CLINICAL STUDY PROTOCOL

Study Title:	A Phase 2A Randomized Double-Blind Placebo-Controlled Trial to Evaluate the Efficacy and Safety of Varoglutamstat (PQ912) in Patients with Early Alzheimer's Disease with a Stage Gate to Phase 2B (VIVA-MIND)
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Protocol Number:	PBD 01187
US IND #:	143871
ADCS Reference #:	ADC-058-PBD
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Study Summary

Title	A Phase 2A Randomized Double-Blind Placebo-Controlled Trial to Evaluate the Efficacy and Safety of Varoglutamstat (PQ912) in Patients with Early Alzheimer's Disease (AD) with a Stage Gate to Phase 2B (VIVA-MIND)
Rationale	Varoglutamstat (PQ912) is an inhibitor of the enzyme glutaminyl cyclase (QC) and its isoenzyme (isoQC). In preclinical models, varoglutamstat effectively reduces levels of post-translationally modified forms of Aβ into pGlu-Aβ (Glutamate 3/11 cyclization) and cytokine monocyte chemoattractant protein 1 (CCL2) into pGlu-CCL2 (Glutamine cyclisation). These post-translationally modified pyroglutamated proteins appear to be particularly important pathogenically in human AD. pGlu-Aβ is neuro and synaptotoxic, proinflammatory, promoting its self-aggregation into oligomers and resisting degradation, while CCL2 and pGlu-CCL2 act as proinflammatory cytokines linked to the negative neuroinflammatory mechanisms in AD. Varoglutamstat has completed a phase 1 program with a wide dose range exploration and a phase 2A clinical trial (SAPHIR) with PK/PD results that are consistent with the preclinical and phase 1 predictive models. The SAPHIR trial provides preliminary evidence in early AD patients of efficacy on disease-relevant cerebrospinal fluid (CSF) biomarkers, favorable electrophysiological findings with reduced theta power on quantitative electroencephalogram (qEEG), and a potentially early cognitive signal while demonstrating predictable and measurable target engagement.
Target Population	The below inclusion criteria apply to individuals enrolled in this phase 2A protocol, and will apply to individuals enrolled in phase 2B as well: Male and postmenopausal or surgically sterile females, aged 50 to 89 years (inclusive at screening), meeting criteria for Mild Cognitive Impairment (MCI) due to AD or Mild probable AD according to NIA-AA guidelines (Appendix II), and according to a molecular diagnostic CSF biomarker profile of AD including: $A\beta_{1-42} \leq 1030$ pg/mL AND p-tau-181 > 27 pg/mL, OR p-tau-181/$A\beta_{1-42}$ > 0.023 (Roche Elecsys® assays [1-3]). Major inclusion criteria include the following test scores at screening: MMSE score 20-30 [inclusive], MoCA score < 26, and CDR global score of 0.5 or 1. Eligible participants should be receiving a stable dose of acetylcholinesterase inhibitors (AchEI) and/or memantine for at least four months prior to screening and be expected to remain on same dose(s) for trial duration. Those participants with contraindications or failed treatment with either AchEI and/or memantine (with at least a 6-week wash-out period from screening) will also be eligible for inclusion. Participants being treated with aducanumab (Aduhelm™) or any Amyloid Beta Antibody (such as for example lecanemab (Leqembi™) are not eligible for inclusion.

Number of Participants and Transition from Phase 2A to 2B	Approximately 180 participants will be randomized using a 1:1 allocation to active treatment or placebo in phase 2A. Following phase 2A, interim futility data (ADNI Battery Composite (ABC) and EEG theta power) will be reviewed and a stage-gate decision for whether or not to continue to phase 2B will be undertaken. In phase 2B, there will be additional enrollment of approximately 234 participants, using the same inclusion criteria. Phase 2B enrollment will not begin until there is a "go" decision from phase 2A. Newly-enrolled phase 2B participants will also be randomized using a 1:1 allocation to active treatment or placebo. Thus, a total of 180 + 234 = 414 participants will be randomized in the two phases combined. Participants will be balanced in their randomization so that no more than 40% have MCI and at least 60% have Mild probable AD according to NIA-AA Diagnostic Guidelines (Appendix II). Participants who are randomized but drop out prior to the start of study drug will be replaced.
Number of Clinical Sites	Recruitment and enrollment of participants will occur through the Alzheimer's Disease Cooperative Study (ADCS) network across approximately 30 clinical sites in phase 2A, and approximately 55 clinical sites in phase 2B.
Study Design & Statistical Plan	This is a phase 2A multi-center, randomized, double blind, placebo-controlled, parallel group study of varoglutamstat, with a stage gate to phase 2B. In phase 2A there will be adaptive dosing evaluation of three dose levels with exposure to varoglutamstat or placebo for a minimum of 24 weeks, with preliminary evaluation of both cognitive function and pharmacodynamic changes on EEG spectral analysis. In the event that the stage gate for phase 2B is passed , then phase 2B will assesses efficacy and longer-term safety in a larger study group, i.e., 414 participants through 72 weeks of treatment. The final analysis will use a mixed effects repeated measures model, with no imputation for missing data. Sensitivity analyses will be performed.
Dose Strategy & Treatment Duration	**<u>Phase 2A – Adaptive Dose Finding</u>** Participants will be enrolled sequentially into one of three dose cohorts, labeled A, B, and C, with 60 participants per cohort (n=30 active and n=30 placebo) as follows: • **Cohort A** will enroll first. Participants will take 150 mg BID for 4 weeks, then 300 mg BID for 4 weeks, and then 600 mg BID (highest dose) until the first occurrence of either: a) the 600 mg dose meets its stopping rule, at which time all Cohort A participants will down titrate to 300mg BID; or b) the stage-gate decision to Phase 2B is reached.

	• **Cohort B** will enroll after cohort A has accrued. Participants enrolled to Cohort B will take 150 mg BID for 4 weeks, and then 300 mg BID (middle dose) until the first occurrence of either: a) the 600 mg dose has been selected within Cohort A, at which time cohort B subjects will titrate up to 600 mg BID (after at least 4 weeks at 300 mg BID); or b) the 300 mg dose meets its stopping rule, at which time all subjects on a 300 mg BID dose will down-titrate to 150 mg BID; or c) the stage-gate decision to Phase 2B is reached, whichever comes first. • **Cohort C** will accrue after cohort B has accrued. Participants will take 150 mg BID (lowest dose) until the first occurrence of either: a) the 600 mg dose has been selected within Cohort A *or* the 300 mg dose has been selected within Cohort B, at which time Cohort C subjects will titrate up to the selected dose; or b) the 150 mg dose meets its stopping rule, in which case all medication will cease and the trial will end for safety reasons; or c) or the stage-gate decision to Phase 2B is reached, whichever comes first. Each dose cohort will be tested for safety and tolerability using a continuously monitored stopping boundary. If a dose cohort meets discontinuation criteria, participants tolerating that dose or a higher dose will drop down to the next dose level being evaluated in phase 2A. Cohorts will also be tested for sufficient plasma exposure and target occupancy (TO) starting at week 4 and every 4-8 weeks thereafter until participants have reached 8 weeks of originally assigned full dose treatment, according to the visit schedule in Appendix I. A final phase 2A plasma and CSF PK sample will be undertaken at week 24. Additional phase 2B plasma PK samples will be taken at week 48 and 72, and CSF PK sample will be taken at week 72. Varoglutamstat plasma levels will be measured just prior to the morning dose and 2 to 6 hours after the morning dose for each time point. Participants in phase 2A will continue to undergo study assessments and dosing schedule according to Appendix I, until a stage-gate decision is reached. This may be up to 72 weeks on treatment for early-enrolled participants.

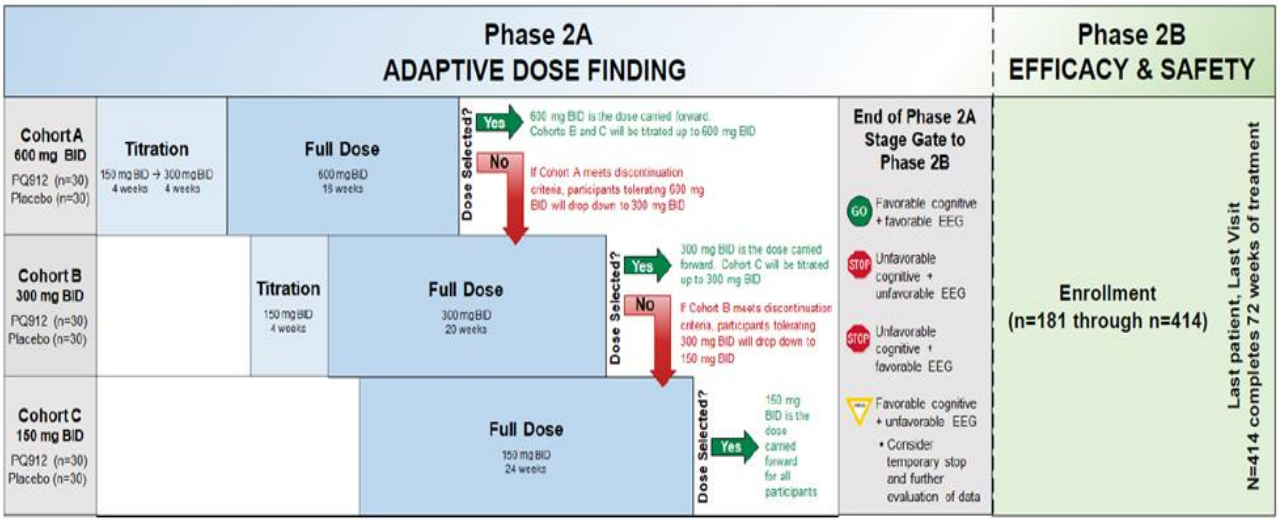
Stage Gate Analysis & Dose Expansion	**<u>Phase 2A->2B Stage Gate (determined by an interim analysis)</u>** There will be an interim analysis for futility at the end of phase 2A, using two outcome measures, namely an ADNI Battery Composite (ABC) (see Section 9.1.1) and EEG theta power. If the ABC measure records NO (indicating evidence of negative cognitive effects), the trial will stop. If both the ABC and the EEG theta power measures record YES (no evidence of negative cognitive effects, and positive evidence of benefit on the EEG measure), the trial will continue. Otherwise (i.e. in the case that the ABC measure records YES and the EEG theta power measure records NO), the stopping rule will be indeterminate, the trial will pause, and further analysis may be undertaken prior to a final decision being reached by the overseeing Study Steering Committee (SSC) and the study sponsor. **<u>Phase 2B – Dose Expansion</u>** Phase 2B will follow once the phase 2A stage-gate decision has been reached. Participants in phase 2B will be dosed at the optimal dose level selected in phase 2A; participants in cohorts A and B may be titrated up to this dose. All newly-enrolled participants in phase 2B will undergo an initial titration period as defined for that dose level in phase 2A. The placebo group allocation will remain unchanged. The randomization rate remains 1:1 between varoglutamstat and placebo throughout the accrual period. Total enrollment of the trial will be completed when an additional 234 participants are randomized (final enrolled n=414). Total duration of study treatment is 72 weeks, followed by a 4-week post-treatment observation period.
OBJECTIVES – PHASE 2A	
Primary	The primary objectives in phase 2A are: • To determine the highest safe and well-tolerated dose over the safety evaluation period (from first dose to completion of 8 weeks at the originally assigned full dose), which will include assessment of the proportion of participants who experience any Adverse Event of Special Interest (AESI) • To evaluate early evidence for efficacy of varoglutamstat as measured by the ADNI Battery Composite (ABC) and by pharmacodynamics changes on EEG spectral analysis over a 24-week treatment period
PK	The PK objective in phase 2A is to measure varoglutamstat levels in plasma and to establish the sufficiency of target occupancy (TO) of QC in plasma following at least 8 weeks of treatment at the dose levels being tested. This PK testing starts at week 4 and continues every 4-8 weeks throughout phase 2A. This plasma PK data will be provided to the DSMB for consideration in parallel with safety data. Following consideration of safety, tolerability, and PK, the DSMB will provide a recommendation to the SSC on whether the conditions to advance through the stage-gate have been met as well as the final dose selection for phase 2B.
OBJECTIVES – PHASE 2B	

Primary	The primary objective in phase 2B is to evaluate the efficacy of varoglutamstat as measured by the CDR-SB over a 72-week treatment period.
Secondary	**Key Efficacy Objective** The key secondary objective in phase 2B is to evaluate the efficacy of varoglutamstat as measured by CFC2, a cognitive-functional composite, over a 72-week treatment period. **Other Efficacy Objectives** Other secondary efficacy objectives in phase 2B are to evaluate the efficacy of varoglutamstat as measured by the: (1) composite sum of standardized scores from a set of ADNI neuropsychological test measures, the ADNI Battery Composite (ABC); (2) qEEG (global relative theta wave power); (3) Functional Activities Questionnaire (FAQ); (4) Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog-13); and (5) Neuropsychiatric Inventory (NPI).
Safety & Tolerability	Assessment of the longer-term safety and tolerability of varoglutamstat also serve as secondary objectives in phase 2B of this study. Such measures include the Columbia-Suicide Severity Rating Scale (C-SSRS), drug discontinuation rates, mortality rates, frequency of AEs, AESIs and serious adverse events (SAEs) by grade, attribution, and system organ class, with focus on skin and subcutaneous AEs and gastrointestinal AEs.
PK	The pharmacokinetics (PK) of varoglutamstat in phase 2B will be assessed by measuring varoglutamstat levels in plasma and in CSF.
Exploratory	Exploratory objectives are to investigate treatment effects of varoglutamstat as assessed by the changes in: (1) brain volume measured by cranial MRI; (2) Mini-Mental State Examination (MMSE); (3) Montreal Cognitive Assessment (MoCA); (4) CSF biomarkers (Aβ1-42, t-tau, p-tau-181, sTREM2, YKL-40, neurogranin, SNAP-25, neurofilament light chain (NfL) and VILIP-1); (5) qEEG connectivity network measures; (6) AD Composite Score (ADCOMS); (7) ADAS-Cog-Exec; (8) relative QC activity in CSF; and (9) comparing treatment to placebo arms on changes in the primary outcome measure (CDR-SB), key secondary outcome measure (CFC2), and in the TO measure, within subgroups defined separately by: (i) APOE genotype (E4 carrier vs non E4 carrier), and (ii) MCI vs. Mild probable AD.
ENDPOINTS – PHASE 2A	

Primary	The primary endpoints in phase 2A are: • The proportion of participants, for each dose, who experience any AESI during the safety evaluation period, from first dose to completion of 8 weeks at the full originally assigned dose. • The within-participant change from baseline to week 24 in the composite sum of standardized scores from the ADNI Battery Composite (ABC, 9-item, see Section 9.1.1), compared between active arm and placebo. • The within-participant change from baseline to week 24 in quantitative EEG (global relative theta wave power), compared between active and placebo.
Safety & Tolerability	Secondary safety and tolerability endpoints are: • Rates of all AEs (SAEs, TEAEs, AESIs) • Drug discontinuation rates • Mortality rates • Suicidality on the C-SSRS • Changes on brain MRI scans as determined by site Principal Investigator (PI) with a local reading (ARIA-E, ARIA-H, infarcts) • Frequency and severity of abnormalities on physical examinations, vital signs, health status, ECG and safety labs
PK	The PK endpoints in phase 2A are the mean values of varoglutamstat plasma levels for each cohort following at least 8 weeks of treatment at the dose levels being tested. These PK endpoints will be measured pre-and post-dose starting at week 4 and every 4-8 weeks throughout phase 2A. The estimated TO will support the decision of the dose carried forward to phase 2B.
ENDPOINTS – PHASE 2B	
Primary	The primary endpoint in phase 2B is the within-participant change from baseline to week 72 in CDR-SB, compared between active arm and placebo.

Secondary	**Key Efficacy Endpoint** The key secondary efficacy endpoint in phase 2B is the within-participant change from baseline to week 72 in the efficacy of varoglutamstat assessed by CFC2, compared between active and placebo. **Other Secondary Efficacy Endpoints** • The within-participant change from baseline to week 72 in the composite sum of standardized scores from the ADNI Battery Composite, (ABC, 9-item, see Section 9.1.1) compared between active arm and placebo. • The within-participant change from baseline to week 72 in qEEG (global relative theta wave power), compared between active and placebo. • The within-participant change from baseline to week 72 in Functional Activities Questionnaire (FAQ), compared between active and placebo. • The within-participant change from baseline to week 72 in ADAS-Cog-13, compared between active and placebo. • The within-participant change from baseline to week 72 in Neuropsychiatric Inventory (NPI), compared between active and placebo.
PK	The PK in phase 2B will be measured in both plasma and CSF and will be analyzed at the end of the study. This will include measures of target occupancy in both plasma and CSF as well as the ratio of plasma to CSF varoglutamstat.
Safety & Tolerability	The safety and tolerability endpoints include the change in the following measures: (1) AEs and SAEs by grade, attribution, and system organ class, with special focus on skin and subcutaneous AEsand gastro-intestinal AEs; (2) C-SSRS; (3) drug discontinuation rates; and (4) mortality rates.
Exploratory	Exploratory endpoints include the within-participant change between active and placebo in: (1) brain volume measured by cranial MRI; (2) Mini-Mental State Examination (MMSE); (3) Montreal Cognitive Assessment (MoCA); (4) CSF biomarkers (Aβ1-42, t-tau, p-tau-181, sTREM2, YKL-40, neurogranin, SNAP-25, NfL and VILIP-1); (5) qEEG connectivity network; (6) AD Composite Score (ADCOMS); (7) ADAS-Cog-Exec; (8) relative change from screening (100%) to week 72 in QC activity in CSF; and (9) changes in the primary outcome measure (CDR-SB), key secondary outcome measure (CFC2), and in the TO measure, within subgroups defined separately by: (i) APOE genotype (E4 carrier vs non E4 carrier), and (ii) MCI vs Mild probable AD.

STUDY SCHEMATIC



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List of Abbreviations:

Aβ	Amyloid Beta
AβO	Amyloid Beta Oligomers
ABC	ADNI Battery Composite
AchEI	Acetylcholinesterase Inhibitor
AD	Alzheimer's Disease
ADCOMS	AD Composite Score
ADAS-Cog-	Alzheimer's Disease Assessment Scale-Cognitive Subscale - 13-item
ADNI	Alzheimer's Disease Neuroimaging Initiative
AEC	Amplitude Envelope Correlation
AICD	Automatic Implanted Cardioverter Defibrillator
AUC	Area Under the Curve
AE	Adverse Event
AESI	Adverse Event of Special Interest
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ARIA-E	Amyloid-Related Imaging Abnormalities Related To Underlying Vasogenic
ARIA-H	Amyloid-Related Imaging Abnormalities Related To Hemosiderin Deposits
ASA	Acetylsalicylic Acid
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BID	Twice per day
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
C-SSRS	Columbia-Suicide Severity Rating Scale
CBD	Cannabidiol
CCL2	C-C motif chemokine ligand 2
CDR-SB	Clinical Dementia Rating- Sum of Boxes
CFC2	Cognitive-Functional Component 2
CFR	Code of Federal Regulations
CIED	Cardiac Implantable Electronic Devices
CJD	Creutzfeldt-Jakob Disease
C_{max}	Maximum Concentration
CONSORT	Consolidated Standards of Reporting Trials
CPK	Creatinine Phosphokinase
CSF	Cerebrospinal Fluid
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DDI	Drug–Drug Interaction
DMP	Data Management Plan
DSMB	Data Safety & Monitoring Board
eCRF	Electronic Case Report Form
EC50	Concentration Producing 50% of Maximal Effect

ECG	Electrocardiogram
EDC	Electronic Data Capture
EEG	Electroencephalogram
eGFR	Estimated Glomerular Filtration Rate
EMA	European Medicines Agency
FAQ	Functional Activities Questionnaire
FDA	Food & Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HAV	Hepatitis A Virus
hERG	Human Ether-a-Go-Go-Related Gene
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High-Density Lipoprotein
HIPAA	Health Insurance Portability & Accountability Act
HLA	Human Leukocyte Antigen
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
HPT	Hypothalamic-Pituitary-Thyroid
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
Ikr	Rapid Delayed Rectifier Channel
INR	International Normalized Ratio
IRB	Institutional Review Board
isoQC	Iso-glutaminyl Cyclase
ITT	Intent-To-Treat
kg (unit)	Kilogram
Ki	Inhibitory Constant of PQ912 for QC which is known with 25 nM
LAR	Legally Authorized Representative
LBD	Lewy Bodies Dementia
LDH	Lactate Dehydrogenase
LDL	Low-Density Lipoprotein
LP	Lumbar Puncture
LPS	Lipopolysaccharide
MCI	Mild Cognitive Impairment
MedDRA	Medical Dictionary for Regulatory Activities
MHIS	Modified Hachinski Ischemic Scale
mITT	Modified Intent-To-Treat
mg (unit)	Milligram

ml (unit)	Milliliter
MMRM	Mixed-Effect Model Repeated Measure model
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NfL	Neurofilament Light (Protein)
ng (unit)	Nanogram
nM (unit)	Nanomolar
NIA-AA	National Institute on Aging – Alzheimer's Association
NCRAD	National Centralized Repository for Alzheimer's Disease
NGAL	Neutrophil gelatinase-associated lipocalin
NOAEL	No-observed-adverse-effect-level
NOEL	No-observed-effect-level
NPH	Normal Pressure Hydrocephalus
NPI	NeuropsychiatricInventory
OHRP	Office of Human Research Protection
P-tau	Phosphorylated tau
PD	Pharmacodynamic
PDE4	Phosphodiesterase 4
pE-Aβ	Pyroglumated form of β-Amyloid
PET	Positron Emission Tomography
PHI	Personal Health Information
PI	Principal Investigator
PK	Pharmacokinetic
PLI	Phase Lag Index
PLT	Phase Lag Time
PP	Per Protocol
PQ912	Varoglutamstat
PRN	Pro Re Nata (As Needed)
PSP	Progressive Supranuclear Palsy
PT	Preferred Term
PTT	Partial thromboplastin time
QC	Glutaminyl Cyclase
qEEG	Quantitative Electroencephalogram
Quarc	Quantitative Anatomical Regional Change
RAVLT	Rey Auditory Verbal Learning Test
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SSC	Study Steering Committee
sTREM2	Soluble Variant Triggering Receptor Expressed on Myeloid Cells 2
SUSAR	Serious Unexpected Suspected Adverse Event
SOC	MedDRA System Organ Class

T-tau	Total tau
T_3	Triiodothyronine
T_4	Thyroxine
TBL	Total Bilirubin
TCR	T Cell Receptor
TEAE	Treatment Emergent Adverse Events
TK	Toxicokinetic
T_{max}	Time of maximum concentration of drug after dosing
TO	Target Occupancy
TSH	Thyroid-Stimulating Hormone
ULN	Upper Limit of Normal
VILIP	Visinin-Like Protein
vMRI	Volumetric Magnetic Resonance Imaging
WBC	White Blood Cell
WBV	Whole Brain Volume

1 INTRODUCTION

1.1 Background

More than 5 million people are afflicted with Alzheimer's disease (AD) and related dementias in the United States, and by 2050 this number could rise as high as 16 million. [4] Recent data suggests that AD affects twice as many females as males and is thought to be associated with multiple biological and genetic factors. The emotional and financial burden of AD to patients, family members, and society is enormous, and is predicted to grow exponentially as the median population age increases. The potential to preserve, or even improve, cognition in adults at high risk of cognitive decline due to AD clearly has important implications, not only for the affected individual, but also for the support system that bears the social and financial burdens of long-term caregiving.

There are currently approved medications including acetylcholinesterase inhibitors (AchEIs) and memantine. These medications are approved for symptomatic treatment of AD. They have small effect sizes and generally limited clinical benefits. In June 2021, FDA granted an accelerated approval of aducanumab (Aduhelm^{TM})[5], an intravenous infused monoclonal antibody, for the treatment of early AD with contingency that continued approval will require verification of clinical benefit in a confirmatory clinical trial. Its indication is based on its ability to reduce amyloid beta plaques, with reasonable likelihood that this will predict clinical benefit. It is not currently a standard of care in the community, and the position of payers and insurers has not yet been ascertained. There remains an urgent need to find effective treatments for AD that can arrest or reverse the disease and are clinically efficacious.

