, race and ethnicity, weight, height, and BMI) will be summarized by treatment using descriptive statistics.

9.4.3.2 Pharmacokinetic Analysis

Pharmacokinetic plasma samples will be used for the analysis of B-124a and exploratory work may be performed for identification and analysis of B-124a and additional metabolites. The PK parameters will be determined by a noncompartmental analysis of plasma concentrations for B-124a.

No inferential statistical analyses will be performed. In general, PK data will be described and summarized using descriptive statistics and listed by subject (as applicable). Individual table and summary tables using descriptive statistics (N, median, mean, standard deviation, percent coefficient of variation, minimum, and maximum) will be presented for plasma concentrations and PK parameters. The individual, mean, and median plots of plasma concentrations will also be provided.

In Arms 1 and 2, dose proportionality for C_{max} and area under the concentration-time curve (AUC) may be explored using the random intercept power model, with subjects as a random effect, using the following equation:[11,12]

$$ln\ (AUC\ or\ C_{max}) = \alpha + \beta\ ln\ (dose)$$

where α is the intercept and β is the slope parameter. Dose proportionality will be assumed if β is close to 1 and its 90% confidence interval (CI) is entirely contained within the 80% to 125% interval corrected for dose range.[11,12]

9.4.3.3 Pharmacodynamic Analysis

The by-time point analysis of QTc data will be performed for Arms 1 and 2. The analysis for QTcF will be based on a linear mixed-effects model. A similar analysis will be performed for HR and PR, QRS, RR, and QT intervals. Details of the analyses will be provided in the SAP.

9.4.3.4 Pharmacokinetic/Pharmacodynamic Analysis

An exposure-response analysis will be attempted to examine the relationship between QTc and plasma concentrations of B-124a (Arm 1 only) from the PK/QTc population using a linear mixed-effects modeling approach.

The predicted effect and its 2-sided 90% CI for placebo-corrected change-from-baseline in corrected QT interval (ΔΔQTc) at the geometric mean C_{max} of B-124a for all time points will be obtained from the model. If the upper bound of the 90% CI of the predicted effect is below 10 msec, B-124a will be deemed as having no clinically relevant QT effect over the range of exposures studied.

9.4.3.5 Pharmacogenomic Analysis

Pharmacogenomic data could be included as part of a covariate analysis in a population PK analysis but will not be reported in the clinical study report.

9.4.3.6 Exploratory Endpoint Analysis

To explore the relationship between B-124a plasma exposure and change in BP, qEEG measures, and neurological scale scores (ie, CADSS and MOAA/S), exploratory PK/PD and/or exposure-response analyses will be attempted to understand the potential activity of B-124a and dissociation effect in healthy subjects. Additional details will be provided in a separate analysis plan.

9.5 Interim Analysis and Adaptive Design

Not applicable.

9.5.1 Data Monitoring Committee

Not applicable.

9.5.2 Dosing Review Committee

Once all the data for dose selection for the cohort are available, the unblinded safety, tolerability, PK (PK sampling data through 24 hours postdose), and/or PD data collected from each cohort will be provided to the DRC for review. The investigator will remain blinded. The unblinded data from each cohort will be evaluated by the DRC to determine

if the dose for the next cohort will be escalated as planned, if the dose from the previous cohort will be repeated, if the dose will be increased, or if the dose will be decreased. Dose escalation may be modified or stopped based upon the sponsor's and/or investigator's clinical judgment at any time. Dose escalation will continue until a non-tolerated dose is reached and the MTD is established or the last cohort is completed. The DRC will consist of OPDC and Rugen trial personnel, including GPV, Clinical Management, Clinical Pharmacology, Medical, and Biostatistical representatives, CRO Medical representatives, as well as other staff, as needed. See Section 4.1.1 for additional details.

10 Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Trial Oversight Considerations

10.1.1 Ethics and Responsibility

This trial must be conducted in compliance with the protocol, FDA regulations, applicable International Council for Harmonisation (ICH) GCP guidance, international ethical principles derived from the Declaration of Helsinki and CIOMS guidelines, and applicable local laws and regulations. Each trial site will seek approval/favorable opinion by an IRB according to regional requirements, and the investigator will provide that documentation to the sponsor. The IRB will evaluate the ethical, scientific, and medical appropriateness of the trial. Further, in preparing and handling the eCRF, IRE, and any safety information, the investigator, subinvestigator, and their staff will take measures to ensure adequate care in protecting subject privacy. To this end, a subject ID will be used to identify each subject.

