PATH-1 STUDY PROTOCOL

A PHASE 1b DOSE RANGE FINDING STUDY OF PHENSERINE COMPARED TO DONEPEZIL IN PARTICIPANTS WITH EARLY OR MILD ALZHEIMER'S DISEASE

Short Title: Phenserine on the **A**lzheimer's **T**reatment **H**orizon, Study 1
Acronym: PATH-1
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Approval Date:

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10.12.2024

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Sponsor functions including Medical Monitors, Coordinating Investigator, and Principal Investigator Names and Contact Information can be found in Appendix 11

Protocol Amendment Summary of Changes Table

DOCUMENT HISTORY	
Document	**Date**
Protocol, protocol version 1.2	**09-Dec-2024**
Protocol, protocol version 1.1	25-Nov-2024
Original Protocol, protocol version 1.0	12-Sep-2024

Revised protocol version 1.2 (09-Dec-2024)
The version 1.2 of the PATH-1-protocol includes all changes introduced within the scope of the request for information RFI-CT-2023-510282-10-00-IN-004 (part I) from the Norwegian Medical Product Agency (DMP) from the 04 December 2024.

Revised protocol version 1.1 (25-Nov-2024)
The version 1.1 of the PATH-1-protocol includes all changes introduced within the scope of the request for information RFI-CT-2023-510282-10-00-IN-002 (part I) and -003 (part II) from the Norwegian Medical Product Agency (DMP) and Regional Committees for Medical and Health Research Ethics (REK) from the 11 November 2024.

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Tables and figures

List of Abbreviations

Abbreviation	Definition
Aβ	Amyloid-beta
Aβ1-40	Amyloid-beta 1-40
Aβ1-42	Amyloid-beta 1-42
AChE	Acetylcholinesterase
AChEI	Acetylcholinesterase Inhibitor
AD	Alzheimer's Disease
ADL	Activities of Daily Living
AE	Adverse Event
ALT	Alanine Aminotransferase
APP	Amyloid Precursor Protein
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BDNF	Brain-Derived Neurotrophic Factor
bd	Twice daily (bis die)
CI	Coordinating Investigator
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CSSRS	Columbia-Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
CYP3A4	Cytochrome P450 3A4
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EV	Eudravigilance
FDG-PET	Fluorodeoxyglucose Positron Emission Tomography
FLAME	Factors of Longitudinal Attention, Memory, and Executive Function - validated computerized tests
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
IB	Investigator’s Brochure
IEC	Independent Ethics Committee
INR	International Normalized Ratio

Abbreviation	Definition
IRB	Institutional Review Board
IUD	Intrauterine Device
IUS	Intrauterine System
MCI	Mild Cognitive Impairment
MoCA	Montreal Cognitive Assessment
Nfl	Neurofilament Light Chain
NIA	National Institute on Aging
NIH	National Institutes of Health
od	Once daily (omni die)
PD	Pharmacodynamic
PET	Positron Emission Tomography
PK	Pharmacokinetic
PPI	Patient and Public Involvement
PROTECT	An online cohort study platform
P-Tau	Phosphorylated Tau
p-tau	Phosphorylated Tau
p-tau217	Phosphorylated Tau 217
qds	Four times a day (quater die sumendus)
RCT	Randomized Controlled Trial
rCBF	Regional Cerebral Blood Flow
rCMRGlc	Regional Cerebral Metabolic Rate for Glucose
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TDS	3 times a day (ter die sumendum)
Tmax	Time to Maximum Concentration
t1/2 elim	Half-Life of Elimination
ULN	Upper Limit of Normal
WHO	World Health Organization
WOCBP	Woman of Childbearing Potential

1. Protocol Summary

In this open-label Phase 1b study, eligible participants with early or mild Alzheimer's disease (AD) will be randomized 6:2 to receive phenserine or donepezil for a total treatment duration of 8 weeks. Participants will dose escalate at 2- to 4-week intervals (depending on their treatment assignment) to achieve a maintenance dose level that is well tolerated. The objectives of the study are to characterize phenserine's pharmacodynamic activity in comparison to donepezil as evaluated by changes in exosome biomarkers and effects on cognition. In addition, safety, tolerability and pharmacokinetic assessments will be evaluated. The data from the study will be used to select an appropriate dose range for a subsequent Phase 2 proof-of-concept study to characterize phenserine's efficacy and safety in people with early or mild Alzheimer's Disease (AD) over a longer treatment duration.
The protocol is further summarized in the following synopsis and in the study schema that is presented in Figure 1. In addition, a detailed Schedule of Activities is presented in Table 1.

1.1. Synopsis

Protocol Title:
A phase 1b dose range finding study of Phenserine compared to Donepezil in participants with early or mild Alzheimer's disease.

Brief Title: Phenserine on the Alzheimer's Treatment Horizon, Study 1

Participants will be randomized into two groups: one group will receive phenserine, and the other will receive donepezil. The phenserine group will begin with a dosage of 5 mg twice daily, with the dose being gradually increased every two weeks as tolerated until the maximum dose of 10 mg three times daily is reached. The donepezil group will start at 5 mg once daily, with the possibility of escalation to 10 mg once daily at Week 4, again aiming for a 45% cholinesterase inhibition as tolerated.
The study is designed to last 8 weeks, with a planned total enrollment of 16 individuals from various centers across Norway. Participants will return for follow-up visits every two weeks, during which pharmacodynamic, pharmacokinetic, and safety assessments will be conducted. The final safety follow-up will occur after the completion of dosing for those who complete the study. Early termination visits will include a comprehensive safety follow-up for those who discontinue the study prematurely.

The primary objective of this study is to assess the effects of phenserine compared to donepezil on exosome biomarkers of cell death in individuals with early or mild AD. Additionally, the study aims to evaluate the safety and tolerability profile of phenserine at ascending doses up to 10 mg three times daily (TDS) in comparison to donepezil at doses up to 10 mg once daily (OD). Furthermore, the study aims to analyze steady-state blood levels of phenserine to characterize and compare dose-response relationships for pharmacodynamic outcomes and key safety assessments. Finally, the study will explore changes in specific biomarkers of Alzheimer's Disease (AD) in cerebrospinal fluid (CSF) and blood plasma, as well as assess phenserine's potential short-term effects on cognition using the FLAME Memory Composite and other cognitive sub-tests.

Participants will be enrolled at six centers across Norway. The study will also be supported by the PROTECT platform, which allows for the recruitment of individuals over 50 years of age who have demonstrated cognitive decline, making them suitable candidates for this study.

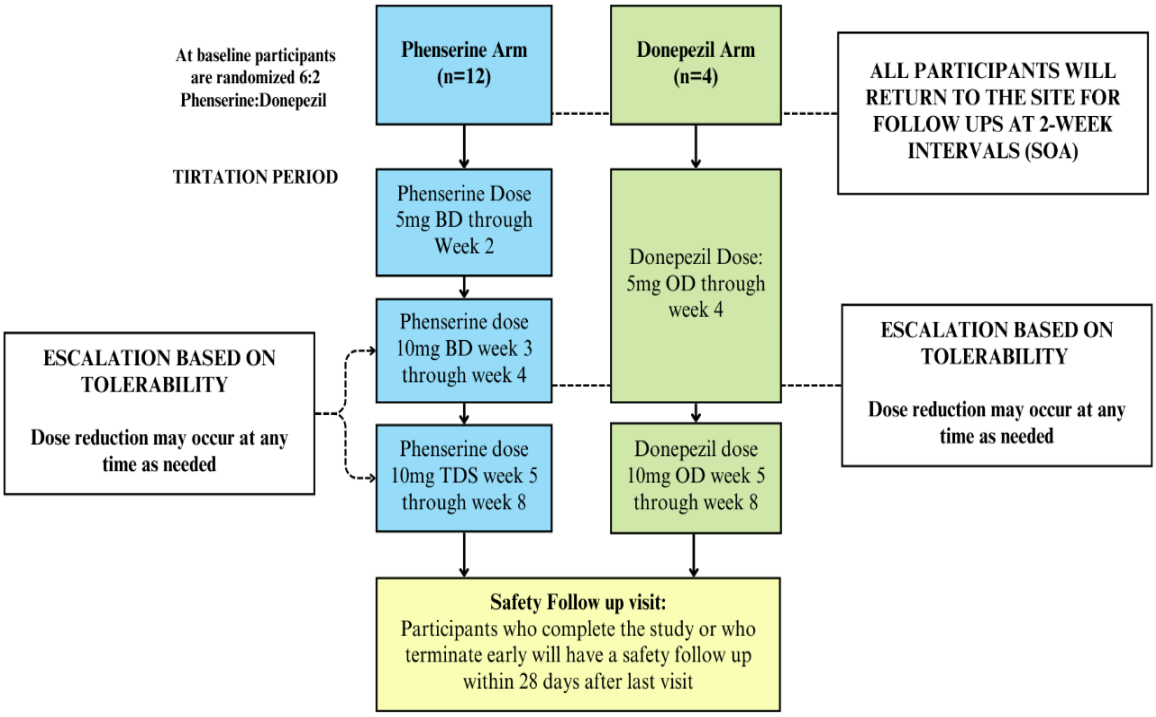
The anticipated duration of participation for each patient is 8 weeks, with the overall study expected to be completed within 9 months, accounting for a 6-month enrollment period followed by the treatment phase.

Study Population and Statistical Methods
A total of 16 participants are expected to be enrolled in this dose-ranging study. The primary population for analysis will be a modified intent-to-treat group, including all participants who received at least one dose of study medication and provided at least one follow-up exosome sample.

This study will provide critical data on the pharmacodynamic and pharmacokinetic profiles of phenserine, which will guide future research and potential therapeutic strategies for early to mild AD.

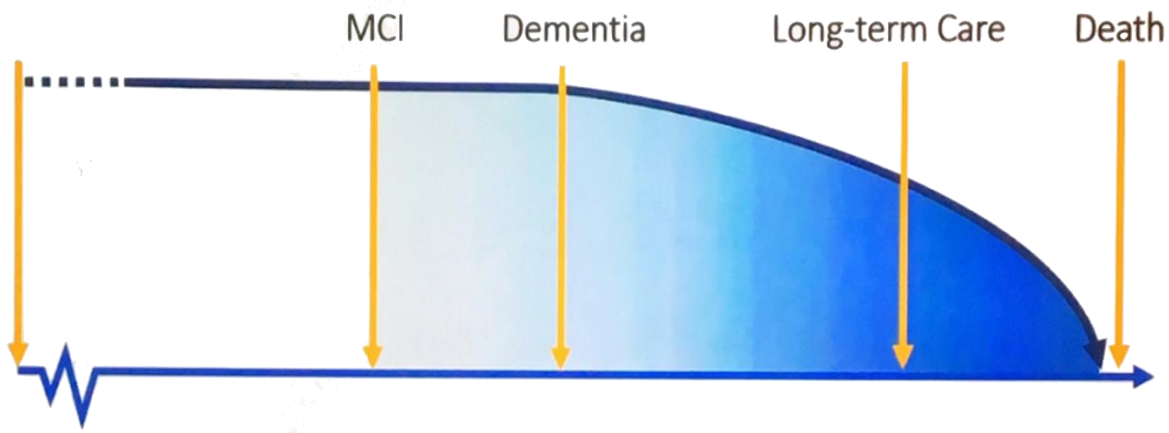
1.2. Schema

Figure 1. Study Schema



Study design, detailing the randomization of participants into two arms: the Phenserine Arm (n=12) and the Donepezil Arm (n=4). Participants in the Phenserine Arm will start at 5 mg twice daily (BD) with dose escalations at specified intervals up to 10mg three times daily (TDS).

Figure 2. FDA Framework for Pre-Clinical and Clinical AD



Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Characteristic pathophysiologic changes of AD but no evidence of clinical impact.	Characteristic pathophysiologic changes of AD and subtle detectable abnormalities on sensitive neurophysiological measures, but no functional impairment.	Characteristic physiologic changes of AD, subtle or more apparent detectable abnormalities on sensitive neurophysiological measures and mild but detectable functional impairment.	Mild AD dementia functional impairment.	Moderate AD dementia: functional impairment.	Severe Ad dementia: functional impairment.

1.3. Schedule of Activities (SoA)

Table 1. Schedule of Activities

	Screening (within 28 days before baseline)	**Treatment Period**					**Safety Follow-up[1]/EOS (within 28 days after last visit)**
		Baseline	**W2**	**W4**	**W6**	**W8**	
Days ±Visit window	**-28 to 0**	**0**	**(14± 3)**	**(28± 3)**	**(42± 3)**	**(56± 3)**	**(56± 28)**
Informed consent	X						
Demographics	X						
Medical history[2]	X						
Concomitant medications	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X
Neurological Exam	X						X
ECG	X	X	X	X	X	X	X
MMSE	X						
MoCA		X				X	
CDR	X						
MRI[3]	X						
Suicidal screening using C-SSRS, modified[4]	X			X		X	X
Inclusion/Exclusion criteria	X	X					
Randomization		X					
Dispense of IMP[5]		X	X	X	X		
Adverse events		X	X	X	X	X	
AchE sample collection		X	X	X	X	X	

Dose review (for escalation or de-escalation)			X	X	X		
Exosome Biomarker Collection		X	X	X	X	X	
Blood and CSF-based biomarkers[6]	X					X[7]	
PK sample collection			X	X	X	X	
Safety laboratory test[8]	X		X	X	X	X	X
Urinalysis	X		X	X	X	X	X
FSH test[9]	X						
Pregnancy test[10]	X		X		X		
FLAME Cognitive battery		X				X	

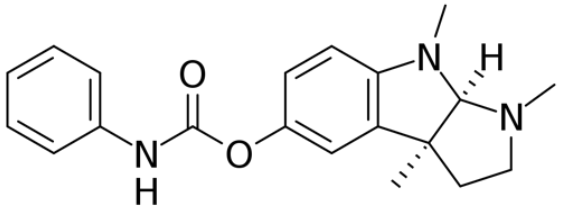
[1] A safety follow-up visit will be conducted within ~4-weeks after the last study-drug dose for all participants who complete the study or who terminate early.
[2] Includes prior medication use.
[3]Only if no MRI scan available within last 2 years.
[4]The C-SSRS will only be conducted at the follow-up/EOS visit, if the patient experienced suicidal thoughts after the study treatment period.
[5] Before any dose escalation. According to tolerability and laboratory tests, the dosage will be reassessed, and participants may need to return to the center for a reduced dose.
[6]CSF samples will only be collected from patients who specifically consent to CSF sample collection and analysis. If CSF biomarker data are available then there is no need for a new CSF collection at screening
[7]CSF collection at W8 is voluntary and requires an additional informed consent.
[8]Renal and liver function will be taken every visit. Full Safety laboratory battery will be taken at screening and safety follow-up.
[9] Women considered post-menopausal will have an FSH test to confirm their status during the screening period and prior to dosing.
[10] Pregnancy testing is required for all women of childbearing potential within one day prior to receiving the first dose of study drug.

