STUDY DRUG:
CY6463

PROTOCOL NUMBER:
C6463-202

STUDY TITLE:
A Phase 2a study evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of CY6463 when administered to participants with Alzheimer's disease and vascular pathology

REGULATORY AGENCY IDENTIFIERS:
IND: 138999
NCT: 04798989

SPONSOR:
Cyclerion Therapeutics, Inc.
245 First Street, 18th Floor
Cambridge, MA 02142 USA

[redacted]

Approval Dates	
Original Protocol:	29 January 2021
Amendment 1:	05 March 2021
Amendment 2:	28 April 2021
Amendment 3:	16 December 2021

Confidentiality Statement

1. PROTOCOL SUMMARY

1.1. Synopsis

<table>
<tr><td colspan="3">Name of sponsor: Cyclerion Therapeutics, Inc.</td></tr>
<tr><td colspan="3">Name of investigational product: CY6463 (alias IW-6463)</td></tr>
<tr><td colspan="3">Name of active ingredient: CY6463</td></tr>
<tr><td>Protocol number: C6463-202</td><td>Phase: 2a</td><td>Country: United States (US)</td></tr>
<tr><td colspan="3">Study title: A Phase 2a study evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of CY6463 when administered to participants with Alzheimer’s disease and vascular pathology</td></tr>
<tr><td colspan="3">Primary objective and endpoint:
Objective: To evaluate the safety and tolerability of CY6463 when administered to participants with Alzheimer’s disease with vascular pathology (ADv)

Endpoint: Incidence of treatment-emergent adverse events (TEAEs) from study drug initiation through Follow-up</td></tr>
<tr><td colspan="3">Study design:
C6463-202 is a multicenter, randomized, placebo-controlled, double-blind, sponsor unblinded study evaluating CY6463 vs placebo in participants with ADv.
Participants will be randomized in a 1:1 ratio to receive either CY6463 or placebo once daily (QD) for approximately 87 sequential days. They will complete a total of 7 scheduled visits over the course of the study per the Schedule of Events, from Screening through Follow-up.
For scheduled visits that occur on a dosing day, participants will take their study drug dose during the visit to allow for appropriate timing of pre- and postdose assessments. In conjunction with these Treatment period visits, participants will fast for ≥4 hours before clinical laboratory samples are collected. All other study drug doses will be self-administered at home. Participants will be instructed to take their QD dose at a consistent time (preferably ±1 hour) throughout the Treatment period. CY6463 may be taken with or without food.</td></tr>
<tr><td colspan="2">Randomized participants (planned): ~30</td><td>Study centers (planned): up to 10</td></tr>
<tr><td colspan="3">Diagnosis and main criteria for inclusion: Individuals 60 years of age and older with mild cognitive impairment and clinical and/or imaging findings supporting the diagnosis of Alzheimer’s disease and who satisfy all other eligibility criteria, with a goal of including those with subcortical, small-vessel disease and excluding those with large-vessel disease and/or history of large infarcts.</td></tr>
<tr><td colspan="3">Investigational product, dosage, and mode of administration:
CY6463 tablets (5 mg) taken orally on a 15-mg QD regimen</td></tr>
<tr><td colspan="3">Reference therapy:
Placebo-to-match CY6463 tablets taken orally on a 3-tablet QD regimen</td></tr>
<tr><td colspan="3">Duration of study drug dosing and overall study participation for each individual:
Each participant will receive study drug (CY6463 or placebo) for approximately 12 weeks (~87 days).
Overall individual study duration will be approximately 5 months, including the Screening, Treatment, and Follow-up periods.</td></tr>
</table>

Safety assessments:

AEs, clinical safety laboratory tests, vital signs, physical examinations, 12-lead electrocardiograms

Statistical methods:

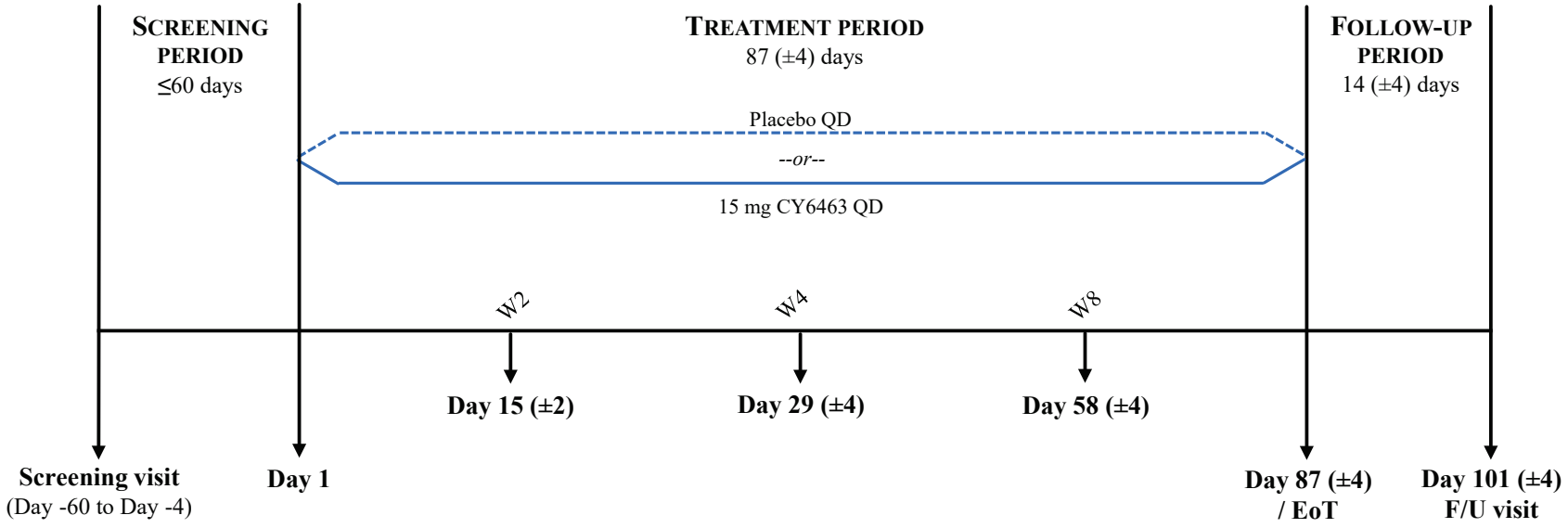
Sample size justification: A sample size of approximately 30 randomized participants was determined outside of statistical considerations and is considered sufficient to address the primary research objectives.

General considerations: Corresponding with the primary objective, TEAE incidence (number and percentage of participants with TEAEs) will be reported for each treatment group (placebo, CY6463).

Continuous variables will be summarized using number of participants, mean, SD, minimum, median, interquartile range, and maximum values. Categorical variables will be summarized using the frequency and percentage of participants in each category. Percentages will be based on the total number of participants with non-missing values. If values are missing, the number missing will also be presented, but without a percentage. Data summaries will be presented by treatment group. All available data will be included.

1.2. Study Schematic

Figure 1: Study Design Schematic



↓=visit; EoT=end of treatment; F/U=Follow-up; QD=once daily; W=Week

1.3. Schedule of Events and Assessments

Table 1 provides the timing of the events and assessments to be conducted in this study. Also refer to the appropriate sections of this protocol and accompanying study manuals (eg, laboratory, pharmacy, imaging) for more information.

Table 1: Schedule of Events

Period →	**Screening**	**Treatment**					**Follow-up**
Study Day →	**Day -60 to -4**	**Day 1**	**Day 15**	**Day 29**	**Day 58**	**Day 87/EoT [a]**	**Day 101**
Allowable visit window (days) →			**±2**	**±4**	**±4**	**±4**	**±4**
Procedures ↓							
ICF signed	X						
Eligibility evaluation	X	X					
MMSE (see Section 9.2)	X	X [b]				X	
Demography	X						
Medical history	X	X					
Complete physical exam (see Section 9.4)	X						
Symptom-directed physical exam		X	X	X	X	X	X
Weight (W), height (H)	W, H	W	W	W	W	W	W
12-lead ECG [c]	X	X [b]	X	X	X	X	
Vital signs (RR, O_2, BP/pulse, including orthostatic) (see Section 9.6)	X	X	X	X	X	X	X
Urinalysis (UTI screening) [d]	X						
Blood sample (genetics; see Section 9.7.1)	X						
Blood sample (chemistry, coag/hematol, lipids)	X	X [b]	X	X	X	X	X
Blood sample (biomarkers)		X [b]	X	X	X	X	X
Blood sample (PK)			X	X	X	predose post: 2,4,6h (±15m)[e]	
C-SSRS (see Section 9.8)	X	All visits (including unscheduled)					
Participant diary (see Section 9.9)	Issued	*Each visit:* Monitored and filed as source document, new diary issued					Returned
Adverse event monitoring	X	X	X	X	X	X	X

Table 1: Schedule of Events (Continued)

Period →	Screening	Treatment					Follow-up
Study Day →	Day -60 to -4	Day 1	Day 15	Day 29	Day 58	Day 87/EoT [a]	Day 101
Allowable visit window (days) →			±2	±4	±4	±4	±4
Procedures ↓							
Prior & concomitant meds (see Sec. 8.7)	X	X	X	X	X	X	X
Cognitive battery (see Section 9.11)	X [f]	X [b]	X	X	X	X	X
EEG, ERP (see Section 9.12)	X	X [b]	predose, and at 2h (±30m) postdose				X
Neuroimaging (rs ASL, fMRI) (see Sec. 9.13)	X [g]	X [b]	4 (±1½) h post			4 (±1½) h post	
CSF sampling (biomarkers & PK)	X [h]					6h (±15m) post [e]	
Study drug dosing [i], supply, accountability		X	X	X	X	X	

Note: All assessments on dosing days are performed predose unless otherwise noted. Assessments for all scheduled study visits can be performed across multiple days **provided that:** the assessments occur within the stated visit window, the predose assessments are performed before the daily dose is taken, and postdose assessments occur at the stated time per protocol. For example, the Day 15 visit assessments could be conducted across 2 days, such that on Day 14, all predose assessments could be completed prior to administration of the Day 14 dose, with the EEG performed at 2 hours postdose; and then on Day 15, the participant could undergo the MRI 4 hours after taking the Day 15 dose. Also see footnote b.

ABCA7=adenosine 5triphosphate (ATP)-binding cassette sub-family A, member 7; ApoE4=apolipoprotein E4; ASL=arterial spin labeling; BP=blood pressure; coag=coagulation; CSF=cerebral spinal fluid; C-SSRS=Columbia-Suicide Severity Rating Scale; ECG=electrocardiogram; EEG=electroencephalography; EoT=end of treatment; ERP=event-related potential; fMRI=functional magnetic resonance imaging; h=hour(s); H=height; hematol=hematology; ICF=informed consent form; m=minute(s); meds=medications; MMSE=Mini-Mental State Examination; O_2=oxygen saturation; PK=pharmacokinetic(s); post=postdose; pre=predose; RR=respiratory rate; rs=resting state; UTI=urinary tract infection; W=weight; X=predose

[a] EoT visit assessments also to be conducted (if possible) for participants who prematurely discontinue from study drug. See Section 7.6.