Financial aspects, subject insurance, and the publication policy for the trial will be documented in the agreement between the sponsor and the investigator.

10.1.2 Informed Consent

Informed consent will be freely obtained from all subjects (or their guardian or legally acceptable representative, as applicable for local laws). The ICF will be approved by the same IRB that approves this protocol.

Each ICF will comply with the ICH GCP guidelines and local regulatory requirements. The investigator will ensure that the sponsor reviews and authorizes any written site-specific ICF used in the trial before submission to the IRB. In support of the site's standard process for administering informed consent, this trial will also allow for an electronic informed consent form (eICF) as a tool within applicable regions and trial sites. The eICF utilizes the IRB-approved site-specific ICF to offer subjects an enhanced platform to review and understand their rights as a research subject as well as required trial procedures. When possible, trial sites will have subjects review and sign the eICF prior to starting any trial procedures; however, if local regulations do not allow for use of the electronic format, subjects may continue in the trial utilizing the standard paper and wet ink signature process.

Investigators may discuss trial availability and the possibility for entry with a potential subject without first obtaining consent. However, informed consent must be obtained and documented before initiation of any procedures that are performed solely for the purpose of determining eligibility for this trial, including withdrawal from current medication(s).

Potential subjects are free to refuse entry into the trial, or withdraw from the trial at any time, without justification, and there will be no consequences to their further care.

Prospective trial subjects will be provided with controlled access to the eICF application by trial site staff. When the trial site staff and the subject agree that the subject has enough information to make an informed decision to participate, the subject will electronically sign in the eICF application and an electronic date and timestamp will be applied to the signature. The subject will be given a printed, signed copy of the ICF. Any other parties required by the IRB (trial site staff, witnesses, or legally authorized representative) are also required to sign electronically and these signatures will be stored with the eICF in accordance with the ICH GCP Guideline and local regulatory requirements/guidelines. These signatures cannot be altered, removed, or copied.

Once appropriate essential information has been provided and fully explained in layman's language to the subject by the investigator (or a qualified designee), and it has been documented that the subject has had the opportunity to ask questions, the IRB-approved written ICF will be signed and dated by both the subject and the person obtaining consent (investigator or designee), as well as by any other parties required by the IRB. The subject will receive a copy of the signed ICF; the original shall be kept on file by the investigator.

Subjects may be asked to sign additional ICFs if the protocol is amended and the changes to the protocol result in additional information that needs to be provided to the subjects, so that they can make a knowledgeable and voluntary decision on continued trial participation. Female partners of male subjects who become pregnant during the course of the trial may be asked to sign additional ICFs in order to collect additional information regarding the nonsubject partner and fetus.

Subjects will also provide consent for the PGx sample as part of the main ICF.

10.1.3 Confidentiality

All information generated in this trial will be considered confidential and will not be disclosed to anyone not directly concerned with the trial without the sponsor's prior written permission. Subject confidentiality requirements of the region(s) where the trial is conducted will be met. However, authorized regulatory officials and sponsor personnel (or their representatives) may be allowed full access to inspect and copy the records, consistent with local requirements. All IMPs, subject bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the sponsor.

Subjects will be identified only by unique subject ID in the eCRF. If further subject identification is required, subjects' full names may be made known to a regulatory agency or other authorized officials if necessary, subject to local regulations.

10.1.4 Quality Control and Quality Assurance

10.1.4.1 Monitoring

The sponsor has ethical, legal, and scientific obligations to follow this trial in accordance with established research principles, the applicable ICH GCP guidance, and applicable regulatory requirements and local laws. As part of a concerted effort to fulfill these obligations (maintain current personal knowledge of the progress of the trial), the sponsor's monitors will visit the site during the trial, as well as communicate frequently via telephone, e-mail, and written communications. In addition, all investigators and trial site personnel will undergo initial and ongoing training for this particular trial, and this training will be clearly documented.

10.1.4.2 Auditing

The sponsor's Quality Assurance Unit (or representative) may conduct trial site audits. Audits will include, but are not limited to, IMP supply, presence of required documents, the informed consent process, site operations, delegation of authority and training, and a review of the eCRF with source documents, as applicable. The investigator will agree to cooperate and participate with audits.