2. Introduction

Phenserine is a next generation AChE inhibitor being developed for the treatment of AD. Unlike currently marketed AChE inhibitors, it has additional mechanisms of action that also include a mediating effect on cell death pathways and anti-amyloid activity, which may confer disease-modifying effects in people with AD.

Phenserine's names, designations, and chemical structure are summarized below.

INN: Phenserine tartrate
Other Names: -eseroline phenylcarbamate
CAS No. 101246-66-6
Trade name(s): Not applicable
IUPAC name: (3a'S,8aR)-1,3a,8-Trimethyl-1H,2H,3H,3aH,8H,8aH-pyrrolo[2,3-b] indol-5-yl N-phenylcarbamate
Molecular Formula: C20H23N3O2
Molecular Weight: 337.4 g/mol
Structural Formula:



Phenserine was originally identified and developed by the United States (U.S.) National Institute of Aging (NIA), part of the U.S National Institute of Health (NIH). It has previously been evaluated in a total of 11 studies, including 2 initial safety and tolerability studies that included PK and PD assessments in healthy normal subjects. In addition, bioavailability and food effect studies were conducted to assess PK parameters of an early immediate release 10-mg tablet formulation versus an oral solution. A U.S. corporate sponsor, Axonyx Inc., subsequently purchased the rights to the compound and conducted additional clinical studies, including additional PK and safety studies using 10-mg and 15-mg tablet formulations under fasted and fed conditions in healthy subjects. In addition, eight Phase 2-3 studies were conducted in patients with probable mild-moderate AD. In total, the drug has been evaluated in over 1300 people with AD. All studies for which data are publicly available are summarized in the Investigator's Brochure.

It is imperative to address the growing global health challenge of dementia. According to the 2023 World Alzheimer's Report (1), it is anticipated that, globally, the number of people living with dementia will increase to 139 million by 2050, a 2.5-fold increase over the estimated number in 2019. Similarly, the annual costs associated with dementia are expected to more than double to $2.8 trillion dollars by 2030, compared to US$1.3 trillion in 2019. The World Health Organization (WHO) estimated in 2023 that there are more than 55 million people with dementia

worldwide, and that each year, there are nearly 10 million newly diagnosed cases, In addition, it is estimated that at least 15% of people aged 60 or above have Mild Cognitive Impairment (MCI), and that between 8 and 15% of these will progress to dementia each year, most commonly to AD(2). Alzheimer's disease (AD) is a devastating, progressive neurodegenerative disorder with a massive personal and financial impact on individuals, families and society. With ageing populations and the rapid rise in the number of people with AD and MCI, increasing the pipeline to accelerate the development of effective treatments is considered an urgent priority.

2.1. Study Rationale

This protocol aims to assess the safety and tolerability of phenserine compared to donepezil across various doses administered over a 8-week period in participants with early or mild AD. The study will also evaluate the pharmacodynamic and pharmacokinetic activity of the two drugs to determine dose-response relationships and identify an appropriate dose range for a subsequent Phase 2 study of phenserine.
This study seeks to evaluate the effects of an individualized maximum tolerated dose of phenserine with adequate AChE inhibition to establish an appropriate dose range for future efficacy trials.
The main objective is to test phenserine's disease modifying effect, and therefore we will include a comparator that controls for the known cholinesterease inhibition of phenserine, eg donepezil. This approach allows us to control for the symptomatic effects of cholinesterase inhibition, and to provide direct comparative data on safety and tolerability relative to a well-known AChE inhibitor.
The study duration is relatively short. This is justified by the following rationale: The cholinesterase inhibition effect occurs rapidly within a few hours after intake, and is sustained for about 6 hours. Thus the 4 week duration of the highest dose is sufficient to demonstrate this effect. The time of onset for the effect on cell death markers and other disease mechanisms is expected to start within 4 weeks but definitely within the full 8 weeks study period.
Donepezil, the most used cholinesterase inhibitor in AD with dementia, is included as comparison to control for the cholinesterase inhibition of phenserine, since the main rationale for the PATH1 trial is to explore whether phenserine has an additional disease-modifying effect, including effect on markers of cell-death.

2.2. Background

In 2018, and in parallel with the conduct of the ELAD study that evaluated liraglutide (an established medication for Type 2 diabetes) for its neuroprotective effects in people with mild to moderate AD, we initiated a new selection process to identify additional drug candidates for re-purposing in AD, MCI and other forms of dementia. Drug repurposing, defined as "the application of established drug compounds to new therapeutic indications," offers a route to drug development that is accessible to academic institutions, government and research council programs, and charities and not-for-profit organizations, complementing the work of pharmaceutical and biotechnology companies. Repurposing an existing drug offers an attractive

way of enhancing traditional drug development and accelerating new treatments for people with AD dementia and MCI into the clinic.

The international expert panel that participated in the 2018 assessment applied the same approach as in the earlier 2012 selection process. A total of five compounds or classes of compounds were nominated for further consideration by the panel. These compounds were ACE inhibitors, antiviral drugs, disease-modifying antirheumatic drugs (DMARDs), fasudil and phenserine. Following several rounds of prioritization, the panel came to a clear consensus that the three highest priority candidates for repurposing in AD, MCI and other dementias were phenserine, fasudil, and antiviral drugs. This protocol is designed to assess the safety and tolerability of phenserine compared to donepezil across a range of doses administered over a 8-week dosing period in participants with early or mild AD. The pharmacodynamic and pharmacokinetic activity of the two drugs will also be evaluated to determine dose-response relationships to identify an appropriate dose range for a subsequent Phase 2 study of phenserine.

Phenserine was initially developed as an acetylcholinesterase inhibitor (AChEI) (3), but there are several mechanisms by which phenserine may act on neuronal and synaptic loss (4), a key common pathway evident in AD. A range of pre-clinical studies indicate that phenserine suppresses interleukin-1b, reduces glutamate-induced excitotoxicity, protects against oxidative toxicity, reduces A-beta levels, improves neural precursor cell viability, elevates neurotrophic brain-derived neurotrophic factor, and inhibits amyloid-beta precursor protein synthesis (4). In the only published phase 2 randomized controlled trial (RCT), phenserine (10-15 mg b.i.d.) conferred improvement in cognition in people with AD receiving 12 weeks of the higher dose of phenserine. Safety and tolerability were very good. The proportion of withdrawals due to adverse events was 6% in the placebo arm and the highest dose groups, indicating that the optimal dose was probably not achieved for most patients. There were also methodological problems, prompting the authors to conclude that the full potential of phenserine for AD has not yet been fully evaluated (3).

The most common side-effects of phenserine are those associated with cholinesterase inhibition, i.e., nausea, vomiting and diarrhoea. The best predictor of cholinesterase linked gastrointestinal effects is the level of cholinesterase inhibition achieved, with the optimal therapeutic threshold being 50% inhibition (5). Given that the completed clinical trials with phenserine did not select patients based on biomarker confirmation of AD (meaning that a considerable proportion of participants likely did not have AD), and that the maximum dose did not lead to side-effects and thus many participants did not likely achieve a sufficiently high dose level, this study aims to evaluate the effects of an individualized maximum tolerated phenserine dose with adequate level of AChE inhibition to determine an appropriate dose range for subsequent efficacy trials.

2.3. Benefit/Risk Assessment

The risk-benefit profile of phenserine is supported by nonclinical and clinical data from its early development as a cholinesterase inhibitor for the treatment of AD. In total, phenserine has been evaluated in 11 clinical trials, including initial safety and tolerability studies that included PK and PD assessments at different dose regimes (5mg, 10mg, 15mg) (6) in fed and fasted healthy normal subjects. In addition, phenserine's safety and efficacy profile for the treatment of AD were evaluated in eight Phase 2-3 randomized controlled trials (all of which were conducted entirely in the Europe) that enrolled 1,344 people with mild to moderate AD, including 645 participants who received phenserine for 6- to 12-months treatment duration. Although these clinical trials failed to show efficacy, design and methodological issues were identified by the company and subsequently verified by independent assessors as having confounded the analyses, the outcomes, and the conclusions. All available clinical data from these and other studies of phenserine are further described in the IB. These included subtherapeutic total daily doses, as evidenced by the drug's efficacy *and* safety profile. Importantly, the safety data from the completed trials support additional well-designed studies to better identify efficacious doses and evaluate potential disease-modifying effects in people with early or mild AD symptoms.

In addition to the reported clinical data, a standard package of nonclinical safety studies was performed to support the clinical program as it advanced. Completed studies include Good Laboratory Practice (GLP) 12-week subchronic studies in rats and dogs as well as chronic toxicity studies of up to 52 weeks treatment duration in rats, dogs and non-human primates and a carcinogenicity study in p53+ mice. Although the reports for the completed clinical and nonclinical studies are not all publicly available, the data were reviewed by relevant health authorities prior to allowing chronic studies in humans to proceed. All studies for which data are publicly available are summarized in the Investigator's Brochure. The risks and potential benefits of phenserine expected in this study are further summarized below based on publicly available information. In addition, more detailed information about phenserine's pharmacology and safety is detailed in the Investigator's Brochure (IB).

2.3.1. Risk Assessment

Several elements are included in the protocol to ensure safety of participants. Medical conditions or drugs with clinically important interaction with cholinesterase inhibitors are listed in the Exclusion criteria. In addition, frequent (bi-weekly) clinical and laboratory safety assessments are conducted. Specific conditions and changes needing particular attention are listed, including stopping criteria for changes in liver and kidney function tests.

In the 13-week GLP study in male and female rats, the effects of phenserine were evaluated in a at doses of 0, 5, 10, and 15 mg/kg/day (10 males and females per dose group). Evidence of cholinergic stimulation was observed at all dose levels and included tremors, increased salivation, staining around the mouth and excessive lacrimation. Clinical toxicity included decreased food consumption and decreased weight gain in the two high-dose groups. No consistent changes were observed in hematologic or clinical chemistry evaluations. Urinalyses showed a dose-related trend in decreased urine volume, increased specific gravity, decreased pH, ketonuria and alubuminuria. These findings were considered secondary to decreased food

consumption and decreased weight gain. Macroscopic and microscopic examinations of body tissues postmortem showed only isolated findings, and no findings were considered drug related. In the 13-week GLP dog study, phenserine was administered once-daily to 8 animals per dose group (4 male, 4 female). All animals survived the study. Clinical signs of pharmacologic effect, including excessive salivation and tremors, were observed in animals receiving 1.5 or 5 mg/kg/day. There were no significant differences in body weight or food consumption at any dose level. In addition, no significant or drug-related findings were observed following fundoscopic ophthalmic examinations; electrocardiographic evaluations; hematologic, blood chemistry, and urinalysis measures; and macroscopic and microscopic examinations of all animals.

In another 1-Month Oral Dose Rat Study, with dose regimen: once daily oral doses of 0, 5, 10, 15, 20 and 25 mg/kg/day in 5 male rats per group, The most prominent cholinergic adverse effect was hypersalivation, which peaked within 1 hour of dosing and rapidly declined thereafter. There was a small reduction in food consumption seen at 5mg/kg which could be indicative of nausea. In the 3 highest dose groups 3 rats died (1 each at 15, 20, and 25 mg/kg). No consistent changes were observed in hematology or clinical chemistry parameters at termination. Gross examination of body tissues obtained postmortem showed only isolated findings, and there was no evidence of any drug effects (The 5mg/Kg dose is equivalent to 55-60mg in humans)

NIA (National Institute on Aging) and/or Axonyx (a biopharmaceutical company focused on the development of treatments for Alzheimer's disease) also conducted a standard panel of genotoxicity and safety pharmacology studies of phenserine to support clinical trials in the US and Europe.
Limited information regarding the metabolism of phenserine is in the public domain, but in Grieg et al., 2005, it is indicated that phenserine's metabolism principally involves N-demethylation and hydroxylation, and is mediated predominantly through the CYP3A4 pathway.