[b] If the Screening safety laboratory and ECG assessments are conducted within 1 week before Day 1 and the participant meets all eligibility requirements, the safety laboratory and ECG assessments do not need to be repeated on Day 1. In addition, the Day 1 predose MMSE, ECG, biomarker and safety laboratory sampling, cognitive battery, and the EEG, ERP, and neuroimaging can be conducted up to 3 days before Day 1.

[c] ECGs should be obtained after the participant has rested supine for ≥5 minutes. When timing coincides, ECGs and vital signs can be assessed together, but ECGs and vital signs should be obtained before blood draws (or ≥10 m after a blood draw, if necessary). See Section 9.5.

[d] Because of the potential for UTIs to cause sudden confusion or delirium, a urine sample will be tested for infection at Screening. Standard of care will be administered for a positive test result.

[e] The 6-hour postdose CSF and blood samples on Day 87 must be collected on the same day and at approximately the same time for an individual participant.

[f] Includes 2 training sessions for the Cogstate digital battery assessments; the trainings may be split over multiple visits (eg, Screening and Day 1). If split over multiple visits, the second training session must be completed prior to administration of the cognitive battery on Day 1. If completed at the same visit, the sessions must be separated by ≥15 m. **Note:** Only 1 training session is required for the letter fluency and category fluency assessments; this training will be completed during the predose/Screening period.

[g] Neuroimaging at Screening visit is limited to MRI scan needed for study eligibility; a scan obtained ≤6 months before Screening is acceptable.

[h] An aliquot from the screening CSF sample collected for the baseline biomarker assessment can be used to assess study eligibility (see Inclusion 5).

[i] For in-clinic dosing: study drug will be administered under medical supervision and the participant's mouth will be examined to ensure ingestion of study drug.

2. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

LIST OF TABLES

LIST OF FIGURES

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Abbreviation/Term	Definition
ABCA7	adenosine 5'-triphosphate (ATP)-binding cassette sub-family A, member 7
AD	Alzheimer's disease
ADv	Alzheimer's disease with vascular pathology
AE	adverse event
AESI	adverse event of special interest
ApoE4	apolipoprotein E4
ASL	arterial spin labeling
[redacted]	
BMI	body mass index
BP	blood pressure
cGMP	cyclic guanosine 3',5'-monophosphate
CNS	central nervous system
CRO	clinical research organization
CSF	cerebral spinal fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
CY6463	IW-6463
DRC	Data Review Committee
ECG	electrocardiogram
eCRF	electronic case report form
EEG	electroencephalography
EoT	end of treatment
ERP	event-related potential
fMRI	functional magnetic resonance imaging
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	intent-to-treat

Abbreviation/Term	Definition
IW-6463	CY6463
IWRS	interactive voice/web response system
LAR	legally authorized representative
MedDRA	Medical Dictionary for Regulatory Activities
min	minute(s)
MMRM	mixed-effect model repeated measures
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
NO	nitric oxide
PD	pharmacodynamic(s)
PDE5	phosphodiesterase 5
PK	pharmacokinetic(s)
PT	preferred term
QD	once daily
qEEG	quantitative electroencephalography
QT	the time between the beginning of the QRS (ventricular polarization) complex and the end of the T-wave
QTcF	QT interval corrected for HR using Fridericia's formula
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
sGC	soluble guanylate cyclase
SOC	system organ class
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
US	United States

4. INTRODUCTION

4.1. Name and Description of Study Drug

CY6463 (also known as IW-6463) is an orally administered central nervous system (CNS)-penetrant stimulator of soluble guanylate cyclase (sGC), a signaling enzyme that catalyzes the formation of cyclic guanosine 3',5'-monophosphate (cGMP) from guanosine triphosphate in response to nitric oxide (NO) binding. Intracellular cGMP regulates vascular tone and regional blood flow, fibrosis, and inflammation, and has been implicated in neuronal survival and cognitive function.

CY6463 is being investigated as a treatment for individuals with Alzheimer's disease (AD). It is intended for administration only to eligible participants in accordance with this protocol. See Section 8 for additional study drug product details.

4.2. Description of Study Population and Rationale

AD is a progressive degenerative disorder that affects 40 to 50 million individuals worldwide.(1) A report released in 2020 estimates more than 5 million Americans ages 65 and older are living with AD—and that number is expected to nearly triple by the year 2050.(2) Due to its significant impact on memory and other mental functions, AD is emotionally and financially devastating to both patients and their families/caregivers, with an estimated global cost in the $1-trillion range.(3) Recent drug development in this therapeutic area has been focused on clearing the disease's pathological hallmarks (beta amyloid plaques and tau protein-containing tangles), but these approaches have not yet translated into viable therapies. Currently available treatments show only a modest effect on symptoms and do not address the underlying disease pathology. Therefore, new approaches to treatment and new therapies are urgently needed.

The role of vascular pathology in AD has recently received increased attention and, more specifically, the role of the NO-sGC-cGMP pathway in the pathogenesis of this disease.(4-6) In AD and related dementias, endothelial cell loss and NO dysregulation are major contributing factors to cognitive impairment and progression and result in reduced cerebral blood flow, vascular leakage, inflammation, and neuronal dysfunction and loss.(4, 6, 7) Because this pathway has been shown to have impacts on cognition, neuroprotection, neuroinflammation, and bioenergetics, it has the potential to serve as a target for new therapies.

As a CNS-penetrant sGC stimulator, CY6463 has the potential to preferentially modulate the NO-sGC-cGMP pathway and provide therapeutic benefit for individuals with AD. This study will focus on individuals with AD with vascular pathology (ADv)—a subset of the larger AD population who have vascular pathology and cardiovascular risk factors. It is hypothesized that the ADv population is likely to receive more benefit and respond more favorably to CY6463 due to CY6463's impact on vascular biology.

Individuals 60 years of age and older with mild cognitive impairment and clinical and/or imaging findings supporting the diagnosis of AD and vascular pathology and who satisfy all other eligibility criteria will be enrolled. This study will aim to include participants with subcortical, small-vessel disease and to exclude those with large-vessel disease and/or history of large infarcts.

4.3. Findings from Nonclinical and Clinical Studies

4.3.1. Nonclinical Studies

Findings from nonclinical studies support the hypothesis that sGC stimulation may have beneficial effects across several key aspects of AD pathology and thus may serve as a viable treatment option for patients. Refer to the most recent CY6463/IW-6463 Investigator's Brochure (IB) for more detail.

4.3.2. Clinical Studies

Safety and pharmacokinetic (PK) data from study C6463-101 in healthy adults together with safety, PK, and pharmacodynamic (PD) data from study C6463-102 in healthy elderly adults support further clinical investigation of CY6463. Information regarding these studies is available in the IB.

4.4. Risk/Benefit

Results from the nonclinical efficacy and safety assessment program support a favorable risk/benefit assessment. Clinical evaluation of CY6463 thus far has been limited to healthy participants; direct benefit to a patient population has not yet been determined.

Evaluation of CY6463 in individuals with ADv is justified based on the minimal risk profile reported in healthy participants (including elderly) who have received CY6463, along with the potential benefit based on the pharmacological effects observed in nonclinical studies. Indirect benefits to participants include increased medical care/attention, a better understanding of their disease, and satisfaction knowing that their participation will contribute to AD research and potential therapeutics. Refer to the IB for more information.

Procedures and assessments included in this protocol are generally consistent with standard of care for monitoring and/or diagnosing disease in patients with AD and with vascular pathology. Although magnetic resonance imaging (MRI), blood sampling, and lumbar punctures represent the highest burden of the study assessments for participants, these assessments present minimal risk and a low probability of harm. The duration and frequency of MRI procedures, blood sampling volumes and frequency, and the number of lumbar punctures have been minimized to reduce the risk to study participants and to collect only the information necessary to support further development of CY6463.

Study stopping rules are discussed in Section 6.5.

4.5. Compliance Statement

See Appendix 1.

5. OBJECTIVES AND ENDPOINTS

Objective	Endpoints
Primary (Safety):	
• To evaluate the safety and tolerability of CY6463 when administered to participants with ADv	• Incidence of TEAEs from study drug initiation through Follow-up
Exploratory (Pharmacokinetics):	
• To evaluate the PK of CY6463 when administered to participants with ADv	• Plasma and CSF concentrations of CY6463 at assessed timepoints • PK parameters as data permit, including but not limited to T_{max}, C_{max}, C_{trough}, and the CSF:plasma concentration ratio
Exploratory (Pharmacodynamics):	
• To evaluate the effect of CY6463 on brain neurophysiology in participants with ADv	• Change from baseline to Day 87 in qEEG and ERP parameters
• To evaluate the effect of CY6463 on CBF in participants with ADv	• Change from baseline in CBF on Day 87 as measured by ASL
• To evaluate the effect of CY6463 on brain activity in participants with ADv	• Change from baseline on Day 87 in fMRI BOLD signal
• To evaluate the effect of CY6463 on brain small-vessel pathology in participants with ADv	• Change from baseline in white matter structural integrity on Day 87 as measured by MRI
• To evaluate the effect of CY6463 on biomarkers in participants with ADv	• Change from baseline on Day 87 in CSF and plasma biomarkers levels
• To evaluate the effect of CY6463 on cognitive performance in participants with ADv	• Change from baseline on Day 87 in cognitive performance assessments • Change from baseline on Day 87 in MMSE score

ADv=Alzheimer's disease with vascular pathology; ASL=arterial spin labeling; BOLD=blood-oxygen-level-dependent; CBF=cerebral blood flow; C_{max}=maximum observed plasma concentration; CSF=cerebral spinal fluid; C_{trough}=trough plasma concentration observed at the end of a dosing interval [collected before the next administration]; ERP=event-related potential; fMRI=functional magnetic resonance imaging; MMSE=Mini-Mental State Examination; MRI=magnetic resonance imaging; PK=pharmacokinetic(s); qEEG=quantitative electroencephalography; TEAE=treatment-emergent adverse event; T_{max}=time to C_{max}

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

C6463-202 is a multicenter, randomized, placebo-controlled, double-blind, sponsor unblinded study evaluating the safety, tolerability, PK, and PD of CY6463 compared with placebo in participants with ADv.

Participants will be randomized per Section 6.3.1 to receive approximately 87 sequential days of either CY6463 or placebo once daily (QD), and will complete a total of 7 scheduled visits over the course of the study from Screening through Follow-up. For all scheduled visits that occur on a dosing day, participants will take their dose during the visit to allow for appropriate timing of pre- and postdose assessments. All other doses will be self-administered at home. Total study duration for each participant will be approximately 5 months.