Vivoryon Therapeutics N.V. [Vivoryon] has developed a novel drug candidate, varoglutamstat (PQ912), for the potential treatment of early AD. Varoglutamstat is a first-in-class, highly specific and potent small molecule with a unique mechanism of action. Varoglutamstat inhibits the enzyme glutaminyl cyclase (QC) and its isoenzyme iso-QC, resulting in reduced levels of pGlu-Aβ, a posttranslationally modified form of Aβ (Glutamate 3/11 cyclization), as well as pGlu-CCL2, a post-translationally modified form of cytokine monocyte chemoattractant protein 2 (CCL2) (Glutamine cyclization). These proteins are significantly overexpressed in AD where pGlu-Aβ has been shown to be synaptotoxic, proinflammatory, promoting of self-aggregation into oligomers, and resistant to degradation, while pGlu-CCL2 has been linked to the presence and severity of neuroinflammation. In preclinical models, these toxic effects can be attenuated with varoglutamstat treatment.

Varoglutamstat has completed an extensive preclinical AD evaluation, a large phase 1 program with robust PK PD evaluation across a range of doses, and a phase 2A clinical trial. [6] It is an excellent candidate to take further into development, as it can address a set of stringent proof-of-concept criteria which include: target validation, suitable dose range for testing with direct PK PD measurement, and biomarkers that can evaluate relevant downstream biological effects. Phase 2A positive results on qEEG with effects on theta power, improvement in working memory (One Back Test) and on CSF biomarkers including neurogranin (measure of synaptic toxicity) and YKL-40 (marker of inflammation) also support further development.

Based on these aggregate results, this clinical study targets early AD with the goal of improving cognition and everyday function and attenuating longer term disease progression through its dual pathways of varoglutamstat's mode of action.

1.2 Preclinical & Scientific Rationale

The cause of AD is thought to be an impairment of the capacity to degrade amyloid beta peptides (Aβ). As a result, Aβ levels accumulate and become substrates for aminopeptidases, resulting in truncated peptides including those carrying an N-terminal glutamate (Aβ (3/11-40/42)). These peptides are substrates for QC. QC, in turn, catalyzes the formation of pyroglutamated forms of Aβ (pE-Aβs), which are hydrophobic and degradation-resistant. These species are prone to initiate the formation of Aβ oligomers (AβO). The (pE) AβO are today regarded as a key culprit of AD. They are highly toxic and assumed to cause reversible synaptic impairment and reduced neuronal connectivity early in AD, which correlates with first memory impairments. Followed by tau-pathology and inflammation, this leads to chronic neurodegeneration. The new concept is to inhibit QC to reduce the level of pE-Aβs, which is expected to alleviate the acute and chronic toxic effects of pE induced AβOs, supporting synaptic regeneration and resulting in a disease modifying effect upon chronic treatment. The investigational product varoglutamstat, a small molecule inhibitor of QC, has been validated in in-vitro and in-vivo studies. The safety and toxicology of varoglutamstat was established in regulatory toxicology studies and the compound was extensively investigated in phase 1 studies in healthy volunteers, including the elderly, and was well tolerated. Varoglutamstat is the first QC inhibitor tested in humans. In addition, a phase 2A trial in early, treatment-naïve AD patients was performed to determine safety and early signs of efficacy in this patient population.

Amyloid deposits in the human brain, which consist primarily of different Aβ peptide species, represent one of the major histopathological hallmarks of AD. The deposition of Aβ is apparently caused by a reduced clearance of the peptides, a process, which initiates a deleterious cascade of events leading to neurodegeneration and dementia. Numerous studies have shown that most of these Aβ peptides are N-terminally truncated and post-translationally modified in AD patients. Among these modifications, the formation of pyroglutamate at the N-terminus is most prominent. Caused by the pE-formation, the peptides are rendered more hydrophobic, which, in turn, increases the amyloidogenicity and stabilizes the peptides towards proteolytic cleavage. Recent studies showed that accumulation of pE-Aβ as well as increased QC expression correlate with disease progression. More recently it has also been shown that pE-Aβ species trigger the formation of highly toxic soluble (pE)-Aβ aggregates (oligomers). pE-Aβ appear to seed Aβ-oligomers [7] and the hypertoxicity of pE-Aβ related oligomers seems to be brought about by differences in the secondary and tertiary structure imposed by pE-Aβ to the aggregates.

In addition, the neurodegenerative processes are characterized by up-regulation of proinflammatory mediators (cytokines). Among those, the chemokine CCL2 (also called monocyte chemoattractant protein 1 (MCP-1)) plays an important role for the stimulation and hyperactivation of glial cells. CCL2 is N-terminally pE-modified at a glutamine residue. Similar to pE-Aβ, the modification confers resistance to degradation and, in addition, mediates receptor activation. Chemokines in turn have been shown to induce expression of QC, which constitutes a vicious cycle between pE-Aβ and neuroinflammation.

The inhibition of the QC enzyme by a selective and specific QC inhibitor prevents the formation of stable pE versions of Aβ and AβOs and subsequently reduces significantly neurodegenerative events leading to a stabilization of cognitive performance. QC inhibition synergistically reduces the neuroinflammatory processes by inhibition of CCL2 modification.

This clinical trial represents a distinct approach from those previously taken in Aβ-lowering treatments by specifically targeting the inhibition of post-translationally modified and particularly neurotoxic pyroglutamated forms of Aβ 1-42, and the neuroinflammatory chemokine CCL2.[8, 9] Evidence is accruing that these pGlu targets and pathways are independent of the therapeutic targets that have been tested with

BACE 1 inhibition as well as Aβ 1-42 directed monoclonal antibodies.[10, 11] Whereas a BACE 1 gene knockout is shown not to affect the levels of soluble and insoluble pGlu-Aβ in APP on transgenic mice, in preclinical models overexpression of the QC enzyme, the protease peptidase cathepsin B and the endoprotease meprin, are associated with increased formation of pGlu-Aβ.[10-13] Furthermore, by not directly targeting the production and clearance of endogenously produced Aβ 1-42, there is no perturbation of its potential contribution to antimicrobial activity, tumor suppression, support of the blood brain barrier, or regulation of synaptic function.[14] Of further interest is donanemab, a mAb, [15] which is specifically directed to pGlu-Aβ, and has achieved a rapid and significant reduction in cortical amyloid plaque burden on PET in AD patients through microglial mediated removal of cerebral amyloid plaques, adding to the potential mechanistic advantages of targeting pGlu-Aβ.

Inhibition of glutaminyl cyclases is considered as a promising new strategy to reduce the formation of neurotoxic Aβ peptides and neuroinflammation in AD, thereby leading to a more acute improvement of early cognitive deficits, a reduction of disease progression and stabilization of clinical signs and symptoms. The therapeutic concept of QC inhibition has the potential of a disease modifying effect in AD.

1.3 Non-clinical data

Data from preclinical studies support the following findings. Additional information can be found in the most recent edition of the Investigator's Brochure. [16]

1.3.1 Pharmacology

Varoglutamstat is a competitive inhibitor of QC and iso-glutaminyl cyclase (isoQC). Both enzymes possess identical substrate specificity but differ in their sub-cellular location. The binding of varoglutamstat to the enzymes (inhibition constant, Ki) from different species is in the lower nanomolar range with about 5 times lower values for isoQC compared to QC. It has been shown that isoQC is the major cyclase in peripheral organs, whereas QC is the major cyclase in the brain. [17-19] QC is therefore thought to be the primary enzyme responsible for cyclisation of several neuropeptides.

Efficacy of varoglutamstat was assessed in different cellular assays with EC50 values (concentration producing 50% of maximal effect) in the high nM to low µM range. In vivo proof of principle for the usefulness of QC inhibitors (varoglutamstat and additional tool compounds) in their intended use to treat AD has been shown in transgenic 'AD mice'. These experiments clearly showed that QC inhibition reduced the level of neurotoxic pE-Aβ species and concomitantly improved impaired cognitive function; a calculated target occupancy of about 60% was found to be sufficient for a clear pharmacological effect. Further experiments demonstrated that lipopolysaccharide (LPS)-induced neuroinflammation in mice was also reduced by QC inhibition.

As QC and isoQC are ubiquitously expressed in the body, secondary pharmacodynamic (PD) effects were investigated. Potential off-target effects on the proximal and/or distal components of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) axis were investigated in mice using varoglutamstat doses at the high end of the pharmacological dose range or beyond. Neither the HPG nor the HPT axis was impacted to a relevant extent. Thus, one can expect a clear dose range where the compound reveals pathology-specific on-target effects (pE-Aβ, pE1-CCL2) only. This result indicates a sufficient on target selectivity of varoglutamstat.

In contrast to the HPG and HPT axes the hypothalamic-pituitary-adrenal (HPA) axis is regulated by different peptide/protein hormones, which are not substrates of QC. Thus, varoglutamstat does not influence

the maturation of any of the regulatory molecules of the HPA axis. This, in turn, is the basis for focusing the assessments on safety pharmacology onto HPT and HPG axes.

1.3.2 Safety Pharmacology

The safety pharmacology of varoglutamstat was studied in a range of in vitro and in vivo studies. Varoglutamstat was investigated in concentrations up to 10 µM in a panel of enzymes (including metal-containing ones) and receptors. Only phosphodiesterase 4 (PDE4) was significantly inhibited at 10 µM but in isolated atria from guinea pigs no signs for functional PDE4 inhibition could be found. Further studies included the core battery of cardiovascular telemetry study in dogs, in vitro human ether-a-go-go-related gene (hERG) study, Irwin test in rat and an assessment of respiratory function in rat. In addition, gastrointestinal motility and the effect on hexobarbital-induced sedation were assessed in mice.

The cardiac potassium channel hERG is responsible for a rapid delayed rectifier current (Ikr) in human ventricles and is assumed to be the most common cause of cardiac action potential prolongation by non-cardiac drugs. The hERG channel trafficking was not influenced by varoglutamstat up to a concentration of 30 µM (EC50 > 90 µM). Other cardiac key ion channels (hKCNQ1/hminK, hKv1.5, hNav1.5, hHCN4, hKv4.3/hKChIP2, Human-type Calcium, hKir2.1) were also not influenced by varoglutamstat at the tested concentration of 10 µM.

In telemetered dogs, systolic, diastolic and mean arterial blood pressure were unaffected by varoglutamstat at any of the doses examined (4, 40 and 240 mg/kg). There was no consistent effect on heart rate as a direct result of varoglutamstat administration; increases noted following administration of 240 mg/kg varoglutamstat are seen as a consequence of the animals feeling unwell following this dose as clinical signs including vomiting, liquid feces and salivation were notable at this dose level. There was no effect on the lead II electrocardiogram (ECG) intervals (RR, PR, QT, QTcF and QTcQ intervals or QRS duration), waveform or rhythm or on core body temperature following administration of varoglutamstat at any of the doses examined.

In conscious rats, single oral doses up to 900 mg/kg of varoglutamstat did not induce any effects on parameters of the respiratory system, (whole body plethysmography) and did not cause any significant physiological or behavioral effects (Irwin test).

There was no effect of varoglutamstat on hexobarbital induced sleeping time in mice up to a single oral concentration of 900 mg/kg.

1.3.3 Pharmacokinetics and Product Metabolism

Varoglutamstat was rapidly absorbed after oral administration in all species tested (mice, rats, dogs and monkeys). Peak concentrations in plasma and in brain were generally observed within 0.25 to 0.5 h post dose. Total body clearance and bioavailability showed major species-specific differences with the latter varying between 15% in monkeys and 70% in dogs. Plasma protein binding of varoglutamstat was similar in all species with the fraction unbound (fu) between 10-20%.

The results of the toxicokinetic (TK) studies in rats and dogs proved that the drug was well absorbed and that the animals were exposed to substantial amounts of varoglutamstat throughout the whole study period. At the highest dose (1200 mg/kg in rats, 360 mg/kg in dogs) peak plasma concentrations in rats were between 20 to 50 µg/mL and in dogs between 80 to 100 µg/mL. Elimination of varoglutamstat was rapid, with approximately 85% of the dose recovered during the first 48 hours after oral administration of radio-

labeled compound to rats. The majority was excreted via fecal route (59%). The metabolite profile of varoglutamstat found in hepatocytes from human, rat, and dog was qualitatively similar. Major metabolites in all species were products of hydroxylation and O-dealkylation.

1.3.4 **Toxicology**

The toxicity of varoglutamstat was assessed in different toxicological studies, using in vitro and in vivo study designs. After initial Dose Range Finding/ Maximum Tolerated Dose (MTD) studies in rats, dogs and monkeys had been performed, 4-week, 13-week and 26/39-week regulatory repeated dose toxicology studies in rats and dogs were performed with daily oral administration of varoglutamstat by gavage.

The results of the 4-week regulatory studies in rats and dogs indicated that kidney may be a possible target organ for varoglutamstat. Therefore, an exploratory study was performed, treating rats for 4 weeks using the maximally feasible dose of 1200 mg/kg/day and applying a set of urinary biomarkers which have been reported in literature and by EMA and US-FDA as early and sensitive biomarkers for renal damage, in addition to the histopathological investigation of the kidneys by a well-known kidney pathologist. In the 13-week repeated dose toxicity study analysis of kidney biomarkers was repeated in addition to the standard protocol. The overall conclusion from the evaluation of the kidney effects in the latter 2 studies was that the minimal or slight effects seen indicate a minor physiological effect probably related to the water balance/flow in the kidneys, without any toxicological relevance. In dogs, the histopathological examinations revealed minimal to slight renal changes in both the 4-week and the 13-week studies, without any aggravation by the prolonged treatment (13 weeks). In some of the animals given the highest dose in these studies (4-week, 360 mg/kg/day) degenerative changes were seen while at lower doses effects of a minor nature, involving a low number of nephrons, were sporadic and were often single and/or unilateral in the histological section. However, these changes were only present in treated dogs and can thus not be excluded to be caused by the treatment with varoglutamstat, although the etiology of such a scattered lesion remains unclear. The urinary biomarkers KIM-1, Clusterin and NGAL were also analyzed in the dog 13-week study and the results support the assessment that the effects seen in dogs do not reflect renal damage. In the 39-week study, only mild, non-adverse effects were seen, supporting that no aggravation over time was identified.

The deaths seen in the repeated dose rat studies were preceded by a deteriorated general condition, weight loss, reduced food consumption and noisy breathing. At necropsy the main finding was distended abdomens and gas filled stomachs. During the evaluation of the 4-week study, no strong causal connection between the observations made clinically and in histology and the deaths could be made. However, in the 13- and 26-week studies an extension of the tissue list for histology was made to include examination of the nasal cavities of the animals. This examination revealed inflammatory changes in the nasopharynx, inflammatory cell infiltration in the lumen, degeneration/necrosis of the epithelium and metaplasia to a squamous epithelium. The rat's forestomach, hosting a rich microflora, showed a slight irritation and it is assumed that thereby a disturbance of the bacterial flora can lead to an extensive gas production severely distended the stomachs. Then during oral gavage treatment, the upwards pressure of the gas can force some varoglutamstat into the nasopharynx and nasal cavities, causing local irritation and inflammation. The obstruction of the upper airways and pressure on the diaphragm and lungs by the distended stomachs can then trigger a cascade of events leading to premature deaths in rats. Thus, it is concluded that the deaths are caused by local reactions/effects on the microflora in the stomach and not a systemic toxicity.

Regarding the rat, the overall conclusion from the studies performed is that no adverse effects in clinical measurements, clinical pathology or histology that are relevant for human exposure has been seen, but only

rat specific adverse effects. Based on this, the no-observed-adverse-effect- level (NOAEL) to be used for basis in human safety assessment is the high dose used in the rat studies, 1200 mg/kg (maximum concentration (Cmax) range 24-46 µg/mL and area under the concentration-time curve (AUC) range 246-650 h*µg/mL in 4-week, 13-week and 26-week studies), as higher doses were not investigated.

In the dog, the major signs were emesis, hypersalivation and a pronounced aversion to dosing (pronounced bitter taste). In the 4-week study, two animals at the high dose level of 360 mg/kg/day varoglutamstat were sacrificed prematurely due to poor clinical condition and weight loss. As in the rats, slight or minimal changes in the stomach and intestines were attributed to a mild irritant effect of varoglutamstat. In all studies the same NOAEL is identified, 120 mg/kg/day varoglutamstat (Cmax range 55-67 µg/mL and AUC range 433-970 h*µg/mL in the 4-week, 13-week and 39-week studies), indicating that no aggravation over time is present.

In the MTD/ dose range finding study in cynomolgus monkeys the dose limiting effect was abundant vomiting. The dose of 180 mg/kg (Cmax=26/19 µg/mL and AUC=374/281 h*µg/mL in M/F at day 13) was tolerated for 14 days and did not induce any adverse effect. Varoglutamstat did not show any evidence of mutagenic activity in two in vitro studies and in one in vivo study.

Varoglutamstat was tested in a standard in vitro genotoxicity test battery comprising a bacterial reverse mutation test (AMES test) and a chromosome aberration test in human lymphocytes. Varoglutamstat caused no mutagenicity or increase in chromosome aberration rate either in the presence or absence of metabolic activation.

There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of varoglutamstat up to the maximum achievable dose level of 1800 mg/kg/day in male rats.

No local tolerance, carcinogenicity or reproductive or developmental toxicology studies have been conducted to date with varoglutamstat. Metabolism of varoglutamstat in the species chosen for preclinical safety testing (rat and dog) has been shown to reflect the metabolite profile in man.

PQ912*HCl was evaluated for phototoxicity in the in vitro 3T3 NRU assay according to ICH S10 guideline at concentrations up to (100 µg/mL). No increase of toxicity was found after UV/Visible light exposure. Therefore, PQ912*HCl was classified as non-phototoxic.

1.3.5 Conclusion of Safety and Toxicology Data

Varoglutamstat has been shown to have a good safety profile in well-established safety pharmacology models, including a full core safety pharmacology battery conducted according to good laboratory practice (GLP). The dose levels used in the studies and the no-observed-effect levels (NOEL, highest dose showing no effects) were significantly above the highest dose level used in phase 1 clinical studies and in phase 2A in patients. [6, 20]

The 4-week toxicology studies indicated the kidney to be a potential target organ of varoglutamstat toxicity, since some slight effects were seen in rats and dogs. However, the effect in rats was not reproducible in an exploratory study or in the 13-week or 26-week studies and the nature of the lesion is considered to be rat specific. In the dog, the kidney effects were of minor nature, but they were only present in treated dogs and can thus not be excluded to be caused by the treatment with varoglutamstat, although the etiology is unclear. However, in the 39-week study no adverse effects on the kidney were revealed.

Taken together results from toxicology and safety pharmacology the preclinical safety assessment does not predict any health risk to humans treated with varoglutamstat.

Based on the higher NOAEL (1200 mg/kg) in the most sensitive species, the rat, the dose levels administered in the clinical phase 2A trial are significantly below the NOAEL value.

1.4 Clinical Data

A phase 1 study [20] has been performed including more than 200 healthy volunteers, including elderly (age range for participants: 18-80 years; elderly 65-80 years). The maximal tolerated dose was not reached with single daily doses of 2 × 1800 mg and multiple doses of 800 mg BID for consecutive 11 days. Average TO of 70% was measured by inhibition of QC activity in CSF, at a dose of 400 mg BID in younger participants. The same average TO was calculated to be achieved with 300 mg BID in elderly participants. [16]

A phase 1 mass balance study was conducted in 6 healthy elderly males to assess the absorption, distribution, metabolism and excretion of varoglutamstat using a single dose of 600 mg (7.6 MBq of [14C]-labeled drug). The PK of total radioactivity, varoglutamstat and 2 known metabolites and the metabolite profiles and identities in plasma were characterized. Urine and feces were collected to assess the excretion of total radioactivity and metabolite profiles.
Human ADME of varoglutamstat was successfully characterized, showing good mass balance, good understanding of drug metabolism, and urinary excretion as the main excretory route of drug-related material. PQ1345 was confirmed to be the only human major metabolite of PQ912.

A phase 2A study, SAPHIR, was the first in-patient study with varoglutamstat, investigating safety, tolerability and efficacy in treatment-naive participants with MCI due to AD or mild dementia due to AD. [6] Participants were treated for 12 weeks with varoglutamstat (first week: 400 mg BID, thereafter 800 mg BID) or placebo.

Summarized below is clinical data from the completed early phase trials of varoglutamstat. Please refer to the Investigator's Brochure for additional clinical information.

1.4.1 Adverse Event Profile

In the phase 1 study, the majority of AEs reported were mild or moderate in severity and resolved without treatment and there were no SAEs reported during the study. Three AEs (2 events of presyncope and one event of pyrexia) were reported during the study; the events of presyncope were not considered to be related to study drug. The event of pyrexia was reported by the 1 participant who was withdrawn from the study due to an AE of papular urticaria; the events appeared to have been associated with each other and both were suspected to be related to study drug.

The most frequently reported drug related AE during the study was headache and gastrointestinal disorders (including nausea, flatulence, constipation, abdominal pain, diarrhea and vomiting). There were no safety concerns for any of the biochemistry, hematology or urinalysis parameters assessed; although 4 participants showed clinically significant changes in clinical laboratory parameters (increases in potassium, erythrocytes and leukocytes). There were no clinically significant findings for vital signs, 12 lead ECGs), cardiac telemetry and physical examinations performed during the study. In addition, there were no safety concerns for the renal biomarkers assessed in elderly participants.

In the phase 2A SAPHIR study, participants received 400 mg BID in the first week of the study and 800 mg BID from week 2 onwards until the end-of-treatment period. A summary of treatment emergent adverse events (TEAEs) is provided in the **Table 1** below.

Table 1: Summary of Treatment Emergent Adverse Events (ITT population) – SAPHIR

		Placebo (N = 60) n (%)	PQ912 (N = 60) n (%)	Total (N = 120) n (%)
N (%) with any TEAE		40 (66.7)	45 (75)	85 (70.8)
Total TEAEs		103	135	238
N (%) with serious TEAEs		3 (5)	8 (13.3)	11 (9.2)
Total serious TEAEs		3	13	16
Total severeTEAEs, grade ≥3		1	9	10
N (%) deaths		0	0	0
N (%) discontinued due to TEAE[$]		0	20 (33.3)	20 (16.7)
N (%) with TEAE by severity	Mild	27 (45)	17 (28.3)	44 (36.7)
	Moderate	12 (20)	20 (33.3)	32 (26.7)
	Severe	1 (1.7)	8 (13.3)	9 (7.5)
N (%) with treatment-related TEAEs[#]		20 (33.3)	37 (61.7)	57 (47.5)
Total treatment-related TEAEs		45 (43.7)	86 (63.7)	131

$ Note: these subjects can still be completers

Adverse events that are treatment-related, possibly or probably treatment-related or unassessable

As shown in Table 1, TEAEs were reported by 45 (75%) participants in the varoglutamstat group and 40 (66.7%) participants in the placebo group. In the varoglutamstat treatment group more TEAEs (86 out of 135, 63.7%) were considered treatment related than in the placebo group (45 out of 103, 43.7%).

In the **Table 2** below the incidences of TEAEs (number of participants) are presented per MedDRA SOC category.

Table 2: Incidences of TEAEs per MedDRA SOC categories (n participants reporting)
As Table 2 shows, participants reported TEAEs most frequently in the SOC categories of gastrointestinal disorders, infections and infestations and skin and subcutaneous tissue disorders.

SOC	Placebo N=60 n	PQ912 N=60 n
Total	40	45
Blood and lymphatic system disorders	1	2
Cardiac disorders	2	2
Endocrine disorders	0	1
Eye disorders	1	1
Gastrointestinal disorders	12	21
General disorders and administration site conditions	6	5
Hepatobiliary disorders	0	3
Infections and infestations	17	17
Injury, poisoning and procedural complications	5	6
Investigations	5	8
Metabolism and nutrition disorders	2	7
Musculoskeletal and connective tissue disorders	8	5
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	1
Nervous system disorders	9	7
Psychiatric disorders	3	4
Renal and urinary disorders	1	3
Reproductive system and breast disorders	1	1
Respiratory, thoracic and mediastinal disorders	1	0
Skin and subcutaneous tissue disorders	5	15
Surgical and medical procedures	2	0
Vascular disorders	2	3

Other AE data from the SAPHIR study showed that participants treated with varoglutamstat reported more TEAEs (135) and TESAEs (13) than placebo treated participants (103 TEAEs, 3 TESAEs) and more participants treated with varoglutamstat discontinued treatment due to AEs, including SAEs and severe AEs. Also, the total number of participants who were non-adherent to randomized treatment for any reason was higher in the varoglutamstat treatment arm than in the placebo treatment arm. TEAEs observed more frequently in varoglutamstat treated participants were related to gastrointestinal, and skin and subcutaneous disorders. It is possible that these observations are associated with the higher dose of 800 mg BID and that lower doses might have shown a more favorable tolerability profile for varoglutamstat.