In early clinical pharmacology studies conducted in normal healthy volunteers, phenserine was shown to be rapidly absorbed and eliminated, and plasma drug concentrations correlated with erythrocyte AChE inhibition. Similarly, in studies that evaluated male and female elderly but otherwise healthy volunteers single doses of phenserine were well tolerated up to 10 mg. In the 20-mg group, dose-limiting side effects associated with cholinesterase inhibition included nausea and vomiting. No clinically significant changes in vital signs or clinical laboratory assessments were observed.
Phenserine showed high and predictable oral bioavailability across the dose levels. Over the 4-fold increase in dose, $AUC_{0\text{-}24}$ values increased by ~25%. T_{max} values ranged from 1.25-1.54 hr, and $t_{1/2\ elim}$ = 6.53 hr. Erythrocyte AChE inhibition reached peak values of 12%, 23% and 46% at 2 hours postdose for the 10, 15, and 20 mg/kg dose groups, respectively, and showed a linear relationship to phenserine plasma concentrations. Other studies have demonstrated that phenserine's effects on cognition and other neuropsychiatric assessments are positively correlated with increased CSF Aβ40 and sAβPP levels as well as improved regional cerebral blood flow (rCBF) and metabolic rate for glucose (*rCMRGlc*). In addition, CSF Aβ42 levels, the Aβ42/40 ratio, P-tau, and T-tau correlated negatively with rCMRglc and cognition (7,8).

In a follow-on study (5) elderly but otherwise healthy volunteers were randomized to receive once daily doses of phenserine immediate release capsules at 5 mg QD, 5 mg BID, 10 mg QD, or 10 mg BID (8 subjects/group) for 6 days. There were no dose-limiting or serious adverse events (AEs) reported for any subject. In addition, there were no clinically significant findings in clinical laboratory measures or ECG assessments across all dose groups. Peak plasma concentrations increased with dose but were similar across the QD and BID dosage regimens for each dose level. In addition, $AUC_{0\text{-}24}$ values remained consistent over the 6-day treatment period confirming that drug accumulation does not occur with repeated dosing. Erythrocyte AChE inhibition of 15% and 30% were observed for the 5 and 10 mg dose groups, respectively, regardless of the once- or twice-daily dosing schedules.

A subsequent NIA-sponsored Phase 2, 12-week RCT in 72 patients indicated that phenserine (10-15 mg b.i.d.) conferred significant improvements in cognitive function and a trend towards improvement in global outcome in people receiving the higher dose, with Cohen's D effect sizes of 0.3-0.4 for symptomatic benefits (6).

A second smaller RCT randomized 20 AD- patients equally to phenserine or placebo for 3 months. The placebo group then received donepezil after 12 weeks in an open design. Participants receiving phenserine had significantly greater improvements in cognition and improvements in cerebral glucose metabolism compared to the group treated with placebo, and the cognitive benefits were maintained after 6 months compared to the group receiving donepezil for the second 3 months of the trial (8).

Although these data are encouraging, the compound has not been assessed in people with early or mild AD, and the potential for disease modification has not previously been evaluated in human clinical trials.

From a safety perspective, in the larger trial with 72 participants, 77% of patients completed the study protocol. The overall discontinuation rate for the 15 mg twice daily group (23.6%) was only slightly higher than for the placebo group (17.1%). Withdrawals due to adverse events were slightly lower in the 15 mg twice daily group (5.9%) than in the placebo group (6.1%). Nausea (9% 10mg bd, 19% 15mg bd) and vomiting (5% at 10mg bd and 11% at 15mg bd) were the most common AEs in the active drug treated groups but did not contribute to dropouts and are comparable to rates for licensed cholinesterase inhibitors (3).

In a further single escalating dose safety study, 32 healthy elderly volunteers received single oral doses of phenserine tartrate up to 20 mg(5). Physical and vital signs were monitored over the ensuing 24 hours. Analyses were performed on plasma samples to determine PK, and PD effects were assessed as a function of AChE levels. No serious adverse events (SAEs) occurred; the most common AEs were headache and vomiting. Evaluation of PK/PD relationships suggested a linear correlation between plasma phenserine concentration and AChE inhibition. Other phase 1 studies indicated that the peak AChE effect occurs 30 minutes after dosing, with a maximum AChE inhibition of about 30% with a 10 mg dose. Food delayed t_{max} from 1.4 hr. to 2.7 hr compared to fasting.

Importantly, there is clear evidence from trials with other cholinesterase inhibitors, that C_{max} is related to the severity of adverse events, and that a total daily dose spread into smaller individual doses has been an effective way of reducing the potential for adverse effects (9). Slow dose titration (10) and administering medications with food have also been important in reducing the potential for adverse events. In the current study, each participant in the phenserine arm will be titrated at 2-weekly intervals from 5 mg twice-daily to up to 10 mg 3-times daily, if tolerated The rationale for this 45% AChEI is based on the results from several research groups that have

aimed to quantify the association between the level of cholinesterase inhibition achieved by donepezil and other ChEIs and clinical response. One of the most active group, prof Nordberg's group at Karolinska Institute, reported (11) that " the optimal CSF AChE inhibition for donepezil was over 45%". This was related to cognitive response. Participants will also be instructed to take each dose with food.
No depressions of any blood cell lines were noted in animal toxicology studies. However, the clinical effects of phenserine on complete blood count (CBC) counts have not been reported in the literature. Nevertheless, a similar molecule based on physostimine, heptylphysostigmine, was found to induce granulocytopenia in two studies that resulted in the suspension of further clinical trials (12).
In summary, the previous studies have shown acceptable tolerability of 15mg BID, eg Winblad et al(3) (255 patients) showed withdrawals due to side-effects in 15mg BID (5.9%) similar to that of placebo (6.1%). In Kadir et al 2008(13), including 20 patients for 3 months followed by 9M open-label, phenserine 15mgx2, ChEI-related gastrointestinal symptoms were observed in three patients, which resolved in two patients after dose reduction to 20mg/d. This is similar rates as reported for licensed doses of cholinesterase inhibitors. Thus, our interpretation is that 30mg is well tolerated.

Several steps have been taken to ensure safety and tolerability, including a) slow stepwise titration, b) divide the daily dose into three doses eg highest single dose is still 10mg, which reduces Cmax which is the main driver of side-effects, c) ensure IMP is taken with food, and d) frequent in-clinic visit with physical examination and safety laboratory measurements. Any participant who escalates to the highest allowed dose and subsequently experiences intolerable adverse effects will be down titrated to their prior dose level. If more than 2 participants experience dose-limiting side effects at the 10 mg tds dose level, no additional participants will be allowed to dose escalate to that level, and the highest dose that all subsequent participants will be allowed to reach is 10 mg bd.

2.3.2. Benefit Assessment

There is an ongoing need to develop disease modifying therapies for AD that are well tolerated and can be widely available to people with AD. Established treatment is limited to, at best, a modest reduction in symptoms but cannot prevent continuous symptomatic deterioration and progressing neuropathology. New disease-modifying anti-amyloid drugs, which can reduce the rate of decline by 25% (14), and are likely to be available soon across Europe, but whilst they will represent an important step forward, their use will be limited by safety and cost. After an initial negative decision, a decision to approve lecanemab was recently made by EMA for lecanemab. However, the effect size is rather small, and safety issues remain, and the drug will not be available for all AD patients. It is still therefore a vital aim to develop effective disease modifying treatments that can be made available to the majority of people with AD, including older and frailer individuals who will not be suitable for the next generation of disease modifying therapies. Although phenserine was previously tested and failed to show efficacy in participants with suspected but unverified mild to moderate AD, follow-up analysis by Axonyx and others identified flaws in the trial design and study conduct that likely contributed to the lack of observed therapeutic benefit..

Although phenserine was initially developed and evaluated as a cholinesterase inhibitor (3), there are several mechanisms by which phenserine may act on neuronal and synaptic loss (15), a key common pathway evident in AD, traumatic brain injury and other neurodegenerative diseases. A range of pre-clinical studies indicate that phenserine suppresses interleukin-1b, reduces glutamate-induced excitotoxicity, protects against H2O2- induced oxidative toxicity, reduces A-beta levels, improves neural precursor cell viability, elevates neurotrophic brain-derived neurotrophic factor, inhibits APP and a-synuclein synthesis, (16).

Whilst these potential actions are of interest, more recent work has suggested that phenserine may confer significant neuroprotection by inhibiting apoptosis through actions on a Pre-Programmed Cell Death Pathway (17). This has been evaluated in several rodent models of neuronal loss, including APP/PSEN1 transgenic AD mice, re-perfusion injury and a weight drop mouse model of traumatic brain injury. In all of these animal studies, treatment with phenserine significantly reduced neurodegenerative lesions, and decreased the neuro-inflammatory response through IBA1 and TNF-α pathways in the hippocampi and cortices of wild type (WT) mice, and in cortices of AD mice(16–19). Synaptic density and Synaptophysin (Syn)-IR were also significantly protected in phenserine-treated AD and TBI animals (16–19).

The mechanisms underlying the protection of neurons were evaluated in cell culture and by using novel exosome technologies to examine key cell pathways from in vivo studies of anoxia and TBI (15,20). For example, Phenserine increased BDNF, Bcl-2, phosphorylated ERK-1/2, and lowered activated caspase-3 protein levels in SH-SY5Y cells exposed to OGD-hypoxia and neuronal injury (18). Despite the failure of Axonyx's previous late-phase clinical program, these new findings support further investigation of phenserine's potential therapeutic utility in AD and potentially other forms of neurodegenerative disease. With this in mind, we have optimized the study design for the PATH-1 study to enhance the study design and methodology to more thoroughly characterize phenserine's potential therapeutic properties.

2.3.3. Optimization of the PATH-1 Protocol for Assessing the Safety and Efficacy of Phenserine in People with Early or Mild AD

The design of the PATH-1 study has been optimized based on the issues identified in the previous Axonyx program (20,21), as follows:

- The eligibility criteria will more definitively identify patients with early or mild AD (based on FDA guidance and on a confirmed significant change on a validated biomarker of AD). This patient population may be more likely to respond to treatment based on a confirmed diagnosis and given the early course of their disease.
- The 6:2 randomization to phenserine or donepezil will ensure adequate early assessment of phenserine's safety and PK, while minimizing a potential placebo effect by offering the other group a well-established and generally effective treatment for people with early or mild AD.
- The titration schema and criteria for establishing a patient-specific maintenance dose will ensure that participants achieve an individually optimized dose based on safety.
- The therapeutic utility of phenserine will be evaluated using novel exosome technologies as well as other established blood- and CSF-biomarkers of AD. In addition, well-established cognitive assessments will provide further characterization of phenserine's effects on AD symptomatology. Together the results of these assessments will be used to select doses and outcome measures for a subsequent Phase 2 study of phenserine in people with early or mild AD.

2.3.4. Overall Benefit Risk Conclusion

Overall, there is strong pre-clinical evidence of phenserine's biological effects that are relevant to the treatment of AD and other neurodegenerative conditions, with a novel effect on apoptosis in addition to cholinesterase inhibition. Previous clinical trials with phenserine suggest a good clinical safety profile and offer some encouraging indications of potential benefit. The protocol has been designed based on the learnings of the failed Axonyx program and will ensure that the patient population is appropriate and that the study assessments more fully characterize phenserine's potential therapeutic utility across more robust measures of pharmacodynamic effects and any correlated improvements in cognitive function.

3. Objectives, and Endpoints

The study will be conducted as a 2 arm, parallel group, randomized RCT of phenserine compared to donepezil in people with early or mild AD. A total of 16 participants will be recruited from sites across Norway and be supported by the PROTECT platform. Eligible participants will be randomized 6:2 to phenserine or donepezil in an unblinded manner.

The end of the research active period for this study is set to January 1, 2028. All analyses within the scope of this study will be completed by this date.

The pharmacodynamic (PD), pharmacokinetic (PK), and safety data from this study are intended to inform dose selection and study design for a subsequent Phase 2 study of phenserine over a longer treatment duration in people with early to mild Alzheimer's disease (AD). The objectives of the study are as follows:

Primary Objective:

To assess the effects of phenserine compared to donepezil on exosome biomarkers of cell death in people with early or mild AD.

Key Secondary Objective:

To evaluate the safety and tolerability profile, of phenserine at ascending doses up to 10 mg tds as compared to donepezil at doses up to 10 mg od in participants with early or mild AD.

Other Secondary Objectives:

To evaluate steady state blood levels of phenserine for the purpose of characterizing and comparing dose-response relationships for pharmacodynamic outcomes and key safety assessments.

To evaluate the proportion of participants in the phenserine arm who achieve the target AChE inhibition of approximately 45%, stratified by dose regimen and the time required to reach the target AChE inhibition of ~45% and the duration participants maintain this inhibition during the treatment period.

To evaluate compliance, the study will measure the extent to which participants take the study medication according to the protocol. Compliance will be assessed using the subject diary and the registration of returned capsules.

Exploratory Objectives:

To evaluate changes in specific biomarkers of Alzheimer's Disease (AD) in cerebrospinal fluid (CSF): Aβ1-40, Aβ1-42, Tau, and p-tau, and in blood plasma: p-tau217 and Nfl.

To assess phenserine's potential short-term effects on specific cognitive tests using the FLAME Memory Composite and other cognitive sub-tests.

To evaluate changes in overall cognitive function using the Montreal Cognitive Assessment (MoCA):

Primary Endpoint:

Changes in exosome biomarkers of pre-programmed cell death, synaptic integrity and function, neuroinflammation, and AD-related protein trafficking in participants treated with phenserine versus those treated with donepezil. Although patients and site staff will be unblinded to the treatment modality administered, they will be blinded to the results of the biomarker evaluations until the study is completed.

Key Secondary Endpoints:

Safety and tolerability of phenserine compared to donepezil in participants with early or mild AD based on the frequencies of reported or observed adverse events (AEs) and serious adverse events (SAE), vital signs, clinical laboratory evaluations, ECGs, and modified CSSRS scores.

Other Secondary Endpoints:

Pharmacokinetic parameters will be assessed at steady state for determination of dose-response relationships for phenserine and donepezil.

Evaluate the proportion of participants in the phenserine arm who achieve the target AChE inhibition of approximately 45%, stratified by dose regime and assess the time required to reach the target AChE inhibition of ~45% and the duration participants maintain this inhibition during the treatment period. Blood samples will be analyzed for Acetylcholinesterase (AchE) activity using a modified Micro-Ellman method (22) normalized to recombinant AChE enzyme activity (refer to Appendix 10).