6.1.1. Screening Period

The Screening period begins with the execution of the informed consent form (ICF) at the Screening visit. After signing the ICF, each participant's study eligibility will be assessed according to the study's Inclusion Criteria and Exclusion Criteria listed in Section 7.

After the Screening visit, participants who are eligible for enrollment must begin complying with the lifestyle restrictions detailed in Section 7.5. They will also begin completing their daily study diary (Section 9.9).

The end of the Screening period will coincide with the start of the Treatment period.

Collection of adverse events (AEs) will begin after the ICF is signed and will continue through the Follow-up period.

6.1.2. Treatment Period

The Treatment period begins on Day 1 (there is no "Day 0") when participants undergo baseline procedures at the study center and receive their first QD dose of study drug. They will return to the study center on approximately Day 87 for the End-of-Treatment (EoT) visit; all other scheduled visits during this period will be completed within the timeframe allowed per Table 1. Throughout the Treatment period, participants will continue completing their daily study diary (Section 9.9).

6.1.3. Follow-up Period

The Follow-up period begins immediately after the EoT visit. During this period, participants will continue to comply with all lifestyle restrictions (Section 7.5) and will continue to complete their daily study diary.

6.1.4. Scientific Rationale for Study Design

An investigator- and participant-blinded, placebo-controlled, randomized design was selected to provide comparable treatment groups and to minimize selection and investigator bias in accordance with the concepts in ICH E10, Choice of Control Groups and Related Issues in Clinical Trials (International Conference on Harmonisation [ICH] of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2001). Placebo was chosen as the control to determine the rate of spontaneously occurring treatment-emergent adverse events (TEAEs), and to reduce the potential for bias in the reporting of AEs.

This study has a 12-week Treatment period to explore the effects of CY6463 compared with placebo control. The treatment duration is believed to be sufficient to evaluate the safety profile of CY6463 as well as observe changes in the study's neurophysiological, PD, and cognitive assessments. In addition, the study includes a follow-up visit 2 weeks after the final dose of study

drug to monitor for any delayed-onset AEs and if any AEs that were ongoing at the EoT visit have resolved. Two weeks represents an interval longer than 5 half-lives of the study drug; at this timepoint, the remaining level of any free study drug will be negligible.

This study is designed to select participants with mild dementia, confirmed AD pathology, cardiovascular risk factors, and evidence of subcortical small-vessel disease. This is a homogeneous population hypothesized to respond more favorably to CY6463 considering the compound's potential impact on vascular biology and neurophysiology.

6.2. Number of Study Centers and Participants

The study will be conducted at up to 10 study sites in the United States (US). It is expected that approximately 30 participants will be randomized.

6.3. Treatment Assignment, Blinding, and Data Review

6.3.1. Randomization Procedure

Participants who are eligible per the criteria in Section 7 will be randomized on Day 1 to a double-blinded regimen of either CY6463 or placebo (1:1 ratio) via central randomization using an interactive voice/web response system (IWRS).

Before the study is initiated, the directions for the system will be provided to each site. The computer-generated randomization schedule will be prepared by an independent statistician not otherwise associated with the study.

6.3.2. Blinding/Unblinding and Data Review

This will be an investigator- and participant-blinded, sponsor-unblinded study. The following practices will apply:

- Sponsor review of unblinded data will facilitate decision making on project progress and potentially trigger plans for additional studies.
- A Sponsor Data Review Committee (DRC), which will consist of the sponsor program director, medical director, senior management (medical) representative, will review unblinded data at the aggregate level for decision-making purposes. The DRC will not have access to specific participant treatment assignments so as to limit potential bias in discussions with the study sites. The sponsor’s biostatisticians and programmers will, however, have access to participants’ randomization assignments for analysis purposes. The remaining members of the core sponsor study team and the entire contract research organization (CRO) study team will be blinded.
- The DRC charter will outline the specific individuals involved, the frequency and activities involved in this review, and measures taken to maintain the integrity of the study.

6.3.2.1. Unblinding for Safety Purposes

Except in a medical emergency, the investigator and study center staff will remain blinded during the conduct of the study and until, at a minimum, all discrepancies in the clinical database

are resolved (ie, at the time of the database lock). Unblinding of a participant's treatment assignment to the investigator and study center staff is restricted to emergency situations in which knowledge of the treatment is necessary for the proper handling of the participant. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the CRO-based medical monitor prior to unblinding a study drug assignment unless this would delay emergency treatment of the participant. Individual treatment assignment unblinding is available to the investigator through the IWRS in the event of an emergency. If the treatment blind for the investigators (and/or study center staff) is broken, the reason and the date should be recorded and signed by the investigator in the source documentation and the electronic case report form (eCRF), and information regarding the unblinding should be submitted as soon as possible (within 24 hours) to the sponsor. In addition, the affected participant will be immediately discontinued from study drug and should follow early EoT procedures (Section 7.6). Unblinding of the sponsor for safety reporting will be detailed in the safety management plan.

6.4. Justification for Dose Regimen

CY6463 (15 mg QD) will be orally administered for approximately 87 days. A dosing duration of approximately 12 weeks is supported by nonclinical toxicology results in rats and dogs. The 15-mg QD dose was selected based on safety, tolerability, and PK data (including cerebral spinal fluid [CSF] concentrations) from Phase 1 studies conducted in healthy adults aged 18 to 79 years; no safety concerns were identified. Among the healthy participants who received CY6463, including at the highest dose levels (50 mg as a single dose, and 15 mg as a QD dose for 14 days in both younger adult and elderly participants), there were no discontinuations due to AEs and no serious adverse events (SAEs) reported. All AEs were considered mild or moderate by the investigator. PK data were linear and predictable, supportive of QD dosing, and were not impacted by food. At steady state, a 15-mg QD dose resulted in maximum concentrations that were above the threshold anticipated to be pharmacologically active in the CSF based on nonclinical studies. Refer to the most recent IB for details.

6.5. Criteria for Study Termination (Study Stopping Rules)

Participant safety will be monitored during the study by the sponsor's medical and safety team who will review emerging safety data in blinded fashion, including clinical laboratory parameters and AEs at appropriate intervals. They will also meet on an ad hoc basis during the study if an unanticipated trend or signal emerges.

The decision to terminate the study for safety reasons will be made by the sponsor. The sponsor will terminate the study if either of the events listed below is reported during the study:

- SAEs in ≥2 participants that are considered study drug related by the sponsor and that code to the same Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC)
- An overall pattern of clinically significant TEAEs or change in any safety parameter that may appear minor in terms of an individual event but, in the opinion of the sponsor, collectively represent a safety concern

See Section 7.6 for discontinuation criteria for individual participants.

6.6. End of Study Definition

The end of the study is defined as completion of the final participant's Follow-up visit, independent of whether study drug was prematurely discontinued in that individual.

7. PARTICIPANT SELECTION, LIFESTYLE RESTRICTIONS, AND EARLY WITHDRAWAL CRITERIA

Prospective approvals of deviations from prespecified recruitment and enrollment criteria, also known as a protocol waiver or exemption, are not permitted.

Please note:

- All Screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a Screening log to record details of each participant screened in order to confirm eligibility or the reason(s) for screening failure, as applicable (see Section 7.4).
  - Laboratory assessments may be repeated if an error is suspected. Laboratory, electrocardiogram (ECG), or blood pressure (BP) values that are outside the range specified in the protocol's eligibility criteria may be repeated, after consultation with the CRO-based medical monitor.
  - After consultation with the Sponsor's medical monitor, the investigator may rescreen a prospective participant should that individual discontinue during the Screening period due to a visit window deviation or other administrative reason. Participants who are rescreened will be required to sign a new ICF.
- When investigators are to determine whether a particular finding from the Screening evaluations is clinically significant, they will consider whether the finding could prevent the individual from performing any of the protocol-specified assessments, represents a condition that should exclude the individual from the study, represents a safety concern if the individual participates in the study, or could confound the study-specified assessments.

7.1. Inclusion Criteria

Each participant must meet <u>each</u> of the following criteria to be eligible for entering this study.

1. Provides written informed consent prior to the performance of any protocol-specified procedure or, if unable to provide informed consent due to cognitive status, provides assent to participate, with a legally authorized representative (LAR) providing written informed consent on behalf of the participant.

   Note:

   - If the participant requires a caregiver, a stable caregiver/study partner must also agree to participate as a designated caregiver in the study by agreeing, for example, to

accompany the participant to study visits, manage at-home study drug dosing compliance, and assist with diary completion. See Appendix 1.

- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of the ICF may be used for screening purposes where noted, provided that the procedures meet the protocol-specified criteria and were performed within the timeframe defined in the Schedule of Events and/or in the respective eligibility criteria.

2. 60 years of age or older on the day of consent
3. Meets core clinical criteria for probable AD dementia according to the 2011 National Institute on Aging–Alzheimer's Associated guidelines (8); see Appendix 4. Can be based on medical history.
4. Mini-Mental State Examination (MMSE) score of 20 to 26 (inclusive)
5. Confirmation of AD pathophysiology either by a prior positive amyloid positron-emission tomography scan or a prior CSF sample (evidence of Aβ/tau deposition). If AD pathophysiology status is unknown, an aliquot from the screening CSF sample collected for the baseline biomarker assessment can be used to determine AD pathophysiology.
6. Presence of ≥2 of the following cardiovascular risk factors:
   a. Hypertension confirmed either by: diagnosis in medical history, use of ≥1 ongoing medication to treat hypertension, or consistent (as determined by the investigator) history of systolic and/or diastolic BP values >130 and >80 mmHg, respectively
   b. Type 2 diabetes or prediabetes confirmed either by: diagnosis in medical history, use of ≥1 ongoing medication to treat diabetes, or consistent (as determined by the investigator) history of clinical laboratory values of A1c ≥5.7% and/or fasting plasma glucose ≥100 mg/dL
   c. Hypercholesterolemia confirmed either by: diagnosis in medical history, ongoing use of ≥1 medication to treat lipid imbalance, or consistent (as determined by the investigator) history of clinical values of total cholesterol >200 mg/dL, low-density lipoprotein cholesterol >130 mg/dL, and/or triglycerides >150 mg/dL
   d. Body mass index (BMI) ≥25 kg/m^2
7. MRI (existing MRI obtained ≤6 months before Screening visit is acceptable) findings of mild-to-moderate subcortical small-vessel disease as determined by white-matter lesions with a Fazekas (9) score of 1 (ie, punctate foci) or 2 (ie, beginning confluence of foci)
8. If receiving concomitant or chronic medication(s), has had no change for ≥4 weeks before study drug initiation and has no plans to alter the regimen(s) during the study
9. Agrees to adhere to lifestyle restrictions (see Section 7.5) from the time of the ICF signature until the end of the Follow-up visit
10. *If male*, agrees to refrain from donating sperm from the Screening visit through 90 days after taking the final study drug dose

11. *If male*, agrees to use ≥1 of the following effective contraception methods from the signing of the ICF until ≥90 days after taking the final study drug dose:
    a. If in line with the preferred and usual lifestyle, will abstain from heterosexual intercourse from the signing the ICF until ≥90 days after taking the final study drug dose

       —*or*—

    b. If heterosexually active:
       - Use a combination of 2 birth control methods (≥1 must be highly effective). Examples include condom with spermicide + intrauterine device; hormonal contraceptive associated with the inhibition of ovulation [eg, oral or transdermal patch or implant] + barrier method; surgery [bilateral tubal ligation/cauterization or vasectomy] + barrier method) –*or*—
       - Partner is surgically sterile (bilateral oophorectomy, hysterectomy, or tubal sterilization [tie, clip, band, or burn]) or is postmenopausal
12. If *female*, is postmenopausal/not of reproductive potential, defined as:
    a. Amenorrheic for ≥12 consecutive months (without an alternative medical cause) and follicle-stimulating hormone level >40 mIU/mL (or within the testing laboratory's menopausal range) at Screening
    b. Amenorrheic for ≥24 consecutive months (in the absence of medications known to induce amenorrhea)
    c. Surgically sterile (bilateral oophorectomy, hysterectomy, or tubal sterilization [tie, clip, band, or burn])
13. Agrees to not receive an investigational therapy or device in any other study while participating in this study, through the Follow-up visit
14. Agrees to the study procedures, including undergoing lumbar puncture for CSF samples

7.2. Exclusion Criteria

A participant who meets any of the following criteria will be excluded from entering this study.