Regarding the safety of varoglutamstat 800 mg BID treatment: no clinically relevant findings were noted for vital signs, ECG, physical or neurological examination or brain MRI scans. Shifts from normal to deviant values were more frequently seen during treatment with varoglutamstat for hemoglobin, hematocrit,

and alkaline phosphatase but they tended to normalize towards the end of treatment and normalized at the follow-up visit.

Regarding time to onset, the majority of safety and tolerability findings started between treatment Weeks 3 and 8 with very few new findings or recovery between Weeks 8 and 12.

1.4.2 Potential for Drug-Drug Interactions

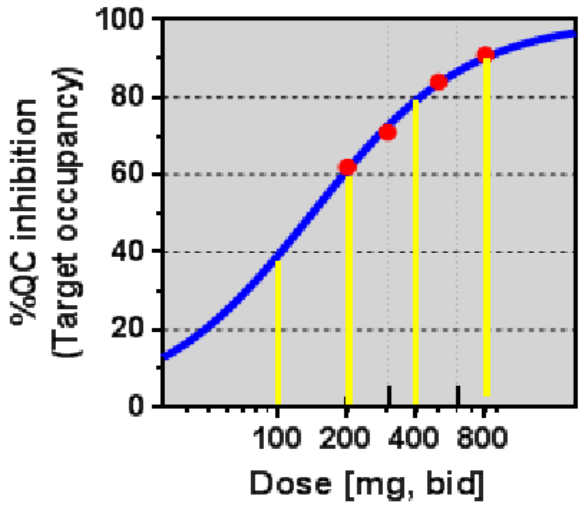
A drug-drug interaction study of varoglutamstat 800 mg BID was conducted to investigate the PK interaction potential with substrates metabolized by the enzymes cytochrome P450 (CYP) 3A4 and CYP2C19. The results showed that varoglutamstat has no influence on the metabolic pathway of the CYP3A4 enzyme, but is a moderate inhibitor of the CYP2C19 pathway leading to a 2-3 fold increase of the Cmax and AUC of products metabolized predominantly through the CYP2C19 pathway. Therefore, the concomitant administration with strong inhibitors of the CYP2C19 like fluconazole, fluvoxamine and ticlopidine and with CYP2C19 substrates with a narrow therapeutic margin like S-mephenytoin, repaglinide, phenytoin, phenobarbital and indomethacin must be avoided and replaced by alternative products before starting varoglutamstat exposure. Concomitant treatment with other medical products classified as moderate inhibitors of the CYP2C19 enzyme like omeprazole, fluoxetine, esomeprazole, moclobemide and oxiconazole is possible and should be carried out with caution. In case new AEs would have occurred, it was recommended to reduce the dose of the concomitantly administered product by 50%.

CYP2C19 was identified *in vitro* to be the major varoglutamstat metabolizing isoform followed by CYP3A and others. Therefore, concomitant administration of moderate inducers of the CYP2C19 and CYP3A4 enzyme like rifampin must be avoided because the concentration of varoglutamstat would be reduced below effective levels. Furthermore, concomitant administration of strong inhibitors of the CYP3A4 enzyme is to be avoided in participants identified as poor metabolizers of CYP2C19 at baseline.

1.4.3 Dose Selection

For phase 2A of this clinical trial, we have selected the dose range of 150 mg BID, 300 mg BID, and 600 mg BID based on the PK/PD relationships established in the phase 1 and phase 2A SAPHIR studies that indicate that each dose could achieve a minimum mean threshold level of > 50% QC inhibition in a population of older individuals. [20] As seen in the dose response curve (**Figure 1**) of aggregate PK PD data, extrapolation of the lowest dose of 150 mg BID will achieve a mean QC inhibition of about 50%, the highest dose of 600 mg BID above 85% inhibition, and the middle dose of 300 mg BID a mean of ~ 70% inhibition. [16]

Figure 1: Dose Response, aggregate PK PD data of varoglutamstat



In the SAPHIR study, at 800 mg BID there were significant differences on primary composite endpoints of safety and tolerability, indicating that while this dose achieved the predicted excellent median TO, it cannot be advanced further in development and the dose range will need to be lower. The approach in phase 2A of this study is to enroll a sentinel cohort of participants randomized 1:1 to varoglutamstat or placebo using a sequential dose design. Participants will be serially enrolled into one of three dose cohorts, with up to 60 participants per cohort (30 randomized to varoglutamstat, and 30 to placebo). The two higher doses (cohort A, 600 mg BID; cohort B, 300 mg BID) will each have a dose titration period, and all participants will be treated for a minimum of 8 weeks at originally assigned full dose in phase 2A unless the Pocock safety boundary is crossed or a higher dose is selected. We have selected the 8-week time window following the safety and tolerability survival curves from SAPHIR which identified the important TEAEs to be gastrointestinal, and skin and subcutaneous disorders, and the time to non-adherence to varoglutamstat as occurring primarily up to but not beyond week 8. This stopping boundary focuses on a defined set of sensitive and specific AE's, based on preliminary data, and is designed to drop a dose very quickly in case of low tolerability. The highest tolerated dose which passes safety review will be proposed for phase 2B following sufficient plasma exposure of varoglutamstat confirming the sufficiency of target occupancy. The AUC in CSF can be estimated from plasma AUC with reasonable accuracy.[21]

1.4.4 Potential Risk to Fetal Development

No studies intended to evaluate reproductive or developmental toxicity have been performed with varoglutamstat at this stage of development.

2 STUDY DESIGN

This is a phase 2A multi-center, randomized, double-blind, placebo-controlled, parallel group clinical trial in participants with early AD, with a stage gate to phase 2B.

Phase 2A will determine the highest dose of varoglutamstat that is safe and well tolerated with sufficient plasma exposure and a calculated TO in CSF. During this phase, continuous safety evaluation using a pre-defined safety stopping boundary will help determine which dose will be carried forward into phase 2B. In addition, at the end of phase 2A, an interim analysis of both cognitive function using an ADNI Battery Composite (ABC) and pharmacodynamic changes on EEG spectral analysis will be conducted to inform a stage gate decision on whether to proceed with phase 2B.

In the event that the stage gate for phase 2B is reached, the study will continue and phase 2B will assess efficacy and longer-term safety of varoglutamstat in a larger group of participants through 72 weeks of treatment. Throughout the remainder of the trial, all participants will be randomized to this optimal selected dose from phase 2A or placebo.

3 OBJECTIVES

3.1 Phase 2A

3.1.1 Primary Objective

The primary objectives of phase 2A are:

- To determine the highest safe and well-tolerated dose of varoglutamstat over the safety period (from first dose to completion of 8 weeks at the originally assigned full dose), which will include assessment of the proportion of participants who experience any AESIs, and with sufficient pharmacokinetics and target occupancy (TO)
- To evaluate early efficacy of varoglutamstat as measured by the ADNI Battery Composite (ABC) and pharmacodynamics changes on EEG spectral analysis over a 24-week treatment period

3.1.2 PK Objectives

The PK objective in phase 2A is to measure varoglutamstat levels in plasma and to establish the sufficiency of TO of QC in plasma following at least 8 weeks of treatment at the dose levels being tested. This PK testing starts at week 4 and continues every 4-8 weeks throughout phase 2A. This plasma PK data will be provided to the DSMB for consideration in parallel with safety data. Following consideration of safety, tolerability, and PK, the DSMB will provide a recommendation to the SSC on whether the conditions to advance through the stage-gate have been met as well as the final dose selection for phase 2B.

3.2 Phase 2B

3.2.1 Primary Objective

The primary objective of phase 2B is to assess the efficacy of varoglutamstat as measured by the Clinical Dementia Rating-sum of boxes (CDR-SB), over 72 weeks.

3.2.2 Secondary Objectives

3.2.2.1 Key Efficacy Objective

The key secondary objective in phase 2B is to evaluate the efficacy of varoglutamstat as measured by CFC2, a cognitive-functional composite, over 72 weeks.

3.2.2.2 Other Secondary Efficacy Objectives

Other secondary efficacy objectives in phase 2B are to evaluate the efficacy of varoglutamstat as measured by the:

- Composite sum of standardized scores from the ADNI Battery Composite (ABC)
- Quantitative EEG (global relative theta wave power)
- Functional Activities Questionnaire (FAQ)
- Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS-Cog-13)
- Neuropsychiatric Inventory (NPI)

3.2.3 Safety and Tolerability Objectives

The safety and tolerability of varoglutamstat in phase 2B will be assessed by the following measures:

- Rates of all AEs (SAEs, TEAEs, AESIs)
- Drug discontinuation rates
- Mortality rates
- Suicidality on the C-SSRS
- Imaging abnormalities on brain MRIs as determined by the site PI with a local reading, including ARIA-H, ARIA-E, infarcts
- Frequency and severity of abnormalities on physical examinations, vital signs, health status, ECG and safety labs

3.2.4 PK Objectives

The pharmacokinetics of varoglutamstat in phase 2B will be assessed by the measurement of:

- Varoglutamstat levels in plasma
- Varoglutamstat levels in CSF

3.2.5 Exploratory Objectives

The exploratory objectives in phase 2B are to investigate longitudinal treatment effects of varoglutamstat as assessed by the changes in:

- Brain volume measured by cranial MRI
- Mini-Mental Status Examination (MMSE)
- Montreal Cognitive Assessment (MoCA)
- CSF disease-relevant biomarkers including: Aβ1-42, t-tau, p-tau-181, sTREM2, YKL40, neurogranin, SNAP25, NfL and VILIP-1
- qEEG network connectivity measures
- AD Composite Score (ADCOMS)
- ADAS-Cog-Exec
- Relative QC activity in CSF

Exploratory objectives also include comparing treatment to placebo on changes in the primary outcomes measure (CDR-SB), key secondary outcome measure (CFC2), and in the TO measure, within subgroups defined separately by: (i) APOE genotype (E4 carrier vs non E4 carrier), and (ii) MCI vs. Mild probable AD, as well as comparing plasma-based amyloid biomarker results using the PrecivityAD® test with CSF-based amyloid and p-tau test results using the Elecys® test.

4 ENDPOINTS

4.1 Phase 2A

4.1.1 Primary Endpoints

Primary efficacy endpoints are:

- The within-participant change from baseline to week 24 in the composite sum of standardized scores from the ADNI Battery Composite, (ABC, 9-item, see Section 9.1.1), compared between active arm and placebo.
- The within-participant change from baseline to week 24 in qEEG (global relative theta wave power), compared between active and placebo.

Primary safety endpoints are:

The primary safety endpoint in phase 2A is based on a composite measure of AESIs which are predefined by severity within the primary MedDRA System organ classes (SOCs) of Skin or subcutaneous tissue disorders or hepatobiliary disorders as described below. The endpoint will be determined by the proportion of participants, for each dose, who experience any AESI within the 8-week dose-selection period. The safety evaluation period for dose selection and the safety stopping rule is defined as from the first dose to completion of 8 weeks on the originally assigned full dose of varoglutamstat, or to the time of drug discontinuation, whichever happens first. There is continuous monitoring with a Pocock sequential boundary for safety stopping.

AESIs will be monitored throughout the study, including during phase 2b and up to 30 days (inclusive) after the study drug has been discontinued (please see section 11.1.). Events occurring after the safety evaluation period (through 8 weeks on the originally assigned full dose in phase 2A) will not contribute to the phase 2A Pocock sequential boundary analyses.

An AESI is defined as the occurrence (occurrence of an AESI means the date when the criteria of an AESI are fulfilled, not the overall onset of an AE without meeting the criteria of an AESI) of any of the following treatment-emergent adverse events within the primary MedDRA System organ classes (SOCs) of Skin or subcutaneous tissue disorders or hepatobiliary disorders:

- Discontinuation of participant due to an AE (any severity, including SAEs)
- Adverse event in the primary MedDRA System organ class (SOC) of hepatobiliary disorders with severity 3 and above according to Common Terminology Criteria for Adverse Events (CTCAE v 5.0) regardless of discontinuation
- Discontinuation of participant due to an extreme lab parameter related to the liver or bile organ system:
    - ALT or AST >8xULN
    - ALT or AST >5xULN for more than 2 weeks
    - ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
    - ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
- Adverse event in the primary MedDRA System organ class (SOC) of Skin or subcutaneous tissue disorders with severity grade 3 and above according to CTCAE v 5.0

Details of the CTCAE v5.0 can be found at the following link:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf.

4.1.2 Safety & Tolerability Endpoints

Secondary safety and tolerability endpoints are:

- Rates of all AEs (SAEs, TEAEs, AESIs)
- Drug discontinuation rates
- Mortality rates
- Suicidality on the C-SSRS
- Changes on brain MRI scans as determined by the site PI with a local reading (ARIA-E, ARIA-H, infarcts)
- Frequency and severity of abnormalities on physical examinations, vital signs, health status, ECG and safety labs

4.1.3 PK Endpoints

The PK endpoints in phase 2A are the derived mean values of varoglutamstat plasma levels for each cohort following at least 8 weeks of treatment at the dose levels being tested. These PK endpoints will be measured pre-and post-dose starting at week 4 and every 4-8 weeks throughout phase 2A. The estimated TO will support the decision of the dose carried forward to phase 2B.

4.1.4 Stage-Gate Analysis

Following phase 2A, interim futility data (ADNI Battery Composite (ABC) and EEG theta power) will be reviewed and a stage-gate decision for whether or not to continue to phase 2B will be undertaken (see Section 12.1.4). Analyses of additional measures may be undertaken in the case of an indeterminate stage-gate result on ABC and EEG theta power.

4.2 Phase 2B

4.2.1 Primary Endpoint

The primary efficacy endpoint in phase 2B is the within-participant change in CDR-SB from baseline to week 72, compared between the varoglutamstat treatment group and the placebo group. CDR-SB has shown good reproducibility across multiple studies, with more robust placebo arm decline than other measures in the MCI and mild AD dementia populations. [22]

4.2.2 Secondary Endpoints

4.2.2.1 Key Efficacy Endpoint

The key secondary endpoint in phase 2B is the within-participant change in CFC2 from baseline to week 72, compared between the varoglutamstat treatment group and the placebo group. CFC2 was the best performing endpoint in a recent study of MCI and early AD. [22] This trial will serve as an external prospective validation of this measure.

4.2.2.2 Other Secondary Efficacy Endpoints

The efficacy of varoglutamstat will be assessed by the within-participant changes from baseline to week 72, compared between the treatment group and the placebo group, on the following:

- Composite sum of standardized scores from the ADNI Battery Composite (ABC, 9-item, see Section 9.1.1):
- Quantitative EEG (global relative theta wave power)
- Functional Activities Questionnaire (FAQ)
- Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS-Cog-13)
- Neuropsychiatric Inventory (NPI)

4.2.3 PK Endpoints

The PK in phase 2B will be measured in both plasma and CSF and will be analyzed at the end of the study. This will include measures of target occupancy in both plasma and CSF as well as the ratio of plasma to CSF varoglutamstat.

4.2.4 Safety and Tolerability Endpoints

The following safety and tolerability measures will be assessed for differences between the varoglutamstat treatment group and the placebo group:

- Rates of all AEs (SAEs, TEAEs, AESIs)
- Drug discontinuation rates
- Mortality rates
- Suicidality on the C-SSRS
- Changes on brain MRI scans as determined by the site PI with a local reading (ARIA-E, ARIA-H, infarcts)
- Frequency and severity of abnormalities on physical examinations, vital signs, health status, ECG and safety labs

4.2.5 Exploratory Endpoints

The following exploratory endpoints in phase 2B include the within-participant longitudinal change between active and placebo in:

- Brain volume measured by cranial MRI
- Mini-Mental Status Examination (MMSE)
- Montreal Cognitive Assessment (MoCA)
- CSF disease-relevant biomarkers including: Aβ1-42, t-tau, p-tau-181, sTREM2, YKL40, neurogranin, SNAP25, NfL and VILIP-1
- qEEG network connectivity
- AD Composite Score (ADCOMS)
- ADAS-Cog-Exec
- Relative change from screening (100%) to week 72 in QC activity in CSF
- Changes in the primary outcome measure (CDR-SB), key secondary outcome measure (CFC2), and in the TO measure, within subgroups defined separately by: (i) APOE genotype (E4 carrier vs non E4 carrier), and (ii) MCI vs Mild probable AD.

Exploratory endpoints in phase 2B also include comparison of plasma-based amyloid biomarker test results using the PrecivityAD® test with CSF-based amyloid and p-tau test results using the Elecys® test. [23, 24]

5 ETHICS AND REGULATORY CONSIDERATIONS

5.1 Good Clinical Practice

This study will be conducted in accordance with:

- Principles of the Declaration of Helsinki (revised version of Fortaleza, Brazil October of 2013).
- Good Clinical Practice (GCP) guidelines, as defined by the International Conference on Harmonization (ICH) Guideline, Topic E6(R2), the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50) – Protection of Human Subjects and Part 56.
- Institutional Review Boards (IRBs), Health Insurance Portability and Accountability Act (HIPAA), and all other applicable local regulatory requirements and laws.

Study personnel involved in conducting this study will be qualified by education, training and experience to perform their respective task(s) in accordance with GCP.

5.2 Institutional Review Board

Institutional Review Boards and Research Ethics Boards must be constituted, and their authority delegated through the institution's normal process of governance according to applicable State and Federal requirements for each participating location. Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment materials by an appropriate IRB registered with the Office for Human Research Protections (OHRP). The protocol will be submitted for approval to the appropriate IRB for each study site. The study will not commence at any site until written approval to enroll is obtained from ADCS Regulatory Affairs.

The investigator must obtain approval from the IRB for all protocol amendments and, when warranted, changes to the informed consent document. Protocol and informed consent form amendments can be made only with the prior approval of the ADCS and Sponsor. The investigator may not implement any protocol deviation except where necessary to eliminate an immediate hazard to study participants, or when change(s) involve only logistical or administrative aspects of the trial, i.e. change of monitor(s) or telephone number(s) (ICH GCP 4.5.2). The investigator shall notify the IRB of deviations from the protocol or serious adverse events occurring at the site, in accordance with local and central IRB procedures.

5.3 Informed Consent and HIPAA Compliance

No study-specific procedure will be undertaken on an individual patient until that patient or the patient's legally authorized representative (LAR) has given written informed consent to take part in the study. The study partner must also participate in the consenting process.

It will be made clear to each potential participant, their LAR if applicable, and study partner that informed consent may be withdrawn at any time without needing to give a reason and that such withdrawal will not compromise the relationship between the patient and the Investigator nor the patient's future treatment.

Informed consent will be obtained in accordance with US 21 CFR 50.25 and ICH Good Clinical Practice. Applicable HIPAA privacy notifications will be implemented, and HIPAA authorizations

signed before protocol procedures are carried out. Information should be given in both oral and written form.

Consent forms must be in a language fully comprehensible to the prospective participants and/or their LARs, and study partners, and ample opportunity must be given to inquire about the details of the study. Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient or by the patient's LAR, and by the person who conducted the informed consent discussion. Patients or their LAR must be provided a copy of the signed ICF. Study partners may also be required to sign the informed consent prior to study participation at the discretion of the responsible IRB reviewing this research.

The consent for storage will include consent to access stored data, biological samples, and imaging data for secondary analyses. Consent forms will specify that DNA and biomarker samples are for research purposes only; the tests on the DNA and biomarker samples are not diagnostic in nature and participants will not receive results. CYP2C19 status will be disclosed to investigators.

Abnormal findings during the study including clinical laboratory results and MRI scan findings of clinical significance can be shared with participants or their treating physician per site clinician discretion with consent of the participant.

5.4 Patient Confidentiality │ HIPAA

Information about study participants will be kept confidential and managed according to the requirements of HIPAA. Those regulations require a signed patient HIPAA Authorization informing the patient of the following:

- What protected health information (PHI) will be collected from patients in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research patient to revoke their authorization for use of their PHI

If a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. Each site PI, under the guidance of his/her IRB, is responsible for ensuring that all applicable HIPAA regulations and state laws are met.

5.5 Potential Risks and Benefits Associated with this Study

5.5.1 Potential Risks

Risks associated with study participation are the potential for adverse reactions to the study drug (see Section 1.4.1), concomitant medications, invasive study assessments like blood draws and lumbar puncture, and risks related to the process of undergoing brain MRI scans and neuropsychological testing.

5.5.2 Potential Benefits

Participants in this study may experience an improvement in their AD symptoms, even though such improvement cannot be predicted with any certainty. This study is expected to benefit the AD community by furthering the development of a new therapy and providing more information to those studying potential treatments for AD.

6 STUDY DRUG

6.1 Study Drug

The study drug will be presented as either varoglutamstat or matching placebo tablets.

Participants are required to take the assigned number of tablets BID (once in the morning and once in the evening), preferably with a meal. The first tablet intake will occur in the morning of the next day following receipt of initial dispensing of study drug supply to the participant. The intake of the second dose should occur in the evening on the same day. Participants should then continue with the twice daily treatment regimen, taking the tablets at approximately the same time of day throughout the study, until directed otherwise by the treating investigator or other qualified study personnel. Tablets are to be swallowed whole and not to be crushed or broken.

6.2 Coding, Packing and Labeling

Varoglutamstat (150 mg) or placebo tablets, manufactured by CCI [redacted], will be contained in blister packs. Participants will receive a boxed supply for each dosing period between study visits. CCI [redacted] will be the drug depot shipping study drug to the site.

The study drug packaging will be labeled with a unique identifier for dispensing and drug accountability. Labels will be in accordance with all applicable regulatory requirements for the labeling of active pharmaceutical ingredients. Labels will contain the drug name, protocol number, storage conditions and a caution statement that the drug is for clinical investigational use only.

The study drug will be securely stored at the study site in accordance with the conditions specified on the label, separately from other drugs. The study drug may not be used for any purpose other than this study.

6.3 Randomization and Study Drug Ordering System

Each participant will be randomly allocated in a 1:1 ratio into one of the 2 groups: treatment with varoglutamstat or placebo. A randomization schedule will be generated and incorporated into an electronic Interactive Response Technology (IRT) system and the treatment group will be assigned as the site randomizes the participant. The randomization will be stratified on diagnostic classification (mild AD dementia or MCI due to AD, by NIA-AA working group 2011 criteria), and site. A permuted blocks randomization will be used, with block size 2 and 4. Participants will be balanced in their randomization so that no more than 40% have MCI and at least 60% have Mild probable AD.

To complete participant randomization, the Investigator will use the study EDC system to enter screening information on each participant. The Investigator must confirm study eligibility prior to randomization. Participants will be randomized at the baseline visit, once screening is completed and it is determined that the participant is eligible for the study. For those participants who qualify, the system will issue a study drug kit number. Participants who are randomized but drop out prior to the start of study drug will be replaced.

6.4 Blinding

This is a double-blind placebo-controlled trial. Participants and study personnel will be blinded to both treatment arm (study drug or placebo) and dosage. Only after the DSMB has selected the dose for the rest of the study, sites will be notified what the selected optimal dose is, but will remain blinded to the treatment arm. The blind will be maintained by use of matching placebo.

All participants will receive 4 tablets twice a day irrespective of their cohort. The tablets will be provided to participants in blister packs. The ratio of study drug tablets to placebo tablets will vary across cohorts (i.e. based on dose level of each cohort).

Sites will be informed when the dose decision in phase 2A is determined, when the selected optimal dose is to be implemented, and what the selected optimal dose is. At that time, study personnel will be notified but will remain blinded to the treatment arm (study drug or placebo). After the dose decision in phase 2A is determined, a new supply of study drug will be provided at the next study visit for all participants in the study.