Compliance will be recorded, based on drug count and subject diary, including the proportion of participants complying with the treatment schedule. Compliance is defined as drug intake within 80-120% of the scheduled dosing.

Exploratory Endpoints:

Assess changes in Alzheimer's Disease (AD) biomarkers in cerebrospinal fluid (CSF): Aβ1-40, Aβ1-42, Tau, and p-tau, and in blood plasma: p-tau217 and Nfl.

Neuropsychology assessments will be conducted using the FLAME computer-based domain composites for memory, executive function, attention and sustained attention to evaluate changes over the 8-week treatment period in the phenserine treated participants compared to those who received donepezil. In addition, evaluate overall cognitive changes using the Montreal Cognitive Assessment (MoCA).

3.1. Table 2. Objectives and endpoints

Objectives	Endpoints
Primary	
To assess the effects of phenserine compared to donepezil on exosome biomarkers of cell death in people with early or mild AD.	Changes in endosomal biomarkers of pre-programmed cell death, synaptic integrity and function, neuroinflammation, and AD-related protein trafficking in participants treated with phenserine versus those treated with donepezil. Although patients and site staff will be unblinded to the treatment modality administered, they will be blinded to the results of the biomarker evaluations until the study is completed.
Secondary	
To evaluate the safety and tolerability profile of phenserine at ascending doses up to 10 mg tds as compared to donepezil at doses up to 10 mg od in participants with early or mild AD.	Safety and tolerability of phenserine compared to donepezil in participants with early or mild AD based on the frequencies of reported or observed adverse events (AEs) and serious adverse events (SAE), vital signs, clinical laboratory evaluations, ECGs, modified CSSRS scores.
Other secondary	
To evaluate steady state blood levels of phenserine and donepezil for the purpose of characterizing and comparing dose-response relationships for pharmacodynamic outcomes and key safety assessments.	Pharmacokinetic parameters will be assessed at steady state for determination of dose-response relationships for phenserine and donepezil
To evaluate the proportion of participants in the phenserine arm who achieve the target cholinesterase inhibition of approximately 45% across different dosing regimens, as well as the time required to reach the target and the duration of maintaining this inhibition.	Proportion of participants in the phenserine arm achieving the target cholinesterase inhibition of ~45%, stratified by dosing regimen and the time to reach the target inhibition and the duration of maintaining this target will be assessed and recorded
To evaluate compliance, the study will measure the extent to which participants	Compliance will be recorded, based on drug count and subject diary, including the proportion of

take the study medication according to the protocol. Compliance will be assessed using the subject diary and the registration of returned capsules.	participants complying with the treatment schedule. Compliance is defined as drug intake within 80-120% of the scheduled dosing.
Exploratory	
To evaluate disease modification by assessing changes in specific biomarkers of Alzheimer's Disease (AD) in cerebrospinal fluid (CSF): Aβ1-40, Aβ1-42, Tau, and p-tau, and in blood plasma: p-tau217 and Nfl.	Assess changes in Alzheimer's Disease (AD) biomarkers in cerebrospinal fluid (CSF): Aβ1-40, Aβ1-42, Tau, and p-tau, and in blood plasma: p-tau217 and Nfl.
To assess phenserine's potential short-term effects on specific cognitive tasks using the FLAME Memory Composite and other cognitive sub-tests	Neuropsychology assessments will be conducted using the FLAME computer-based domain composites for memory, executive function, attention and sustained attention to evaluate changes over the 8-week treatment period in the phenserine treated participants compared to those who received donepezil.
To assess effect on global cognition	The Montreal Cognitive Assessment (MoCA) will be administered at baseline and at the end of the study to evaluate changes in overall cognitive function across multiple domains, including visuospatial abilities, language, attention, memory, and executive function.

3.2. Outcome Measures

3.2.1. Primary Outcome Measure

The primary outcome measure will assess changes in four panels of exosome biomarkers, including markers of pre-programmed cell death, synaptic integrity and function, neuroinflammation, and AD-related protein trafficking in participants treated with phenserine versus those treated with donepezil. Panel details are presented in separate standardized operationalized procedures (SOPs).

Although participants and site staff will be unblinded to the treatment modality administered, they will be blinded to the results of the exosome and blood/CSF biomarker evaluations until the study is completed.

The pre-programmed cell death panel (PNCDD) will investigate key factors such as BAX, Bcl-2, which will provide insights into preprogrammed cell death processes (4,17) and any changes in the profile observed following treatment with phenserine or donepezil. The second panel focuses on synaptic markers, including synaptotagmin, offering a nuanced understanding of synaptic integrity and function. The third panel is centered on evaluating inflammatory biomarkers, including TNF-α, IL-1β, IL-6, and IL10, and the fourth panel will evaluate AD-specific markers, including Aβ1-42, and will elucidate kay molecular signatures associated with AD pathology. This comprehensive evaluation of mechanistic biomarkers through exosome technologies aims to deliver a detailed understanding of the impact of phenserine versus donepezil on various facets of AD neuropathology, contributing to the trial's primary objective.

3.2.2. Secondary Outcome Measures

The key secondary outcome in this study is the safety and tolerability profile of phenserine at ascending doses up to 10 mg tds as compared to donepezil at doses up to 10 mg od in participants with early or mild AD. Safety data will be collected through measurements of vital signs, physical and neurological examinations, clinical safety laboratory tests (including hematologic analyses for urea and electrolytes, creatinine, liver function tests, and full blood count as well as urinalysis), ECG parameters, and suicidality using a modified shortened version of the Columbia Suicide Severity Rating Scale (C-SSRS) (23).
Adverse events will be recorded following completion of informed consent. Qualified personnel will code adverse events using the lowest level term according to MedDRA (version 19.0) and by duration, severity grade, relationship to the study drug, the action(s) taken, and outcome.

Information about all serious adverse events will be collected. A serious adverse event (SAE) is an undesirable sign, symptom, or medical condition that is fatal or life-threatening, requires hospitalisation, results in persistent or significant disability/incapacity, constitutes a congenital anomaly or birth defect, or is medically significant. A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any adverse reaction that is classed as serious and suspected to be caused by the investigational medicine that is not consistent with previously established safety information (i.e., it is suspected and unexpected). To ensure participants' safety, each SAE will be reported to the data monitoring committee within 24 hours (h) of the trial staff learning of its occurrence and SUSARs will be reported in accordance with regulatory requirements.

Tolerability will primarily be assessed based on reported AChEI-related adverse events (24)during the dose escalation phase of the study. Based on these measurements, doses will be escalated for the phenserine arm to achieve a maximum tolerated dose.

Assessments will be completed at site before the participant is cleared for the next dose level. This decision will be made based on tolerability and safety blood testing and ECG. If laboratory data coming after the visit indicate the need to revise this decision, then the participant will be contacted. The responsible study physician will be responsible for this decision, if needed, after consultation with the local PI, the medical monitor, or the study PI.

PK parameters will also be assessed as a secondary outcome measure. Blood samples for PK analyses will be collected at steady state for determination of dose-response relationships for phenserine and donepezil.

Compliance will be recorded as the proportion who comply with the intake of IMP, defined as between 80-120% of the scheduled dosing. Compliance will be measured by counting the returned capsules and a subject diary.

3.2.3. Exploratory Outcome Measures

Neuropsychology assessments will be conducted using the FLAME computer-based domain composites for memory, executive function, attention and sustained attention to evaluate changes over the 8-week treatment period in the phenserine-treated participants compared to those who received donepezil. The results of these assessments will be compared to changes observed on exosome biomarkers to identify similar trends across the treatment arms and in individual participants.

In addition, the Montreal Cognitive Assessment (MoCA) (24) will be used at baseline and end-of-treatment to assess for any changes in cognition over the course of the treatment period.

CSF or blood AD or Tau markers or amyloid PET will be analyzed for typical AD features as an inclusion criterion (see Section 5). Blood will also be collected at the Week 8 visit to evaluate any changes in blood-based biomarkers across the two groups. Among consenting participants, CSF will also be collected and stored for evaluation of CSF-specific biomarker changes. All procedures will be performed according to highly standardized procedures in accordance with the defined biobanking pre-analytical standard operating procedures (SOPs) of the BIOMARKAPD project (25).

4. Study Design

4.1. Overall Design

Consented patients will undergo a screening visit within 28 days of the planned baseline visit. (For women of childbearing potential, a negative serum pregnancy test will be required within 1 day prior to receiving the first dose of study drug). Only those patients who complete screening and meet study eligibility criteria at baseline will be randomized 6:2 to receive phenserine or donepezil in an open-label manner. The first dose of study medication will be taken in the clinic, and the participant will instructed on how to continue dosing at home until their next visit.

Participants will visit the clinic for safety and other planned study assessments at 2-week intervals through the 8-week treatment period, as detailed in the Schedule of Activities (Table 1).

Patients who withdraw or discontinue from the study before Week 8 will have an early termination visit within one week of discontinuation that includes safety follow-up. For participants who complete the study, a final follow-up safety assessment will be conducted within one week after completion of dosing.

4.2. Scientific Rationale for Study Design

The randomized, controlled design is required to fully understand the safety and efficacy of phenserine in this cohort. Eligibility requires MCI or mild dementia due to AD based on biomarker confirmation. To ensure safety, the design implements drug titration at different time-points after a detailed unblinded safety-review by the DSMB. The DSMB's role will focus on overall participant safety, Individual dose titration decisions will be made by the site study clinician and Principal Investigator (PI), based on pre-defined criteria. The outcomes include exosome biomarkers, detailed cognitive testing, as well as ADL and fluid biomarker changes.

4.2.1. Patient Input into Design

This project has been presented to the WiseAge reference panel which reviews every study conducted at SESAM, SUS. The feedback has been positive, the panel agrees that there is an unmet need to find new disease-modifying treatments for AD. The panel also underlined the need for safety assessments given the longer duration of treatment compared to previous studies with phenserine and recommended that lumbar puncture and CSF analysis be optional if that is possible. See Appendix 6. for description regarding user involvement.

4.3. Justification for Dose

In the only published phase 2 RCT of phenserine, improvement in cognition was observed in people with AD who received phenserine 15 mg bid for 12 weeks of treatment. In addition, phenserine was generally safe and well tolerated. The proportion of participants who withdrew from the study due to adverse events was just 6% in the placebo arm and the highest dose groups indicating that adequate cholinesterase inhibition in the active arms was likely not achieved. Other methodological issues were identified with the study, prompting the authors to conclude that the full potential of phenserine for AD had not yet been fully evaluated (20)

The most common side-effects observed in prior studies of phenserine were like those associated with other cholinesterase inhibitors and included nausea, vomiting and diarrhoea. The intended maximum dose in this study is similar to the previous clinical studies, and as outlined in the Risk assessment (section 3.2.1) and the study procedures, the distribution in three daily doses, the frequent clinical follow-up assessments, the gradual titration requiring acceptable tolerability, ensures that the safety of the participants is acceptable.

4.4. Study treatment administration

At each visit, participants will receive a clear explanation of the study intervention, including dosing instructions, to ensure accurate understanding and adherence. They will also be provided with a subject diary (see section 11.7 – Appendix 7) to track dosing times assess compliance with the regime and any side effects or adverse events experienced. In addition, participants will be instructed to return all medication bottles at each visit, including any unused medication. The returned medication will be counted and documented by the study staff to cross-verify compliance with diary entries.

A compliance rate between 80% and 120% is considered as acceptable for drug adherence.

4.4.1. Missed Dose Instructions for phenserine

- If a dose is missed: Participants are instructed to take the dose if it is within 2 hours since the scheduled time. If more than 2 hours have passed since the scheduled dosing time, the participant is instructed to omit the missed dose and continue with the next scheduled dose.. The participant is encouraged to mark the missed dose in the diary.
- Maintaining Safe Dosing Intervals: To prevent excessive dosing, participants should always ensure that a minimum interval is maintained between doses. This interval is 8 hours for three-times-daily dosing, 12 hours for two-times-daily dosing, and 24 hours for once-daily dosing

4.4.2. Missed dose instruction for donepezil

- In accordance with SmPC.

4.4.3. Study Partner Role in Compliance

Due to the varying dosing schedules during titration, study partners are asked to support participant compliance by:

- Reminding participants of scheduled doses.
- Monitoring and reporting any side effects.
- Assisting in documenting any missed or delayed doses in the subject diary.

The study partner will be instructed by the study personnel at site on how to support the participant in completing the subject diary and how they can be supportive in terms of compliance.

4.4.4. Dose escalation:

Based on tolerability, including adverse events and laboratory testing, the study clinician will decide whether the dose will be escalated as per the study plan. If clinical tolerability is deemed acceptable, the participant will receive the next dose level. If not the previous dosing regime will be continued. The detailed handling of this situation is outlined in a dedicated SOP.

4.5. End-of-Study Definition

The end of the study is defined as the date of the last visit of the last participant in the study. The date for the end of study is set to 31 December 2025. However, the date for the research active period is set to 01 January 2028. All analyses within the scope of this study will be completed by the 01 January 2028.

A participant is considered to have completed the study if the participant has completed all periods of the study including the safety follow-up visit 1-week after the last dose (table 1). Safety follow-up visit conducted within 28 days after the final dose of phenserine at the 8 week visit is considered sufficient since bioavailability when administered orally has a half-life for AChE plasma inhibition was 8.25 hr (26).

4.6. Early Termination of the Study

Reasons for early termination of the clinical trial may include:

- Ethical concerns,
- insufficient recruitment of participants,
- early evidence of benefit or harm of the investigational medicinal product,
- patient safety appears to be at risk,
- the sponsor deems it necessary to terminate the study for safety reasons,
- the study proves to be not feasible.