1. Severe visual, auditory, social, or cognitive impairment as determined by the investigator that may affect the ability to adhere to protocol requirements or to complete required assessments
2. Diagnosis of dementia-related CNS disorder other than AD or vascular dementia (eg, Parkinson's disease, Huntington's disease, frontotemporal dementia, schizophrenia, Lewy body dementia)
3. Evidence of symptomatic large-vessel disease, symptomatic carotid artery disease (≥40% occlusion) per ultrasound, large-vessel infarcts, strategic lacunar infarcts (ie, focal lesions found in specific sites critical for cognition and/or behavior), or infarcts >15 mm in size, as determined by MRI or medical history

4. History of significant CNS trauma (eg, cerebrovascular accident or traumatic head injury) or infection (eg, human immunodeficiency virus, syphilis) that has affected brain function, per investigator opinion
5. Hypotension, defined as systolic BP ≤90 mmHg or diastolic BP ≤60 mmHg, at Screening or predose on Day 1. (Each BP recording will be the average of 3 consecutive BP readings conducted at intervals of ≥1 minute; see Section 9.6)
6. Orthostatic hypotension at Screening or predose on Day 1. (Orthostatic hypotension is defined as a decrease in systolic BP of ≥20 mmHg, or a decrease in diastolic BP of ≥10 mmHg, between 1–3 minutes after assuming a standing position vs BP while in a sitting or supine position.)
7. Any contraindication to MRI procedures
8. Any contraindication to lumbar puncture procedure, as assessed by the investigator, possibly including abnormalities in lumbar spine previously observed on imaging, history of clinically significant back pain, back pathology, spinal surgery, back injury, noncommunicating hydrocephalus or an intracranial mass lesion, or allergy to lidocaine (if planned to be used as a local anesthetic during procedure)
9. Unable to participate in electroencephalography (EEG) protocol due to auditory challenges or inability to tolerate EEG cap or headphones, as determined by the investigator
10. Any uncontrolled or unstable chronic disease, as determined by the investigator
11. Clinically significant renal impairment requiring dialysis and/or history of renal transplant
12. Suicidality, defined as active suicidal thoughts during the 6 months prior to Screening visit or at Day 1 (eg, Type 4 or 5 on the Columbia-Suicide Severity Rating Scale [C-SSRS]), or history of suicide attempt in previous 2 years, or at serious suicide risk, in investigator's judgment
13. Resides in a nursing home or assisted care facility with need for continuous direct medical care and nursing supervision. *Exception*: Participant may reside in such facility provided that continuous direct medical care is not required, a qualified caregiver is available for co-participation, and the participant is physically able to attend in-clinic study visits.
14. Family history of short QT syndrome or long QT syndrome
15. Clinically significant cardiac involvement or an ECG with corrected QT interval using Fridericia's formula (QTcF interval) >450 ms for men or >470 ms for women.
    Note: If the first ECG QTcF interval obtained is out of range, the ECG should be repeated twice and the average of the 3 results should be used to determine eligibility.
16. Any surgery or hospitalization in the 4 weeks prior to enrollment (*Exception:* minor dermatologic procedures)
17. Recent history (eg, within prior 2 years) of 1 or more CNS bleeding event requiring intervention and/or hospitalization

18. Any of the following at Screening or predose on Day 1:
    - Alanine transaminase >2.0× upper limit of normal (ULN) as defined by laboratory
    - Aspartate transaminase >2.0×ULN as defined by laboratory
    - Total bilirubin >1.5×ULN as defined by laboratory (isolated bilirubin >1.5×ULN is acceptable if total bilirubin is fractionated and direct bilirubin <35%)
    - Current or recent (within prior 12 months) hepatic disease
19. Current use of aspirin ≥325 mg/day, any P2Y12 inhibitor, any anticoagulant medication, specific inhibitors of phosphodiesterase 5 (PDE5), nonspecific inhibitors of PDE5 (including dipyridamole and theophylline), any supplement for the treatment of erectile dysfunction, riociguat, vericiguat, any nitrate, [redacted] medications are prohibited from the Screening visit through the duration of the study. See Appendix 3.
20. History or presence of severe gastrointestinal dysmotility (eg, dyspepsia, stomach aches, nausea, vomiting, recurrent pancreatitis, constipation) as determined by the investigator that may impact compliance and/or oral drug administration, absorption, and exposure
21. Participated in a study evaluating an investigational therapy within 1 month or 5 half-lives of that investigational drug, whichever is longer, before Day 1
22. Clinically significant hypersensitivity or allergy to any of the inactive ingredients contained in the active or placebo drug products (lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, silicon dioxide, magnesium stearate, talc, polyethylene glycol, polyvinyl alcohol, and titanium dioxide).
23. Donated or lost >500 mL blood within 3 months of the Screening visit and/or donated any plasma within 2 weeks of the Screening visit
24. History of cancer. *Exceptions*: localized cutaneous basal or squamous cell carcinoma in the last 5 years, low-grade localized prostate/cervical cancers, or previous localized prostate/cervical cancers that have a low likelihood of recurrence
25. Is not suited for study participation in the clinical judgment of the investigator

7.3. Trial Population Diversity

The US population is characterized by significant racial and ethnic diversity and includes several minority groups that constitute substantial and growing proportions of the population. For example, according to the 2010 US census (10), 16.3% of Americans reported having Hispanic/Latino ethnicity, those who self-identified as Black represented 14% of the population, and those who identified as Asian represented 6%. People who identified as White represented just 72% of the population.

AD is disproportionately experienced by minority ethnoracial groups in the US.(2, 11) The *2020 Alzheimer's Disease Facts and Figures* (2) states: "…older Blacks/African Americans and Hispanics/Latinos are disproportionately more likely than older Whites to have Alzheimer's or other dementias. Most studies indicate that older Blacks/African Americans are about twice as likely to have Alzheimer's or other dementias as older Whites. Some studies indicate older

Hispanics/Latinos are about one and one-half times as likely to have Alzheimer's or other dementias as older Whites." While some disproportionality may be due to genetic variation, the greater driver is thought to be differences in socioeconomic factors and in disproportional comorbid health conditions associated with increased AD risk. Notably, the disproportionality of those comorbid conditions, including diabetes and cardiovascular disease, is also likely driven by socioeconomic differences.(2, 11, 12) In addition, racial and ethnic groups may also experience and respond to treatments differently due to the same differential factors.

It is important for clinical trial populations to reflect the targeted patient population and/or to enrich for participants expected to respond differentially based on scientific rationale. Appropriately representative trial populations are an important facet of clinical trial designs and contribute to the scientifically rigorous evaluation of a drug to ensure that the safety and efficacy profile of a treatment is accurate.

This study is expected to randomize approximately 30 participants with ADv in the US. To be eligible, participants must have evidence of AD and of vascular pathology as well as ≥2 of 4 cardiovascular risk factors (hypertension, type 2 diabetes, hypercholesterolemia, and/or BMI ≥25 kg/m^2)—criteria that are disproportionately experienced by some minority groups. Although recruiting a trial population representative of the US ADv population is limited by the small size of this study, efforts to enroll an appropriately diverse study population may include, but are not limited to, opening site(s) in racially/ethnically representative communities, enlisting investigators representative of the population, participating in outreach activities in representative communities, and ensuring that participant- and community-focused materials are culturally relevant and accessible to a broad range of backgrounds.

7.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized to study drug. The investigator will maintain a screening log to record details of all prospective participants screened and to confirm eligibility or record reasons for screening failure, as applicable. A minimal set of screen failure information is required to ensure transparent reporting of participants who screen fail, to respond to queries from regulatory authorities, and to meet the Consolidated Standards of Reporting Trials publishing requirements. Minimal information includes demography, reason(s) for screen failure, eligibility criteria met and/or not met, and AE details.

7.5. Lifestyle Restrictions

Participants will be instructed to follow the lifestyle restrictions below.

7.5.1. Meals and Diet

Participants will fast (water is allowed) for at least 4 hours before clinical laboratory assessments at all scheduled visits except the Screening visit.

7.5.2. Caffeine, Alcohol, and Tobacco/Nicotine

Caffeine: Participants should not consume excessive amounts of caffeine, defined as >800 mg per day, from 7 days before the first dose of the study drug through the Follow-up visit.

Participants should abstain from any caffeine-containing products for 24 hours before each scheduled visit. Caffeine quantities are defined below.

Item (serving)	Caffeine content
Coffee (8 oz/237 mL)	80–100 mg
Energy drink (8 oz/237 mL)	40–250 mg
Tea, green or black (8 oz/237 mL)	30–50 mg
Cola (12 oz/355 mL)	30–40 mg
Dark chocolate (1 oz/28 g) or milk chocolate (2 oz/57 g)	12 mg

Source: US Department of Agriculture, Agricultural Research Service. Food Data Central. 2019 [fdc.nal.udsa.gov]

Alcohol: Participants should not consume alcohol within 24 hours of each scheduled visit. At other times throughout the study, participants should not consume >2 units of alcohol daily on average (1 unit=14 g pure alcohol [eg, 12 oz regular beer; 5 oz table wine; or 1.5 oz distilled spirits]).

Tobacco/Nicotine: Participants should abstain from the use of tobacco- or nicotine-containing products (including e-cigarettes and patches) until completion of the Follow-up visit.

7.5.3. Contraception Requirements for Male Participants of Reproductive Potential

Male participants of reproductive potential must use effective contraception as defined in Inclusion Criteria 10 and 11 (Section 7.1), as applicable.