Regulatory authorities may inspect the investigator site during or after the trial. The investigator will cooperate with such inspections and will contact the sponsor immediately if such an inspection occurs.

10.1.5 Protocol Deviations

In the event of a significant deviation from the protocol due to an emergency, accident, or mistake (eg, violation of informed consent process, IMP dispensing or subject dosing error, treatment assignment error, subject enrolled in violation of eligibility criteria or concomitant medication criteria), the investigator or designee will contact the sponsor or designee at the earliest possible time by telephone or via e-mail. The investigator and sponsor (or designee) will come as quickly as possible to a joint decision regarding the subject's continuation in the trial. This decision will be documented by the investigator and the sponsor (or designee) and reviewed by the site monitor.

Any major protocol deviation will be recorded in the eCRF along with the start date and details of the deviation.

10.1.6 Records Management

10.1.6.1 Source Documents

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents will include but are not limited to medical records, electronic data, logs, and recorded data from automated instruments or applications. All source documents pertaining to this trial will be maintained by the investigators and made available for direct inspection by authorized persons.

Investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to source data/documents by authorized persons as defined in the ICF. In all cases, subject confidentiality must be maintained in accordance with local regulatory requirements.

10.1.6.2 Data Collection

During each subject's visit to the site, an investigator or their designee participating in the trial will record information to document all significant observations. At a minimum, these notes will contain:

- Documentation of the informed consent process, including any revised consents;
- Documentation of the investigator's decision to enroll the subject into the trial, the review of all inclusion/exclusion criteria prior to IMP administration, and confirmation of the subject's actual participation in the trial;
- The date of the visit and the corresponding Visit or Day in the trial schedule;
- General subject status remarks, including any *significant* medical findings. The severity, frequency, duration, action taken, and outcome of any AEs and the investigator's assessment of relationship to IMP must also be recorded;
- Any changes in concomitant medications or dosages;

- A general reference to the procedures completed, including dosing and IMP compliance;
- The signature (or initials) and date of the investigator (or designee) who made an entry in the medical record.

In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes as described above.

Source documents and source data will be captured electronically (where permitted by local regulation) in this trial and will meet the same fundamental elements of data quality (eg, attributable, legible, contemporaneous, original, and accurate) as paper records. These data will be collected into a system that is fully validated according to 21 Code of Federal Regulations (CFR) Part 11. Changes to the data will be captured by an automatic audit trail.

Designated trial site staff will not be given access to the electronic source system until they have been appropriately trained. Information to be originally captured and reviewed electronically shall include details of the subject visit and the protocol required assessments performed as a part of these visits, medical history, AEs, and concomitant medications. Because this trial is using an electronic source record as the original point of data capture, there is no additional data entry step for the trial site for data collected directly into the application, rather, the electronic source record directly populates the trial database.

Some data may be captured via paper and then entered into the eSource system. These and any other data treated in this manner will be source data verified per the monitoring plan and the location of the source data (ie, eSource, paper, or a local electronic system) will be documented before the trial start. Any changes to the information in paper source documents will be initialed and dated on the day the change is made by a trial site staff member authorized to make the change. Changes will be made by striking a single line through erroneous data (so as not to obliterate the original data), and clearly entering the correct data (eg, ~~wrong data~~ right data). If the reason for the change is not apparent, a brief explanation for the change will be written in the source documentation by the investigator or their designee.

Another exception will be safety laboratory or central ECG data, where the official source documentation will be considered the report issued by the analyzing laboratory.

Remote monitoring of the original electronic source records will take place; however, on-site monitoring inspections will continue to take place in order to review data entry of source documentation directly captured on paper and transcribed into the system, to ensure protocol adherence, to assess trial site operational capabilities, and to perform other monitoring activities that cannot be performed remotely.

At the end of the trial, the investigator must certify that the data entered into the eSource application are complete and accurate. After database lock, the investigator will receive an electronic copy of the subject data.

10.1.6.3 File Management at the Trial Site

The investigator will ensure that the trial site file is maintained in accordance with applicable ICH GCP guidance and as required by applicable local regulations. The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

10.1.6.4 Records Retention at the Trial Site

The FDA regulations require all investigators participating in clinical drug trials to maintain detailed clinical data for one of the following periods:

- A period of at least 2 years after the date on which a New Drug Application is approved by the FDA;
- A period of 2 years after the sponsor has notified the FDA that investigation with this drug is discontinued.