Only in the case of an emergency, when knowledge of whether the participant has received the investigational product is essential for the clinical management or welfare of the participant, may the Investigator unblind a participant's treatment assignment. Procedures for emergency unblinding are initiated by contacting the ADCS Medical Monitor.

6.5 Phase 2A Titration, Dosing Schedule, and Adaptive Dose Decision

Participants will be enrolled sequentially into one of three dose cohorts, labeled A, B, and C, with 60 participants (n=30 active, n=30 placebo) per cohort.

6.5.1 Phase 2A Dosing Schedule and Titration

6.5.1.1 Dose Titration

There will be up to three doses studied, 600 mg BID, 300 mg BID and 150 mg BID. A subject will spend at least 4 weeks at the 300 mg dose before taking the 600 mg dose. A subject will spend at least 4 weeks at the 150 mg dose before taking the 300 mg dose. A subject may start taking medication at the 150 mg dose.

6.5.1.2 Dose Selection

Doses will be investigated for safety and tolerability in three potentially overlapping dose cohorts, starting with the highest dose (see Section 6.5.1.3). Each dose will have an 8 week period at full dose, during which participants will be closely monitored for tolerability with a safety stopping rule (see Section 12.1.2). If a given dose meets its stopping rule, all subjects on that dose will be down-titrated to the next dose. The first dose which completes its dose-selection period at full dose without crossing the safety boundary, will be selected as the optimal dose for all further participant treatment. Down-titration for an individual subject is not allowable in phase 2A if there is
significant intolerability (see Section 6.9.1).

After dose selection, all participants currently on treatment will be titrated to that dose. Treatment will continue for a minimum of 24 weeks at the selected dose including the titration phase; earlier participants may be treated at the selected dose for up to 72 weeks, depending on the final dose selected.

6.5.1.3 **Phase 2A Dose Cohorts**

Participants will be enrolled sequentially into one of three dose cohorts, labeled A, B and C, with 60 participants (n=30 active, n=30 placebo) per cohort.

- **Cohort A** will enroll first. Participants will take 150 mg BID for 4 weeks, then 300 mg BID for 4 weeks, and then 600 mg BID (highest dose) until the first occurrence of either: a) the 600 mg dose meets its stopping rule, at which time all Cohort A participants will down titrate to 300mg BID; or b) the stage-gate to Phase 2B is reached.
- **Cohort B** will enroll after cohort A has accrued. Participants enrolled to Cohort B will take 150 mg BID for 4 weeks, and then 300 mg BID (middle dose) until the first occurrence of either: a) the 600 mg dose has been selected within Cohort A, at which time cohort B subjects will titrate up to 600 mg BID (after at least 4 weeks at 300 mg BID); or b) the 300 mg dose meets its stopping rule, at which time all subjects on a 300 mg BID or higher dose will down-titrate to 150 mg BID; or c) the stage-gate to Phase 2B, whichever comes first.
- **Cohort C (150 mg BID)** will accrue after cohort B has accrued. Participants will take 150 mg BID (lowest dose) until the first occurrence of either: a) the 600 mg dose has been selected within Cohort A *or* the 300 mg dose has been selected within Cohort B, at which time Cohort C subjects will titrate up to the selected dose; or b) the 150 mg dose meets its stopping rule, in which case all medication will cease and the trial will end for safety reasons; or c) or the stage-gate to Phase 2B is reached, whichever comes first.

6.6 **Phase 2A Adaptive Dose Decision**

As described above (Section 6.5.1.2), the phase 2A dose decision will be taken when cohort A completes its dose-selection period at full dose without crossing the safety boundary, **or alternatively**, if cohort A meets the stopping rule and the dose is discontinued, when cohort B completes its dose-selection period at full dose without crossing the safety boundary, **or alternatively**, if both cohorts A and B meet the stopping rule, when cohort C completes its treatment period at full dose (see Section 12 for further statistical detail).

Given that the highest doses are being tested first, the optimal dose selection in phase 2A could be reached when cohort A completes the dose-selection period, or alternatively, at the next highest tolerated dose when cohort B completes the dose-selection period, or finally when cohort C completes the dose-selection period. The treatment in phase 2A will extend for at least 24 total weeks including titration phase, for all participants at the selected optimal dose, irrespective of cohort assignment.

The following are four possible scenarios that pertain to this dose decision:

1. If the selected dose is 600 mg BID (cohort A), cohorts B and C will be titrated up to this dose.
2. If the selected dose is 300 mg BID (cohort B), all cohort A participants which are still receiving study medication (including those that did not experience an AESI (i.e. tolerated the 600 mg BID dose) will be reduced to 300 mg BID and cohort C will be increased to 300 mg BID. Participants in cohort C must have received at least 4 weeks of 150 mg BID before increasing their dose to 300 mg BID.
3. If the selected dose is 150 mg BID (cohort C), all participants in both cohorts A and B which are still receiving study medication (including those that did not experience a AESI and tolerated their assigned dose) will be reduced to 150 mg BID.

4. If cohort C meets the stopping rule, then the trial is halted.

6.7 Phase 2B Titration Period

Newly-enrolled participants in phase 2B will receive the optimal dose level determined to be carried forward during phase 2A. If the optimal dose selected is 150 mg BID, participants will not be required to undergo an initial dose titration. If the optimal dose selected is 600 mg BID or 300 mg BID, newly-enrolled participants will undergo an initial titration period as follows:

- **600 mg BID**: Participants will first receive 150 mg BID for 4 weeks, then 300 mg BID for 4 weeks, then 600 mg BID for the remainder of the study.
- **300 mg BID:** Participants will first receive 150 mg BID for 4 weeks, and then 300 mg BID for the remainder of the study.

6.8 Daily Dosing Regimen

Twice daily, participants are required to take the assigned number of tablets. The first tablet intake will occur in the morning of the next day following receipt of initial dispensing of study drug supply to the participant. The intake of the second dose should occur in the evening of the same day. Participants should then continue with the twice daily treatment regimen, taking the tablets at approximately the same time of day throughout the study, until directed otherwise by the treating investigator or other qualified study personnel. It is preferable that each dose of study medication be taken with a meal. Tablets are to be swallowed whole and not to be crushed or broken.

6.9 Dose Adjustments

All dose modifications and study drug interruptions must be recorded in the electronic Case Report Form (eCRF). Phase 2A and phase 2B have different allowances for dose adjustments, as outlined below.

6.9.1 Phase 2A

No dose reductions are allowed in phase 2A; however, dose interruptions are permitted at the discretion of the site investigator. Following dose interruption, participants should resume treatment at the dose being taken prior to interruption. Should interruption of study treatment last 3 days or more, the site investigator must consult with the ADCS Medical Monitor.

In phase 2A, study treatment should be immediately and permanently discontinued if any of the following AESIs occur when skin or subcutaneous tissues and liver are involved and the AE is either:

- Skin or subcutaneous tissue severity grade 3 or more according to CTCAE v 5.0 being either: Erythema, rash, Urticaria or any skin event involving bullous changes of the skin.

  The decision to continue or discontinue treatment with study treatment for all other AESIs of the primary MedDRA System organ class (SOC) of Skin or subcutaneous tissue disorders should be taken by the investigator together with the medical monitor and the Sponsor.

- Liver: severity grade 3 or more according to CTCAE v 5.0
- Abnormal laboratory values involving liver:
  - ALT or AST >8X ULN
  - ALT or AST >5X ULN for more than 2 weeks

- ALT or AST >3X ULN and total bilirubin levels >2X ULN or INR >1.5
- ALT or AST >3X ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

Details for the CTCAE v 5.0 can be found at the following link:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf.

As detailed in Section 12.4, participants must take at least 80% of the assigned study treatment to be included in the Per Protocol (PP) analysis population.

6.9.2 **Phase 2B**

If a participant experiences an adverse event for which the investigator believes a dose reduction or dose interruption is warranted, the investigator may halve the dose or temporarily suspend dosing until he/she assesses it is safe to return to the assigned dose.

Specifically, 300 mg BID may be reduced to 150 mg BID (i.e. 1 tablet twice daily) and 600 mg BID may be reduced to either 300 mg BID (i.e. 2 tablets twice daily) or 150 mg BID (i.e. 1 tablet twice daily). On the other hand, if the dose carried forward into phase B is 150 mg BID, a reduction to 150 mg daily dose cannot be undertaken for more than a short period of time (participants should maintain at least 80% compliance of the study treatment) because the exposure will not result in a sufficient target occupancy. In this case, if the dose of 150 mg BID cannot be tolerated study treatment will need to be permanently discontinued.

The site Investigator is to ensure the following each time a dose modification occurs:

Dose interruptions are permitted in phase 2B. The site investigator is to make the decision on whether to temporarily suspend study treatment. However, should the interruption of study treatment last 14 days or more, the site Investigator must consult with the ADCS Medical Monitor.

In phase 2B, study treatment should be <u>immediately and permanently discontinued</u> if any of the following AESIs occur when skin or subcutaneous tissues and liver are involved and the AE is either:

- Skin or subcutaneous tissue: severity grade 3 or more according to CTCAE v 5.0 being either: Erythema, rash, Urticaria or any skin event involving bullous changes of the skin.

  The decision to continue or discontinue treatment with study treatment for all other AESIs of the primary MedDRA System organ class (SOC) of Skin or subcutaneous tissue disorders should be taken by the investigator together with the medical monitor and the Sponsor.

- Liver: severity grade 3 or more according to CTCAE v 5.0
- Abnormal laboratory values involving liver:
  - ALT or AST >8X ULN
  - ALT or AST >5X ULN for more than 2 weeks
  - ALT or AST >3X ULN and total bilirubin levels >2X ULN or INR >1.5
  - ALT or AST >3X ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

Details for the CTCAE v 5.0 can be found at the following link: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf.

Early discontinuation/withdrawal procedures are outlined in Section 10.

Similar to phase 2A and as detailed in Section 12.4, participants must take at least 80% compliance of the assigned study treatment in order to be included in the Per Protocol (PP) population.

6.10 Missed Doses

If a participant misses a morning dose of study drug, the dose should be skipped (i.e., should not be made up) and the dosing resumed **in the evening of that same day** at the normal time and dosage.

If a participant misses an evening dose of study drug, the dose should be skipped (i.e., should not be made up) and dosing resumed **on the morning of the following day** at the normal times and dosage.

6.11 Drug Accountability

The Investigator or his/her designated representatives will dispense study drug only to participants enrolled in the study.

The Investigator (or, as appropriate, pharmacist/individual who is designated by the Investigator/institution) must maintain records of the delivery of the study drug to the trial site, the inventory at the site, the use by each participant, and the destruction or return of unused study drug.

At each visit, participants and their study partners should bring in all unused drug and empty or partially full containers. At the conclusion of the study and/or after study drug accountability has been verified by a ADCS clinical monitor, each site will be permitted to destroy or return all unused study drug and empty or partially full containers collected from each participant. Destruction at the study site must be performed in accordance with the participating site SOP.

7 PATIENT SELECTION AND CONCOMITANT MEDICATIONS

7.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria to be eligible for enrollment:

1. Age 50 to 89 (inclusive) at screening.
2. Meeting Criteria for having Mild Cognitive Impairment (MCI) due to AD or Mild probable AD according to workgroups of the Diagnostic Guidelines of the National Institute on Aging and Alzheimer's Association (NIA-AA) (see Appendix II).
    a. The differentiation of dementia from MCI 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.
    b. The differentiation of mild versus moderate AD is determined by CDR and MMSE. Those with scores of CDR global >1 or MMSE <20 will be considered to have moderate disease

and will be ineligible. Those with CDR global of 1 and MMSE ≥20 will be considered to be mild AD per protocol.

3. Mini-Mental State Examination (MMSE) score 20-30 inclusive at screening.
4. Montreal Cognitive Assessment score (MoCA) < 26 at screening.
5. Clinical Dementia Rating global score 0.5 or 1 at screening.
6. Positive CSF AD biomarker signature:
    a. Abnormal $A\beta_{1-42} \leq 1031$ pg/mL AND Abnormal p-tau-181 > 27 pg/mL, OR p-tau-181/$A\beta_{1-42}$ > 0.023 (Roche Gen 2 Elecsys® assays [1-3]) or
    b. For participants in whom CSF sampling is not feasible due to medical or technical reasons, a previous positive amyloid Positron-Emission Tomography (PET) scan with an FDA approved agent and a formal read, or a previous positive AD biomarker result consistent with the laboratory-based criteria for that particular assay, are eligible for inclusion. Previous PET scan or biomarker results will not qualify if the subject has since been exposed to an anti-amyloid agent.
7. Participants will have been treated with a stable dosage regimen of acetylcholinesterase inhibitors (AchEI) and/or memantine for at least 4 months prior to screening. Participants should be expected to remain on a stable dosage regimen of these medications for the duration of the trial. Participants being treated with aducanumab (Aduhelm™) or any Amyloid Beta Antibody (such as lecanemab (Leqembi™)) are **not** eligible for inclusion (*see exclusion criteria 22 and 23 below)*.
    a. Participants who are not being treated with AchEI and/or memantine at the time of screening because they have contraindications to these medications, or because they have previously failed treatment with these medications, are also eligible for inclusion, if it is expected that they will not be treated with these medications for the duration of the trial.
    b. Use of medical food specific to AD if intake discontinued at least 2 months before enrollment, OR if dose has been stable for at least 6 months before enrollment.
8. Modified Hachinski score of ≤ 4 at screening.
9. Cranial MRI scan consistent with a diagnosis of AD within 6 months of screening.
10. Participants must have a study partner who has frequent interaction with them (approximately >3-4 times per week), will be present for all clinic visits, and can assist in compliance with study procedures.
11. Female participants must be post-menopausal for at least one year or surgically sterile (bilateral tubal ligation, hysterectomy or bilateral oophorectomy) for at least 6 months prior to screening. Male participants with female partners of childbearing potential must be willing to and practice birth control (e.g. condoms, abstinence, etc.) during study treatment and until 28 days after the last dose of study drug.
12. Body mass index (BMI) ≤ 35 kg/m^2 at screening.
13. Ability (patients and their study partners) to read, speak and understand English or Spanish to ensure compliance with cognitive testing and study visit procedures.

14. Living in the community (includes assisted living facilities but excludes long-term care nursing facilities).

15. Ambulatory, or able to walk with an assistive device, such as a cane or walker.

16. Provision of informed consent from the participant (or the participant's legally authorized representative (LAR) if unable to provide consent) and the study partner.

7.2 Exclusion Criteria

Participants that meet any of the following criteria must not be included in the study:

1. Use of prohibited medications as defined in Section 7.4.2.
2. Significant neurodegenerative diseases, other than AD, and causes of dementias, including Parkinson's disease and Huntington's disease, vascular dementia, CJD (Creutzfeldt-Jakob disease), LBD (Lewy Body dementia), PSP (Progressive Supranuclear Palsy), AIDS (Acquired Immunodeficiency Syndrome), or NPH (normal pressure hydrocephalus).
3. Meeting Diagnostic Criteria for Possible AD according to workgroups of the Diagnostic Guidelines of the NIA-AA.
4. Hepatic impairment defined as Child-Pugh class A or more severe liver impairment.
5. Any of the following abnormal laboratory parameters:
    a. Alanine aminotransferase (ALT/SGPT) >3X the upper limit of normal (ULN)
    b. Aspartate aminotransferase (AST/SGOT) values >3X ULN
    c. Total bilirubin >1X ULN (Gilbert's syndrome *does not* exclude a participant)
    d. Hemoglobin < 11 g/dL
    e. Creatinine clearance (eGFR) ≤ 30 ml/min/1.73m^2
    f. Serum creatinine >1.5X ULN
6. History of moderate or severe skin reactions to medications or current moderate or severe disease of the skin and subcutaneous tissues.
7. Current serious or unstable illness including cardiovascular disease, resistant hypertension, hepatic, renal, gastroenterologic, respiratory, endocrinologic, neurologic, psychiatric, immunologic, or hematologic disease or other conditions that, in the investigator's or sponsor's opinion, could interfere with the interpretation of safety and assessment of efficacy in this study.
8. History of a major depressive episode within the past 6 months of screening.
9. History of diagnosis of schizophrenia.
10. History of uncontrolled bipolar disorder within past five years of screening.
11. History of seizures within past two years of screening.
12. Contraindication to lumbar puncture.
13. Contraindication to MRI, including but not limited to:
    a. Clinical history or examination finding that, in the judgment of the investigator and/or the local radiologist, would pose a potential safety risk to the participant being considered for an MRI;
    b. Implant devices not compatible in the magnetic resonance environment, such as: Automatic Implanted Cardioverter Defibrillators (AICDs); cochlear implants; cerebral aneurysm clips; implanted infusion pumps; implanted nerve stimulators; metallic splinters in the eye; other magnetic, electronic, or mechanical implants; Pacemakers are permitted only if labeled as conditional cardiac implantable electronic devices (CIEDs), have been approved for MRI use by the FDA, verified by the pacemaker's manufacturer as approved for MRI use, and determined to be suitable by the site's radiologist and principal investigator.

14. Cranial MRI at screening shows evidence of infection, tumor, cortical infarction, significant amyloid-related imaging abnormality (ARIA) namely > 4 microbleeds or superficial siderosis, or multiple lacunes in prefrontal or critical memory regions as determined by the site PI with a local reading; inconclusive findings may be subject to review by the ADCS Imaging Core.
15. Uncontrolled diabetes with HbA1c value >8.0 %.
16. Hepatitis B, C, or HIV infection.
17. Untreated or insufficiently treated hypothyroidism, vitamin B12 or folate deficiency.
18. Clinically significant laboratory abnormalities that may influence study assessments as determined by the investigator.
19. ECG obtained during screening that, in the opinion of the investigator, is clinically significant with regard to participation in the study.
20. Cancer or a malignant tumor within the past 3 years. Participants who underwent potentially curative therapy with no evidence of recurrence for >3 years *are not excluded*. Participants with stable prostate cancer or non-melanoma skin cancers *are not excluded*.
21. Participation in another clinical trial for an investigational agent (other than monoclonal antibody) and having taken at least one dose of study drug, unless confirmed as having been on placebo, within 90 days prior to the baseline visit. The end of a previous investigational trial is defined as the date of the last dose of an investigational agent.
22. Monoclonal antibody treatment with anti-amyloid or anti-tau agents intended to address the pathophysiologic processes associated with AD within the previous 180 days prior to the baseline visit.
23. Participants who are planning to receive treatment with aducanumab or any Amyloid Beta Antibody (such as lecanemab (Leqembi™)) during the course of the VIVA-MIND study.

7.3 Concomitant AD Medication

Participants will have been treated with a stable dosage regimen of AchEIs and/or memantine for at least 4 months prior to screening. Participants should be expected to remain on a stable dosage regimen of these medications for the duration of the trial.

Participants who have received monoclonal antibody treatment within the previous 180 days prior to the baseline visit are not allowed on study. In addition, participants who are planning to receive treatment with aducanumab or any Amyloid Beta Antibody (such as lecanemab (Leqembi™)) during the course of the VIVA-MIND study are excluded from participation.

Participants that are treatment naïve because AchEIs and memantine are not FDA-approved for MCI will **not** be excluded on this basis.

Participants who are **not** being treated with AchEIs and/or memantine at the time of screening because they have contraindications to these medications or because they have previously failed treatment with these medications, are also eligible for inclusion, if it is expected that they will **not** be treated with these medications for the duration of the trial. Otherwise, drug naïve participants will not be allowed into the trial. In the event that a participant's dose of AchEI and/or memantine is increased, or if a participant reaches an FDA indication for treatment with AchEI and/or memantine during their period of study participation, these medications will be permitted as part of their concomitant treatment.

7.4 Other Concomitant Medication

7.4.1 Permitted Concomitant Medications

The following concomitant medications are permitted:

- Use of Souvenaid is allowed if Souvenaid is discontinued at least 2 months prior to baseline, or if the participant is on stable dose for at least 6 months prior to baseline and is willing to continue during the study on the same dose and frequency.

Any concomitant medications must be recorded in the eCRF, noting the name of medication, the dose, duration, and indication. However, any of these permitted concomitant medications used on an intermittent basis should not be taken less than 12 hours prior to any scheduled visits that include any cognitive tests.

7.4.2 Prohibited Concomitant Medications

Varoglutamstat is a moderate inhibitor of the CYP2C19 enzyme. Therefore, the concomitant administration with strong inhibitors or inducers of the CYP2C19 enzyme or substrates with a narrow therapeutic margin **are not permitted**. Participant's medication, if medically appropriate, must be changed or stopped with at least a 2-week washout period before starting treatment with varoglutamstat.

The table below (**Table 3**) provides a listing of concomitant medications associated with CYP2C19 that are prohibited due to potential drug-drug interaction effects with varoglutamstat.

Table 3: Strong inhibitors or inducers of the CYP2C19 enzyme or substrates with a narrow therapeutic margin (PROHIBITED)

Fluconazole Fluvoxamine Ticlopidine Rifampin S-mephenytoin Phenytoin Phenobarbital Indomethacin Lansoprazole	**≥ 2 week washout before baseline**

The concomitant administration of varoglutamstat with all other moderate or weak inhibitors or products predominantly metabolized through the CYP2C19 enzyme **should be done with caution**. In case of new onset AEs, it is recommended to reduce the dose of the concomitant medication affected by the moderate inhibition of CYP2C19 by 50%.

The table below (**Table 4**) provides a listing of concomitant medications associated with CYP2C19 that must be used with caution due to potential drug-drug interaction effects with varoglutamstat.

Table 4: Moderate or weak inhibitors of the CYP2C19 enzyme or products predominantly metabolized through the CYP2C19 enzyme (USE WITH CAUTION)

Esomeprazole
Fluoxetine
Omeprazole
Moclobemide
Voriconazole
Carbamazepine
Cimetidine
Felbamate
Etravirine
Ketoconazole
Oral contraceptives

Concomitant administration with strong inhibitors of the CYP3A4 enzyme **are prohibited** only in those participants who are identified as poor metabolizers of CYP2C19 through genotype testing at baseline.

The table below (**Table 5**) provides a listing of concomitant medications associated with CYP3A4 that are prohibited due to potential DDI risk with varoglutamstat.

Table 5: Strong inhibitors of the CYP3A4 enzyme (PROHIBITED in participants identified as poor metabolizers of CYP2C19 at baseline)

Boceprevir
Clarithromycin
Cobicistat
Danoprevir and ritonavir
Elvitegravir and ritonavir
Grapefruit juice or anything derived from grapefruit
Idelalisib
Indinavir and ritonavir
Itraconazole
Ketoconazole
Lopinavir and ritonavir
Nefazodone
Nelfinavir
Paritaprevir and ritonavir and (ombitasvir and/or dasabuvir)
Posaconazole
Ritonavir
Saquinavir and ritonavir
Telaprevir
Tipranavir and ritonavir
Telithromycin
Troleandomycin
Voriconazole

The following concomitant medications are not permitted from screening through the end of the study:

- Treatment with aducanumab (Aduhelm™)
- Treatment with Amyloid Beta Antibodies (such as lecanemab (Leqembi™))
- Treatment with immunosuppressive medications (e.g. systemic corticosteroids in a dose equivalent to more than 10 mg of prednisone/day) within the last 90 days prior to baseline. Topical and nasal corticosteroids and inhaled corticosteroids for asthma are permitted.
- Treatment with chemotherapeutic agents for diseases other than malignancies within the last year prior to baseline.
- Concomitant treatment with St. John's Wort (a washout phase of at least 2 weeks prior to baseline is required).
- Any concomitant treatment which may impair cognitive function requires a washout phase of at least 5 half-lives of the treatment prior to screening.
- The following requirements apply to all other medications not intended to treat AD:
    - Participants must be on stable dose for at least 4 weeks prior to baseline, except for medications which are administered as short courses of treatment (e.g. anti-infective) or which are to be used as needed (PRN).
    - Medications which are central nervous system active and may affect cognitive function are not permitted during a period of 72 hours prior to neuropsychological testing.
    - Hypnotics are not permitted during a period of 72 hours prior to EEG recording or cognitive testing.
    - Participants who initiate treatment or undertake dose adjustment with drugs not intended for treatment of cognitive impairment during the study may continue in the study if in the opinion of the investigator this will not interfere with study procedures or safety.
    - CBD products (especially ingested or inhaled) should be avoided. If the patient is already taking them (regularly or on a per needed basis), a washout period of at least one week is required before proceeding with baseline.