Details regarding pregnancy testing and reporting, including in female partners of male participants, are provided in Section 9.10.4.

7.5.4. Physical Activity

Participants will abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during the study but should maintain the same level of physical activity from Screening through the final Follow-up visit.

7.6. Discontinuation of Study Drug in Individual Participants

Participants will be informed that they may withdraw from the study (ie, withdraw their consent) at any time without prejudice to their care. Attempts should be made to determine the reason for the withdrawal, if possible.

- The participant (or the LAR, if applicable; see Eligibility Inclusion 1) may request destruction of any samples collected but not yet tested; the investigator must document this request in the study records.
- If consent is withdrawn, the sponsor may retain and continue to use any data collected prior to the withdrawal of consent.

A participant may be discontinued from **study drug** by the investigator or sponsor due to any of the following:

- In the investigator's opinion it is not in the best interest of that individual to continue
- An AE (see Section 8.2 for dose modification in individuals) such as:
  - A symptomatic hypotension-related AE (eg, syncope) that requires intervention and is considered related to study drug
  - An ECG change from baseline that is considered to be clinically meaningful by the investigator (eg, abnormal Q waves, prolonged QT)

Whenever possible, participants who prematurely discontinue from study drug but have not withdrawn consent should complete the EoT (except for study drug dosing) and Follow-up visits and associated assessments (see Table 1).

Participants who discontinue due to an AE will be followed until resolution of all of their AEs or until the unresolved AEs are judged by the Investigator to have stabilized.

A participant may be discontinued from **study participation** by the investigator or sponsor at any time for any reason, such as the participant being lost to follow-up (see Section 7.7).

A premature discontinuation from the study will occur when a randomized individual ceases study participation prior to completing all study visits. The sponsor will be notified of any premature study discontinuation. The date the participant is withdrawn from the study and the reason for discontinuation will be recorded on the study termination form of the eCRF.

Unless consent is withdrawn, the sponsor and/or sponsor's designee may contact a participant after the individual's completion or discontinuation from the study to inquire about health status and/or experiences in the study.

7.7. Lost to Follow-up

The following actions will be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule.
- Before a participant is deemed lost to follow-up, study center personnel must make every effort to regain contact with the participant (ie, where possible, 3 telephone calls and, if necessary, a certified letter sent to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's source document.
- If the participant continues to be unreachable, study center personnel or designee will attempt to determine the vital status of the participant within legal and ethical boundaries for all randomized participants, including those who did not receive study drug. Public sources may be searched for vital status information. If vital status is determined to be "deceased," this will be documented and the participant will not be

considered lost to follow-up. Sponsor personnel will not be involved in any attempts to collect vital status.

Should the participant continue to be unreachable and the vital status is unknown, or the participant is determined to be living, he/she will be considered lost to follow-up from the study.

7.8. Replacement of Randomized Participants

If participants prematurely discontinue or are considered lost to follow-up, additional participants may be recruited and enrolled at the discretion of the sponsor.

8. STUDY DRUG AND CONCOMITANT MEDICATION ADMINISTRATION

8.1. Study Drug Regimen

All eligible participants will be randomized to either CY6463 (15 mg QD) or matching placebo QD; see Table 2. Study drug may be taken with or without food. Participants will be instructed to take their daily study drug dose each morning at a consistent time (preferably ±1 hour) throughout the Treatment period. Participants are to record the date and time of each at-home dose administration in their study diary (see Section 9.9).

***Exception/Note*:** For all scheduled visits that occur on a dosing day, participants will take their dose during the visit to allow for appropriate timing of pre- and post-dose assessments. Attempts should be made to schedule these visits such that dosing can occur at the participant's approximate daily dosing time. In conjunction with these visits, participants will fast for ≥4 hours before clinical laboratory samples are collected.

Table 2: Study Drug Identification and Dosing Regimen

	Study Drug	
Product name	CY6463	Placebo to match the CY6463 tablet
Dosage form & description	round, white to off-white solid tablet	round, white to off-white solid tablet
Unit dose strength	5-mg tablet	N/A
Regimen*	15 mg (3×5-mg tablet) taken QD	3×PTM tablets taken QD
Route of administration	oral	oral
Sourcing	Provided centrally to the study center pharmacy by the sponsor/designee	
Packaging & labeling	Supplied in white, induction-sealed 60-cc high-density polyethylene bottles that are fitted with a polypropylene child-resistant cap. Each bottle contains a count of 35 tablets and a 3-g silica gel desiccant pack and is labeled as required per country requirement.	

N/A=not applicable; PTM=placebo to match; QD=once daily; study drug=CY6463 or matching placebo
* Regimen to be assigned centrally, via randomization.

Study drug supply and dosing compliance assessment details are provided in Section 8.3.

8.2. Dose Modification in Individual Participants

For a participant who does not tolerate the 15-mg QD dose (ie, three 5-mg tablets) as evidenced by an associated AE, the participant's dose may be de-escalated to 10-mg QD dose (ie, two 5-mg tablets).

- The decision to de-escalate study drug will be made on a per-participant basis jointly by the investigator and the CRO-based medical monitor, in consultation with the sponsor's medical monitor as needed; the modification will be recorded in the source documents and eCRF.
- Initiation of the 10-mg QD dose should occur only after the AE(s) prompting the dose reduction has/have either resolved or improved sufficiently to be considered mild in severity or, if the AE is a worsening of a pre-existing condition, has returned to baseline status.

If the 10-mg QD dose is also not tolerated, the participant will be discontinued from study drug for the remainder of the Treatment period due to the AE. See Section 7.6 for additional study drug discontinuation criteria and procedures.

8.3. Study Drug Supply and Dosing Compliance

The appropriate amount of study drug will be dispensed to randomized participants on Day 1. Drug will be resupplied as needed.

When participants are dosed at the study center, they will receive study drug under medical supervision, directly from the investigator or designee who will examine the participant's mouth to ensure that study drug was ingested. The study drug dose and the participant's identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study drug. The date and time of each dose administered in the clinic will be recorded in the source documents.

Participants and/or their caregivers/study partners, as applicable, will be asked to record the date and time of each at-home study drug administration in their daily study diary (Section 9.9). They will be asked to bring their diary to each scheduled visit. The diaries will be checked for dosing compliance; as applicable, participants will be reminded of the need for daily compliance with dosing.

During the Treatment period, participants will also be asked to bring their study drug supply in the original bottles to each scheduled visit. Study drug dosing compliance will be based on tablet counts conducted during the Treatment period and will be documented in the source documents and relevant eCRF. Deviation(s) from the assigned regimen will be recorded, including dates for dosing delays and/or dose reductions.

8.4. Treatment and Tracking of Overdose

For this study, any reported daily dose of study drug >15 mg QD will be considered an overdose whether or not associated with an AE(s).

In the event of an overdose, the investigator/treating physician should:

- Immediately contact the CRO-based medical monitor
- In consultation with the CRO-based medical monitor, evaluate the participant to determine whether study drug should be interrupted, whether the subsequent dose should be reduced, and/or if plasma PK sample should be obtained
- Closely monitor the participant for any AE(s)
- Document the quantity of the excess dose as well as the duration of the overdose

8.5. Continued Access to Study Drug after the End of the Study

Continued access to study drug after cessation of an individual's study participation is not planned.

8.6. Study Drug Preparation, Handling, Storage, and Accountability

Study drug must be stored in a secure, monitored (manual or automated) area in accordance with the labeled storage conditions and with access limited to the investigator and authorized study center staff.

Study drug should be kept at room temperature (ie, 50°–86°F) at all times. Participants will be advised to report any excursion outside of this temperature range to study center staff. Study drug bottles must be kept tightly closed.

8.6.1. Accountability

The investigator/designee must confirm that appropriate temperature conditions have been maintained during transit for all study drug received and that any discrepancies are reported and resolved before use of the study drug.

Only participants who are eligible per this protocol for study drug administration may receive study drug. Only authorized study center staff may supply study drug to the participants.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). A copy of the final drug accountability log will be provided to the sponsor or designee.

8.6.2. Disposal

Refer to the study's pharmacy manual for study drug disposal instructions. No study drug is to be destroyed without prior written permission of the sponsor.

8.7. Concomitant Medications and Therapies

Any medication or vaccine (including over-the-counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements, or other specific categories of interest) that the

participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

Note: Prior therapies, medications, vaccinations, surgeries, etc. (ie, before initiation of study drug) must be differentiated from concomitant therapies (ie, those after initiation of study drug) to facilitate understanding of any AEs that occur after the start of study drug.

See Section 8.7.1 for information on medications that are prohibited in this study. In addition, participants will not initiate the use of additional medication (including nonprescription "over-the-counter" preparations) for treatment of AD during the study other than the study drug.

The CRO-based medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

8.7.1. Prohibited Medications

Comprehensive drug-drug interaction studies with CY6463 have not been conducted in humans. See Appendix 3 for a complete list of medications that are prohibited. Participants and/or their caregivers/study partners should be reminded of the restrictions on food, beverages, supplements, and other substances as noted in Section 7.5.

The decision and need to switch medications and/or dose-reduce concomitant medications prior to or during study drug exposure will be made by the investigator in consultation with the study center pharmacist (if available) or the CRO-based medical monitor to assess the risk/benefit of administering the concomitant medication.

9. STUDY ASSESSMENTS AND PROCEDURES

Study assessments will be conducted according to the timing and sequence indicated in the Schedule of Events. Details regarding those procedures are provided below and in the study manuals (eg, laboratory, pharmacy, imaging).

During any unscheduled visit, safety assessments will be conducted per investigator discretion; results will be documented in the source document and the appropriate eCRF.

9.1. Informed Consent

Before a potential study participant undergoes any study-specific evaluations or procedures, the participant (and/or participant's LAR/guardian, if applicable) must provide written, informed consent. See Appendix 1 for more information.

9.2. MMSE

The Mini-Mental State Examination (MMSE) consists of 11 questions that together test 5 areas of cognitive function: orientation, registration, attention, and calculation, recall, and language.

The maximum score is 30. A score of 23 or lower is indicative of cognitive impairment, and 17 or lower indicates severe impairment.

9.3. Demography, Medical History, and Baseline Medications

At the Screening visit, demographic characteristics (eg, age, sex, race/ethnicity, apolipoprotein E4 [ApoE4] status if known, and highest level of education completed) will be recorded along with a complete medical history that will include the participant's diagnosis of AD, evidence of vascular pathology, smoking history, prior and current medications (see Section 8.7), surgeries, and any additional relevant medical history.

9.4. Physical Examination

At Screening, a complete physical examination will be performed by the investigator and documented on the appropriate eCRF. All other examinations may be symptom directed at the investigator's discretion.