The investigator must not dispose of any records relevant to this trial without either (1) written permission from the sponsor or (2) provision of an opportunity for the sponsor to collect such records. The investigator will be responsible to maintain adequate and accurate electronic or hard copy source documents of all observations and data generated during this trial including any data clarification forms received from the sponsor. Such documentation is subject to inspection by the sponsor and relevant regulatory authorities. If the investigator withdraws from the trial (eg, due to relocation or retirement), all trial-related records should be transferred to a mutually agreed-upon designee within a sponsor-specified timeframe. Notice of such transfer will be given to the sponsor in writing.

10.1.6.5 Publication Authorship Requirements

Authorship for any Rugen/Otsuka-sponsored publications resulting from the conduct of this trial will be based on International Committee of Medical Journal Editors (ICMJE) authorship criteria (http://www.icmje.org/recommendations). According to ICMJE guidelines, one may be considered an author only if the following criteria are met:

1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2) Drafting the work or revising it critically for important intellectual content; AND
3) Final approval of the version to be published; AND
4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All authors must meet the above criteria, and all who qualify for authorship based on the above criteria should be listed as authors.

Investigators or other trial subjects who do not qualify for authorship may be acknowledged in publications resulting from the trial. By agreeing to participate in the trial, investigators or other trial subjects consent to such acknowledgement in any publications resulting from its conduct.

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10.2-1 will be performed.

Table 10.2-1 Clinical Laboratory Assessments

Hematology: Hematocrit Hemoglobin Mean corpuscular hemoglobin concentration Mean corpuscular volume Platelets RBC count WBC count (absolute and differential) Urinalysis: Color Glucose Microscopic analysis, if indicated, WBC/RBC counts per high powered field Occult blood pH Protein Specific gravity Specimen appearance Drug Screen (all items in urine except where noted): Alcohol (urine alcohol test is preferred but breath alcohol test may be used if necessary) Amphetamines Barbiturates Benzodiazepines Cannabinoids Cocaine Cotinine (urine or serum) Methadone Opiates Phencyclidine Propoxyphene	Serum Chemistry: Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bilirubin Blood urea nitrogen Calcium Cholesterol Creatinine Gamma glutamyl transferase Glucose Lactate dehydrogenase Potassium Sodium Total protein Triglycerides Additional Tests: Activated partial thromboplastin time Follicle-stimulating hormone (for confirmation of postmenopausal females) Prothrombin time-international normalized ratio Serology T_3 and T_4 Thyroid-stimulating hormone Urine drug screen Urine (and confirmatory serum, if needed) pregnancy test for FOCBP

RBC = red blood cell; T_3 = triiodothyronine; T_4 = thyroxine; WBC = white blood cell.

10.3 Appendix 3: Contraceptive Guidance and Collection of Pregnancy Information

Females of childbearing potential are females whose menstruation has started and who are not documented as sterile (eg, have had a bilateral oophorectomy, or hysterectomy, or who have been postmenopausal for at least 12 months). Females of nonchildbearing potential do not meet the definition of FOCBP.

For heterosexually active males and FOCBP, or their partners, there must be a documented agreement that the subject and their partner will take effective measures (ie, 2 different approved methods of birth control or remains abstinent) to prevent pregnancy during the course of the trial and for 90 days (for males) or 30 days (for FOCBP) after the last dose of IMP. Abstinence will be permitted if it is confirmed and documented at every trial visit. Unless the subject is sterile (ie, females who have had a bilateral oophorectomy, have had a hysterectomy, or have been postmenopausal for at least 12 consecutive months; or males who have had a bilateral orchiectomy) or remains abstinent during the trial and for 90 days (for males) or 30 days (for FOCBP) after the last dose of IMP, 2 of the following approved methods of birth control must be used: vasectomy, tubal ligation, nonhormonal intrauterine device, condom, sponge, or occlusive cap (vaginal diaphragm or cervical/vault cap). Any single method of birth control, including vasectomy and tubal ligation, may fail, leading to pregnancy. The contraceptive method will be documented in the eCRF. Male subjects must also agree not to donate sperm from trial screening through 90 days after the last dose of IMP.