The sponsor shall discuss the decision to terminate the study with the steering committee. The study may be terminated at individual sites if GCP requirements are not met or if the respective sites are not working in accordance with GCP guidelines.

5. Study Population

5.1. Inclusion Criteria

1. A diagnosis of AD based on the most recent NIA-AA diagnostic criteria for AD (27).
2. A significant change on a validated AD amyloid or tau biomarker (as determined either by visual reading of amyloid PET scans [using any of the approved ligands], or CSF Aβ 1-42 or blood p-tau 217 levels [cut-off as determined by the individual laboratory]).
3. MCI (FDA stage 3) or mild dementia (FDA stage 4) based on a CDR Global rating of 0.5 (MCI) or 1.0 (mild dementia) (28)
4. An MRI scan within the past two years that has no findings inconsistent with AD.
5. Participants who have recently participated in other clinical trials or have been under treatment with memantine or acetylcholinesterase inhibitors (e.g., Donepezil, Rivastigmine, Galantamine) must undergo a washout period of at least 4 weeks prior to the start of the study.
6. Capacity to give informed consent based on the clinical judgement of an experienced clinician.
7. The participant has an individual who is in regular, daily contact via phone or in-person visits and who can act as a reliable study partner and provide meaningful input into rating scales.
8. Age ≥50 years.
9. Fluency in Norwegian and evidence of adequate premorbid intellectual functioning.
10. Capable of participating in all scheduled evaluations and complete all required tests.
11. Females of childbearing potential and males must commit to use highly effective methods of birth control (see highly effective birth control for male and females described in 11.4 appendix 4) from signing informed consent form until at least 30 days after last administration of phenserine or donepezil.

5.2. Exclusion Criteria

1. Significant cerebrovascular disease, as indicated by clinical history, neurological examination, or on MRI (including cortical infarction or deep white matter or periventricular white matter hyperintensities with a Fazekas scale score of 3 (29).
2. Current treatment with a cholinesterase inhibitor or memantine.
3. Hypersensitivity to AChE inhibitors or related compounds: Known hypersensitivity to donepezil, piperidine derivatives, or any formulation components.
4. Participants undergoing or planning procedures requiring anesthesia with depolarizing neuromuscular blockers (e.g., succinylcholine) due to the risk of prolonged paralysis or apnea when combined with AChE inhibitors.
5. Active peptic ulcer disease or gastrointestinal bleeding, or a history of gastrointestinal ulcers or bleeding.
6. Severe cardiac conditions: Significant arrhythmias, sick sinus syndrome, supraventricular conduction abnormalities, or other cardiac rhythm disorders that could pose a risk with cholinesterase inhibitors.

7. Severe respiratory disease: Chronic obstructive pulmonary disease (COPD) or poorly controlled asthma.
8. History of urinary obstruction or bladder issues, particularly those requiring catheterization.
9. Current clinically significant depression or other mental disorders likely to affect cognition or interfere with study participation.
10. Participants using sedating drugs, if unavoidable, will be excluded from the study. However, short-acting sleep medications can be used if taken as recommended and if the participant has maintained a stable regimen for at least 3 months prior to the start of the study.
11. Current participation in any other drug trial(s).
12. Currently ongoing life-threatening disease, such as metastatic cancer, advanced cardiovascular disease, advanced respiratory disease, terminal kidney disease, or advanced stages of an infectious disease.
13. Any current or past neurological disease unrelated to AD and with cognitive sequelae.

5.3. Lifestyle Considerations

- No lifestyle/dietary restrictions are required.

5.4. Screen Failures

A screen failure occurs when a participant who has consented to participate in the clinical study is not subsequently assigned to study intervention/entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the CONSORT publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE. Depending on the cause of screen failure, the Centre PI will determine whether re-screening is allowed. For example, if there is prohibited drug treatment, changes in the drug regimen can be made, providing considered safe by treating physician and study physician.

5.5. Criteria for Temporarily Delaying

The study procedures will be temporarily delayed if there is emerging new evidence concerning the safety of phenserine.

6. Study Intervention(s) and Concomitant Therapy

6.1. Study Intervention(s) Administered

In this study, phenserine will be formulated as a capsule, with dosages 5mg and 10mg. Participants randomized to the phenserine arm will start at 5 mg twice daily (bd) with escalations every two weeks, as tolerated until a maximum dose of 10 mg three times daily (tds).

Participants randomized to the donepezil arm will start at 5 mg tablet once daily (od) with escalation, to 10 mg od from Week 5, as tolerated.

6.1.1. Table 3. Study Intervention(s) Administered

Intervention Name	Phenserine tartrate	Phenserine tartrate	Phenserine tartrate	Donepezil	Donepezil
Intervention Description	First two weeks,	W3-W4	W5-W8	First four weeks	W5-W8
Type	drug	drug	drug	drug	drug
Dose Formulation	capsule	capsule	capsule	tablet	tablet
Unit Dose Strength(s)	5 mg	10 mg	10 mg	5 mg	10 mg
Dosage Level(s)	5 mg bd	10 mg bd	10 mg tds	5 mg od	10 mg od
Route of Administration	oral	oral	oral	oral	oral
Use	interventio n	intervention	intervention	Comparator	Comparator
IMP and NIMP/AxMP.	IMP	IMP	IMP	IMP	IMP
Sourcing	Provided centrally by the sponsor	Provided centrally by the sponsor	Provided centrally by the sponsor	Provided centrally by the sponsor	Provided centrally by the sponsor
Packaging and Labeling	KRAGTA B	KRAGTAB	KRAGTAB	Local Pharmacy*	Local pharmacy*

*Donepezil will be purchased commercially, eg with standard labelling, and stored locally at pharmacy or study centre.

6.1.2. Table 4. Study Arm(s)

Arm Title	Start dose	Maintenance dose	Donepezil	Maintenance of Donepezil
Arm Type	intervention	Intervention	Comparator	Comparator
Arm Description	Participants will receive 5mg mg bd on Week 1-2	Participants will receive 10 mg bd from Week 3-4 10 mg TDS from Week 5-8	Participants will receive 5mg od from week 1-4	Participants will 10mg od from week 5

6.2. Preparation, Handling, Storage, and Accountability

Preparation, handling, and storage of the IMP have been described in detail in the IB. In summary, the phenserine tartrate capsules manufactured under Good Manufacturing Practice (GMP) conditions. Each capsule contains 5 mg, 10mg and of phenserine tartrate, formulated with excipients to ensure stability and bioavailability.

The substance donepezil (under the brand name Aricept®) is a resale product. No additional manufacturing or labeling measures is necessary for this study.
The IMP, donepezil will be available in 5 mg and 10 mg strengths (table 3) and will be obtained from the study personnel at the local pharmacy by prescription. Please refer to section 11.8 (Appendix 8) for detailed information on prescription procedure for dispensing donepezil. We have based the use of donepezil as a treatment in the study on the original product from Pfizer, but it is also possible to use generic products of donepezil if this is what the local pharmacy has for dispensing. Participants randomized to treatment with donepezil will follow the treatment plan according to the patient information leaflet. These tablets are approved for clinical use and comply with all relevant safety and efficacy standards. Also participants allocated to treatment with donepezil will be asked to fill a subject diary for drug compliance. The subject diary template is available in appendix 7 below.

Capsules and tablets should be handled with care to avoid exposure to moisture or excessive heat. Personnel handling the medicines must follow standard safety procedures, including wearing gloves and protective clothing if necessary.

All capsules and tablets must be accounted for using a drug accountability log. Each site is responsible for tracking the receipt, dispensing, and return of capsules/tablets, ensuring accurate records are maintained for compliance with regulatory requirements.

6.3. Assignment to Study Intervention

In this study, participants will be randomized 6:2 to phenserine or donepezil in an open-label manner. Drug Dispensing and Dosing Instructions

Randomized subjects will be dispensed a single bottle of phenserine or donepezil drug product on Day 0 following all baseline assessments and confirmation of eligibility. Each participant will take the first dose in clinic and be instructed on how to continue dosing at home through the next two weeks (5 mg bd for the phenserine group and 5 mg od for the donepezil group). As shown in Figure 1, at the end-of-Week 2 visit, participants will undergo safety and tolerability assessments, and will be tested to determine their cholinesterase inhibition level. Based on tolerability, participants in the phenserine treatment group may be titrated to the next higher dose level (10 mg bd) for the next two weeks of treatment, while participants in the donepezil arm will continue the 5 mg od regimen. Adequate supplies will continue to be provided to the participants at this visit and in future visits to ensure they have adequate study drug (with an appropriate overage) to ensure that there are no disruptions in their daily dosing regimen through to the next study visit. At the end-of-Week 4 visit, and based on safety/tolerability assessments, participants in the phenserine arm may be escalated to 10 mg tds through to the end-of-Week 8 visit and participants in the donepezil arm may escalate to 10 mg od and remain at that dose through the remainder of the study (i.e., end of Week 8, as long as it is tolerated. Participants in both arms will return for follow-up assessments at the end-of-Week 6.. At the end-of-Week 8 visit, all participants will undergo final on-drug study assessments and will then return to the site for a final safety follow-up visit within 1 week of the last study-drug dose.

6.3.1. Allocation- procedure to randomize a participant

Both the subjects and the site staff will know which treatment they are assigned. Participants and site staff, will be blinded to the results of the exosomal and blood/CSF biomarker evaluations until the study is completed.
Participants will be identified by a unique study ID. Personal identifiable data (such as name, address, and other contact details) will be collected, but will be stored separately from the research data and will be destroyed as per applicable regulations when the project is concluded. Data will be managed by the University Hospital Stavanger clinical trials unit, following all relevant data protection guidelines and all relevant Clinical Trial Regulations. Data will be collected and stored electronically in accordance with the Data Protection Act 2018 and ICH GCP E6 R2. Data will be cleaned and validated appropriately, with a full Data Protection Impact Assessment (DPIA) undertaken along with development of a comprehensive Data management Plan (DMP) before the first participant is recruited.

The computer-generated randomized allocation sequence will be produced by an independent, unblinded statistician and imported into the Viedoc Electronic Data Management System (EDCS). The unblinded statistician will generate randomisation lists using using R v4.2 as implemented in https://icostatistics.shinyapps.io/randlist/ Participants will be screened for eligibility and first included by study physicians after informed consent. After study inclusion the participant will be randomized via Viedoc. The participant will be allocated treatment arm via Viedoc and receive the assigned IMP treatment at the first baseline visit by a study nurse. The study nurse will record the date of IMP distribution and the time and date of the first dose administered in Viedoc.

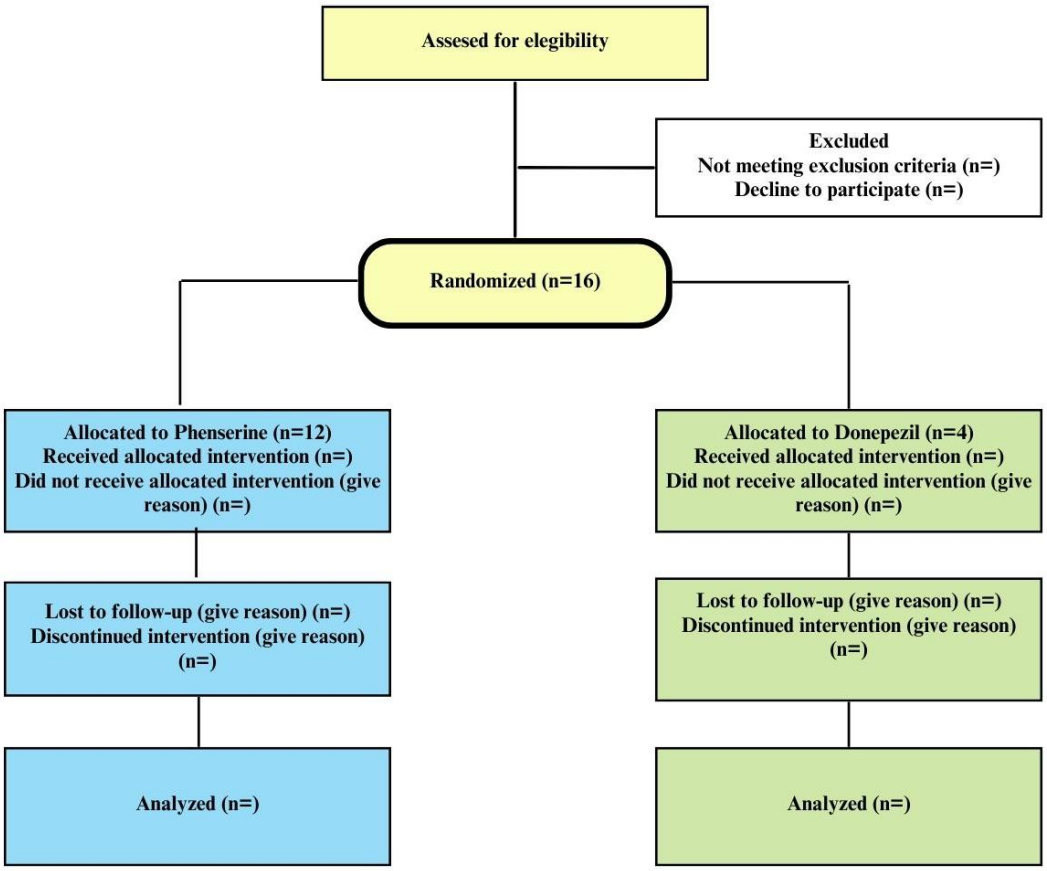
6.3.2. Subject identification

Patients who meet the inclusion criteria will be identified by a study investigator and will receive a brief information letter about the study. They will be explicitly informed that participation is entirely voluntary and that their decision, whether to participate or not, will not adversely affect their care at the hospital.