A complete physical examination should include examination and assessment of the following:

General appearance	Lymph nodes	Nervous system
Cardiovascular system	Head, eyes, ears, nose, and throat	Skin
Respiratory system	Neck	Mental status
Abdomen/liver/spleen	Musculoskeletal system	

Breast, genitourinary, and rectal examinations are optional and may be performed at the discretion of the investigator.

Height (cm) will be measured only at Screening. Weight (kg) will be recorded throughout the study per Table 1. BMI will be calculated and recorded at Screening.

9.5. Electrocardiograms (ECGs)

All ECGs will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. The ECG should be obtained after the participant has rested supine for ≥5 minutes.

If a QTc result (corrected using Fridericia's formula) is outside of the normal range (>450 ms), the ECG should be repeated twice and the average of the 3 results calculated.

All ECGs will be evaluated by an investigator or qualified designee for the presence of abnormalities. Results will be reported as "normal," "abnormal clinically significant," or "abnormal not clinically significant." Abnormalities of clinical significance will be recorded as an AE.

If a confirmed clinically significant abnormal result is obtained, the study center should follow standard institutional procedure until the result resolves to baseline. If concerns remain, escalate the issue to the sponsor's medical monitor.

9.6. Vital Signs

Vital signs will be measured before blood collection(s) and will include oral temperature (°C), respiratory rate, peripheral oxygen saturation, and seated-to-standing BP and pulse rate. All measures should be preceded by ≥5 minutes of rest for the participant in a quiet setting without distractions (eg, television, cell phones).

Oxygen saturation measurements should be taken by pulse oximeter on room air.

BP and pulse will be measured using a completely automated device. The same device make/model should be used for an individual participant throughout the study, if possible. Manual techniques will be used only if an automated device is not available.

- *Supine/semi-recumbent BP measurements*: 3 consecutive BP readings will be recorded in the source documents at intervals of approximately 1 minute. The average of the 3 BP readings will be recorded in the eCRF.
- *Orthostatic BP and pulse measurements*: After the supine/semi-recumbent measurements are collected, the participant will assume a sitting position for 1 to 3 minutes, and finally assume a standing position for 2 (±1) minutes before the standing BP and pulse values are measured (single measure); these values will be recorded in the source document.
- The supine/semi-recumbent average and the standing values will be used to calculate and record orthostatic BP and pulse in the eCRF.

9.7. Laboratory Assessments

9.7.1. Genetics

Participants who do **not** have ApoE4 genetic status recorded in their medical records will be tested for ApoE4 status and for ABCA7 (adenosine 5-triphosphate [ATP]-binding cassette sub-family A, member 7) status (if not already in their medical record), and potentially for additional genetic polymorphisms associated with endothelial dysfunction at Screening. Collection instructions are provided in the study's laboratory manual.

9.7.2. Safety Laboratory Assessments

Tests detailed in Table 3 will be performed per Table 1 and sent to the central laboratory for analysis. See the study's laboratory manual for collection and processing details.

Except at Screening, participants will fast for ≥4 hours prior to safety laboratory sample collections.

On **Day 1** prior to randomization, clinical safety laboratory results (clinical chemistry, coagulation, and hematology) from the Day 1 predose sample (see Table 1 footnote b) must be reviewed for clinical significance to confirm eligibility and suitability for this study; local laboratory values may be used for this purpose provided that a separate set of the Day 1 samples is sent to the Central laboratory for processing and for recording in the eCRF. Laboratory parameter requirements for study eligibility are detailed in Section 7.1 and Section 7.2.

Throughout the study, the investigator is responsible for reviewing each laboratory report and for determining the clinical significance of any results that fall outside of the laboratory normal ranges; this review must be documented and filed with the source documents. Additional tests may be performed at any time during the study per investigator discretion or as required by local regulations. Laboratory assessments may also be repeated if an error is suspected.

An abnormal laboratory value not already part of a diagnosis and that requires a change in the study drug or is considered clinically significant by the investigator is to be recorded as an AE.

All laboratory values considered abnormal and clinically significant during study participation, including the Follow-up visit, should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or the CRO-based medical monitor.

Table 3: Protocol-Required Laboratory Assessments

Assessment	Parameter			
Hematology	Hematocrit	RBC indices:	WBC count w/differential:	
	Hemoglobin	--Mean corpuscular hemoglobin (MCH)	--Basophils	--Monocytes
	Platelet count	--Mean corpuscular volume (MCV)	--Eosinophils	--Neutrophils
	RBC count	--%Reticulocytes	--Lymphocytes	
Clinical chemistry (serum)	Albumin	BUN	GGT	
	Alkaline phosphatase	Calcium	Glucose (fasting)	
	ALT/SGPT	Creatinine	Potassium	
	AST/SGOT	Creatine kinase	Protein (total)	
	Bilirubin (total, direct)	Cystatin C	Sodium	
Coagulation	aPTT	INR	Prothrombin time	
Lipids	Total cholesterol	HDL-c	LDL-c	Triglycerides

Note: Events of ALT ≥3×upper limit of normal (ULN) and bilirubin ≥2×ULN (>35% direct bilirubin) or ALT ≥3×ULN and INR>1.5, which may indicate severe liver injury (possible Hy's Law), will be reported as an SAE.
ALT=alanine aminotransferase; aPTT=activated partial thromboplastin time; AST=aspartate aminotransferase; BUN=blood urea nitrogen; GGT=gamma glutamyl transferase; HDL-c=high-density lipoprotein cholesterol; INR=international normalized ratio; LDL-c=low-density lipoprotein cholesterol; RBC=red blood cell; SGOT=serum glutamic-oxaloacetic transaminase; SGPT=serum glutamic-pyruvic transaminase; WBC=white blood cell

9.7.3. PK and Biomarker Evaluations (Plasma and CSF)

Samples will be collected per the Schedule of Events and the study laboratory manual. The actual date and time (24-h clock time) of each sample collection will be recorded in the source documents and eCRF. Specific instructions for the collection and handling of biological samples are provided in the study's laboratory manual.

Samples collected for biomarker analysis will be used to further understand the ADv disease process, pathways associated with the disease state, and/or the mechanism of action of CY6463 and its PD effect.

[redacted]

9.7.3.1. Plasma Sampling

Sparse whole-blood samples will be collected for measurement of plasma concentrations of CY6463 using a validated liquid chromatography-tandem mass spectrometry bioanalytical method. Blood samples will also be collected to evaluate changes in concentrations of select disease and PD biomarkers.

9.7.3.2. CSF Sampling

CSF samples will be collected for measurement of CY6463 concentrations and for changes in the concentrations of select disease and PD biomarkers.

9.8. Suicidal Risk Monitoring (C-SSRS)

CY6463 is considered to be a CNS-active investigational drug. Although CY6463 and other similar drugs in this class have not been associated with an increased risk of suicidal thinking or behavior, the sponsor considers it important to monitor for such ideation or behavior before and during this clinical study. Therefore, participants should be appropriately monitored and closely observed for suicidal ideation and behavior or any other unusual change in behavior. The C-SSRS (13) will be administered per the Schedule of Events by an individual with appropriate clinical training who has taken the specific rater training for the scale, which will be provided by an agent of the sponsor prior to study start. If at any post-Screening visit there are "YES" answers on Items 4, 5 or on any behavioral question of the C-SSRS, an immediate risk assessment should be conducted with the CRO-based medical monitor to determine whether it is safe for the participant to continue in the study.

9.9. Participant Diary for Recording Daily Dosing, Concomitant Medications, and Changes in Health Status

At the Screening visit, each participant will be issued a study diary in which they (or their LAR/guardian or caregiver/study partner, as applicable) will be asked to record on a daily basis the name of any new and/or changes in concomitant medication(s) they take, along with the date, time, and dose strength of that medication(s), and any changes in health status that they experience (including the dates, times, and brief descriptions). They will be asked to continue entering this information each day throughout the study until the Follow-up visit. The diaries are intended to serve as reminders to the participant and caregiver/study partner and will constitute official source documents. During the Treatment period, participants will be instructed to also record the date and time of each study drug dose taken at home.

Participants will be asked to bring their diary to each scheduled visit. Study staff will monitor the diaries during the visit, record any relevant data in the eCRF, and will retain the diaries as part of the participants' source files. A new diary will be issued at each visit, with the final diary collected at the Follow-up visit.

9.10. Adverse Events, SAEs, and Other Safety Reporting

The definitions of AEs, adverse events of special interest (AESIs), and SAEs can be found in Appendix 2.

Immediate safety concerns should be discussed with the study's designated CRO-based medical monitor upon occurrence or awareness. Changes in the participant's health status since the previous visit or previous study drug administration must be checked, including laboratory results, as applicable.

9.10.1. Time Period, Frequency, and Reporting Information

AEs, AESIs, and SAEs will be recorded on the AE eCRF starting from the signing of the ICF until the Follow-up visit after the final study drug dose.

- SAEs will be reported within 24 hours of the investigator's first awareness of the event
- Nonserious AESIs will be reported within 5 business days of the investigator's first awareness of the event

9.10.1.1. Follow-up of AEs, AESIs, and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AESIs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (see Section 7.7). The investigator will submit any updated SAE information within 24 hours of it becoming available, using the AE eCRF.

9.10.1.2. Post-Study Events

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of an individual's study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and considers the event to be reasonably related to the study drug or study participation, he/she must promptly notify the sponsor.

9.10.2. Method of Detecting AEs and SAEs

AEs will be reported by the participant (or, when appropriate, by the participant's LAR, caregiver, study partner) spontaneously and/or in response to open-ended questioning from the study personnel or revealed by observation. Open-ended and nonleading verbal questioning of the participant will be the preferred method to inquire about AE occurrences. Care will be taken not to introduce bias when detecting events.

See Appendix 2 for details regarding the recording, evaluation, and causality assessment of AEs, AESIs, and SAEs.

9.10.3. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study drug under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study drug under clinical investigation. The sponsor

will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority and the investigators. The principal investigator at each site will be responsible for notifying the relevant institutional review board (IRB)/independent ethics committee (IEC) of these additional SAEs, according to local standard operating procedures.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and sponsor policy and forwarded to investigators, as necessary.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

9.10.4. Pregnancy Reporting and Monitoring

Study drug will be immediately discontinued in a female participant upon confirmation of a positive pregnancy test result and will be reported to the sponsor within 24 hours of first awareness. Pregnancy in a female partner of male participant will be reported to the sponsor within 24 hours after obtaining the necessary signed ICF from the female partner. The participant/consenting pregnant female partner will be followed to determine the outcome of the pregnancy.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such. Elective abortions without complications should not be handled as AEs.

Any post-study pregnancy-related SAE considered reasonably related to the study drug by the investigator will be reported to the sponsor. While the investigator is not obligated to actively seek this information in former study participants or their pregnant female partners, he or she may learn of an SAE through spontaneous reporting.

9.11. Cognitive Performance Assessments

The following Cogstate AD battery of cognitive function tests will be completed digitally.