Before enrolling males and females in this clinical trial, investigators must review the below information about trial participation as part of the ICF process. The topics should generally include:

- General information
- Informed consent form
- Pregnancy prevention information
- Drug interactions with hormonal contraceptives
- Contraceptives in current use
- Follow-up of a reported pregnancy

Before trial enrollment, males and FOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. Subjects must sign the ICF confirming that the above-mentioned risk factors and the consequences were discussed.

A urine pregnancy test for human chorionic gonadotropin (hCG) will be performed at screening and at check-in to the inpatient facility on all FOCBP. If a urine test is performed and is positive, the investigator will follow-up with a confirmatory serum test.

During the trial, all FOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (eg, missed or late menstrual cycle). Male subjects must be instructed to contact the investigator immediately, during the trial, if their partner suspects that they might be pregnant (eg, missed or late menstrual cycle).

If a subject is suspected to be pregnant before she receives IMP, the IMP administration must be withheld until the results of serum pregnancy tests are available. If the pregnancy is confirmed, the subject must not receive the IMP and must not be enrolled in the trial. If pregnancy is suspected while the subject is taking IMP, the IMP must be withheld immediately (if reasonable, taking into consideration any potential withdrawal risks) until the result of the pregnancy test is known. If pregnancy is confirmed, the IMP will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety) and the subject will be withdrawn from the trial. Exceptions to trial discontinuation may be considered for life-threatening conditions only after consultations with the IRE contact (see the cover page of this protocol for contact information).

The investigator must immediately notify the sponsor (within 24 hours) of any pregnancy associated with IMP exposure during the trial and for 30 days (for female subjects) or 90 days (for female partners of male subjects) after the last dose of IMP, and record the event on the IRE form and forward it to the sponsor. The sponsor will forward the Pregnancy Surveillance Form(s) to the investigator for monitoring the outcome of the pregnancy.

Protocol required procedures for trial discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (eg, x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report to the sponsor, on the Pregnancy Surveillance Form(s), follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants will be followed for a minimum of 6 months from the date of birth.

10.4 Appendix 4: Protocol Amendments

The investigator will not make any changes to this protocol without the sponsor's prior written consent and subsequent approval/favorable opinion by the IRB. Any permanent change to the protocol, whether an overall change or a change for specific trial site(s), must be handled as a protocol amendment. Any amendment will be written by the sponsor. Each amendment will be submitted to the IRB, as required by local regulations. Except for "administrative" or "nonsubstantial" amendments, investigators will wait for IRB approval/favorable opinion of the amended protocol before implementing the change(s). Administrative amendments are defined as having no effect on the safety of subjects, conduct or management of the trial, trial design, or the quality or safety of IMP(s) used in the trial. A protocol change intended to eliminate an apparent immediate hazard to subjects should be implemented immediately after agreement by the sponsor and investigator, followed by IRB notification within local applicable timelines. The sponsor will submit protocol amendments to the applicable regulatory agencies within local applicable timelines.

When the IRB, investigators, and/or the sponsor conclude that the protocol amendment substantially alters the trial design and/or increases the potential risk to the subject, the currently approved written ICF will require similar modification. In such cases, after approval/favorable opinion of the new ICF by the IRB, repeat written informed consent will be obtained from subjects enrolled in the trial before expecting continued participation and before the amendment-specified changes in the trial are implemented.

10.4.1 Protocol Amendment(s)/Administrative Change(s)

10.4.1.1 Protocol Amendment 1

Amendment 1 Approval Date: 05 Aug 2021

PURPOSE:

The purpose of this protocol amendment is to include additional stopping criteria, increase the sample size in Arm 2, note that the IMP should be destroyed by the trial site rather than returned to the sponsor, clarify the time frame for pregnancy reporting, and remove the appendices for identifying values of potential clinical relevance. In addition, minor editorial revisions were made.

BACKGROUND:

These changes to Protocol X06-201-00001, issued 25 May 2021, were made because:

- Stopping criteria were added to address concerns around potential neurotoxicity.
- The sample size in the food effect arm was increased from 8 to 12 subjects to sufficiently characterize the effect of food on the PK of B-124a.
- IMP return was changed to IMP destruction for compliance with Otsuka's standard procedures.
- The time frame for pregnancy reporting was modified for consistency with the rest of the protocol.
- The appendices for identifying values of potential clinical relevance were removed because the criteria for identifying laboratory values, vital signs, and ECGs of potential clinical relevance will be outlined in the SAP.