Participants who are actively being treated with low dose acetylsalicylic acid (ASA) may undergo lumbar puncture, at the discretion of the investigator. Participants who are actively being treated with dual antiplatelet therapy or with any anticoagulation medications (e.g. heparin, warfarin, thrombin inhibitors, Factor Xa inhibitors) should **not** undergo lumbar puncture. If not contraindicated, participants who are being treated with low dose ASA and/or other antiplatelet/anticoagulant medications may have these medications held for an appropriate period of time (based on the half-life of the medication and/or pharmacodynamic effects on hematological parameters), at the discretion of the investigator, prior to performing lumbar puncture. Stop and start dates must be documented on the appropriate Concomitant Medication CRF. Platelet counts should be >100,000, and PTT and INR levels must be returned to normal before the lumbar puncture is performed. The ADCS Medical Monitor should be contacted if there are any questions.

If, during the clinical study, the administration of a non-permitted concomitant medication becomes necessary, the ADCS Medical Monitor must be contacted to determine if the participant needs to be prematurely discontinued from active treatment (see Section 10).

8 STUDY PROCEDURES

8.1 Study Visits

The schedule of study visits and procedures to be performed at each visit are outlined below and a table can be found in Appendix I.

8.1.1 Screening (within 90 days prior to baseline)

Sites should make every attempt to complete screening procedures within a period of 60 days from the time of informed consent. However, sites will be allowed a period of up to 90 days from informed consent if additional time is needed to complete the lumbar puncture and receive CSF AD biomarker results.

Participants who the Investigator considers to be appropriate for the study, will have the study explained to them, their LAR if applicable, and their study partner by the Investigator or his/her designee and will be given a copy of the written Informed Consent Form (ICF). When they have had sufficient time to study this information and the opportunity to ask any questions they wish, they will be invited to give their consent to participation by signing the ICF. The study partner may also be required to sign the ICF at the discretion of the responsible IRB reviewing this research.

Once the participant or LAR (and study partner if required) have given consent, the following procedures will be undertaken during the screening period.

- Confirmation of MCI due to AD or Mild probable AD according to workgroups of the Diagnostic Guidelines of the NIA-AA (see Appendix II).
- Mini-Mental State Examination (MMSE)
- Montreal Cognitive Assessment (MoCA)
- Clinical Dementia Rating (CDR)
- Documentation of demographics, medical history and education
- Modified Hachinski Ischemic Scale (MHIS)
- Neurological exam
- Physical examination
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Height
- Documentation of concurrent pathologies and concomitant treatment
- Documentation of adverse events from time of consent
- Blood sample collection for:
    - Clinical safety laboratory tests (see Section 8.3)
        - Hematology
        - Chemistry
        - Other: testosterone, TSH, T3, T4, HbA1c
    - HIV, HBsAg, HCV antibody, syphilis
    - Biobanking for future biomarker analysis
    - PrecivityAD® biomarker assays (Aβ1-42, p-tau181, p-tau181/ Aβ1-42, and p-tau217)
- Urinalysis (see Section 8.3)
- Resting 12-lead ECG
- Cranial MRI per protocol imaging requirements
    - If a participant has not had an MRI performed within 6 months of screening (i.e. within 6 months from the date of informed consent), then an MRI must be performed as part of the screening requirements for this study, per the imaging protocol, and should be one of the last screening procedures performed to determine final eligibility in order to prevent patients from undergoing unnecessary MRIs.

  - If a participant has had an MRI within 6 months of screening (i.e. within 6 months from the date of informed consent) but the MRI does not follow the study-specific imaging protocol, that MRI can be used to help determine eligibility; however, another MRI must be performed per the imaging protocol, and must occur as close to, and prior to, the baseline visit, after all other eligibility criteria have been confirmed.
- Lumbar puncture* to measure diagnostic, exploratory biomarkers and QC activity and PQ912 concentration
- Post lumbar puncture safety telephone follow-up (1 to 3 days after lumbar puncture)
- C-SSRS

*NOTE: The lumbar puncture should be done ***after*** the MRI scan ***if*** performed on the same day. If the lumbar puncture is performed on a **separate day** from the MRI and occurs **before** the MRI, then there must be at least a 3-day window between the lumbar puncture and the MRI. If the lumbar puncture is performed on a separate day from the MRI and occurs **after** the MRI, then there is no window (waiting period) between the MRI and lumbar puncture. The volumetric MRI (vMRI) should occur after all other eligibility criteria have been confirmed.

Following completion of all screening assessments, results for participants who meet all inclusion and none of the exclusion criteria will be submitted via the study EDC system. The Investigator must confirm all study eligibility criteria are met prior to participant randomization.

Re-screening

Re-screening can be undertaken when the reason for screen failure has been identified and corrected. A full re-screen (re-consent participant, assign new Participant ID and perform all screening assessments) may be conducted if more than twelve weeks have passed since the last assessment of screen failure.

If the participant screen fails a second time, any further re-screening must be discussed on a case-by-case basis with ADCS Medical Safety, and approved.

For re-screening, vMRI taken at the previous screening (screen failure) can be used if it will be less than 3 months old at the time of randomization. Otherwise, the vMRI needs to be repeated.

If the lumbar puncture was done before screen failure, sites should inquire with ADCS Clinical Operations on whether a lumbar puncture should be re-taken when the patient is re-screened and randomized.

8.1.2 Baseline (Week 0)

At baseline, eligible participants will be randomized into the study. Each will undergo the following procedures **prior** to first dose of study drug:

- Verification of Inclusion/Exclusion criteria
- Randomization in EDC
- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events

- Blood samples for:
    - Safety laboratory tests (hematology and serum chemistry, see Section 8.3)
    - Biobanking for future biomarker analysis
    - Genotyping: *APOE* (e4 +/-), HLA (HLA-A, HLA-B, HLA-C, DRB1, DQB1), CYP2C19
- Urinalysis (see Section 8.3)
- C-SSRS
- ADAS-Cog-13
- NPI
- FAQ
- ADNI Battery Composite (ABC, 9-item, see Section 9.1.1)
- Research Satisfaction Survey
- qEEG **– all phase 2A participants, substudy participants only in phase 2B**

Following the completion of the baseline visit procedures, all participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return at week 4 (± 7 days).

In phase 2A, all participants will undergo qEEG at the baseline visit. The EEG procedure can be scheduled for the day of the baseline visit or for the day before. It is preferable to schedule and conduct the EEG in the morning between 8.00 am to 11.00 am local time when the participant is most well-rested.

In phase 2B, newly-enrolled participants will be consented to participate in a qEEG substudy. Participants that consent to the substudy in phase 2B will undergo a qEEG at the baseline visit. Enrollment to the qEEG substudy of phase 2B will be completed when the target sample size is achieved ***and*** all baseline qEEGs have been centrally confirmed to be of acceptable quality. Site recruitment efforts for the qEEG substudy in phase 2B should continue until otherwise directed by ADCS personnel.

The qEEG must be performed by qualified study personnel and the data must be de-identified and submitted for central review and analysis by a blinded third-party vendor. Site personnel will be informed as to whether a participant's qEEG is deemed acceptable.

8.1.3 Week 4 (± 7 days)

All participants returning for week 4 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
    - Safety laboratory tests (hematology and serum chemistry, as listed in Section 8.3)
    - Pharmacokinetics – **all participants;** plasma sample to be taken once prior to the participant receiving their routine morning dose of study drug at Week 4 and again 2 to 6 hours after this routine morning dose at Week 4. Date and time of PK sampling and of study drug intake on the day of visits and day prior should be documented, along with time of last meal.

  - Biobanking for future biomarker analysis
- C-SSRS

At this visit, participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 4 weeks (± 7 days).

8.1.4 **Week 8 (± 7 days)**

All participants returning for week 8 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
  - Safety laboratory tests (hematology and serum chemistry, as listed in Section 8.3)
  - Pharmacokinetics- **all participants;** once prior to the participant receiving their routine morning dose of study drug at Week 8 and again 2 to 6 hours after this routine morning dose at Week 8. Date and time of PK sampling and of study drug intake on the day of visits and day prior should be documented, along with time of last meal.
  - Biobanking for future biomarker analysis
- Urinalysis
- C-SSRS

At this visit, participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 4 weeks (± 7 days).

8.1.5 **Week 12 (± 7 days)**

All participants returning for week 12 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
  - Safety laboratory tests (hematology and serum chemistry, as listed in Section 8.3)
- C-SSRS
- CDR-SB
- ADAS-Cog-13

Participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 4 weeks (± 7 days).

8.1.6 **Week 16 (± 7 days)**

All participants returning for week 16 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
    - Safety laboratory tests (hematology and serum chemistry, see Section 8.3)
    - Testosterone, TSH, T3, T4, HbA1c
    - Biobanking for future biomarker analysis
    - Pharmacokinetics – **cohort A and B participants only;** plasma sample to be taken once prior to the participant receiving their routine morning dose of study drug at Week 16 and again 2 to 6 hours after this routine morning dose at Week 16. Date and time of PK sampling and of study drug intake on the day of visits and day prior should be documented, along with time of last meal.
- Urinalysis
- C-SSRS

Participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 8 weeks (± 7 days).

8.1.7 **Week 24 (± 7 days)**

All participants returning for week 24 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
    - Safety laboratory tests (hematology and serum chemistry, see Section 8.3)
    - Testosterone, TSH, T3, T4, HbA1c
    - Biobanking for future biomarker analysis
    - Pharmacokinetics – **all participants;** plasma sample to be taken once prior to the participant receiving their routine morning dose of study drug at Week 24 and again 2 to 6 hours after this routine morning dose at Week 24. Date and time of PK sampling and of study drug intake on the day of visits and day prior should be documented, along with time of last meal.
      *NOTE: PK samples should be drawn at the time of lumbar puncture.
- Urinalysis
- Resting 12-lead ECG
- Cranial MRI per protocol imaging requirements
- C-SSRS

- ADAS-Cog-13
- CDR-SB
- NPI
- FAQ
- ADNI Battery Composite (ABC, 9-item, see Section 9.1.1)
- MMSE
- MoCA
- Research Satisfaction Survey
- Lumbar puncture to measure diagnostic, exploratory biomarkers and QC activity and PQ912 concentration– **phase 2A participants only**
- Post lumbar puncture safety telephone follow-up (1 to 3 days after lumbar puncture) - **phase 2A participants only**
- qEEG - **all phase 2A participants, substudy participants only in phase 2B**

At this visit, participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 12 weeks (± 7 days).

8.1.8 Week 36 (± 7 days)

All participants returning for week 36 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
    - Safety laboratory tests (hematology and serum chemistry, as listed in Section 8.3)
- Urinalysis
- C-SSRS
- ADAS-Cog-13

At this visit, participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 12 weeks (± 7 days).

8.1.9 Week 48 (± 7 days)

All participants returning for week 48 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
    - Safety laboratory tests (hematology and serum chemistry, see Section 8.3)
    - Testosterone, TSH, T3, T4, HbA1c

  - Biobanking for future biomarker analysis
  - Pharmacokinetics – **all participants**; plasma sample to be taken once prior to the participant receiving their routine morning dose of study drug at Week 48 and again 2 to 6 hours after this routine morning dose at Week 48. Date and time of PK sampling and of doses on the day of visits and day prior should be documented, along with time of last meal.
- Urinalysis
- C-SSRS
- ADAS-Cog-13
- CDR-SB
- NPI
- FAQ
- ADNI Battery Composite (ABC, 9-item, see Section 9.1.1)
- MMSE
- MoCA
- Research Satisfaction Survey

At this visit, participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 12 weeks (± 7 days).

8.1.10 **Week 60 (± 7 days)**

All participants returning for week 60 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
  - Safety laboratory tests (hematology and serum chemistry, as listed in Section 8.3)
- C-SSRS
- ADAS-Cog-13

At each visit, participants will receive a supply of study drug with instructions on how to take it. Participants will be given an appointment to return in 12 weeks (± 7 days).

8.1.11 **Week 72 or Early Termination (± 7 days)**

All participants returning for week 72 will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment
- Documentation of adverse events
- Blood samples for:
  - Safety laboratory tests (hematology and serum chemistry, see Section 8.3)
  - Testosterone, TSH, T3, T4, HbA1c

  - Biobanking for future biomarker analysis
  - Pharmacokinetics – **all participants**; plasma sample to be taken once prior to the participant receiving their routine morning dose of study drug at Week 72 and again 2 to 6 hours after the routine morning dose at Week 72. Date and time of PK sampling and of study drug intake on the day of visits and day prior should be documented, along with time of last meal.
- Urinalysis
- Resting 12-lead ECG
- qEEG - **all phase 2A participants; phase 2B substudy participants only**
- Lumbar puncture* (± 7 days) to measure diagnostic biomarkers, exploratory biomarkers, levels of PQ912 and QC activity
- Post lumbar puncture safety telephone follow-up (1 to 3 days after lumbar puncture)
- C-SSRS
- CDR-SB
- ADAS-Cog-13
- Cranial MRI (± 14 days)
- NPI
- FAQ
- ADNI Battery Composite (ABC, 9-item, see Section 9.1.1)
- MMSE
- MoCA
- Research Satisfaction Survey
- Treatment Blinding Questionnaire

*NOTE: PK samples should be drawn at the time of lumbar puncture. The lumbar puncture should be done ***after*** the MRI scan ***if*** performed on the same day. If the lumbar puncture is performed on a **separate day** from the MRI and occurs **before** the MRI, then there must be at least a 3-day window between the lumbar puncture and the MRI. If the lumbar puncture is performed on a **separate day** from the MRI and occurs **after** the MRI, then there is no window (waiting period) between the MRI and lumbar puncture. If a participant is terminating early, they do not need to undergo this lumbar puncture. However, every effort will be made to encourage the participant's ongoing voluntary participation and to explain that CSF collection may provide informative information in the future for the field of AD. Participants will be given an appointment to return in 4 weeks (± 7 days).

Participants who terminate the study early will undergo a Post-Treatment Safety Follow-Up visit 4 weeks (± 7 days) following their Early Termination visit, and will undergo the same assessments as listed under section 8.1.12.

8.1.12 Week 76 or Post-Treatment Safety Follow-up (± 7 days)

All participants returning for either week 76 or the Post-Treatment Safety Follow-Up visit at 4 weeks (± 7 days) following their Early Termination visit will undergo the following procedures:

- Physical exam
- Vital signs (sitting blood pressure, pulse, temperature, respiration rate)
- Weight
- Documentation of concomitant treatment

- Documentation of adverse events
- Blood samples for
    - Safety laboratory tests (hematology and serum chemistry, see Section 8.3)
    - Testosterone, TSH, T3, T4, HbA1c
- Urinalysis

8.2 Quantitative EEG (qEEG) and theta power (Phase 2A, primary outcome measure)

In all phase 2A participants, and in a substudy of phase 2B participants, an EEG with spectral analyses will evaluate pharmacoEEG response to varoglutamstat through a 20-minute resting state EEG recorded against a common reference at 21 electrode positions, and the global relative theta wave power (4-8 Hz) will be calculated by averaging values of all 21 electrodes. Global relative theta wave power (4-8 Hz) will serve as a primary efficacy outcome in phase 2A, and as a secondary outcome measure in phase 2B. EEG network connectivity will serve as exploratory measures, and these include: coherence [25-27]; amplitude envelope correlation (AEC) [28]; phase lag index (PLI) [29]; phase lag time (PLT); and triggered cortical potential (TCR, amplitude and latency).

8.3 Laboratory Safety Assessments

Clinical Safety Laboratory tests will be performed by the designated Central Laboratory at the University of Rochester Medical Center (URMC). It is up to the investigator's discretion to assess the CSF for routine safety measurements, according to the appropriate local medical standards of the site.

Clinical Safety Laboratory assessments include the following:

Hematology:
Hemoglobin, hematocrit, platelet count, RBC, WBC, differential count and absolute neutrophil count

Chemistry:
Sodium, potassium, chloride, calcium, ALT, AST, LDH, alkaline phosphatase, GGT, phosphorous, bicarbonate, CPK, total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, glucose, creatinine, BUN, estimated Glomerular Filtration Rate (eGFR), uric acid, total cholesterol, LDL, HDL, triglycerides, B12 (screening), folate (screening)

Other:
Testosterone, TSH, T3, T4, hemoglobin A1c (HbA1c)

Urinalysis:
pH, specific gravity, protein, glucose, ketones, urobilinogen, bilirubin, blood, leucocytes and nitrite

Serology (screening):
Human Immunodeficiency Virus (HIV), Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and syphilis

An approximate blood volume of 23 mL at screening, 30 mL at baseline, 14 mL at Weeks 4, 8, 12, 36, and 60, and 18 mL at Week 16, 24, 48, 72 (or Early Termination) and 76 (or Post-Treatment Safety Follow-Up)

will be collected for laboratory safety analyses. Full details of sampling (blood and urine), sample preparation and storage methods will be given in the laboratory manual.

8.4 CSF, Plasma and Serum Assessments

An approximate CSF volume of 8 ml (absolute minimum requirement) to 15 ml (preferred minimum) at screening, week 24 (for phase 2A participants) and at the end of study visit will be collected. CSF samples should be collected at the same time of day, either morning (between 8 and 10AM) or afternoon (between 1 and 3PM). CSF samples should be collected by gravity method only.

To the extent possible, LPs should be completed using a non-traumatic needle (*e.g.* Sprotte needle or equivalent), although this is left to the discretion of the site. To minimize transfers and contact with additional surfaces, CSF collection should be completed by gravity. If for technical reasons this is not possible, LP via fluoroscopy and/or collection of fluid via aspiration will be permitted, following approval from ADCS Clinical Operations team. If fluoroscopy is used, details of the procedure and the risks associated with the additional use of radiation may need to be included in your site's Informed Consent.

Blood samples for varoglutamstat levels in plasma should be drawn at the time of lumbar puncture at week 24 and 72 (between 2 to 6 hours after the morning dose). According to studies on human pharmacokinetics in elderly volunteers after repeated dosing with 200 to 800 mg varoglutamstat, taken BID in a fasted state, the elimination phase of varoglutamstat is very robust, as described by an effective half-life (t1/2α; accounting for approximately 90% of total AUC) of approximately 3 hours (CVs of 15 to 20%) [16]. Tmax is approximately 1 hour after drug intake and delayed for 0.5 to 1 hour when the drug is taken together with food. The suggested time frame for collection of plasma following varoglutamstat dosing (2 to 6 hours after drug intake) covers up to 2 half-lives after Tmax. Considering the known PK parameter from the phase 1 study together with the collected sparse exposure information should be adequate to get an estimate of the AUC with reasonable accuracy by population PK model. Measuring CSF levels at the same time points allows for assessment of blood-brain distribution. Both plasma PK and CSF levels in combination with the known enzyme kinetic parameters allow for prediction of the mean target occupancy in CSF.

Date and time of varoglutamstat doses on the day of lumbar puncture and day prior should be collected on source document worksheets, for entry into the EDC system.

Anti-platelet medications may be discontinued at the PI's discretion for lumbar puncture (see Section 7.4.2).

The unused portion of CSF, plasma and serum will be stored at a central biorepository, the National Cell Repository for Alzheimer's Disease (NCRAD), for future research.

Details of the CSF sampling are contained in the Study Procedures Manual.

8.4.1 QC inhibition and varoglutamstat levels in plasma

Blood collection to measure plasma varoglutamstat levels and metabolite levels will occur at the following time points: week 4 (all participants), week 8 (all participants), week 16 (cohorts A and B participants only), week 24 (all participants), week 48 (all participants) and week 72 (all participants). Samples should be taken just prior to the routine morning dose of study drug at each visit where PK samples are collected, and again between 2 and 6 hours after the routine morning dose of study drug at each of these visits. CCI [redacted], has established and validated the assays to quantify varoglutamstat

and its main metabolites. Varoglutamstat levels in plasma will be used to estimate TO, and will be provided to the DSMB for consideration in parallel with safety data in phase 2A. The TO from CSF concentrations will also be calculated at the end of the trial to confirm the plasma derived estimates. To reach 50% inhibition in CSF, a concentration at Ki (25 nM) in CSF is needed. To achieve this, a 20-fold higher mean concentration in the periphery (500 nM ≙ 170 ng/ml) is pursued to fulfil the go-no-go decision criterion (see Appendix III).

Samples for measurement of QC activity in serum should be taken at screening, week 4, week 8, week 16 (cohorts A and B only), week 24, week 48 and week 72. QC inhibition in serum is a customized measure developed by Probiodrug/Vivoryon and then established for higher throughput by CCI [redacted]. Measurement of changes in QC activity (inhibition) is the most direct pharmacodynamic marker. The measurement of QC activity in serum will be used for exploratory analysis only, such as correlations between serum QC activity and CSF QC activity.

Participants should take their doses at their routine time on the days of these visits. Date and time of study drug intake, the time of last meal, and the time of the blood draw (just prior to intake of morning dose and between 2 and 6 hours after intake of the morning dose) should be recorded on the source document worksheets for the day of the study visit and for the day prior to the study visit.

PK samples should be drawn at the time of lumbar puncture for those visits at which this procedure is scheduled to occur. Date and time of doses on the day of lumbar puncture at these visits should be recorded on source document worksheets for entry into the EDC system.

8.4.2 QC inhibition and PQ912 levels in CSF

Collection of CSF samples to measure QC inhibition will occur at screening, week 24 (phase 2A participants), and at week 72. Samples will be transferred to CCI [redacted] for analysis. QC inhibition in CSF is a customized measure developed by Vivoryon. It is the most direct pharmacodynamic marker.

Collection of CSF samples to measure varoglutamstat levels will occur at week 72. CCI [redacted], established and validated the bioanalytical methods to quantify PQ912 and its main metabolite, PQ1345, which have been used for recent preclinical and clinical evaluations (See Investigators Brochure, in Investigational Product Section). Varoglutamstat levels in CSF will be used to calculate TO in CSF (see Appendix III).

8.4.3 CSF biomarkers

CSF biomarkers will be centrally analyzed and include measures of AD pathology (Aβ40, Aβ42, t-tau, P-tau-181); inflammation (sTREM2, YKL 40); synaptic function (neurogranin, SNAP 25); and axonal injury (NFL and VILIP-1).

8.4.4 Blood-Based Biomarkers Sub-Study

During the Phase 2A portion of the VIVA-MIND trial, the PrecivityAD® test from CCI [redacted] will be compared to the CSF-based Elecsys® biomarker assay, which will be considered as the gold standard.

The PrecivityAD® assay utilizes a plasma mass spectrometry method coupled with a diagnostic algorithm that incorporates APOE genotyping and has reached a breakthrough device designation from the FDA.This

sub-study is being conducted to establish whether the necessary agreement rates to support enrolling participants primarily according to plasma-based biomarker criteria in phase 2B can be achieved.

8.4.5 Genotyping

Genotyping of CYP2C19 and of the Apolipoprotein E (*APOE*) alleles, and HLA testing, will be performed in blood obtained at baseline and processed by the central lab. *APOE* (e4 +/-) genotype is associated with the risk and age of onset of AD. Results of *APOE* genotyping (homozygous or heterozygous for the following alleles: E2, E3, E4) will be used to explore a relationship between *APOE* genotype clinical course and response to treatment.

Polymorphism of CYP2C19 will be assessed to correlate with varoglutamstat pharmacokinetics and to identify poor versus intermediate/fast metabolizer. HLA testing (e.g., HLA-A, HLA-B, HLA-C, DRB1, DQB1) will be conducted in order to correlate potential (serious) adverse event types to HLA patient subtypes.

The DNA sample will be stored at NCRAD for additional sample sharing that may include further genetic testing.

Samples will be de-identified to preserve the confidentiality of participants. Participants can request in writing any time to have their samples destroyed.

Results of this testing are for research purposes only and will not be disclosed to the participant or study partner.

8.5 Physical and Neurological Examination

A physical examination will be performed by a medically qualified professional at every study visit. A review of the major body systems will be performed, and vital signs will be assessed. A neurological examination will only be performed at screening and will include assessment of motor strength, sensory, deep tendon, tremor, cerebellar, cranial, and mental status.