- **Detection test** (3 min; measures processing speed): A playing card is presented face down. The participant is asked to press “yes” as soon as the card flips over.
- **Identification test** (3 min; measures attention): A playing card is presented face down. When the card flips over, the participant must decide whether or not it is red. If the card is red, they press “yes” and if it is not, they press “no.” The participant works as quickly and accurately as possible.
- **One-card learning test** (6 min; measures visual memory): A playing card is presented face up. The participant must decide whether they have seen the card before in the test. Accordingly, they will press “yes” or “no” as quickly and accurately as possible.

- **One back test** (4 min; measures working memory): A playing card is presented face up. The participant must decide whether the card is the same as the previous card. If the card is the same, the participant should press "yes" and if it is not, the participant should press "no." The participant is encouraged to work as quickly and as accurately as possible.
- **International shopping list test** (5 min; measures verbal learning): After being read a shopping list, the participant is asked to recall as many items from the list as possible.
- **International shopping list test—delayed recall** (5 min; measures verbal memory): After some time has passed, the participant is asked to recall again as many items from the shopping list as possible.
- **Continuous paired associate learning test** (7 min; measures visual memory): The participant must learn and remember the pictures hidden beneath different locations on the screen.
- **Modified Groton maze learning test** (7 min; measures executive function): A 10×10 grid of tiles is presented on the screen. A 28-step pathway is hidden among the tiles. A blue tile indicates the start. A tile with red circles indicates the finish. The participant must move 1 step at a time from the start toward the finish by touching a tile next to their current location. If the correct move is made, a green checkmark appears. If the move is incorrect, a red cross appears. Once completed, the participant is returned to the start location to repeat the test by remembering the pathway that was just completed.

The following assessments of semantic and phonemic fluency will also be completed.

- **Letter fluency** (3 min; measures verbal fluency): Using the 3-letter set of F, A, and S, the participant is given 1 minute to name as many words as possible beginning with each one of the letters in turn, for a total of 3 minutes. The score is the total number of unique correct words.
- **Category fluency** (1 min; measures verbal fluency): Using a single category of animals, the participant is given 1 minute to name as many unique words as possible within that category. The score is the total number of unique correct words.

9.12. Neurophysiological Assessments

Details of EEG procedures will be described outside of this study protocol. Because the postdose EEG assessment will be approximately 2 hours after the predose EEG assessment, the electrodes should remain in place to reduce variability and ensure consistent testing conditions.

9.12.1. Resting State qEEG (Eyes Open/Closed)

Resting state quantitative electroencephalography (qEEG) recordings will be performed with alternating periods of eyes open and eyes closed. During part of the recording session, participants will face a featureless wall and will be instructed not to stare, not to move their head and eyes, and to suppress eye blinks.

9.12.2. Auditory Oddball Assessment for Event-Related Potential (ERP)

Participants will be seated wearing the EEG cap and headphones and instructed to sit still and relax. During this task, participants will be presented with a series of auditory tones. Of these tones, the majority are presented as frequent stimuli while some are presented as deviant/infrequent stimuli. Participants will be instructed to pay attention to the tones and identify the infrequent/deviant tones.

9.13. Neuroimaging Assessments

Exploratory neuroimaging modalities will be used to measure the potential PD effects of CY6463 in the brain. Modalities include arterial spin labeling (ASL) and functional magnetic resonance imaging (fMRI).

Regional and overall changes in cerebral blood flow will be measured using ASL during resting state. Brain activity/reactivity will be measured using fMRI, a relative measure based on the blood-oxygen-dependent effect performed during resting state. MRI will also be used to detect subtle changes in brain tissue by quantitatively measuring changes in cellular microstructure and white matter structural integrity.

Details of imaging acquisition procedures will be described outside of this study protocol.

10. STATISTICAL CONSIDERATIONS

See Section 5 for a list of the study objectives and their corresponding endpoints.

10.1. Statistical Hypotheses

The primary objective of the study is to evaluate the safety and tolerability of CY6463 (15 mg QD) after 12 weeks of treatment. No formal statistical hypotheses will be conducted to assess this objective.

If appropriate, comparisons between CY6463 and placebo will be made to investigate the exploratory objectives. Such tests will be 2-sided with a 10% significance level. Due to the exploratory nature of this study, there will be no adjustment of the p-values for multiple testing. All reported p-values will be considered nominal.

10.2. Sample Size Determination

A sample size of approximately 30 randomized participants was determined outside of statistical considerations and is considered sufficient to address the primary research objectives.

10.3. Populations for Analyses

The following analysis populations are defined for this study.

Population	Description
Screened	All participants who signed the ICF
ITT	All randomized participants
Safety	All randomized participants who took ≥1 dose of study drug
PK	All randomized participants with ≥1 evaluable postdose PK assessment
PD evaluable	All randomized participants who completed the baseline and EoT visit assessments, had ≥80% compliance with study drug dosing, and did not have any major protocol deviations that could affect the PD assessments

EoT=end of treatment; ICF=informed consent form; ITT=intent-to-treat; PD=pharmacodynamic(s); PK=pharmacokinetic(s)

10.3.1. Determination of PD Evaluable Population

The PD evaluable population will be identified and documented based on a review of protocol deviations before database lock by members of the study team, including but not limited to the medical monitor and study biostatistician. The categories of major protocol deviations to be reviewed include, but are not limited to, participants who:

- Did not meet key inclusion/exclusion criteria
- Received disallowed concomitant medication that could meaningfully impact results
- Had treatment compliance rate <80%

The number and percentage of participants excluded from the PD evaluable population will be summarized by type of deviation and by treatment arm.

10.4. Statistical Analyses

This section summarizes the planned statistical analyses of the endpoints listed in Section 5. A more detailed and technical description of theses analyses will be included in the statistical analysis plan (SAP).

10.4.1. General Considerations

Continuous variables will be summarized using the number of participants, mean, SD, minimum, median, interquartile range, and maximum values. Categorical variables will be summarized using the frequency and percentage of participants in each category. Percentages will be based on the total number of participants with non-missing values. If there are missing values, the number missing will also be presented, but without a percentage. Data summaries will be presented by treatment group (placebo, CY6463). All available data will be included.

10.4.2. Primary Endpoint(s)

Corresponding with the primary objective of evaluating the safety and tolerability of CY6463, TEAE incidence (number and percentage of participants with TEAEs) will be reported for each

treatment group for the Safety population. A TEAE is defined as an AE that started on or after the administration of randomized study drug through the last day of the Follow-up period.

10.4.3. Exploratory Endpoint(s)

Plasma and CSF concentration levels of CY6463 will be summarized for the PK population at each nominal timepoint on scheduled study visits. Additionally, the following descriptive statistics will be presented: number and percentage of participants with values that are below the assay's limit of quantification; geometric mean; and coefficient of variation, calculated as 100*SD/mean. PK parameters, if available, will also be summarized using descriptive statistics for the PK population.

All other exploratory endpoints will be summarized by treatment group for each assessment timepoint using the ITT population. Baseline values used to calculate the change from baseline in these endpoints will be defined as the last non-missing assessment prior to the first administration of study drug, usually the predose assessment on Day 1.

Analyses of change from baseline exploratory endpoints will be conducted using the ITT population. A mixed-effect model repeated measures (MMRM) analysis with change from baseline as the response variable, treatment, visit, and treatment-by-visit interaction as fixed effects and the respective baseline value as the covariate with unstructured as the variance-covariance structure will be performed. Treatment differences between the CY6463 group and the placebo group will be estimated for the overall treatment period and at each assessment timepoint. Least square means and least square mean differences between the CY6463 group and placebo and their associated 90% confidence intervals will be presented. Participants who are missing both baseline and all treatment period values will be excluded. The missing at random assumption of MMRM will be utilized, and no imputation will be performed for missing observations.

The summary and analyses of the non-PK exploratory endpoints will also be performed using the PD evaluable population.

10.4.4. Safety Analyses

All safety analyses will be performed on the Safety population.

AEs will be coded using the latest version of MedDRA available at the start of the study. TEAEs will be summarized for each treatment group by SOC and preferred term (PT), and additionally by severity and relationship to study drug. In addition, the incidence of TEAEs leading to premature discontinuation of study drug and SAEs will be summarized by treatment group, SOC, and PT. Listings of pretreatment AEs, TEAEs, severe TEAEs, AESIs, study drug-related TEAEs, SAEs, TEAEs leading to study discontinuation, and AEs leading to death (if any) will be provided.

Descriptive statistics for observed values and changes from baseline values at each assessment time point will be presented by treatment group for each clinical laboratory, vital signs, and ECG parameters.

The number and percentage of participants with suicidal ideation, suicidal behavior, and self-injurious behavior without suicidal intent will be summarized at each assessment timepoint by treatment group.

10.4.5. Other Analyses

Potential relationships between CY6463 plasma concentrations and PD exploratory assessments may be assessed.

10.5. Interim Analyses

No formal interim analysis is planned for this study. Study data will be reviewed by an unblinded DRC as specified in Section 6.3.2.

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APPENDIX 1. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Direct Access to Source Data/Documents

Study Monitoring

Before an investigational site can enter a participant into the study, a representative of the sponsor will visit (in person or remotely, as necessary) the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the sponsor or its representatives. This will be documented in a clinical study agreement between the sponsor and the investigator.

During the study, a monitor from the sponsor or its representative will have regular contact with each study center for the following purposes:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the study team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that study drug accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRFs with the participant's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each participant (eg, clinic charts).
- Record and report any protocol deviations not previously sent to the sponsor
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to the sponsor and that those SAEs that met criteria for regulating reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

Audits and Inspections

Authorized representatives of the sponsor, a regulatory authority, an IEC or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, good clinical practice (GCP) guidelines of the ICH, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

The principal investigator at each study centers must obtain IRB/IEC approval for the study. Initial IRB/IEC approval and all materials approved by the IRB/IEC for this study, including participant ICF(s) and recruitment materials, must be maintained by the investigator and made available for inspection.

Quality Control and Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor may conduct a quality assurance audit. See Audits and Inspections, above.

Ethics

Ethics Review

The final study protocol, including the final version of the ICF(s), must be approved or given a favorable opinion in writing by the respective study center's IRB/IEC. The investigator must submit written IRB/IEC approval to the sponsor/sponsor's designee before enrolling a participant into the study.

The principal investigator of the respective study center is responsible for informing the IRB/IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB/IEC must approve all participant-facing materials, including but not limited to advertising used to recruit participants for the study. The protocol must be re-approved by the IRB/IEC upon receipt of amendments and annually, as local regulations require.

The principal investigator of a respective study center is also responsible for providing the IRB/IEC with reports of any reportable serious adverse drug reactions from any other study conducted with the study drug. The sponsor/sponsor's designee will provide this information to the principal investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB/IEC according to local regulations and guidelines.

Ethical Study Conduct

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements and sponsor policy.