MODIFICATIONS TO PROTOCOL:

General Revisions:

- Added that based on the available toxicokinetic data, the highest dose will be limited to 29 mg, or 934 ng/mL and 3040 ng·h/mL based on the C_{max} and AUC of B-124a, respectively, whichever is reached first. Also added that any dose higher than 29 mg or any dose level that would produce plasma concentrations higher than those cited above will not be tested until additional data to support higher doses and/or plasma concentrations are available.
- Clarified that the maximum exposure will be less than 10-fold those achieved at the highest dose of 30 mg/kg (rather than 100 mg/kg) in the rat neurotoxicity study.
- Added stopping criteria of exposure greater than a C_{max} of 934 ng/mL or AUC of 3040 ng·h/mL and/or a dose higher than 29 mg.
- Changed the sample size in Arm 2 from 8 to 12 subjects.
- Changed the total sample size from 64 to 68 subjects.

- Added that the IMP should be destroyed by the trial site if approved by the sponsor and if the IMP destruction meets all local regulations, and that this will be documented in the IMP accountability records.
- Removed the return of IMP to the sponsor.
- Clarified that the investigator must immediately notify the sponsor of any pregnancy associated with IMP exposure during the trial and for 30 days (for female subjects) or 90 days (for female partners of male subjects) after the last dose of IMP.
- Removed Appendix 4 (Criteria for Identifying Laboratory Values of Potential Clinical Relevance), Appendix 5 (for Identifying Vital Signs of Potential Clinical Relevance), and Appendix 6 (Criteria for Identifying ECG Measurements of Potential Clinical Relevance).

ADDITIONAL RISK TO THE SUBJECT:

There is no additional risk to the subjects.

10.4.1.2 Protocol Amendment 2

Amendment 2 Approval Date: 17 Dec 2021

PURPOSE:

The purpose of this protocol amendment is to remove the dosing restrictions and stopping criteria that were added in Amendment 1; add additional PK, vital signs, and ABPM time points for Cohorts 3 to 7 in Arm 1; clarify the Day 8 vital signs and ABPM time point in Arm 1; correct the description of the randomization numbers; update text describing the CADSS; and remove AESI from the definition of an IRE.

BACKGROUND:

Changes to X06-201-00001 Protocol Amendment 1, issued 05 Aug 2021, were made due to the following:

- Additional stopping criteria were removed since neurotoxicity data to support higher doses and/or plasma concentrations are now available.
- Additional time points for PK blood collection, vital signs measurement, and ABPM collection were added for Cohorts 3 to 7 in Arm 1 based on the data from earlier dose cohorts.
- The descriptions of randomization numbers and of the CADSS were updated based on Note to Files issued after the approval of the Protocol Amendment 1.
- Adverse events of special interest were removed from the definition of an IRE as no AESIs have been identified for the IMP to be administered during this trial.

MODIFICATIONS TO PROTOCOL:

General Revisions:

- Removed text around the highest dose being limited to 29 mg, or 934 ng/mL and 3040 ng·h/mL based on the C_{max} and AUC of B-124a, respectively, whichever is reached first.
- Removed the stopping criteria of exposure greater than a C_{max} of 934 ng/mL or AUC of 3040 ng·h/mL and/or a dose higher than 29 mg.
- Removed text describing the maximum exposure based on the highest dose in the rat neurotoxicity study.
- Added additional PK blood collections time points for Cohorts 3 to 7 in Arm 1 on Day 5: 96 hours postdose, Day 6: 120 hours postdose, Day 7: 144 hours postdose, and Day 8: 168 hours postdose. In addition, deleted the example text in parenthesis (including “reduced” and “eg, the Day 4 sampling time point may be excluded”) from the text describing that the PK sampling time points in subsequent dosing cohorts in Arm 1 may be changed.