8.6 Electrocardiogram

A standard 12-lead resting ECG will be performed during screening, week 24, and week 72. The Investigator or designee will review the 12-lead ECG and findings will be recorded in the eCRF as normal, abnormal but not clinically significant, or abnormal and clinically significant. Any clinically significant abnormalities on ECGs recorded after administration of the investigational product will also be documented as AEs and entered on the AE page of the eCRF. Clinically significant abnormalities on ECGs prior to administration of the investigational product, will be recorded as medical history.

8.7 Cranial MRI Assessments

Brain structural change is seen in normal aging, but is accelerated in neurodegenerative disease, including AD. Atrophy in AD arises from neuron and synapse loss that begins in the entorhinal cortex. The pathology then spreads throughout the limbic regions of the temporal lobe, including the hippocampal formation. Subsequently, neuron loss and atrophy are observed throughout neocortical association areas in temporal, parietal and frontal lobes.

vMRI allows the *in vivo* assessment of brain structure volume and provides a measure of atrophy rate. Results from vMRI studies suggest that the patterns of atrophy in AD, which mirror the pathological

progression of the disease, can reliably be detected and tracked across time. Atrophy of the medial temporal lobe, including hippocampus and entorhinal cortex, has long been described in vMRI studies of AD. Hippocampal volume derived from MRI correlates with histological hippocampal volume and degree of neuronal loss and AD pathology, and entorhinal cortical thickness change appears to be an early and sensitive indicator of neurodegeneration associated with AD [30, 31]. Longitudinal MRI measures of regional and whole-brain volumetric change provide a valuable complement to cognitive measures in that they are not influenced by temporary symptomatic improvements, and they provide an early index of the study drug's ability to reach the target organ and have an effect on AD-related atrophy.

Participants will undergo vMRI scans of the brain at screening, week 24 and week 72 in order to assess clinical safety and to assess for changes in brain volumes that may be associated with clinical change due to treatment with varoglutamstat.

vMRI scans will use the same imaging protocol, which will include a localizer scan, a 3D T1- weighted sagittal acquisition (MPRAGE or IR-SPGR), a T2-weighted FLAIR axial acquisition, a T2* gradient recalled echo axial acquisition for magnetic susceptibility, and a diffusion weighted axial acquisition to assess for restricted diffusion.

Images will be checked for image quality and adherence to scanning protocols. 3D T1- weighted datasets passing quality checks will be corrected for spatial distortion and for intensity variation. Screening and follow-up datasets for each participant will be spatially registered to one another using rigid-body registration followed by nonlinear registration and neuroanatomic parcellation to quantify whole-brain and subregional volumetric change on a patient-by patient basis.

Whole brain volume (WBV, excluding cerebellum), bilateral ventricular volume and bilateral hippocampal volume will be measured. Quantitative anatomical regional change (Quarc) will be used as the computational MR image processing application. Detail for the statistical computations is given in the Statistical Analytical Plan (SAP).

If performed on the same day as a lumbar puncture, the vMRI should be conducted before the lumbar puncture. Otherwise, at least a 3-day window between vMRI and the lumbar puncture is required. Scanners that have passed the study's qualification procedures will be used. Participants must be scanned by the same scanner throughout the study.

Participants with a contraindication to MRI at the time of screening are deemed ineligible to participate in this study. Participants may continue to participate on the study if they have already been randomized but develop a contraindication to MRI during the course of the study.

9 STUDY-SPECIFIC INSTRUMENTS

9.1 Cognitive Measures

9.1.1 ADNI Battery Composite (ABC) (Phase 2A, primary outcome measure)

The ADNI Battery Composite (ABC) score is comprised of the 9 measures from the ADNI Neuropsychological Test Battery listed below. A subset of 5 of these measures (indicated by*) can be administered remotely and represent a contingency for remote telehealth assessments if this accommodation is needed due to the COVID-19 pandemic. Modeling of ADNI data indicate that power for the 5-item and 9-item ABC are comparable.

- Rey Auditory Verbal Learning Immediate Recall*
- Rey Auditory Verbal Learning Delayed Recall*
- Number Span Forward*
- Number Span Backward*
- Category Fluency (average of Animal and Vegetable Fluency)*
- Trail Making A, Time to Completion (negative of score)
- Trail Making B, Time to Completion (negative of score)
- Digit Symbol Substitution, Total Correct
- Boston Naming Test (30 item), Total Correct

Each test will be scored so that higher is better (i.e. the negative value will be taken for tests where higher is worse, as indicated). For each subject and each test, the test score will be standardized by subtracting the overall baseline mean, and dividing by the baseline standard deviation. The baseline mean and standard deviation statistics for each measure will be computed using the entire enrolled study population. Then the ABC score for each subject will be the sum of the standardized test values (either the 5- or 9-item ABC score depending on circumstance at the time the trial launches).

9.1.1.1 Rey Auditory Verbal Learning Test

The Rey Auditory Verbal Learning Test is a 15-item list learning test that will be used to assess verbal learning and memory. [32] Testing consists of five learning trials, an interference list (single trial), and delayed recall of the initial word list.

9.1.1.2 Boston Naming Test (30-item)

An abbreviated version of the Boston Naming Test will be administered to assess visual confrontation naming. [33] Participants are asked to identify (i.e., name) 30 line drawings of objects (odd-numbered items from the 60-item Boston Naming Test).

9.1.1.3 Trail Making Test (Trails A and B)

The Trail Making Test is a test of processing speed and executive function. [34] Trail Making A requires participants to draw a line to connect 25 numbered circles in ascending numerical order as quickly as possible. Trail Making B requires participants to draw a line to connect 25 circles containing either numbers (1 through 13) or letters (A through L) in alternating and ascending order (e.g., 1 to A; 2 to B) as quickly as possible.

9.1.1.4 Verbal Fluency – Category Fluency

Category fluency assesses semantic memory and language fluency. [35] Participants are asked to name as many different exemplars as possible within 60 seconds in each of two semantic categories: animals and vegetables.

9.1.1.5 Digit Symbol Substitution

The Digit Symbol Substitution test assesses executive function and psychomotor speed. [36] Participants are asked to complete an array of symbol-digit pairings based upon a presented key as quickly as possible for 90 seconds.

9.1.1.6 Number Span Forward and Backward

Number Span assesses two different working memory constructs: Forward Number Span measures the capacity for retaining information very briefly for the purpose of repeating it exactly, while Backward Number Span measures the ability not only to retain the information but also to mentally manipulate the numbers and recite them in reverse sequence. [37] Numbers for both forward and backward span tests are presented with sequences ranging from 2 to 9 numbers. Two trials are administered at each sequence length. Two scores are reported for each task: number of correct trials and longest sequence repeated correctly prior to failing two consecutive trials of the same length.

9.1.2 ADAS Cognitive Subscale-13 (ADAS-Cog-13)

The ADAS-Cog-13 is a structured scale that evaluates memory (word recall, word recognition), reasoning (following commands), language (naming, comprehension), orientation, ideational praxis (placing letter in envelope) and constructional praxis (copying geometric designs). Ratings of spoken language, language comprehension, word finding difficulty, and ability to remember test instructions are also obtained. [38] The test is scored in terms of errors, with higher scores reflecting poorer performance and greater impairment. Scores can range from 0 (best) to 70 (worse). The ADAS-Cog-13-item scale [39] includes all original ADAS-Cog items with the addition of a number cancellation task and a delayed free recall task, for a total of 85 points. The purpose of these additional items is to increase the number of cognitive domains and range of symptom severity without a substantial increase in the time required for administration.

9.1.3 Mini-Mental State Examination (MMSE)

The MMSE is a frequently used screening instrument for AD drug trials. It evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two intersecting pentagons. [40] A lower score indicates more cognitive impairment. The highest (best) score is 30.

9.1.4 Montreal Cognitive Assessment (MoCA)

The MoCA is a brief mental status exam which was designed to be more sensitive to MCI and early dementia than the MMSE. [41] It assesses numerous cognitive domains, including attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Like the MMSE, the highest (best) score is 30. Administering both the MoCA and the MMSE in this trial will allow comparisons of the utility within the setting of a clinical trial.

9.1.5 AD Composite Score (ADCOMS)

The ADCOMS is a composite clinical outcome designed for prodromal and mild AD dementia trials. It is comprised of 4 ADAS-Cog subscales (Delayed word recall, Orientation, Word recognition, Word finding difficulty); 2 items from the MMSE (Orientation time; Intersecting pentagons); and all 6 items from the CDR-SB, and it has demonstrated improved sensitivity to clinical decline over individual scales. [42] This measure is derived from the primary and secondary outcomes.

9.1.6 Cognitive Functional Composite-2 (CFC2)

The Cognitive Functional Composite-2 (CFC2) is a novel composite outcome measure of cognition and everyday function that has been derived from measures within ADNI and optimized for detecting change

in early AD. [18] CFC2 is comprised of selected subtests from the ADAS-Cog (Word Recall, Delayed Word Recall, and Orientation); CDR Sum of the Cognitive Boxes (Memory, Orientation, Judgement & Problem Solving); and the FAQ.

9.1.7 ADAS-Cog-Exec

The ADAS-Cog-Exec is a cognitive composite outcome that was designed to improve the performance characteristics of the ADAS-Cog for MCI and early AD clinical trials and to enhance measurement sensitivity to detect changes in executive functioning [43]. It is an optimally-weighted composite of scores on ADAS-Cog13 Word Recall, Delayed Word Recall, Orientation, and Number Cancellation subtests; Trail-Making A & B, Digit Symbol Substitution and Category Fluency; and cognitive components of the CDR (Memory, Orientation, Judgement & Problem Solving). Modeling based on data from ADNI-1 demonstrated that the ADAS-Cog-Exec is superior to the ADAS-Cog13 in detecting cognitive change over 12 months in participants with MCI, resulting in improved statistical power.

9.2 Behavioral and Functional Measures

9.2.1 Clinical Dementia Rating (CDR) Scale – Sum of Boxes (SB)

The CDR-SB [44] is a validated composite rating of cognition and everyday functioning used in longitudinal AD research which incorporates both informant input and direct assessment of performance. It assesses through semi structured interview 3 cognitive domains including memory, orientation, and judgement/problem solving and 3 everyday functional domains including community affairs, home and hobbies and personal care. There are 5 levels of impairment from none CDR=0 to severe CDR=3. The individual domain scores are added to create a sum of the box scores.

9.2.2 Functional Activities Questionnaire (FAQ)

The FAQ, a 10-item questionnaire, is a validated interview completed by the study partner, and which rates the study patient on their ability to carry out ten complex activities of daily living: Each item is rated on a scale from 0 to 3. [45] Scores are summed across items to provide a total disability score (higher scores = greater impairment; maximum score = 30).

9.2.3 Neuropsychiatric Inventory (NPI)

The NPI is a well-validated, reliable, multi-item instrument to assess psychopathology in AD dementia based on the results of an interview with the study partner. [46] The NPI evaluates both the frequency and severity of 10 neuropsychiatric features, including delusions, hallucinations, agitation/aggression, dysphoria, anxiety, euphoria, apathy, disinhibition, irritability and lability, and aberrant motor behavior, as well as evaluates sleep and appetite/eating disorders. Frequency assessments range from 1 (occasionally, less than once per week) to 4 (very frequently, once or more per day or continuously). Severity assessments range from 1 (mild) to 3 (severe). The score for each subscale is the product of severity and frequency and the total score is the sum of all subscales.

9.2.4 Modified Hachinski Ischemic Scale (MHIS)

This brief questionnaire, conducted by a clinician, incorporates information regarding medical history, cognitive symptoms and features of stroke, reported by a study partner as well as the neurological examination, and neuroimaging studies. [47]

9.2.5 Columbia-Suicide Severity Rating Scale (C-SSRS)

Consistent with FDA guidance [48], the C-SSRS will be implemented throughout the study. The C-SSRS captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the corresponding assessment period. [49] The scale includes suggested questions to elicit the type of information needed to determine if a suicide-related thought or behavior occurred. The first time the scale is administered in this study, the C-SSRS "Screening/Baseline" version will be used, and the findings will constitute the baseline assessment. The C-SSRS "Since Last Visit" scale will be used for all subsequent assessments. If the investigator determines that a participant is at risk of suicide or self-harm, appropriate measures to ensure the participant's safety and to obtain mental health evaluation must be implemented. The event should be recorded as either an AE or SAE as determined by the investigator and reported within 24 hours to the Sponsor.

9.3 Research Satisfaction Survey

A Research Satisfaction Survey will be administered to the participant and study partner to evaluate satisfaction with the study. The survey may reveal specific aspects of the study that participants dislike which can inform efforts to improve their experiences when participating in future studies. Past studies show that participant input and feedback is important for retention. [50]

9.4 Treatment Blinding Questionnaire

Following the week 72 clinic assessment (or early termination visit if participant completes study before week 72), the site Investigator and Raters will each complete a Treatment Blinding Questionnaire to document knowledge of intervention group assignment per participant.

10 EARLY DISCONTINUATION/WITHDRAWAL PROCEDURES

The entire study may be discontinued at the discretion of ADCS or Vivoryon. In these circumstances the Investigator will arrange for all ongoing participants to be seen and for their study drug to be discontinued as soon as is safely possible.

Participants are free to withdraw from study participation at any time, for any reason, and without prejudice.

Discontinuation of study treatment and/or the participation of an individual patient in the study will be terminated in the following circumstances:

1. Withdrawal of informed consent by the participant or LAR. If the study partner withdraws his/her consent to participate then attempts will be made to find a replacement. If a replacement study partner cannot be found, sites should inform Clinical Operations before a participant is discontinued from the study. In any event the patient will be continued in the study in so far as possible. Participants who withdraw consent will be advised by the Investigator regarding subsequent treatment and investigation.
2. Treatment of a participant with a non-permitted concomitant medication may necessitate discontinuation from study drug and will be determined by the Investigator in conjunction with the ADCS Medical Monitor.
3. Adverse event or other significant medical condition which, in the opinion of the Investigator render it necessary to discontinue study drug (see Section 6.9).

4. The participant experiences a medical emergency that necessitates unblinding their treatment assignment. (see Section 6.4)
5. Any other occurrence that, in the Investigator's opinion, makes continued participation contrary to the participant's best interests.
6. Movement of participant into a long-term care nursing facility. Movement into an assisted living facility is not cause for discontinuation from the study.

Participants who discontinue study treatment for any reason will have the opportunity to continue on the protocol with further visits per protocol to the end of the study with their ongoing consent. Their continued participation will be encouraged.

The Investigators at each site will make every reasonable effort to maximize participant retention, even if the study treatment is discontinued before week 72. However, if an investigator removes a participant from study, or if a participant declines all further study participation during the study, an Early Termination Visit will be completed as close as possible to the time of study discontinuation. The Early Termination Visit may contain the same assessments as week 72, to allow collection of the main outcome measures. The Post-Treatment Safety Follow-Up visit (4 weeks after the early termination/end of treatment) should also be conducted. For further detail please refer to the Study Procedures Manual.

11 DEFINITION OF ADVERSE EVENTS

An adverse event (AE) or adverse experience is any untoward medical occurrence in a study participant who is administered a medicinal product, that does not necessarily have a causal relationship with the study treatment, and that occurs after informed consent is signed and up to and including 30 days after the study drug has been discontinued. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Pre-existing conditions, which increase in frequency or severity or worsen in nature during, or as a consequence of, use of a drug in human clinical trials, will also be considered adverse experiences. Adverse events may also include pre- or post-treatment complications that occur as a result of protocol-mandated procedures (e.g., invasive procedures such as a lumbar puncture).

An AE **does not** include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is the AE.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
- Worsening of symptoms associated with expected decline in AD is not considered an AE in this study.
- Overdose of either study drug or concomitant medication without any signs or symptoms unless the participant is hospitalized for observation. Overdoses should be reported as outlined in Section 11.4.

A TEAE is defined as any AE that developed, worsened, or became serious after first dose of study drug and up to and including 30 days after the last dose of study drug.
An AESI is predefined as listed in Section 4.1.1, e.g.,by severity within the primary MedDRA System organ classes (SOCs) of Skin or subcutaneous tissue disorders or hepatobiliary disorders. Adverse events of the gastro-intestinal SOC were not selected as AESI because in the prior Phase 2a SAPHIR study GI events

were of short duration, mostly of minor severity and led rarely to discontinuation of study medication or study. Also GI adverse events occurred frequently in the control arm, can have multiple origins including viral infections, food contamination and therefore are not suitable for dose decisions.

11.1 Evaluation and Reporting of Adverse Events

All AEs (i.e. a new event or an exacerbation of a pre-existing condition) that occur from the time of consent and up to and including 30 days after the study drug has been discontinued must be recorded as an AE on the AE eCRF. The Investigator must follow all AEs until the AE resolves, or until the Investigator and/or the Medical Monitor determine the event is chronic or clinically stable. If an AE remains unresolved at the conclusion of the study, the Investigator and Medical Monitor will make a clinical assessment to determine whether continued follow-up of the AE is warranted. All participants who have received at least one exposure to study therapy will be evaluated for safety of study treatment.

The Investigator should attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs/symptoms.

Investigators will be provided with training on safety assessments that are specific to this trial. Participants and their study partners will be informed that they should contact their study site immediately (via telephone number or at a clinical study visit) if signs or symptoms of skin or subcutaneous tissue adverse events (itching, reddening, blister) occur or signs of hepatobiliary adverse events occur (itching, fatigue, yellow skin or eyes). In case of doubt the participant and caregiver are advised to stop the intake of study drug until a visit (planned or un-planned) at the study site has been performed.

11.2 Assessment of Adverse Events

All AEs must be promptly documented on the Adverse Event eCRF and assessed by the Investigator. Details of the event must include the dates of onset and resolution, severity, relationship to study drug, seriousness, and whether the event caused the participant to withdraw from the study, outcome and timing with regard to administration of the study drug.

Severity: Unless the AE is considered an AESI, severity should be graded and recorded according to the table below (unlike other AEs, AESI are graded according to CTCAE v5 – please see section on AESI collecion and reporting).

Severity	Definition
Mild	Awareness of event but easily tolerated
Moderate	Discomfort enough to cause interference with usual activity
Severe	Inability to carry out usual activity, incapacitating, requires medical intervention

Relationship: The relationship of the Adverse Event to the study drug will be determined by the Principal Investigator, and assessed using the following definitions:

Relatedness	Description

Not Related	There is no evidence of a causal relationship and a causal relationship cannot be reasonably attributed to the study treatment or procedures. The event is clearly due to extraneous causes.
Unlikely Related	A poor temporal relationship exists between the event onset and administration of intervention. The event could easily be explained by the participant's clinical state, intercurrent illness, or concomitant therapies.
Possibly Related	A relationship cannot be ruled out with certainty and the event may be related. There is some evidence to suggest a causal relationship, but the influence of other factors may have contributed to the event, such as the participant's clinical condition or concomitant treatment.
Probably Related	The event is likely related to the intervention. There is evidence to suggest a causal relationship, such as reasonable temporal sequence from treatment administration or procedure. The influence of other factors is unlikely.
Definitely Related	The event is clearly related to the intervention. There is clear evidence to suggest a causal relationship. The influence of other factors can be ruled out.

These criteria, in addition to good clinical judgment, should be used as a guide for determining the causal assessment. If it is felt that the event is not related to study drug therapy, then an alternative explanation should be provided.

11.3 Collection and reporting of Adverse Events of Special Interest (AESIs)

Adverse Events of Special Interest (AESIs) are a key safety outcome in this study. They represent AEs thought to be specific to varoglutamstat (PQ912) and are central to the study's phase 2A primary objective of determining the highest safe and well-tolerated dose amongst three dose levels being tested (please see Section 4.1.1). Their specification is based on accumulated data from an early phase 1 (SAD & MAD) study on healthy young and elderly subjects, and a phase 2A study in patients with MCI and early AD (the SAPHIR study).

AESIs are collected until the end of the safety reporting period, up to 30 days (inclusive) after the study drug has been discontinued (please see Section 11.1). For the purposes of dose selection during phase 2A, AESIs will be included in the calculation of the Pocock boundary interval (please see Section 4.1.1) if they occur (occurrence of an AESI means the date when the criteria of an AESI are fulfilled, not the overall onset of an AE without meeting the criteria of an AESI) between the point of initial exposure to the IP, through titration, to the completion of 8 weeks on the originally assigned full dose (*i.e.* week 16 for Cohort A, week 12 for Cohort B, and week 8 for Cohort C). This interval is called the safety evaluation period for dose selection. The Pocock boundary is only valid until the first dose selection criterion is met (i.e., the highest safe dose is selected).

Per Section 4.1.1, an AESI is occurrence of any of the following treatment-emergent adverse events within the primary MedDRA System organ classes (SOCs) of Skin or subcutaneous tissue disorders or

hepatobiliary disorders:

- Discontinuation of participant due to an AE (any severity, including SAEs)
- Adverse event in the primary MedDRA System organ class (SOC) of hepatobiliary disorders with severity 3 and above according to Common Terminology Criteria for Adverse Events (CTCAE v 5.0) regardless of discontinuation
- Discontinuation of participant due to an extreme lab parameter related to the liver or bile organ system:
    - ALT or AST >8xULN
    - ALT or AST >5xULN for more than 2 weeks
    - ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
    - ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
- Adverse event in the primary MedDRA System organ classes (SOC) of Skin or subcutaneous tissue disorders with severity grade 3 and above according to CTCAE v 5.0

Details of the CTCAE v5.0 can be found at the following link:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf.

When identified, AESIs should be entered into the EDC by the site PI, within 24 hours of the Investigator becoming aware that the event fulfils the criteria of an AESI, through the AE eCRF. A specific check box asking if the AE constitutes an AESI is to be completed. All additional information including seriousness and relatedness are also to be completed for AESIs. In completing the AESI check box the severity grading system will provide choices reflecting CTCAE conventions. This checkbox will also alert Medical Safety, who will follow-up with the site within 24 hours of awareness and complete a "Yes/No" field on the eCRF to indicate agreement or not with the site's characterization. In the event of a disagreement, additional information may be requested from the site to help adjudicate.

In addition, regardless of actual seriousness, a separate SAE/AESI Report Form is to be completed (within 24 hours after confirmation by Medical Safety in VIVA-MIND EDC) which should include the date the AE became an AESI (e.g., change in severity grading, discontinuation), and sent to the pharmacovigilance vendor CCI via fax or email.

11.4 Collection and Reporting of Serious Adverse Events

Following written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specific procedures. The reporting period ends 30 days (inclusive) after discontinuation of dosing. In addition, the investigator should report any SAE occurring after this time period that is believed to be related to study drug or protocol-specific procedures.

An SAE is an AE from this study that results in any of the following outcomes:

- Death
- Life-threatening situation (participant is at immediate risk of death)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect in the offspring of a patient who received study drug

- Considered significant by the investigator for any other reason

An SAE report should be completed on an SAE/AESI reporting form for any event where doubt exists regarding its status of seriousness.

If the investigator believes that an SAE is not related to the study drug but is potentially related to the conditions of the study (such as a withdrawal of previous therapy or a complication related to study procedure), the relationship should be specified in the narrative section of the SAE/AESI Report Form.

SAEs, whether related or not related to study drug, overdose, and potential drug induced liver injury must be reported to the ADCS and to the pharmacovigilance vendor, CCI, within 24 hours of the Investigator becoming aware of the event. For this study we will be capturing SAEs through the study EDC system for reporting to the ADCS and SPM[2]. For reporting to CCI, sites will be required to complete and submit an SAE/AESI report form via email or fax. Sites should refer to the Study Procedures Manual for further instructions on reporting an SAE to both the ADCS and CCI. In addition, all applicable SAEs must be reported by the Site Investigator to the IRB of record according to IRB's reporting requirements for such events.

The Site Investigator, or designated staff, is responsible for reporting all SAEs and all Other Important Medical Events to the ADCS and CCI, immediately or no later than 24 hours after awareness of the event. They must enter SAE information into the study EDC system (i.e., event term, start stop dates, causality, and severity) according to study-specific eCRF Completion Guidelines. The ADCS Medical Monitor, ADCS Project Director, and CCI will be notified via email upon entry of an SAE into the EDC system. The DSMB may at any time request additional information from the ADCS in relation to a reported event.

If only limited information is initially available, follow-up reports are required. If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent using the same procedure used for the transmission of the initial SAE and the same event term should be used. In addition, new or revised event information must be entered into the EDC and submitted on the SAE/AESI report form to CCI at the same time.