Informed Consent

The principal investigator(s) at each study center will ensure that each participant (and/or LAR and the participant's caregiver/study partner, as applicable [see Eligibility Inclusion 1]) is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of study participation. A participant will not undergo any study procedure unless and until both the participant (and/or the participant's LAR, if applicable) sign the ICF(s). The ICF(s) will be written in such a way that at minimum, the LAR and caregiver/study partner, as applicable, understand the implications of study participation. The participant (and

LAR/caregiver/study partner, as applicable) will be given the opportunity to ask questions and allowed time to consider the information provided. Participants must also be notified that they are free to discontinue from the study at any time.

The principal investigator(s) must maintain the original, signed ICF(s). A copy of the signed ICF(s) must be given to the participant (and to the LAR/caregiver/study partner, as applicable).

Participants who are rescreened are required to sign a new ICF.

Data Handling and Recordkeeping

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information that would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the ICF.
- The participant must be informed that his/her medical records may be examined by the sponsor's quality assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Inspection of Records

The sponsor or sponsor’s designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, participant charts and study source documents, and other records relative to study conduct.

Retention of Records

Principal investigators must maintain all documentation relating to the conduct of this study for a period of 2 years after the last marketing application approval of CY6463 or, if not approved, 2 years after discontinuance of its development. If it becomes necessary for the sponsor or a regulatory authority to review documentation relating to the study, the investigator will permit access to such records.

Publication Policy

All data generated in this study will be the property of the sponsor. An integrated clinical and statistical report will be prepared at the completion of the study.

Please refer to the study center clinical trial agreement for publication and disclosure information.

APPENDIX 2. ADVERSE EVENTS, AESIS, AND SAES: DEFINITIONS AND PROCEDURES FOR RECORDING AND EVALUATING

Definition of Terms

Adverse Event (AE)

Per ICH, AEs are defined as any untoward medical occurrence in a patient or clinical study participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Events Meeting the AE Definition
● Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, that are considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease) ● Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition ● New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study ● Signs, symptoms, or the clinical sequelae of a suspected intervention-intervention interaction ● Signs, symptoms, or the clinical sequelae of a suspected overdose of either study drug or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent; such overdoses should be reported regardless of sequelae.
Events NOT Meeting the AE Definition
● Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition ● The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition ● Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE ● Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital) ● Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen

Serious Adverse Event (SAE)

An SAE is an AE occurring during any study phase (ie, at baseline or during Treatment, washout, or Follow-up), and at any dose of study drug that fulfills 1 or more of the following:

- Results in death
- Is immediately life-threatening (The term "life-threatening" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity: a substantial disruption of a person's ability to conduct normal life functions
- Results in a congenital abnormality or birth defect
- Is an important medical event that may require medical intervention to prevent 1 of the outcomes listed above.

Adverse Events of Special Interest (AESIs)

Based on the mechanism of action of sGC and anticipated vasodilation effects and based on the fact that CY6463 is a CNS-penetrant stimulator of sGC, the sponsor will collect AESIs in a timely manner. The AESIs relate to symptomatic hypotensive events and/or tachycardia, dizziness, syncope, and TEAEs related to change of neurobehaviors (ie, suicidality or euphoria).

In addition, because the marketed sGC stimulator riociguat includes bleeding events as a warning and precaution on its prescribing information (14), bleeding events will also be considered AESIs for CY6463 in this study. The specific list of AESI terms will be provided in the safety management plan.

An AESI classification means that although an event might be nonserious, it will nonetheless be reported to the sponsor by completing the AE eCRF and filling in the AESI box within 5 business days of the investigator's first awareness of the AESI event.

Treatment-Emergent Adverse Event (TEAE)

A TEAE is defined as an AE that started on or after the administration of randomized study drug through the last day of the Follow-up period.

Recording of AEs and/or SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. The investigator will then record all relevant information in the AE eCRF page.

- It is **not** acceptable for the investigator to send photocopies of the participant's medical records in lieu of completion of the AE eCRF.
- There may be instances when copies of medical records for certain cases are requested by the Safety CRO. In this case, all participant identifiers, with the

exception of the participant number, will be redacted on the copies of the medical records before submission to the Safety CRO.

AE terms should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate the event based on signs, symptoms, and/or other clinical information Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE. The investigator will complete the AE CRF in its entirety, including details regarding event onset (date and time), resolution (date and time), intensity, causality, action taken, serious outcome (if applicable), whether or not any medications were given in response to the event and whether or not the event caused the participant to discontinue the study.

Assessment of Intensity

Intensity will be assessed by the investigator according to the following scale:

- **Mild:** An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities
- **Moderate:** An event that causes discomfort sufficient to interfere with normal everyday activities
- **Severe:** An event that prevents normal everyday activities

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria of this Appendix. An AE of severe intensity may or may not be considered serious.

Relationship to Study Drug

For all AEs, the investigator must provide an assessment of causal relationship to study drug. The causality assessment will be recorded in the participant's source documentation and on the AE eCRF. The investigator may change his/her opinion of causality in light of follow-up information and amend the eCRF with the updated causality assessment.

Causal relationship will be assessed according to the criteria below.

Related:	An event where there is at least a reasonable possibility of a causal relationship between the event and the study drug. "Reasonable possibility" means there is evidence to suggest a potential causal relationship between the drug and the event.
Unrelated:	Any other event

APPENDIX 3. PROHIBITED MEDICATIONS, FOODS, AND SUPPLEMENTS

Also see Lifestyle Restrictions (Section 7.5) and Concomitant Medications and Therapies (Section 8.7).

Use of the following is prohibited from the Screening visit through the Follow-up visit, unless noted otherwise below:

- Specific inhibitors of PDE5, including sildenafil, tadalafil, vardenafil
- Nonspecific inhibitors of PDE5, including dipyridamole, theophylline
- Any supplement for the treatment of erectile dysfunction
- Other sGC stimulators, including riociguat and vericiguat
- Nitrates such as nitroglycerin, isosorbide mononitrate, isosorbide dinitrate, sodium nitroprusside, amyl nitrate
- Aspirin ≥325 mg/day
- Any anticoagulant medication
- Any P2Y12 inhibitor, including cangrelor, clopidogrel, prasugrel, ticagrelor, ticlopidine
- Use of any "illicit drug" is not permitted beginning 1 month before Screening through the Follow-up visit
  Note: Medical (prescribed) cannabis and cannabidiol products are permitted except for 24 hours before the Screening, Day 1 and EoT visits, and for 4 hours prior to all other study visits.

[redacted]

APPENDIX 4. NATIONAL INSTITUTE ON AGING / ALZHEIMER'S ASSOCIATION CRITERIA FOR AD DEMENTIA

Source:

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr. CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia. 2011;7(3):263-69. doi:10.1016/j.jalz.2011.03.005A

All-Cause Dementia: Core Clinical Criteria

The diagnosis of dementia is intended to encompass the spectrum of severity, ranging from the mildest to the most severe stages of dementia. The methodology for staging of dementia severity was beyond the charge of the workgroup. Dementia is diagnosed when there are cognitive or behavioral (neuropsychiatric) symptoms that:

1. Interfere with the ability to function at work or at usual activities; and
2. Represent a decline from previous levels of functioning and performing; and
3. Are not explained by delirium or major psychiatric disorder;
4. Cognitive impairment is detected and diagnosed through a combination of (1) history-taking from the patient and a knowledgeable informant and (2) an objective cognitive assessment, either a "bedside" mental status examination or neuropsychological testing. Neuropsychological testing should be performed when the routine history and bedside mental status examination cannot provide a confident diagnosis.
5. The cognitive or behavioral impairment involves a minimum of 2 of the following domains:
    a. Impaired ability to acquire and remember new information—symptoms include: repetitive questions or conversations, misplacing personal belongings, forgetting events or appointments, getting lost on a familiar route.
    b. Impaired reasoning and handling of complex tasks, poor judgment—symptoms include: poor understanding of safety risks, inability to manage finances, poor decision-making ability, inability to plan complex or sequential activities.
    c. Impaired visuospatial abilities—symptoms include: inability to recognize faces or common objects or to find objects in direct view despite good acuity, inability to operate simple implements, or orient clothing to the body.
    d. Impaired language functions (speaking, reading, writing)—symptoms include: difficulty thinking of common words while speaking, hesitations; speech, spelling, and writing errors.
    e. Changes in personality, behavior, or comportment—symptoms include: uncharacteristic mood fluctuations such as agitation, impaired motivation, initiative, apathy, loss of drive, social withdrawal, decreased interest in previous activities, loss of empathy, compulsive or obsessive behaviors, socially unacceptable behaviors.

The differentiation of dementia from mild cognitive impairment (see Albert et al 2011 (15) on the diagnosis of mild cognitive impairment) rests on the determination of whether or not there is significant interference in the ability to function at work or in usual daily activities. This is

inherently a clinical judgment made by a skilled clinician on the basis of the individual circumstances of the patient and the description of daily affairs of the patient obtained from the patient *and* from a knowledgeable informant.

Probable AD Dementia: Core Clinical Criteria

Probable AD dementia is diagnosed when the individual:

1. Meets criteria for all-cause dementia described above, and in addition, has the following characteristics:

   a. Insidious onset. Symptoms have a gradual onset over months to years, not sudden over hours or days;
   b. Clear-cut history of worsening of cognition by report or observation; and
   c. The initial and most prominent cognitive deficits are evident on history and examination in 1 of the following categories.

   - Amnestic presentation: It is the most common syndromic presentation of AD dementia. The deficits should include impairment in learning and recall of recently learned information. There should also be evidence of cognitive dysfunction in at least 1 other cognitive domain, as defined above.
   - Nonamnestic presentations:
     - Language presentation: The most prominent deficits are in word-finding, but deficits in other cognitive domains should be present.
     - Visuospatial presentation: The most prominent deficits are in spatial cognition, including object agnosia, impaired face recognition, simultanagnosia, and alexia. Deficits in other cognitive domains should be present.
     - Executive dysfunction: The most prominent deficits are impaired reasoning, judgment, and problem solving. Deficits in other cognitive domains should be present.

   d. The diagnosis of probable AD dementia ***should not*** be applied when there is evidence of (a) substantial concomitant cerebrovascular disease, defined by a history of a stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) core features of Dementia with Lewy bodies other than dementia itself; or (c) prominent features of behavioral variant frontotemporal dementia; or (d) prominent features of semantic variant primary progressive aphasia or nonfluent/agrammatic variant primary progressive aphasia; or (e) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

Note: All patients who met criteria for "probable AD" by the 1984 NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria (16) would meet the McKhann 2011 (8) criteria for probable AD dementia.

APPENDIX 5. SPONSOR'S SIGNATURE PAGE

Sponsor's Approval

The protocol has been approved by Cyclerion Therapeutics, Inc.

Sponsor's Authorized Officer:

APPENDIX 6. INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for CY6463. I have also read the C6463-202 protocol and agree to conduct the study as outlined.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

_____

Study Center Name (please print)