- Added additional vital sign measurement time points for Cohorts 3 to 7 in Arm 1 on Day 5: 96 hours postdose, Day 6: 120 hours postdose, and Day 7: 144 hours postdose,
- Clarified that the vital sign measurement for all cohorts in Arm 1 on Day 8 will be at 168 hours postdose.
- Added additional ABPM collection time points for Cohorts 3 to 7 in Arm 1 in both the supine and standing positions on Day 5: 96 hours postdose, Day 6: 120 hours postdose, and Day 7: 144 hours postdose.
- Clarified that the ABPM collection for all cohorts in Arm 1 on Day 8 will be at 168 hours postdose.
- Updated text describing the randomization numbers to state subjects in Arm 1 will be assigned a 5-digit number (Cohort 1 will begin with 10001, Cohort 2 will begin with 20001, Cohort 3 will begin with 30001, with a similar pattern for Cohorts 4, 5, 6, and 7) and subjects in Arm 2 will be assigned a 4-digit number beginning with 1001.
- Updated text describing the CADSS as a 28-item scale with 23 subject-rated items and 5 items scored by an observer instead of a 27-item scale with 19 subject-rated items and 8 items scored by an observer Text was also added to state that only subjective items will be utilized in this trial, and text describing the observer component was deleted.
- Removed AESI from the definition for an IRE.

ADDITIONAL RISK TO THE SUBJECT:

There is no additional risk to the subjects.

10.4.1.3 Protocol Amendment 3

Amendment 3 Approval Date: 10 Feb 2022

PURPOSE:

The purpose of this amendment is to allow for dosing of the IMP with 100% cranberry juice or water and to allow for repeat cohorts of a dose level.

BACKGROUND:

Changes to X06-201-00001 Protocol Amendment 2, issued 17 Dec 2021, were made due to the following:

- Dosing with 100% cranberry juice was added to allow flexibility for the dosing vehicle for a given cohort.
- Allowing for repeat cohorts was added in order to further study a dose level if needed.

MODIFICATIONS TO PROTOCOL:

General Revisions:

- Added that doses of IMP may be taken with 240 mL of still water or 240 mL of 100% cranberry juice.
- For Cohort 3 through 7, doses may also be repeated or adjusted based on PK, AEs, and safety assessments.
- For Cohorts 3 through 7, the dose may also be adjusted when administered with 100% cranberry juice.
- For Arm 2, the timepoints for the collection of clinical laboratory samples was clarified to predose on Day 1 and any time on Day 3 and at ET.
- "Country" was removed from the demographics data collected in the eCRF.

ADDITIONAL RISK TO THE SUBJECT:

There is no additional risk to the subjects.

10.4.1.4 Protocol Amendment 4

Amendment 4 Approval Date: 02 Sep 2022

PURPOSE:

The purpose of this amendment is to add another single ascending oral dose arm (similar to Arm 1) to the study to assess 3 to 4 cohorts using the liquid-filled capsule formulation of B-124a and removal of the food effect arm (which was the previous Arm 2).

BACKGROUND:

Changes to X06-201-00001 Protocol Amendment 3, issued 10 Feb 2022, were made due to the following:

- Arm 1 was completed with the API capsule formulation; the Sponsor used that preliminary data to inform a new formulation which has been added to the trial and the food effect arm was removed.

MODIFICATIONS TO PROTOCOL:

General Revisions:

- Removal of the food effect arm (Arm 2).
- Replaced the Arm 2 to assess 3 to 4 dose levels of the liquid-filled capsule formulation of B-124a.

ADDITIONAL RISK TO THE SUBJECT:

There is no additional risk to the subjects.

11 References

1 Newport DJ, Carpenter LL, McDonald WM, Potash JB, Tohen M, Nemeroff CB, et al. Ketamine and other NMDA antagonists: Early clinical trials and possible mechanisms in depression. Am J Psychiatry. 2015;172(10):950-966.

2 Guidance for Industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research (CBER). September 2007.

3 Garner R, Gopalakrishnan S, McCauley JA, Bednar RA, Gaul SL, Mosser SD, et al. Preclinical pharmacology and pharmacokinetics of CERC-301, a GluN2B-selective N-methyl-D-aspartate receptor antagonist. Pharmacol Res Perspect. 2015;3(6)e00198:1-12.

4 Guidance for Industry. Assessing the effects of food on drugs in INDs and NDAs – clinical pharmacology considerations. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). February 2019.

5 Bremner D, Krystal JH, Putnam FW, Southwick SM, Marmar C, Charney DS, et al. Measurement of dissociative states with the Clinician-Administered Dissociative States Scale (CADSS). J Trauma Stress. 1998;11(1):125-136.