All SAEs should be followed to resolution or stabilization. For any questions relating to SAEs, please contact the ADCS Medical Monitor via telephone or email at the number listed on the protocol face page.

11.5 **Suspected Unexpected Serious Adverse Reactions**

Suspected unexpected serious adverse reactions (SUSARs) are SAEs that are not listed in the Investigator's Brochure as an expected AE and that the Sponsor and/or Investigator assesses as possibly related or probably related or definitely related to study drug or study procedure. United States 21 CFR 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidance or national regulatory requirements in participating countries require the reporting of SUSARs according to their regional specification to regulatory authorities. As the clinical trial sponsor, Vivoryon or a delegate of Vivoryon, is responsible for, and has procedures consistent with regulations that will be followed for, expedited reporting of SUSARs.

11.6 **Overdose Reporting Requirements**

For the purposes of this study, an overdose will be defined by any of the following criteria:

- Any dose of study drug greater than the highest daily dose within a calendar day **that results in an AE or SAE,** OR
- Any dose of more than 1600mg (11 tablets) per day for **more than a single day** irrespective of symptoms, OR
- any dose of more than 3600 mg (24 tablets) **in a single day,** irrespective of symptoms

Any AEs related to the overdose must be reported on the AE and/or SAE page of the eCRF. Signs and symptoms of overdose should be treated according to standard of care.

11. 7 **Clinical Laboratory Abnormalities and Other Abnormal Assessments**

Laboratory abnormalities are usually not recorded as AEs unless considered to be clinically significant by the site clinician. An abnormal laboratory result will be considered an AE if it is associated with clinical signs or symptoms, if the abnormality is of a degree that requires active management (e.g. discontinuation of the study drug, dose modification) or when the event is requiring treatment or other therapeutic intervention (e.g. iron supplements, blood transfusion, etc.).

The Investigator will evaluate the relationship of any significantly abnormal result to protocol treatment and clinical condition, if possible. All clinically significant abnormal laboratory results will be followed until they return to normal or become stabilized.

11. 8 **Pregnancy and Breast Feeding**

Varoglutamstat should not be used during pregnancy or while breast-feeding. The lower age limit for this study is 50 years, and women must be post-menopausal for at least 1 year or surgically sterile (bilateral tubal ligation, hysterectomy or bilateral oophorectomy) for at least 6 months prior to screening to be eligible for this study.

Male participants with female partners of childbearing potential must practice birth control (e.g. condoms, abstinence, etc.) during study treatment and until 28 days after the last dose of study drug.

12 STATISTICAL METHODS

This is a multi-center double-blind placebo-controlled trial of varoglutamstat treatment for approximately 72 weeks. Complete details of the safety, interim and final efficacy analyses will be provided in a separate SAP, which will be maintained by ADCS in consultation with Vivoryon.

12.1 **Phase 2A**

12.1.1 **Overview**

Phase 2A will randomize 180 subjects total 1:1 to treatment with varoglutamstat or placebo. Participants will be enrolled sequentially into one of three dose cohorts labeled A, B, and C, with n=30 active and n= 30 placebo for each dose cohort. There will be three varoglutamstat dose levels, 600 mg BID, 300 mg BID, and 150 mg BID. There is a titration period for dose levels 600 mg BID and 300 mg BID (see Section 6.5.1).

Each dose will be tested for safety and tolerability, using the first 30 participants enrolled at that dose in the active treatment arm, with a continuously monitored stopping boundary during the phase 2A dose selection period (8 weeks at full dose). This stopping boundary focuses on AESIs, a defined set of sensitive and specific AEs (see Section 6.9), based on preliminary data, and is designed to drop a dose very quickly in

case of low tolerability. [51] For example, if the rate of unacceptable AESIs is 20% for a given dose, then with probability 90% this dose will be stopped when (or before) 15 subjects on this dose are through the dose selection period.

In addition, comprehensive safety monitoring will be provided quarterly by the DSMB, with a more general focus on all expected and unexpected AEs, throughout phase 2A.

At the end of phase 2A, the optimal dose of varoglutamstat will be carried forward to efficacy studies in phase 2B, if the interim analysis is passed (the 'stage gate').
There will be an interim analysis for futility at the end of phase 2A, using two outcome measures, the ABC Score and EEG theta power. If the ABC Score measure records NO (indicating evidence of negative cognitive effects), the trial will stop. If both the ABC Score and the EEG theta power measures record YES (no evidence of negative cognitive effects, and positive evidence of benefit on the EEG measure), the trial will continue. Otherwise (i.e., in the case that the ABC Score measure records YES and the EEG theta power measure records NO), the stopping rule will be indeterminate, the trial will pause, and further analysis of additional measures as specified in the SAP may be undertaken prior to a final decision being reached by the overseeing SSC and the study sponsor.

12.1.2 **Safety stopping rule**

The safety stopping rule for a given dose will be assessed using data from the first 30 participants in the active treatment assignment to a given dose will be halted and the dose discontinued if an excessive number of participants in the active arm of the cohort experience a AESI within the dose selection safety reporting period, which is from the time of first dose to completion of 8 weeks at full dose. A Pocock sequential boundary, computed using the exact binomial distribution, [51] will be used to monitor the AESI rate for each dose. That is, in the active arm, out of the first 30 participants assigned to a given dose, when participant number k experiences an AESI reaches the end of 8 weeks at full dose, if the number of participants assigned to that dose who have experienced a AESI within the safety evaluation period is greater than or equal to bk, then the stopping rule will have been met (see **Table 6**). In this case the dose will be discontinued.

Table 6: Accrual will be halted to a dose cohort if the number of participants with a AESI is equal to or exceeds bk out of k participants.

Number of Participants, k	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_k	-	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4
Number of Participants, k	21	22	23	24	25	26	27	28	29	30										
Boundary, b_k	4	4	4	4	4	4	4	4	4	4										

This sequential boundary is equivalent to testing the null hypothesis, after each participant, that the event rate in the active arm of that dose cohort is less than or equal to 2.5%, using an exact one-sided level 0.0054 test. If the null hypothesis is rejected in favor of the alternative hypothesis that the event rate $\geq$ 2.5%, then

the stopping rule will have been met -accrual to that dose will be halted at that point, and the dose will be discontinued.

12.1.3 Dose Selection

If the first 30 subjects assigned to a given dose in the active arm complete 8 weeks at full dose without hitting the stopping boundary, that dose will be selected as safe. The highest dose selected as safe will be the dose selected by the phase 2A portion of the trial. The Pocock boundary is only valid until the first dose selection criterion is met (i.e., the highest safe dose is selected).

If a higher dose is selected as safe, assignment to lower doses will stop, and all current participants on a lower dose will be titrated up to the selected dose according to uptitration schedule. Future accrued participants will be assigned to the selected dose. "If more than one dose cohort is active, and a lower dose hits its stopping boundary, then all participants on a higher dose will be titrated down to the highest dose which has not yet been stopped.

Type I error: This stopping boundary is computed so that the probability of crossing the boundary and dropping the dose is at most 0.01667 when the rate of AESIs is equal to the acceptable rate of 2.5%. Hence, if all 3 cohorts have an acceptable AESI rate of 2.5% or less, all cohorts will continue with at least 95% probability, by the Bonferroni inequality.

Power to stop early: In case of an unacceptable AESI rate of 20%, the cohort will be stopped early with probability 90%. In this case the expected number of participants treated on that cohort is 15. With an unacceptable AESI rate of 10%, the probability of early stopping is 40%, with expected sample size 24 participants. A power table is included in Section 12.2.2.

Additional safety analyses: An exact lower-one sided 95% confidence interval for the AESI rate in each arm of each dose cohort will be computed, which adjusts for the sequential monitoring boundary.[51] Additional safety endpoints include all adverse events, physical exam, vital signs, health status, 12-lead ECG, laboratory determinations and participant withdrawals. Safety and tolerability data as well as demographic data will be summarized in tabular and/or graphical format for each treatment group. The incidence of all laboratory test abnormalities and the median changes from baseline will be tabulated by treatment regimen and time point. The safety population will be used for these analyses.

12.1.4 Interim Efficacy Analysis at the End of Phase 2A

There will be an interim analysis for futility at the end of phase 2A, using two efficacy outcome measures, the ABC Score and EEG theta power. There is a test of hypothesis for each measure, and a stopping rule which depends on the outcomes of these statistical tests.

12.1.4.1 Hypothesis Tests for the Stopping Rule

The ABC Score hypothesis test. The null hypothesis is that within participant change of ABC Score is equal between arms at 24 weeks, against the alternative that decrease is greater for active arm than for the placebo arm. Higher ABC Score is better; hence the alternative indicates harm from the drug. The test will be carried out at a one-sided 40% significance level, using a MMRM model as specified in the analysis plan below. Rejection of the null hypothesis will indicate a statistically significant harm from treatment, and a value of

NO will be recorded from this hypothesis test. Otherwise, a value of YES (no statistically significant evidence of cognitive harm) will be recorded from this hypothesis test.

The EEG theta power hypothesis test. The null hypothesis is that within participant change of theta power is equal between arms at 24 weeks, against the alternative that increase is greater for placebo than for the active arm. Higher theta power is worse; hence the alternative indicates benefit of the drug. The test will be carried out at a one-sided 5% significance level, using a MMRM model as specified in the analysis plan below. Rejection of the null hypothesis will indicate a statistically significant benefit of treatment, and a value of YES will be recorded from this hypothesis test. Otherwise, a value of NO (no statistically significant evidence of benefit) will be recorded from this hypothesis test. The mITT populations will be used for these analyses.

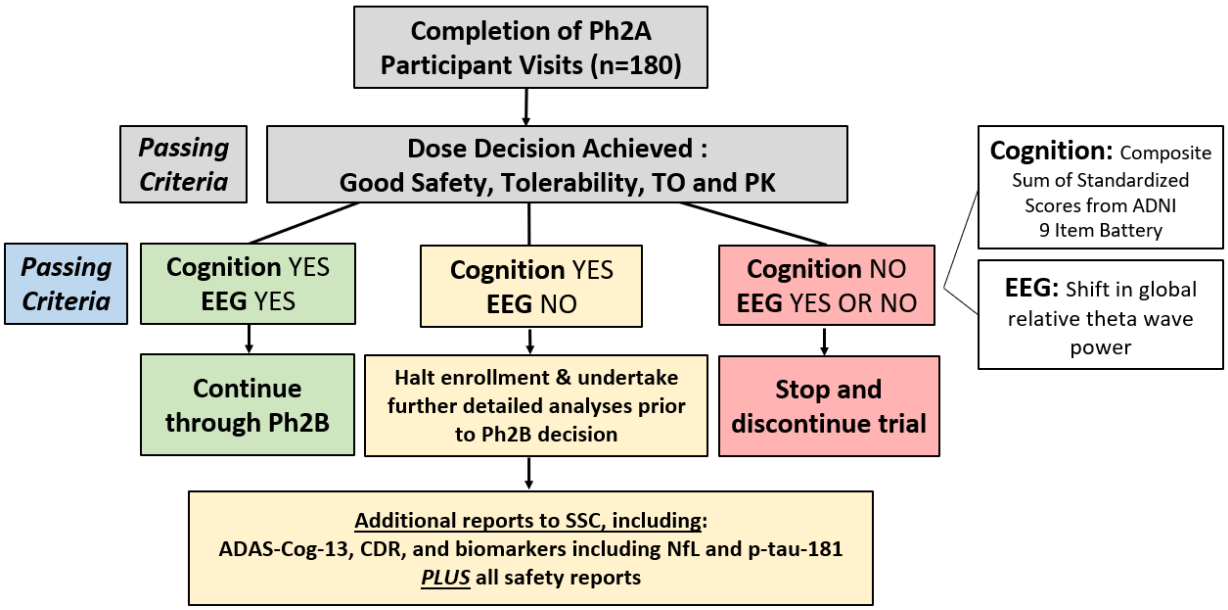
12.1.4.2 The Futility Stopping Rule

If the ABC Score statistical test records NO (indicating significant evidence of negative cognitive effects), the trial will stop. If both the ABC Score and the EEG theta power statistical tests record YES (indicating no significant evidence of negative cognitive effects on the ABC Score measure, and positive statistically significant evidence of benefit on the EEG measure), the trial will continue. Otherwise, the stopping rule will be indeterminate, the trial will pause, and the decision whether to continue the trial will be undertaken jointly by the SSC and the sponsor, as described elsewhere.

12.1.4.3 Scenarios and Operating Characteristics of the Stopping Rule

The characteristics stated here are justified below (**Figure 2**), where details of the implementation of the stopping rule are presented.

Figure 2: Framework of Phase 2A stopping rule



A. Power to stop the trial under evidence of negative cognitive effects. Given assumed sample sizes and using preliminary data as shown below, if the treatment group cognitive change is 19%

worse than the control arm at 24 weeks, the trial has at least 60% probability to stop. If the treatment arm change is 29% worse, the probability rises to at least 70%.

B. Power to continue under cognitive benefit, and EEG benefit. If the cognitive test shows about 40% improvement in the active arm compared to placebo, then the cognitive hypothesis test has more than 90% probability of resulting in a YES, as shown below. If the active arm shows about 45% benefit in the EEG theta power measure compared to the hypothesized placebo arm change, then the EEG hypothesis test has more than 90% chance of returning YES, as shown below from preliminary data. Hence under these assumptions the stopping rule has greater than 80% chance of giving a YES, YES result (assuming statistical independence) and continuing the trial.

C. Power to continue under EEG benefit, and no effect on cognition. If there is no difference between arms on cognitive change, then the cognitive hypothesis test has 60% chance of returning a value of YES (absence of negative cognitive effects). If the active arm shows about 45% less increase in EEG theta power than the hypothesized placebo arm change (a benefit to drug), then the EEG hypothesis test has 90% chance of returning YES. If the errors from the two tests can be treated as independent, then the stopping rule has about 54% ballpark power to proceed under these circumstance. Actual power should be greater, as explained below. In the SAPHIR trial, the active arm showed no change on the EEG theta power, while the placebo arm increased its theta power (resulting in positive EEG outcome).

D. Power to continue under cognitive benefit, and no EEG benefit. If there is no difference between arms on EEG theta power, so that prior results from the SAPHIR trial do not replicate, then there is only 5% chance that the EEG hypothesis test will return a YES. If there is cognitive benefit as in scenario B, the cognitive hypothesis test has 90% chance to return YES. Hence under this scenario, there is about 80% chance of an indeterminate result from the stopping rule.

12.1.5 Analysis Plan

For each of the two outcome measures, the primary analysis will use the within-subject change as the outcome in a mixed effects repeated measures model, using all available outcomes in the modified Intent-to-Treat (mITT) population, with no imputation for missing data. Fixed effects in the model will be *APOE* status (e4 carrier vs. non-carrier), MCI or mild AD dementia, site baseline measure of the outcome, baseline x visit interaction term, active treatment or placebo group, visit , and visit x treatment group interaction. Sites with 5 or fewer subjects will be pooled. The approach will be maximum likelihood. Visit will be treated as a continuous variable (a slopes analysis). The covariance structure will be subject-specific random slopes and intercepts. If the model fails to converge (at the default settings for the software used), then a random intercept model will be used. In case the model fails to converge after modification, model diagnostics will be used to investigate and take corrective action. The process used to arrive at the final model will be fully documented.

For each outcome measure, a one-sided Wald test of hypothesis will be conducted on the visit x treatment group interaction, at the stated significance level. The specific hypothesis tests are indicated above. As a sensitivity analysis, a one-sided two sample t-test at 24 weeks will also be conducted for each measure. Data and model estimates will also be presented graphically.

12.1.5.1 Sample Size, Power, and Operating Characteristics

Operating characteristics below are computed using a one-sided two sample t-test for the 24 week endpoint under the assumption of equal variances. However, in practice we will have additional data at weeks 48 and 72 on each measure for subjects who reach those time points, so the actual analysis will have greater power than presented here, if our technical assumptions are accurate. We assume 90 participants accrued

to each of active drug and placebo arms. We assume dropout to be 5% in the placebo arm and 10% in the active arm by 24 weeks treatment, for a final 24 week sample size of 86 and 81 respectively. Participants who are randomized but drop out prior to the start of study drug will be replaced.

12.1.5.2 Estimated Power for the EEG Theta Power Hypothesis Test

As preliminary data, we obtained theta power measures from 55 placebo arm and 47 active arm participants in the SAPHIR trial, in which participants were treated for 12 weeks with PQ912 (first week: 400 mg BID, thereafter 800 mg BID) or placebo. EEG measures were obtained at baseline and at 12 weeks. Means and standard deviations are below (Table 7).

Table 7: Means and standard deviations of EEG theta power measures in SAPHIR

	Placebo group (N=55)	PQ912 group (N=47)
EEG at Screening	0.16 (sd = 0.076)	0.17 (sd = 0.092)
EEG at 12 weeks	0.176 (sd = 0.082)	0.169 (sd = 0.088)
Within subject Changes	0.0162 (sd = 0.032)	-0.000698 (sd = 0.030)

We assume the mean placebo within-subject change at 12 weeks will roughly double over 24 weeks, to 0.032 units, and assume the standard deviation will be 0.032 units, as seen at 12 weeks. Then, under the given sample size a one-sided two sample t-test at 5% significance level using equal variances has 90% power to reject the null hypothesis of no difference in favor of a benefit to the drug, if the active arm change is 0.018 units, which is about 45% less than the hypothesized placebo arm change. Because in the SAPHIR trial, there was essentially no change in the active arm over 12 weeks, we think this is a reasonable effect size to anticipate.

Table 8: Estimated Power and Effect Size

Power	Effect size	Percentage of diff	Difference, tx - placebo	Control Change from screening	Treatment Change from screening
0.80	0.39	38%	-0.0123	0.03238	0.02
0.85	0.42	41%	-0.0132	0.03238	0.019
0.90	0.45	45%	-0.0145	0.03238	0.018

12.1.5.3 Estimated Power for the ADNI Battery Composite (ABC) Score Hypothesis Test

As preliminary data, we used 523 participants from ADNI 1 with either MCI or mild probable AD diagnosed (MMSE within 20-30) who completed the 9 neuropsychological measures at both baseline and 6 months. We computed the mean and standard deviation for each group separately (MCI or mild AD), and then computed a pooled mean and standard deviation assuming proportions of 40% MCI and 60% mild AD to match the anticipated trial enrollment. The resulting mean within subject change on the ABC Score in the ADNI data over 6 months was 1.015 with standard deviation 2.48, for a Cohen's D of -0.41.

Using these numbers we can construct a one-sided test of hypothesis with desired characteristics. We added Table 8 above for better understanding how to compute the probabilities. We will test the null hypothesis of no difference against the one sided alternative that active arm is doing worse than placebo, at a 40%

significance level. This significance level was arrived at as follows: we desire a test that will produce a YES result with 90% probability under the favorable alternative hypothesis of a 40% improvement in score for the active arm compared to placebo. Using the preliminary data, this corresponds to an active arm decrease of -0.6 points, which is a standardized difference of 0.4/2.48 = 0.16 Cohen's D effect size less change than placebo. Then a one-sided rejection region gives 10% probability of rejection if the true difference is 0.4 points in favor of active treatment, as desired. This led to a significance level under null hypothesis of 40%.

For this test at the 40% significance level, we have 70% power to reject the null and declare evidence of harm, and thus stop the trial, if the true change in treatment group is -1.31 points and a difference between placebo arm and active arm is -0.29, which is 29% worse than placebo. We have 60% probability to stop if the change is -1.20 points, which is 19% worse than placebo, at 24 weeks.

12.2 **Phase 2B**

12.2.1 **Overview**

The highest tolerated dose of varoglutamstat will be carried forward from phase 2A. Phase 2B is a multi-center double-blind placebo-controlled trial of varoglutamstat vs placebo in early AD over a treatment period of 72 weeks (18 months). The primary endpoint is within-subject change in CDR-SB, chosen because of its good sensitivity to show change over 2 years in similar study populations. [22] The accrued sample size is 414 participants (n=207 active, n=207 placebo). Total study duration (phases 2A and 2B) is expected to be conducted over 36 months, and no more than 25% drop out is anticipated.

The study has 80% power to detect an effect size of 0.7 points in CDR-SB. This is about 37% of the expected ~1.9 point mean change in CDR-SB in the placebo arm, assuming 40% of the enrolled study sample has MCI (expected mean change 1.2 points) [52] and 60% has mild AD dementia (expected 2.3-point change). [22] For additional perspective, if the observed effect of varoglutamstat on CDR-SB at the final analysis is 0.5 points, and if variances are similar to published data, [22] the final p-value would be about p=0.04. Details of the final analyses are given below, along with supporting computations.

12.2.2 **Analysis Plan**

Using approximately 207 participants per arm, this study is designed to have at least 80% power to detect a change of 0.7 points in CDR-SB, which is equal in magnitude to about 37% of the decline observed in the placebo arm at 18 months in a similar study population in ADNI. We assume a mean change in placebo arm in CDR-SB at 72 weeks of about 1.9 points (SD 2.28 points), assuming a 40/60 mixture of MCI (mean change 1.2 points) 3 and mild AD dementia participants (2.3 points). [22] The calculation assumes no more than a 25% dropout rate over the study period.

A power table is given below, computed in PASS v14. The computation uses a 2-sided 2-sample t-test at 5% alpha and 80% power, with equal standard deviations. While the final analysis uses a MMRM model rather than a t-test, our comparison of power computations using a MMRM and a t-test shows that both methods give comparable results, using the actual pattern of dropout in ADNI in this patient population.

40/60 MIC, AD		18 m placebo change	SD			
CDR-SOB		1.9	2.28			
Power Computation	treatment effect size, % of placebo change	18 m tx effect, CDR-SOB	cohen's d	n/arm, t-test	inflation for 25% dropout	total n
	0.25	0.48	0.21	330	440	880
	0.27	0.51	0.23	294	392	784
	0.35	0.67	0.29	171	228	456
	0.37	**0.70**	**0.31**	**155**	**207**	**414**
	0.40	0.76	0.33	133	177	355

12.2.2.1 **Analysis of Primary Endpoint**

The primary study hypothesis is that treatment with varoglutamstat will result in a reduction in participant change on total CDR-SB score relative to the placebo group at week 72 in the mITT population.

The primary analysis will use the within subject change in CDR-SB as the outcome in a mixed effects repeated measures model, using all available outcomes in the mITT population, with no imputation for missing data. Fixed effects in the model will be *APOE* status, MCI or mild AD dementia (mild AD: CDR global of 1 and MMSE > 20), site, baseline CDR-SB, treatment group, visit, visitx baseline score interaction, and visit x treatment group interaction. Visit is treated as a categorical variable.

The covariance structure will be specified as follows: a random effect will be included for subject. The within participant covariance will be unstructured. If needed, sites will be pooled (in order of enrollment, starting from minimum enrollment) so that there is a minimum of 5 participantss per site. If the model fails to converge, MCI or mild AD status will be removed from the model. If the model still does not converge, the following structures for the within-subject covariance will be fit, sequentially, until the structure is found that results in convergence of the model: Huynh-Feldt, Toeplitz, Autoregressive, and Compound Symmetry. Error degrees of freedom will be calculated using the Kenward-Roger approximation if an unstructured covariance structure is used ; otherwise, a sandwich estimator will be utilized to estimate the covariance structure and degrees of freedom will be calculated using the between-within method. In case the model fails to converge after all these modifications, model diagnostics will be used to investigate and take corrective action. The process used to arrive at the final model will be fully documented. The primary endpoint will be tested using model-adjusted least squares means at the week 72 visit. Point estimates, standard errors, two-sided 95% confidence intervals, and p-values will be presented.

12.3 **Safety Analysis**

Safety endpoints include AEs, physical exam, vital signs, health status, 12-lead ECG, laboratory determinations and participant withdrawals. Safety and tolerability data as well as demographic data will be summarized in tabular and/or graphical format for each treatment group. The incidence of all laboratory test abnormalities and the median changes from baseline will be tabulated by treatment regimen and time point. The safety population will be used for these analyses.