Participants will be identified by a unique study ID. Personal identifiable data will be stored separately to research data, with only a small select group of key research staff having access, and will be destroyed as per applicable regulations when the project is concluded. Other study staff will only have access to de-identified data. Data will be managed by the University Hospital Stavanger research unit, following all relevant data protection guidelines and all relevant Clinical Trial Regulations. Data will be collected and stored electronically in accordance with the Data Protection Act 2018 and ICH GCP E6 R2. Data will be cleaned and validated appropriately, with a full Data Protection Impact Assessment (DPIA) undertaken along with development of a comprehensive Data management Plan (DMP) before the first participant is recruited. Data collection for this study will be conducted through an Electronic Data Capture (EDC) system known as Viedoc, which is further detailed in Appendix 5.

The informed consent forms will clearly convey to the participants that they have the right to withdraw from the study at any point without any negative impact on their care or treatment. The primary analysis will be based on the modified intention-to-treat population. Participants will be analyzed according to their allocated treatment group irrespective of what treatment they received. Patient throughput for the final analysis will be illustrated using a CONSORT flow diagram (Figure 3).

Figure 3. CONSORT CHART



6.4. Blinding

The study intervention will be open label. No blinding will be performed. Participants and site staff will, however, will be blinded to the results of the exosome and blood/CSF biomarker evaluations until the study is completed.

6.5. Study Intervention Compliance

When participants are dosed at the site, they will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

When participants self-administer study intervention at home, compliance with study intervention will be assessed at each visit, and a subject diary will be completed. Compliance will be assessed by returned capsules/tablets, the subject diary, and during the site visits and documented in the source documents and relevant form. Deviation(s) from the prescribed dosage

regimen should be recorded. Compliance is deemed acceptable if within 80-120% of the scheduled dosing. A record of the quantity of dispensed study intervention to and administered by each participant must be maintained and reconciled with study intervention and compliance records. Intervention start and stop dates, including dates for intervention delays and/or dose reductions will also be recorded.

6.6. Dose Modification

As shown in the Study Schema (Figure 1), participants randomized to the phenserine arm will initiate dosing at 5 mg bd and maintain this dose for the first two weeks of study treatment. At the Week 3 visit, , participants may be escalated to 10 mg bd, then 10mg tds (Figure 1), based on tolerability, including adverse events, clinical assessment, ECG and safety laboratory measurements. Participants who are unable to tolerate a higher dose will have their dose reduced to the next previous dose level and maintained at that dose through the remainder of the study. This clinical decision will be made by the study physician and the study team at each centre at the visit, or when any safety laboratory results are received during the days after the visit. If needed, the medical monitor and the PI will be contacted. All participants are expected to be at their individualized maintenance dose for 4 weeks (beginning of Week 5 through the end of Week 8).

Participants randomized to the donepezil arm, will initiate dosing at 5 mg od and will be evaluated at the Week 4 visit for dose escalation to 10 mg once daily. For participants in the phenserine arm, dosing will follow a stepwise escalation to ensure safety and efficacy:

- **Weeks 1-2:** Participants will begin with 5 mg twice daily (bd).
- **Weeks 3-4:** Participants will escalate to 10 mg twice daily (bd) if the initial dose is well tolerated.
- **Weeks 5-8:** Participants will further escalate to 10 mg three times daily (tds) based on continued tolerability.

Before any dose escalation, the dosage will be reassessed based on tolerability. If necessary, participants may be required to return to the center for a dose reduction. Throughout the study, dose adjustments will be made according to tolerability. Should any dose escalation lead to adverse effects that compromise the participant's well-being or safety, the dose will be reduced to the previously well-tolerated level. Participants will then continue at this adjusted dose for the remainder of the study period. These procedures are outlines in more detailed in the dedicated SOP.

6.7. Treatment of Overdose

In the event of an overdose, supportive care and administration of an anticholinergic will be provided, according to current guidelines. to reverse cholinergic effects. Supportive care includes stabilizing the airway, providing intravenous fluids to maintain hydration and blood pressure, and closely monitoring vital signs and neurological status. If a patient experiences a cholinergic overdose at home, it is crucial to seek immediate medical attention by calling emergency services.

Patients and caregivers will be instructed on recognizing symptoms of cholinergic overdose, severe nausea, vomiting, salivation, diarrhea, muscle weakness and sedation.

6.8. Prior and Concomitant Therapy

Any medication or vaccine (including over the counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

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

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.
Participants must abstain from taking prescription or nonprescription drugs (including vitamins, recreational drugs, and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study intervention until completion of the follow-up visit, unless, in the opinion of the investigator and sponsor, the medication will not interfere with the study.

To ensure participant safety, certain medications or medical conditions known to interact with cholinesterase inhibitors will lead to participant not being included, or excluded is occurring during the study, or used with caution and monitored with particular care during the study:

6.8.1. Contraindications:

- Known hypersensitivity to AChE inhibitors, piperidine derivatives, or any excipients listed in the product formulation. Anaesthesia: AChE inhibitors may exaggerate succinylcholine-type muscle relaxation during anaesthesia and thus surgery requiring anaesthetic will lead to exclusion. Neurological Conditions such as a history of seizures, extrapyramidal symptoms and Neuroleptic Malignant Syndrome

6.8.2. Use with caution

- Cardiovascular conditions, such as QTc prolongation or bradycardia (refer to Exclusion Criteria for detailed monitoring requirements).
- Gastrointestinal risks, including a history of ulcers or concurrent NSAID use (refer to Exclusion Criteria).
- Concomitant Medications: drugs affecting the cholinergic system.

6.8.3. Permitted Medications

- **Paracetamol/Acetaminophen**, at doses of ≤ 2 grams/day, is permitted for use Other concomitant medications may be considered on a case-by-case basis by the investigator in consultation with the medical monitor if required.

6.8.4. Anti-Dementia Drugs

If deemed clinically indicated by the study clinician and the participant, participants currently taking anti-dementia drugs (e.g., donepezil, rivastigmine, galantamine, memantine) can participate in the study after a washout period of at least 4 weeks prior to the start of the study intervention. This is to ensure that there are no residual effects of these medications that could interfere with the study results. The washout period ensures that any pharmacodynamic or pharmacokinetic effects from previous medications are minimized, allowing for a clearer assessment of phenserine and donepezil.

6.8.5. Rescue Medicine

The study site will not supply rescue medication.

7. Monitoring of Study Intervention and Participant Discontinuation/Withdrawal

Participants may choose to withdraw from the study at any time and for any reason. Participants may be withdrawn from the study if it is in their best clinical interest by the site Investigator.

Participants who drop out of the study will be recorded as either defaulters or withdrawn. 'Defaulters' are participants who withdraw their consent to participate in the trial, affecting both treatment and assessment. No further assessments can be made of these participants. 'Withdrawn' are participants who are withdrawn from treatment at the discretion of the chief investigator because of clinical factors, poor compliance, or a change in circumstances. Withdrawn participants will remain in the study and trial assessments will still be undertaken. The reason for dropping out will also be recorded.

Every effort will be made to collect outcome data on all participants who withdraw from treatment for whatever reason. If participants withdraw from treatment, all endpoint assessments will be carried out at the point of drop-out and at the 8-week endpoint unless consent is withdrawn.

This study is unblinded with respect to the treatment assignment so both the participant and site staff will know which treatment modality the participant received.

7.1. Monitoring and discontinuation of Study Intervention

7.1.1. Liver Function Monitoring

1. **Elevated Liver Enzymes (ALT or AST ≤5 × ULN)**:
    a. **Action**: Continue the study intervention with closer monitoring. Repeat liver function tests at the next scheduled visit (2 weeks) or sooner if clinically indicated.
    b. **Clinical Follow-Up**: Investigate potential contributing factors, such as concomitant medications, alcohol intake, or underlying conditions.
    c. **Adjustment**: Consider reducing the dose of the study drug if levels approach 5 × ULN or if clinical symptoms develop.
2. **Isolated Total Bilirubin Elevation (>1 but ≤2 × ULN)**:
    a. **Action**: If ALT or AST levels are normal and there are no signs of liver injury, continue the study intervention with follow-up testing at the next visit.
3. **Clinical Symptoms Without Significant Enzyme Elevation**:
    a. **Action**: If participants report symptoms such as nausea, fatigue, or right upper quadrant discomfort without significant liver enzyme elevation, monitor closely, evaluate for alternate causes, and repeat tests as clinically indicated.

7.1.1.1. Liver Function Stopping Criteria

1. **ALT or AST Elevation**:
    - Discontinue the study intervention if ALT or AST levels are greater than 3 times the upper limit of normal (ULN) in conjunction with an increase in total bilirubin greater than 2 times the ULN (with > 35% direct bilirubin) or if the participant shows signs or symptoms of liver injury (e.g., jaundice).
    - Discontinue if ALT or AST levels are greater than 3 times the ULN and the international normalized ratio (INR) is greater than 1.5 (if INR is measured), which may indicate severe liver injury (possible Hy's law).
2. **Severe Elevation of Liver Enzymes**:
    - Discontinue if ALT or AST levels are greater than 8 times the ULN, regardless of other symptoms.
3. **Persistent Elevation**:
    - Discontinue if ALT or AST levels are persistently greater than 5 times the ULN for more than 2 weeks.
4. **Total Bilirubin Elevation**:
    - Discontinue if total bilirubin is greater than 2 times the ULN, especially if associated with elevated ALT or AST levels.
5. **Signs and Symptoms of Liver Injury**:
    - Discontinue if the participant exhibits clinical signs of liver injury such as jaundice, dark urine, significant right upper quadrant pain, or unexplained nausea/vomiting.
6. **Investigator's Discretion**:
    - Discontinue if the investigator deems it necessary based on their clinical judgment, even if the specific criteria above are not met.

7.1.2. Renal Function Monitoring and Management

1. **Reduced eGFR (≥30 but <90 mL/min/1.73 m²)**:
    a. **Action**: Continue the study intervention with follow-up testing at the next scheduled visit (2 weeks). Encourage hydration if clinically appropriate.
    b. **Clinical Follow-Up**: Assess for reversible causes, such as dehydration, urinary obstruction, or nephrotoxic medications.

7.1.2.1. Renal Function Stopping Criteria

1. Severe Reduction in Renal Function:
    - Discontinue if the estimated glomerular filtration rate (eGFR) falls below 30 mL/min/1.73 m².
2. Acute Kidney Injury:
    - Discontinue if there is evidence of acute kidney injury, defined as:
    - An increase in serum creatinine by 1.5 times the baseline value within 7 days, or
    - A reduction in urine output (oliguria) for more than 6 hours.

3. Signs and Symptoms of Renal Dysfunction:
    - Discontinue if the participant exhibits clinical signs of renal dysfunction, such as significant edema, oliguria, or unexplained fatigue accompanied by abnormal renal markers.
4. Investigator's Discretion:
    - Discontinue if the investigator determines that renal function decline poses a risk to the participant, even if specific thresholds above are not met.

7.1.3. ECG Monitoring and Management:

1. Continuous monitoring of heart rate and rhythm will be conducted every 2 weeks during regular medical evaluations.
2. QTc interval ≥ 460 milliseconds for males or ≥ 470 milliseconds for females will be considered abnormal during the ECG assessments If this happens, frequency of ECG monitoring will be increased by investigator’s discretion and dose reduction or study withdrawal will be considered.
3. Electrolyte levels (e.g., potassium, magnesium) will be monitored to identify factors contributing to QTc prolongation.

7.1.3.1. ECG Stopping Criteria:

1. **Severe Bradycardia or Arrhythmias**:
    - Discontinue if heart rate falls below 50 bpm, significant arrhythmias are detected, or participants experience syncope due to heart block or long sinus pauses.
2. **QTc Prolongation**:
    - QTc >500 ms for both genders.

7.1.4. Gastrointestinal monitoring and management:

1. Assess participants for gastrointestinal symptoms, including abdominal pain, nausea, vomiting, diarrhea, or signs of GI bleeding (e.g., melena, hematemesis), at every 2-week visit.
2. Participants with a history of peptic ulcer disease or taking NSAIDs will have their gastrointestinal status assessed more closely, including monitoring for dyspepsia or signs of ulceration.

7.1.4.1. Gastro intestinal Stopping Criteria:

1. **GI Bleeding or Ulceration**:
    - Discontinue if participants exhibit confirmed GI bleeding (e.g., melena, hematemesis).
2. **Persistent Severe Symptoms**:

- Discontinue if participants experience persistent severe gastrointestinal symptoms (e.g., uncontrolled vomiting, abdominal pain) that do not resolve with symptomatic management.

7.2. Procedures for Participant Discontinuation

In rare instances, it may be necessary for a participant to permanently discontinue the study intervention. If the study intervention is permanently discontinued, the participant should, if at all possible, remain in the study to be evaluated for safety, tolerability, and efficacy outcomes. See the Schedule of Assessments (SoA) for data to be collected at the time of discontinuation of study intervention and follow-up, and for any further evaluations that need to be completed. Participants who discontinue the study intervention should undergo the following procedures:

1. **Documentation of Reason for Discontinuation:**The reason for discontinuation must be documented in detail, including any adverse events, lack of efficacy, non-compliance, or personal reasons.
2. **Outcome data:** Every effort will be made to collect outcome data on all participants who withdraw from treatment for whatever reason. If participants withdraw from treatment, all endpoint assessments will be carried out at the point of drop-out and at the 12-month endpoint unless consent is withdrawn.