6 Kowalski R, Mahon P, Boylan G, McNamara B, Shorten G. Validity of the modified observer's assessment of alertness/sedation scale (MOAA/S) during low dose propofol sedation [abstract]. Eur J Anaesthesiol. 2007;24:26-27.

7 Chernik DA, Gillings D, Laine H, Hendler J, Silver JM, Davidson AB, et al. Validity and reliability of the Observer's Assessment of Alertness/Sedation Scale: study with intravenous midazolam. J Clin Psychopharmacol. 1990;10(4):244-251.

8 Overall JE, Gorham DR. The brief psychiatric rating scale. Psychological Reports. 1962;10:799-812.

9 Cogstate. Featured batteries - Cogstate safety battery [cited 08 Dec 2020]. https://www.cogstate.com/clinical-trials/computerized-cognitive-assessment/featured-batteries/?cn-reloaded=1.

10 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol. 1974;47(3):211-218.

11 Gough K, Hutchison M, Keene O, Byron B, Ellis S, Lacey L, et al. Assessment of dose proportionality: Report from the statisticians in the pharmaceutical industry/pharmacokinetics UK joint working party. Drug Inf J. 1995;29(3):1039-1048.

12 Smith BP, Vandenhende FR, Desarte KA, Farid NA, Welch PA, Callaghan JT, et al. Confidence interval criteria for assessment of dose proportionality. Pharm Res. 2000;17(10):1278-1283.

Agreement

I, the undersigned principal investigator, have read and understand the protocol (including the Investigator's Brochure) and agree that it contains all the ethical, legal, and scientific information necessary to conduct this trial in accordance with the principles of Good Clinical Practices and as described herein and in the sponsor's (or designee's) Clinical Trial Agreement.

I will provide copies of the protocol to all physicians, nurses, and other professional personnel to whom I delegate trial responsibilities. I will discuss the protocol with them to ensure that they are sufficiently informed regarding the investigational new drug, B-124a, the concurrent medications, the efficacy and safety parameters, and the conduct of the trial in general. I am aware that this protocol must be approved by the Institutional Review Board (IRB) responsible for such matters in the clinical trial facility where B-124a will be tested prior to commencement of this trial. I agree to adhere strictly to the attached protocol (unless amended in the manner set forth in the sponsor's Clinical Trial Agreement, at which time I agree to adhere strictly to the protocol as amended).

I understand that this IRB-approved protocol will be submitted to the appropriate regulatory authority/ies by the sponsor. I agree that clinical data entered on eCRF by me and my staff will be utilized by the sponsor in various ways, such as for submission to governmental regulatory authorities and/or in combination with clinical data gathered from other research sites, whenever applicable. I agree to allow sponsor and designee monitors and auditors full access to all medical records at the research facility for subjects screened or enrolled in the trial.

I agree to await IRB approval before implementation of any substantial amendments to this protocol. If, however, there is an immediate hazard to subjects, I will implement the amendment immediately, and provide the information to the IRB within the required local applicable timelines. Administrative changes to the protocol will be transmitted to the IRB for informational purposes only, if required by local regulations.

I agree to provide all subjects with informed consent forms, as required by the applicable regulations and by ICH guidelines. I agree to report to the sponsor any adverse experiences in accordance with the terms of the sponsor's Clinical Trial Agreement and the relevant regional regulation(s) and guideline(s). I further agree to provide all required information regarding financial certification or disclosure to the sponsor for all investigators and subinvestigators in accordance with the terms of the relevant regional regulation(s). I understand that participation in the protocol involves a commitment to publish the data from this trial in a cooperative publication before publication of efficacy and safety results on an individual basis may occur, and I consent to be acknowledged in any such cooperative publications that result.

_____	_____	_____
Principal Investigator Print Name	Signature	Date

This page is a manifestation of an electronically captured signature

SIGNATURE PAGE

Document Name: X06-201-00001 Protocol Amendment 4

Document Number: 1000108451

Document Version: 7.0

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy hh:min) - UTC timezone
Davidson Heather	Clinical Pharmacology Approval	06-Sep-2022 20:16:52
Zhang_Peter	Biostatistics Approval	06-Sep-2022 22:06:37
Sundararajan Kripa	Clinical Approval	06-Sep-2022 16:58:27
Zhang Xiaoyan	Clinical Pharmacology Approval	06-Sep-2022 13:07:51
Gideon Shapiro 	Rugen CSO Approval	07-Sept-2022