12.3.1 Adverse Events

AEs occurring after the start of study drug dosing at baseline (week 0) will be summarized descriptively for the safety population. All AEs will be coded according to system organ class (SOC) and preferred term (PT) using a Medical Dictionary for Regulatory Activities (MedDRA) dictionary. Summary tables showing the number of participants and percent within each category will be generated for each of the following types of AEs and its relationship to study treatment (related to study treatment):

- All events
- Serious events
- Deaths
- Events leading to withdrawal
- Severe events

12.3.2 Laboratory Parameters

Laboratory parameters will be summarized by visit. Frequencies of high and low values with respect to the normal range will be displayed, as will shift tables comparing each treatment visit and baseline visit by time point and treatment group.

12.3.3 Other Safety Parameters

Vital signs and ECG parameters will be summarized across groups by visit using descriptive statistics, and at each outcome visit and at end of study.

Physical examination findings and number of participants will be summarized as the count and percentage of participants by eCRF pre-defined categories at last visit. Concomitant medications will be summarized by treatment group, drug class and PT. Vital signs will be summarized by visit using descriptive statistics.

Overall interpretation results for ECGs and the Investigator interpretation results are collected as normal, abnormal not clinically significant, and abnormal clinically significant. Participants whose interpretation shifts from normal to abnormal will be listed separately including description of the abnormality and any associated comments.

12.4 Analysis Populations

Analysis populations are defined as follows:

- The Safety population will include all randomized participants who took at least one dose of the study drug.
- The Intent-to-Treat (ITT) population will include all randomized participants.
- The mITT population for each efficacy measurewill include all randomized participants who took at least one dose of the study drug, and who have a baseline assessment and at least one follow up assessment of the measure.
- Pharmacokinetic (PK) population will include all randomized participants who have at least one PK sample timepoint.

The primary population for all efficacy analyses is the mITT population. Sensitivity analyses will be performed using the mITT population with multiple imputation. .

The safety population will be used for analyses of safety endpoints.

12.5 Protocol Deviations, Data Blind Review, and Unblinding

Classification of deviations from the protocol as minor or major, and decisions regarding exclusion of participants and/or participant data from the statistical analyses, will be decided on a case-by-case basis without knowledge of the treatment assigned and before the database lock (Data Blind Review). After database lock, the responsible statistician will request the treatment codes, the study will be unblinded, and the statistical analysis will be conducted.

13 RECORDING AND COLLECTION OF DATA

13.1 Case Report Form

The Investigator or designee will record all data collected on the eCRF provided for that purpose. The site will be suitably trained on the use of the eCRF and appropriate site personnel will be provided electronic signature privileges.

All site entries will be made in a secured web site and the Principal Investigator will review the record for completeness. Upon completion of the review, the PI will sign electronically in the signature page of the eCRF.

The Investigator or designee will make necessary eCRF corrections. The investigator must authorize the corrections to the entered data on eCRF.

Completed eCRFs will be submitted according to the ADCS's eCRF Completion Guidelines and reviewed by the ADCS to determine their acceptability. If necessary, data correction requests will be generated for resolution by the study site.

13.2 Study Files and Patient Source Documents

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Authorized representatives of the sponsoring institution may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number to maintain confidentiality.

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include Investigators' Study Files, original participant clinical source documents generated at the study site or completed source document worksheets provided by ADCS. The term "original" means the first recording of the data.

The Investigator will ensure the site master files are maintained, including the study protocol and its amendments, IRB and regulatory approvals with associated correspondence, informed consents, study drug records, staff curriculum vitae, all correspondence, and other appropriate documents.

Participant clinical source documents may include, but are not limited to, participant hospital/clinic records, physicians' and nurses' notes, appointment books, laboratory reports, ECGs, MRI images, pathology and special assessment reports. The Investigator must assure that all original source documents are available to support monitoring activities.

13.3 Rater Training

Site staff will be trained on the assessments and certified on the study specific cognitive, functional and behavioral measures as described in Section 9. The sites will have training at the Investigator meeting and possible online training and didactic training as needed. Throughout the trial the site staff administering the assessments may have limited scales audiotaped in order to ensure ratings are being consistently administered across sites. Scales will be collected on paper and entered via the EDC. Details will be provided with the rater training instructions and in the Cognitive Assessments Manual.

13.4 Monitoring

During the study each site will be monitored at regular intervals by an ADCS clinical research monitor, through a combination of on-site visits and remote monitoring procedures. The monitoring visits must be conducted according to the applicable ICH and GCP guidelines to ensure protocol adherence, quality of data, drug accountability, compliance with regulatory requirements and continued adequacy of the investigational site and its facilities. The Investigator will co-operate in the monitoring process by ensuring the availability of the eCRFs, source documents and other necessary documents at the time of monitoring activities and by prompt attention to any matters brought to his/her attention by the monitor.

13.5 Audit and Inspection

ICH GCP guideline require independent audit of clinical program activities. Such audits may be performed at any time before, during and/or after the study.

In addition, a representative of a national regulatory agency may choose to inspect a study site at any time prior to, during, or after completion of the clinical study.

The Investigator and study staff are responsible for maintaining the site master file containing all study-related regulatory documentation as outlined by ADCS Regulatory Affairs that will be suitable for inspection at any time by ADCS, Vivoryon, its designees, and/or regulatory agencies. The Investigator understands and agrees to give access to the necessary documentation and files.

13.6 Retention of Data

All records connected with this clinical study will be retained for at least two years following the date of an approved marketing application [21 CFR 312.62(c)] for the study drug for the indication for which it is being investigated; or if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified; or for at least 3 years following study termination, whichever is longer. Prior to record disposal, ADCS and Vivoryon may elect to extend the retention period. To ensure that these standards are applied, written permission must be granted from Vivoryon before record disposal. All local laws regarding retention of records must also be followed. Study sites are required to retain all records until written notification allowing destruction is received from the ADCS.

13.7 Reporting of Study Results

The ADCS and the ADCS Principal Investigator will provide annual reports and a final report on the trial to the National Institutes of Health (NIH) as a responsibility of holding its grant funding. Vivoryon will produce an integrated Clinical Study Report for their development of varoglutamstat and their regulatory responsibilities as the IND holder.

13.8 Quality Assurance/Quality Control

ADCS Standard Operating Procedures (SOPs) will be adhered to for all activities relevant to the quality of the study. Documentation of all quality control procedures will be outlined in the Data Management Plan (DMP). Edit checks and listings will be run and used in conjunction with the eCRF pages to support a clinical review of the data. Documentation of all quality control procedures will be outlined in the DMP.

14 DATA SAFETY MONITORING BOARD

The DSMB, which is independent from the ADCS, and which has reporting to the NIA through its project scientist, will provide safety oversight of the trial. It has a standing membership of a chair, and 3 other experts in the field, who have the appropriate background for these responsibilities. Other members may be added depending on the needs of the trial.

The DSMB will meet quarterly as well as ad-hoc in the face of any important safety matters arising. The DSMB will also be informed of SUSARs as they are being reported to regulatory authorities and Investigators. In this trial, they will provide recommendations following each of their meetings and deliberations to the SSC. Based on the review of safety data, the DSMB will make recommendations regarding the conduct of the study to the SSC. These may include continuing the study as designed, amending safety monitoring procedures, modifying the protocol or the informed consent form, or recommending the termination of the study. It is anticipated that the DSMB will holistically evaluate safety signals, in making its recommendation to the Study Steering Committee. There will be a study-specific appendix to the DSMB charter developed for this trial detailing additional DSMB roles and responsibilities for this protocol.

14.1 Additional Phase 2A Safety Monitoring

A formal safety monitoring boundary for the phase 2A dose selection is described throughout this protocol. During phase 2A, the DSMB will review the status of this safety monitoring boundary at each quarterly meeting, which will be included in the regular DSMB report (until the highest safe dose is selected). It is anticipated that the DSMB will be meeting to evaluate each completed dose arm, or earlier if the safety biostatistician identifies that the safety monitoring boundary has been reached or exceeded. Additional details of their responsibilities and review are detailed in the DSMB Charter.

14.2 Additional Phase 2B Safety Monitoring

DSMB will continue to meet regularly, at quarterly meetings during phase 2B to review safety and concerns arising. Additional details of their responsibilities and review are detailed in the DSMB Charter.

15 STUDY STEERING COMMITTEE

The SSC will include the following: a) one designated Chair who is a senior, well-respected leader in the field; b) three other expert members in the field; c) one Sponsor representative; and d) the NIA project scientist as an ex officio member The SSC will be responsible for receiving and considering all recommendations from the DSMB and for taking all final decisions on behalf of the trial, including the stage-gate decision for continuing to phase 2B in cases of indeterminate interim analysis results according to defined study stopping rules. While the stage-gate criteria are pre-specified, the SSC will have the opportunity to consider all available data with adjustment of their decision based on the specific circumstances that may arise. They will have the necessary trial expertise across disciplines including clinical, trial methodology, and statistics. They will be supported by the unblinded safety statistician throughout the trial. It is expected that SSC members may be fully unblinded as need arises during the trial, with the exception of the SSC Sponsor representative, who will remain blinded. In the event that unblinding of study data is required, non-Sponsor SSC members will provide a recommendation to the study sponsor that will preserve their blinded status. All members will provide active disclosures throughout the trial and will otherwise remain at arms-length from the trial at all times. There will be a charter developed for the SSC for this trial detailing its roles and responsibilities. The chair of the SSC will be responsible for keeping trial leadership including the PI/PD informed of significant trial recommendations or decisions so that appropriate protocol amendments, safety updates, and potential communication to participants and IRBs can be timely generated and distributed.

16 PUBLICATIONS POLICY AND SHARING OF DATA

ADCS, in collaboration with Vivoryon, will publish the study results in accordance with the 2010 CONSORT guidelines [53]. See Appendix IV for the CONSORT Checklist and Flowchart.

As there are expected to be too few participants studied at each site for individual site's results to be statistically valid, the results of this study will be disclosed or published only in combined form based upon the statistical analysis performed by ADCS and Vivoryon and will be coordinated by ADCS and Vivoryon. No disclosure of study results will be permitted except as specified in a separate, written agreement between Vivoryon and the Investigator. This study will be registered at www.ClinicalTrials.gov after approval of the designated IRB and prior to enrollment of the first participant, as required for publication by the International Committee of Medical Journal Editors (ICMJE).

Results will be posted on clinicaltrials.gov in accordance with requirements.

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18. APPENDICES

18.1 APPENDIX I – STUDY PLAN AND PROCEDURES AT EACH VISIT

Visit Number	1	2	3		4	5	6	7	8	9	10	11 EOT/Early Term	12 Post Tx Safety Follow Up[17]
Study Visit Time Point	Screening (-90 d)	Baseline (Week 0)	Wk 4 (±7 d)		Wk 8 (±7 d)	Wk 12 (±7 d)	Wk 16 (±7 d)	Wk 24 (±7 d)	Wk 36 (±7 d)	Wk 48 (±7 d)	Wk 60 (±7 d)	Wk 72 (±7 d)	Wk 76 (±7 d)
Informed Consent	X												
Eligibility Review	X	X											
Randomization[1]		X											
Med History/Demographics	X												
Modified Hachinski Ischemic Scale	X												
Weight & Height[2]	X	X	X		X	X	X	X	X	X	X	X	X
Physical Examination	X	X	X		X	X	X	X	X	X	X	X	X
Neurological Examination	X												
Vital Signs[3]	X	X	X		X	X	X	X	X	X	X	X	X
Concomitant Medication	X	X	X		X	X	X	X	X	X	X	X	X
Adverse Events[4]	X	X	X		X	X	X	X	X	X	X	X	X
12-lead ECG (resting)	X							X				X	
B12 and folate (blood tests)	X												
Clinical Safety Blood Tests[5]	X	X	X		X	X	X	X	X	X	X	X	X
Hemoglobin A1c (HbA1c)	X						X	X		X		X	X
Testosterone (blood test)	X						X	X		X		X	X
Thyroid Function (TSH, T4, T3)	X						X	X		X		X	X
Urinalysis[6]	X	X			X		X	X	X	X		X	X
Infectious Disease Serology[7]	X												
APOE (blood test)		X											
HLA (blood test)		X											
CYP2C19 (blood test)		X											
PrecivityAD® biomarker assays	X												
Blood Collection for Biobanking	X	X		X	X		X[8]	X		X		X	
Blood Collection for PQ912 levels[8]				X	X		X	X		X		X	
Blood Collection for QC in Serum[8]	X			X	X		X	X		X		X	

Visit Number	1	2		3	4	5	6	7	8	9	10	11 EOT/Early Term	12 Post Tx Safety Follow Up[17]
Study Visit Time Point	Screening (-90 d)	Baseline (Week 0)		Wk 4 (±7 d)	Wk 8 (±7 d)	Wk 12 (±7 d)	Wk 16 (±7 d)	Wk 24 (±7 d)	Wk 36 (±7 d)	Wk 48 (±7 d)	Wk 60 (±7 d)	Wk 72 (±7 d)	Wk 76 (±7 d)
Cranial MRI[9]	X							X				X	
Lumbar Puncture (LP) for CSF biomarkers[10]	X							X				X	
Post-LP Safety Telephone[11]	X							X				X	
Columbia-Suicidality Severity Rating Scale (C-SSRS)	X	X		X	X	X	X	X	X	X	X	X	
MoCA	X							X		X		X	
MMSE	X							X		X		X	
CDR	X					X		X		X		X	
ADAS-Cog-13		X				X[14]		X	X	X	X	X	
FAQ		X						X		X		X	
ABC - Category Fluency		X						X		X		X	
ABC - Trail Making Test A & B		X						X		X		X	
ABC - Digit Symbol Substitution		X						X		X		X	
ABC - Boston Naming Test		X						X		X		X	
ABC – RAVLT (Immediate & Delayed)		X						X		X		X	
ABC – Number Span Forward & Backward		X						X		X		X	
NPI		X						X		X		X	
Quantitative EEG[12]		X						X				X	
Research Satisfaction Survey		X						X		X		X	
Dispense Study Drug[13]		X		X	X	X	X	X	X	X	X		
Study Drug Instruction Phone Call[15]				X									
Study Drug Accountability				X	X	X	X	X	X	X	X	X	
Treatment Blinding Questionnaire[16]												X	

1 Randomization must occur at the baseline visit after eligibility is confirmed.

2 Height is done at screening only.

3 Vital signs include sitting blood pressure, pulse, temperature, and respiration rate.

4 The reporting period for all AEs and SAEs starts at the screening visit (i.e. when the patient or LAR signs consent). The end of the reporting period for both SAEs and AEs is 30 days after the study drug has been discontinued.

5 Clinical Safety Laboratory Tests will be performed by a Central Laboratory. Assessment includes the following: Hematology (hemoglobin, hematocrit, platelets, RBC, WBC, differential count, and absolute neutrophil count), Chemistry (sodium, potassium, chloride, calcium, ALT, AST, LDH, alkaline phosphatase, GGT, phosphorus, bicarbonate, CPK, total protein, albumin, indirect bilirubin, direct bilirubin, total bilirubin, glucose, creatinine, BUN, uric acid , total cholesterol, LDL, HDL, triglycerides).

6 Urinalysis to include: pH, specific gravity, protein, glucose, ketones, urobilinogen, bilirubin, blood, leucocytes and nitrite

7 Infectious disease serology includes the following: Human Immunodeficiency Virus (HIV), Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and syphilis.

8 A blood sample to measure varoglutamstat (PQ912) level in plasma (PK) should be collected at week 4, week 8, week 24, week 48, and week 72 for all participants. A blood sample to measure glutaminyl cyclase (QC) in serum should also be collected at screening, week 4, week 8, week 24, week 48, and week 72 for all participants. During phase 2A, Cohorts A and B will also have a blood sample drawn at week 16 (at least 8 weeks at the originally assigned full dose).

Participants will have blood drawn just prior to their morning dose at each of these PK timepoints, and again between 2 and 6 hours after this routine morning dose. Date and time of study drug intake on the day of visits and day prior should be collected on source document worksheets, along with time of last meal, for entry into the EDC system. PK (plasma) samples to measure varoglutamstat level should be drawn at the time of lumbar puncture at week 24 and week 72. Date and time of study drug intake on the day of lumbar puncture should be collected on source document worksheets, for entry into the EDC system.

9 All MRIs must be performed per imaging protocol and must use the same scanner throughout study.

- **Screening MRI:** if a patient has **not** had an MRI performed within 6 months of screening (i.e. within 6 months from the date of informed consent), then an MRI must be performed as part of the screening requirements for this study, per the imaging protocol, and should be one of the last screening procedures performed to determine final eligibility in order to prevent patients from undergoing unnecessary MRIs. If a patient **has** had an MRI within 6 months of screening (i.e. within 6 months from the date of informed consent) **but** the MRI does not follow the study-specific imaging protocol, that MRI can be used to help determine eligibility; however, another MRI must be performed per the imaging protocol, and must occur as close to, and prior to, the baseline visit, after all other eligibility criteria have been confirmed.
- **Week 24 MRI:** the protocol window for MRI at week 24 is 7 days before and up to 7 days after the week 24 visit time point. If a participant is terminating early at 36 weeks or after, obtain MRI. If MRI is performed on the same day as a lumbar puncture at week 24, the MRI must be conducted before the lumbar puncture. Otherwise, at least a 3-day window between MRI and the lumbar puncture is required.
- **Week 72 MRI:** the protocol window for MRI at week 72 is 14 days before and up to14 days after the week 72 visit time point. If a participant is terminating early at 36 weeks or after, obtain MRI. If MRI is performed on the same day as a lumbar puncture at week 72, the MRI must be conducted before the lumbar puncture. Otherwise, at least a 3-day window between MRI and the lumbar puncture is required.

10 Visit windows for CSF are: up to 90 days prior to first dose of study drug and within 7 days of the week 24 and 72 study visit. If a patient is terminating early, they do not need to undergo lumbar puncture. Sites should make every attempt to complete screening procedures within a period of 60 days from the time of informed consent. However, sites will be allowed a period of up to 90 days from informed consent if additional time is needed to complete the lumbar puncture and receive CSF AD biomarker results.

11 Post lumbar puncture safety follow up telephone call must occur 1 to 3 days after the lumbar puncture is performed.

12 Quantitative EEG is required for all participants in phase 2A, and substudy participants only in phase 2B. This procedure should be performed by qualified study personnel. The qEEG data will be de-identified and sent to a blinded, third party vendor for central review and analysis. If the quality of the baseline qEEG is deemed unacceptable by the third-party vendor, then the participant will be withdrawn from the substudy and the data will be excluded from the substudy analysis.

13 Participant will be instructed to take the first dose in the morning of the following calendar day.

14 The ADAS-Cog-13 conducted at week 12 will be used as part of the quarterly DSMB review of cognitive safety.

15 Participants will receive a phone call during from site study personnel in order to assess study drug tolerability. If tolerable, then the participant will be instructed to proceed with dose escalation at the cohort's respective time point.

16 Treatment blinding questionnaire to be administered to site PI and Raters.

17 Participants who terminate the study early will undergo a Post-Treatment Safety Follow-Up visit 4 weeks (± 7 days) following their Early Termination visit.

Please note: Unscheduled visits should generally follow the same schedule of events as the Week 4 safety visit. Please consult with ADCS Clinical Operations for further guidance as needed.

18.2 APPENDIX II – NIA/AA DIAGNOSTIC GUIDELINES

Mild Cognitive Impairment (MCI) due to AD [54]

Establish clinical and cognitive criteria:

Cognitive concern reflecting a change in cognition reported by participant or informant or clinician (i.e., historical or observed evidence of decline over time);

Objective evidence of impairment in one or more cognitive domains, including memory (i.e., formal or bedside testing to establish level of cognitive function in multiple domains);

Preservation of independence in functional abilities;

Not demented.

Examine etiology of MCI consistent with AD pathophysiological process:

Rule out vascular, traumatic, medical causes of cognitive decline, where possible;

Provide evidence of longitudinal decline in cognition, when feasible;

Report history consistent with AD genetic factors, where relevant.

Mild Probable AD [55]

Meets core clinical criteria for all-cause dementia. Dementia is diagnosed when there are cognitive or behavioral (neuropsychiatric) symptoms that:

Interfere with the ability to function at work or at usual activities; and

Represent a decline from previous levels of functioning and performing; and

Are not explained by delirium or major psychiatric disorder;

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.

The cognitive or behavioral impairment involves a minimum of two of the following domains:

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.

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.

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.

Impaired language functions (speaking, reading, writing)—symptoms include: difficulty thinking of common words while speaking, hesitations; speech, spelling, and writing errors.

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 MCI 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.

The differentiation of mild versus moderate AD is determined by CDR and MMSE. Those with scores of CDR global >1 or MMSE <20 will be considered to have moderate disease and will be ineligible. Those with CDR global of 1 and MMSE ≥20 will be considered to be mild AD per protocol.

Meets criteria for dementia as described above, and in addition, has the following characteristics:

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

- **Amnestic presentation:** Amnestic presentation includes impairment in learning and recall of recently learned information. There should also be evidence of cognitive dysfunction in at least one other cognitive domain, as listed under the criteria for dementia provided above.
- **Nonamnestic presentations:**
    i. Language presentation: the most prominent deficits are in word-finding, but deficits in other cognitive domains should be present
    ii. 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

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 apahsia; or (e) evidence of another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

18.3 APPENDIX III – PQ912 LEVEL, QC ACTIVITY, CALCULATED TO

Measuring PQ912 levels in plasma and CSF

1. Measurements of PQ912 in plasma

PQ912 levels and main metabolite levels in plasma and CSF will be determined using a validated LC-MS/MS method. These measurements will be done by CCI [redacted].

A blood sample to measure PQ912 concentration in plasma will be collected at week 4, week 8, week 16 (cohorts A and B only), week 24, week 48 and week 72 for all participants. Blood samples at week 24 and week 72 should be drawn at the time of lumbar puncture. Date and time of lumbar puncture and blood collection and date and time of study drug intake on the day of visits and day prior should be documented.

Blood collection should be done just prior to the routine morning dose and again between 2 and 6 hours after routine morning dose at each sampling timepoint.

a. CCI [redacted]

1. Supportive criteria at the end of Phase 2A

In phase 2A, a minimal target occupancy of QC present in CSF of 50% (as a surrogate for brain TO) will be used as go-no-go criteria supportive information on dose decision in the 2A dose optimizing part of this study.

CCI [redacted]

CCI [redacted]

b. PQ912 plasma levels at 4, 8, 16, 24, 48 and 72 weeks

Plasma levels at 4, 8, 16, 24, 48 and 72 weeks will be measured to monitor that there is adequate PQ912 exposure during the remainder of the study. Furthermore, the plasma levels at weeks 24 and 72 will be used as an exploratory parameter to control the PQ912 plasma/CSF ratio and predict possible treatment effects.

2. Measurements of PQ912 in CSF

PQ912 levels in CSF will be measured in the CSF samples taken at week 24 and the end of treatment (EOT, week 72). It is important that the participants are still taking study drug at the time of the week 24 and week 72 lumbar puncture.

a. Calculation of brain (CSF) exposure and estimation CSF TO

CCI [redacted]

Measuring Glutaminyl cyclase activity in CSF

1. Short method description

Measurement of glutaminyl cyclase activity in CSF will be done by CCI [redacted]

CCI [redacted]

CCI

[redacted]

2. Measurements of QC activity in CSF

QC activity in CSF will be assessed during the screening visit, week 24, and at the end of treatment visit (week 72).

Relative QC activity at EOT or % inhibition in the assay is calculated by normalizing EOT activity to the QC activity at screening (without inhibitor) of the same participant.

Equation 4:

$$rEA(\%) = \frac{EA_{EOT}}{EA_{screening}} * 100$$

Equation 5:

$$rInh(\%) = 100 - rEA$$

b. Assessing QC activity in CSF as exploratory endpoint

QC activity in CSF at screening, week 24, and at EOT/week 72 and the respective relative QC activity at EOT/week 72 will be used as secondary endpoint to verify that the QC is inhibited in the CSF of the treatment group.

Furthermore, these QC activities can be used as exploratory biomarkers to answer questions like the following:

- Is there a correlation between QC activity and disease severity?
- Is QC activity a prognostic factor in AD?
- Is QC activity or relative inhibition a predictive factor for PQ912 treatment?
- Does QC activity correlate with other exploratory biomarkers?

18.4 APPENDIX IV – CONSORT CHECKLIST & DIAGRAM (v2010)

Section/Topic	Item No	Checklist item	Reported on page
Title and abstract			
	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	

	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	

Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

CONSORT Flow Diagram (v2010)