As PATH-1 is unblinded there will be no procedures related unblinding of the participants

8. Required Actions and Follow-Up

1. **Reporting**:
    - All events of ALT or AST ≥ 3 × ULN and total bilirubin ≥ 2 × ULN (with > 35% direct bilirubin) or ALT or AST ≥ 3 × ULN and INR > 1.5 or GFR <30 mL/min/1.73 m²) and acute kidney injury (e.g., serum creatinine increase by 1.5 times baseline or oliguria) must be reported to the sponsor in an expedited manner.
    - Additionally, QTc prolongation ≥ 460 milliseconds for males or ≥ 470 milliseconds for females, significant arrhythmias (e.g., Torsade de Pointes), or bradycardia with heart rate <50 bpm and associated symptoms (e.g., syncope) must also be reported within 24 hours. Confirmed gastrointestinal bleeding, such as melena, hematemesis, or peptic ulcers, persistent severe symptoms not resolving with management (e.g., uncontrolled vomiting). should be reported as serious adverse events (SAEs) when applicable. All adverse events must be documented in the case report form (CRF) and reported to the study sponsor regulatory authorities according to local requirements.
2. Follow-Up
    - Repeat laboratory or clinical assessments (e.g., liver enzymes, eGFR, ECGs, or GI evaluations) will be conducted weekly or bi-weekly, depending on the severity of the abnormality, until resolution or stabilization.
    - Comprehensive clinical evaluations, including symptom assessments, will be performed as needed.
3. Continuation of the study intervention
    - Continuation of the study intervention of the study intervention will be considered only if the abnormality resolves or improves to a clinically acceptable level, as determined by the investigator and sponsor.
4. Participant communication: Participants will be informed of the findings, any changes to their treatment plan, and the rationale for either resuming or discontinuing the study intervention.

8.1.1. Temporary Discontinuation

Abnormalities identified during regular monitoring, including but not limited to liver chemistry changes, renal function decline, cardiac abnormalities (e.g., QTc prolongation), or gastrointestinal symptoms, will be assessed by the investigator to determine the appropriate course of action. This may include enhanced monitoring, additional diagnostic evaluations, or discontinuation of the study intervention.

8.1.2. Study Intervention Restart or Rechallenge After Stopping Criteria Are Met

Study intervention restart or rechallenge in participants who have met stopping criteria for liver chemistry, ECG abnormalities, renal dysfunction, or other safety-related are not allowed.

8.2. Lost to Follow up

A participant will be considered lost to follow-up if the participant repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, with telephone calls, and if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, the participant will be considered to have withdrawn from the study.
- Site personnel may look up vital status in The Norwegian Cause of Death Registry or the medical journal.

If vital status is determined as deceased, this will be documented, and the participant will not be considered lost to follow-up.

9. Study Assessments and Procedures

Consented patients will undergo a screening visit within 28 days of the planned baseline visit. For women of childbearing potential, a negative serum pregnancy test will be required within 1 day prior to receiving the first dose of study drug. Only those patients who complete screening and meet study eligibility criteria at baseline will be randomized 6:2 to receive phenserine or donepezil in an open-label manner. The first dose of study medication will be taken in the clinic, and the participants will be instructed on how to continue dosing at home until their next visit.

Participants will visit the clinic for safety and other planned study assessments at 2-week intervals through the 8-week treatment period, as detailed in the Schedule of Activities (Table 1).

Patients who withdraw or discontinue from the study before Week 8 will have an early termination visit within one week of discontinuation that includes safety follow-up. For participants who complete the study, a final follow-up safety assessment will be conducted within one week after completion of dosing.

- Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.
- In the event of a significant study-continuity issue (eg, caused by a pandemic), alternate strategies for participant visits, assessments, medication distribution and monitoring may be implemented by the sponsor or the investigator, as per local health authority/ethics requirements.

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 642 mL.
Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Screening Visit

During the screening visit, participants will undergo a series of assessments to determine their eligibility for the study.

- During this visit, and before any study procedures are performed, informed consent will be obtained from the participant and caregiver to ensure they understand the study's objectives and willingly agree to participate.
    - This includes a medical evaluation with a detailed medical history review including: concomitant medications, physical examination, Vital signs
    - Safety laboratory tests
    - Urinalysis
    - Physical Exam
    - Neurological Exam, including mental status
    - ECG
    - MRI (if not performed within 2 years)
    - Modified C-SSRS
    - Lumbar puncture (Optional, if not performed previously) for CSF analysis of Alzheimer pathology and storage for subsequent biomarker assessment
    - Venepuncture for blood analysis

- Participants will also be evaluated for their cognitive status using MMSE and CDR
- Review of inclusion/exclusion criteria including confirmation of pathology with validated biomarkers
- If participants are taking any anti-dementia medications, they will be instructed on the washout period required before baseline assessments. If considered relevant, re-screen can be performed.

9.2. Randomization

- Participants will be randomized before, or during, the baseline visit.
- Participants will be randomized and allocated study treatment via Viedoc in a 6:2 ratio

9.3. Baseline Visit

At the baseline visit, participants will undergo a comprehensive set of initial assessments. These include:

- Standard medical assessment**:** with the same evaluations as in the screening visit (except neurological examination).
- Participants will be evaluated for their cognitive status using MOCA
- Pregnancy test if applicable
- Adverse events evaluation
- Venepuncture for blood analysis
- Administration of cognitive tests FLAME Memory Composite
- The study drug will be dispensed to the participant

9.4. Week 2 visit (+/- 3 days)

- **Standard medical assesment**: with the same evaluations as in the baseline visit.
- The study drug will be dispensed to the participant
- Adverse events evaluation
- Venepuncture for blood analysis
- Returned bottles and tablets/capsules counted and recorded for compliance.

9.5. Week 4 visit

- **Standard medical assesment**: with the same evaluations as in the baseline visit.
- Modified C-SSRS
- Pregnancy test if applicable
- The study drug will be dispensed to the participant
- Adverse events evaluation
- Venepuncture for blood analysis
- Returned bottles and tablets/capsules counted and recorded for compliance

9.6. Week 6 visit

- **Standard medical assesment**: with the same evaluations as in the baseline visit.
- The study drug will be dispensed to the participant
- Adverse events evaluation
- Venepuncture for blood analysis
- Returned bottles and tablets/capsules counted and recorded for compliance

9.7. Week 8 visit

- **Standard medical assesment**: with the same evaluations as in the baseline visit.
- Modified C-SSRS
- Pregnancy test if applicable
- Adverse events evaluation
- Venepuncture for blood analysis
- Administration of cognitive tests FLAME Memory Composite
- Lumbar puncture for CSF sampling for biomarker analysis (optional)
- Returned bottles and tablets/capsules counted and recorded for compliance.
- .

9.8. Safety Follow-Up Visit

The safety follow-up visit will occur within 4 weeks after the completion of the dosing period. This visit includes:

- **Standard medical assessment**: with the same evaluations as in screening visit.

- Modified C-SSRS (The C-SSRS will only be conducted at the follow-up/ET visit, if the patient experienced suicidal thoughts during the study treatment period.)
- Safety blood tests

9.9. Outcome assessment

Outcome assessments will be conducted at key time points throughout the study, as described in the SoA and in detail in the dedicated SOP. Initial evaluations at the baseline visit include cognitive function tests, vital signs, physical examination, liver function tests, and pharmacokinetic sampling. Follow-up visits at Weeks 2, 4, 6 and 8 will involve ongoing safety and tolerability assessments, vital signs monitoring, liver function tests, additional pharmacokinetic sampling, and cognitive assessment will be completed at baseline and Week 8 to track changes. The final safety follow-up visit will include comprehensive safety evaluations, final vital signs, physical examination, laboratory tests to ensure stability, and final cognitive testing. This approach ensures thorough monitoring and data collection on the efficacy and safety of phenserine compared to donepezil.

9.10. Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA.

9.11. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA.

9.12. Computerized cognitive testing

FLAME computer-based cognitive testing is performed on a highly standardized digital portal, with no input from staff other than setting it up.

We will ensure that all investigators participating in data collection are appropriately trained in administering the FLAME computerized cognitive battery and have a comprehensive understanding of the test's instructions, scoring criteria, and potential challenges in participants.

It is important to familiarize investigators with the study's objectives, ethical guidelines, and the importance of data accuracy.

Assessments are conducted in a quiet and well-lit room within the memory clinic to minimize distractions and ensure participant comfort.

Standardized Instructions:

Begin by providing standardized instructions to participants, explaining the purpose of the assessment and what to expect during the testing.

FLAME Memory Composite:

Administer the FLAME Cognitive Battery following the specific test sequence recommended by the manufacturer.

Ensure that each subtest is administered consistently across all participants, adhering to the standardized instructions provided by the battery.

Monitor participants' responses and progress through the battery, providing necessary guidance or clarification when required.

Data Recording: Record participants' responses and performance with the FLAME computerized cognitive battery accurately, ensuring that all data points are collected in real-time.

Table 5. Tasks Included in Memory Composite score, the Primary outcome:

Task	Timings (minutes)	Description
Picture Recognition (Visual episodic memory)	3.5	At the start of the battery 20 pictures are presented for an equal time on screen. At the end of the battery the original pictures plus the 20 very similar distractor pictures are presented one at a time in a counterbalanced order. For each picture the subject has to indicate whether or not it was the precise picture shown earlier, as quickly and accurately as possible. Each picture remains on the screen until a response is made. The accuracy and speed of each response is recorded.
Self-ordered search (working memory)	5 Minutes Average (however, this task is performance driven so can be as short as 1.5 minutes)	Participants search a series of on-screen boxes to find a hidden symbol. Once found, participants search for the symbol again, remembering that the symbol will never be hidden in the same box twice. The symbol is hidden in every box once per level. After successfully completing a level, a new level opens with more boxes to search than the previous level. The outcome measure is the average number of boxes in the successfully completed trials. Participants are allowed three errors before the test terminates. This task measures working memory
Paired Associate Learning (working memory)	3 Average (however, this task is performance driven so can be as short as 1.5 minutes)	Participants are shown objects, one per "window" in a grid. Then they see the series of objects, one at a time in a random order, and select the correct "window" where the object had previously appeared. This version uses a ratchet- style approach, each successful trial is followed by one with more objects to recall and each unsuccessful trial is followed by the same number of objects as in the unsuccessful attempt. The outcome measure is the average number of correct object-place associations ("paired associates") in the trials that were successfully completed. Participants are allowed three errors before the test terminates. This task measures working memory and learning/episodic memory.
Digit Span (numerical working memory)	3 Minutes Average (however, this task is performance driven so can be as short as 1.5 minutes)	A series of numbers is shown to the participant who then enters the numbers in the same sequence as they appeared using a number keypad. The test uses a ratchet-style approach in which each successful trial is followed by a new sequence that is one digit longer than the last and each unsuccessful trial is followed by a new sequence that has the same number of digits as the unsuccessful trial. This allows an accurate estimate of digit span to be made quickly. The outcome measure is the average number of digits in all successfully completed trials. Participants are allowed three errors before the test terminates.
Total	13	

Table 6. Digital cognitive tests included in the secondary cognitive outcome

Task	Timings (minutes)	Description
Simple Reaction Time	2	The subject is required to respond as quickly as possible when a stimulus is presented in the center of the screen. The subject is informed that the stimuli will be presented one at a time and that they will remain there until a response is made. The speed of each response is recorded.
Digit Vigilance	3	A target digit from 1 to 9 is randomly selected and constantly displayed to the right-hand side of the screen. Digits are then presented one at a time in the center of the screen. The subject is required to respond as quickly as possible every time a digit matches the target digit. Correct detections, the speed of the detections and responses made in error (false alarms) are recorded.
Choice Reaction Time	2	The two possible stimuli in this task that an appear on screen. Equal amounts of each stimuli type will be displayed. The subject is required to respond with the correct response key as quickly as possible every time the stimuli appear on screen. The accuracy and speed of each is response is recorded.
Delayed Visual Recognition (Picture Recognition)	3.5	At the start of the battery 20 pictures are presented for an equal time on screen. At the end of the battery the original pictures plus the 20 very similar distractor pictures are presented one at a time in a counterbalanced order. For each picture the subject has to indicate whether or not it was the precise picture shown earlier, as quickly and accurately as possible. Each picture remains on the screen until a response is made. The accuracy and speed of each response is recorded.
Verbal Reasoning	3	A sentence is displayed at the bottom of the screen whilst a square and a circle are displayed above. The participant needs to respond true or false as to whether the sentence correctly describes the configuration of the circle and square. Participants are given three minutes to solve as many problems as they can. The task measures verbal reasoning.
Total	15	

9.13. Physical Examinations

A brief physical examination will be performed at each visit, including, at a minimum, assessments of:

- Skin Assessment: As part of the assessment, the skin will be examined for any anomalies, such as lesions, rashes, or discolorations.
- Assessment of the Cardiovascular System: This involves evaluating the heart and blood vessels as well as the cardiovascular system. The participant's pulse, blood pressure, and heart sounds will be evaluated.
- Respiratory System Assessment: The examination will include an evaluation of the respiratory system, focusing on lung function assessing the participant's breathing rate and depth, looking for signs of respiratory distress, or listening for breath sounds that could indicate respiratory issues. The state of the lungs will be assessed, usually using auscultation.

- Abdominal Assessment (Liver and Spleen): The abdominal assessment will involve palpation (gentle pressing) of the abdomen to check for any tenderness, masses, or organ enlargement, specifically focusing on the liver and spleen. This helps to detect any hepatosplenic abnormalities or discomfort in the abdominal region.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.13.1. Vital Signs

- Pulse rate, respiratory rate, and blood pressure will be recorded (before blood collection for laboratory tests).
- Blood pressure and pulse measurements will be assessed in a supine position.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (eg, television, cell phones).

9.14. Electrocardiograms

- 12-lead ECG(s) will be obtained as outlined in the SoA (see Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR. QRS, QT, and QTc intervals will be evaluated. Clinical Safety Laboratory Tests

9.15. Clinical Safety Laboratory Tests

- See Appendix 2 for the list of clinical laboratory tests to be performed and the SoA (Section 1.3) for the timing and frequency.
- The investigator must review the laboratory results, document this review, and record any clinically significant changes occurring during the study as an AE. The laboratory results must be retained with source documents.
- Abnormal laboratory findings associated with the underlying disease are not considered clinically significant unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within [insert timeframe] after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.
    - If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.
    - All protocol-required laboratory tests, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA (Section 1.3).
    - If laboratory values from non-protocol-specified laboratory tests performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded.

9.16. Suicidal Ideation and Behavior Risk Monitoring

Participants will be monitored appropriately and observed for suicidal ideation and unusual behavior. Items from the Columbia-Suicide Severity Rating Scale (C-SSRS) will be used as a guide. If signs of suicidal behavior are observed, a risk assessment will be conducted. When informed consent or assent has been given, the informant should be alerted about the need to monitor participants for the emergence of unusual changes in behavior, as well as the emergence of suicidal ideation and behavior and to report such symptoms immediately to the study investigator.

9.17. Adverse Events (AEs) Serious Adverse Events (SAEs), and Other Safety Reporting

The definitions of adverse events (AEs) and serious adverse events (SAEs) can be found in Appendix 3.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs considered related to the study intervention or study procedures, or that caused the participant to discontinue the study intervention (see Section 7). This includes events reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative). Adverse events will be discussed with the local study doctor, and as needed discussed with the medical monitor. Based on the severity considering the safety of the participants, a decision will be made regarding dose reduction, pausing the study drug, or withdrawing the participant from the study.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.

9.17.1. Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the start of treatment until the last follow-up visit at the timepoints specified in the SoA (Section 1.3).

All AEs will be collected from the start of treatment until the last follow-up visit according to the timepoints specified in the SoA (Section 1.3).

Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded as medical history/current medical conditions, not as AEs.

All SAEs will be recorded and reported to the sponsor immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

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

9.17.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrences. All adverse events (AE) will be collected from start of treatment until resolution or

until the investigator assesses AE as “chronic” or “stable”. Each AE is to be classified by the investigator as “serious” or “non-serious”. This classification of the gravity of the event determines the reporting procedures to be followed. The intensity of AE will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) of the U.S. Department of Health and Human Services. If a serious AE (SAE) occurs, reporting will follow local and international regulations, as appropriate. All SAE will monitor for (1) a relationship between SAE and trial medication, (2) expectedness of SAE (derived from reference safety information of approved formulation of phenserine) (3) benefit/risk assessment for the clinical trial did change as a result of SAE, and (4) safety issues, which are sufficient to consider changes in the conduct of the clinical trial. Additionally, the investigator must make a causality assessment for all AEs. In case of a causal relationship with at least reasonable relationship between occurrence of AE and study medication, the AE will be determined as adverse reaction (AR). A case of a serious AR (SAR) that is unexpected with respect to reference safety information of the approved formulation of phenserine will be determined a Suspected Unexpected Serious Adverse Reaction (SUSAR) and requires expedited reporting according to the legal framework (competent authorities, ethic committees, clinical trial sites). Further details are provided in Appendix 3.
A summary of the nonclinical and clinical safety and pharmacokinetics data of phenserine is available in section 6 (page 29) of the Investigator`s Brochure. The section includes a description of serious adverse events and adverse events reported in other clinical trials with phenserine.

9.17.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3.

9.17.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, institutional review boards (IRBs)/independent ethics committees (IECs), and investigators.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it and will notify the IRB/IEC, if appropriate according to local requirements.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

9.17.5. Annual Safety Report (ASR) Responsibility

- In accordance with the Clinical Trial Regulation (CTR, EU 536/2014), Annex III, the sponsor is responsible for the preparation and submission of the Annual Safety Report (ASR) for this clinical trial. The ASR will summarize the safety data collected over the reporting period, including an analysis of adverse events, serious adverse events, and other safety findings.

9.17.6. Pregnancy

- Details of all pregnancies in female participants, will be collected after the start of the study intervention and until the study end.
- If a pregnancy is reported, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the pregnancy.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.
- The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate, and the information will be forwarded to the sponsor.
- Any poststudy pregnancy-related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in Section 8.3.3. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention and be withdrawn from the study.

9.17.7. Cardiovascular and Death Events

Cardiovascular events (eg coronary infarction) or cerebrovascular events (e.g. stroke) will be recorded. A QTc interval ≥ 460 milliseconds for males or ≥ 470 milliseconds for females will be considered abnormal during the ECG assessments.

9.18. Pharmacokinetics

- Plasma PK samples will be collected at W 2, 4, 6, and 8 visits for all IMP participants. Samples will be analyzed for phenserine to determine Cmax and t1/2. Available data will be analyzed by the DSMB to assess the safety of ongoing patients. PK samples will also be collected at the Week 8 visit, at peak dose, from all other patients (and at the final maintenance dose level for those who down-titrate due to tolerability) prior to taking the

morning dose, to establish steady-state concentration and stored for later analyses aimed at exploring the relationship between exposure and tolerability/efficacy outcomes.

- Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded. Samples will be used to evaluate the pharmacokinetics (PK) of phenserine. Each sample will be divided into aliquots as described in the SOP. These samples may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

9.19. Pharmacodynamics

Pharmacodynamic measurements will be performed according to the study's SOP and outlined in the SOA. These assessments will include changes in exosome biomarkers of pre-programmed cell death, synaptic integrity and function, and neuroinflammation will be assessed.

The pre-programmed cell death panel (PNCDD) will investigate key factors such as BAX, Bcl-2, which will provide insights into preprogrammed cell death processes (4,17) and any changes in the profile observed following treatment with phenserine or donepezil. The second panel focuses on synaptic markers, including synaptotagmin, offering a nuanced understanding of synaptic integrity and function. The third panel is centered on evaluating inflammatory biomarkers, including TNF-α, IL-1β, IL-6, and IL10, and the fourth panel will evaluate AD-specific markers, including Aβ1-42, and will elucidate kay molecular signatures associated with AD pathology. This comprehensive evaluation of mechanistic biomarkers through exosome technologies aims to deliver a detailed understanding of the impact of phenserine versus donepezil on various facets of AD neuropathology.

The second panel focuses on synaptic markers, offering a nuanced understanding of synaptic integrity and function.

The third panel is centered on evaluating inflammatory biomarkers.

The fourth panel will evaluate AD-specific markers, elucidating key molecular signatures associated with AD pathology.

Additionally, pharmacodynamic effects related to AChE inhibition will be evaluated. AChE activity will be measured to assess the degree of enzyme inhibition achieved by phenserine compared to donepezil. The inhibition levels will be correlated with both clinical outcomes and other biomarker changes to provide a comprehensive view of the drug's pharmacodynamic profile.

9.20. Genetics

Genetics are not evaluated in this study.

9.21. Biomarkers

Blood and CSF biomarker samples will be collected at Screening and Week 8, while exosomal biomarkers will be assessed at Baseline, Week 2, Week 4, Week 6, and Week 8. This comprehensive evaluation of mechanistic biomarkers through exosome technologies aims to deliver a detailed understanding of the impact of phenserine versus donepezil on various facets of AD neuropathology, contributing to the trial's primary objective.

9.22. Health Economics OR Medical Resource Utilization and Health Economics

Health economics and Medical resource utilization are not evaluated in this study.

10. Statistical Considerations

The Statistical Analysis Plan will be completed before the first participant is randomized.

10.1. Randomization Weighting

The purpose of this study is to determine an appropriate dose range of phenserine for a subsequent Phase 2 study aimed at further characterizing phenserine's therapeutic potential for the treatment of people with early or mild AD. The randomization process is weighted 6:2 for phenserine versus donepezil, to ensure that there is adequate data to assess dose response relationships for phenserine, and to be able to compare with direct data from the donepezil arm and for comparison with donepezil's well-established clinical profile.

Early drop outs

Participants who drop out early from the study (before study drug is taken or before first assessment) will be replaced with other eligible recruitment subjects in order to maintain the target sample size and ensure the statistical power of the study is preserved

10.2. Statistical Hypotheses

- **Primary Hypothesis:**
    - There is a difference between phenserine and donepezil in terms of their effect on the primary endpoint, which involves changes in exosome biomarkers of pre-programmed cell death, synaptic integrity and function, neuroinflammation, and AD-related protein trafficking.
- **Seconday Hypothesis**
    - Phenserine and donepezil do not differ in their safety and tolerability profiles, as measured by the rate and severity of adverse events (AEs).
- **Exploratory Hypothesis:**
    - Phenserine has a different effect compared to donepezil on short-term cognitive performance, as measured by the FLAME Memory Composite and other cognitive sub-tests.
    - Phenserine and donepezil differ in their effects on changes in blood and cerebrospinal fluid (CSF) biomarkers, including Aβ1-40, Aβ1-42, Tau, p-tau, p-tau217, and Nfl

10.3. Statistical Analyses

All primary, secondary and exploratory outcomes will be collected for all participants, except that PK analyses will be performed only for participants randomized to phenserine.

Trial management and data management will be conducted by the University Hospital Stavanger Clinical Trials Unit and the trial statistics will be led by Dr Gareth Williams, King's College London.

Standard safety data (including all AEs, TEAEs, SAEs, important clinical laboratory findings, and AE-related discontinuations) will be summarized for each treatment arm and dose level.

Exosome biomarker values will be natural log transformed to avoid skewness. To assess the effect of phenserine on a given biomarker, a linear mixed-effects model will be used, with treatment groups (phenserine vs donepezil), time, baseline biomarker, and plate to plate variability in exosome concentration (determined by NanoSight) as fixed effects and participant identification treated as a random effect.

To demonstrate that dropout rates are Missing at Random (MAR), we will calculate the correlation between dropout indicators (e.g., whether a participant drops out) and key covariates, including treatment group, baseline characteristics, and other relevant random effect categories. Based on prior observations (liraglutide trail for example), we expect that the likelihood of dropout will largely be explained by observed factors, especially drug treatment status. A logistic regression model will be used, with the dropout indicator as the dependent variable and treatment status, baseline covariates, and any other relevant factors as independent variables. If the model explains a significant portion of the dropout pattern, this would support the MAR assumption. Sensitivity analyses will also be conducted to assess the robustness of this finding under alternative missingness mechanisms.

For clinical secondary and exploratory outcomes, change from baseline will be calculated at the final study assessment (mean, n, standard deviation, standard error, minimum and maximum scores).

The primary outcome is the difference between treatment groups in key proteins measured in neuronally derived exosomes. Based on the previous work of Goetzl et al(30,31) between group differences of 4.9 SD were achieved in these measures. Assuming a difference of half of this magnitude (2.45 sd), a sample size of 4 participants per arm would be needed to give 80% power to detect a difference between groups to the 0.05 level of significance. The additional participants receiving phenserine will provide valuable information for safety reporting.

10.3.1. General Considerations

The statistical analysis plan for this study is designed to assess the efficacy, safety, and pharmacokinetic properties of phenserine compared to donepezil in participants with early or mild Alzheimer's Disease (AD). Analyses will be conducted on a modified intent-to-treat (mITT) population, which includes all randomized participants who receive at least one dose of study medication and have at least one post-baseline assessment. All statistical tests will be two-sided, with a significance level set at 0.05.

10.3.2. Primary Endpoints Analysis

10.3.3. Definition of endpoint(s)

The primary endpoint of this study is the change from baseline in exosome biomarkers, including markers of pre-programmed cell death. These biomarkers plasma are critical indicators of neurodegenerative processes in AD.

10.3.4. Main Analytical Approach

The primary analysis will compare the change in exosome biomarker levels between the phenserine and donepezil treatment groups. The model will adjust for baseline biomarker levels, treatment group, and any other relevant covariates.

10.3.5. Secondary Endpoints Analysis

The secondary endpoints include safety and tolerability, cognitive performance, and pharmacokinetic parameters. Safety and tolerability will be assessed by comparing the incidence and severity of adverse events (AEs) between treatment groups.

10.3.6. Exploratory Endpoints Analysis

Exploratory endpoints focus on changes in blood and CSF biomarkers and their relationship with clinical outcomes.

10.3.7. Safety Analyses

Safety analysis will involve descriptive analysis comparing frequencies between the two treatment groups with frequency AEs and SAEs.

10.4. Sample Size Determination

A sample size of 16 participants for this initial dose-range finding study of phenserine is expected to provide adequate data to inform dose selection and study design for a follow-on Phase 2 trial in a larger sample of participants with early or mild AD.

The primary outcome is the difference in key protein levels measured in neuronally derived exosomes between treatment groups. Previous studies (Goetzl et al.) (30,31)have shown between-group differences of 4.9 standard deviations (SD) in similar measures. Conservatively, this study assumes an effect size of half that magnitude (2.45 SD). Based on this assumption, a sample size of 16+4 4 participants would be required to achieve 80% power to detect a statistically significant difference ($p < 0.05$) between treatment groups.

11. Supporting Documentation and Operational Considerations

11.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

11.1.1. Regulatory and Ethical Considerations

Regulatory compliance

Before data collection commences, the Coordinating Investigator will ensure that appropriate research ethics committee (REC) approvals are in place.

If any amendments are required, the Coordinating Investigator will ensure that information is submitted to the REC in order for that body to issue approval for the amendment. Amendments will only be implemented once approval has been granted.

The Coordinating Investigator will further ensure that this study is conducted in accordance with International Council for Harmonisation (ICH) guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study patient.

The Investigators will also ensure adherence to the basic principles of Good Clinical Practice (GCP).

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) international ethical guidelines
- Applicable ICH Good Clinical Practice (GCP) guidelines
- Applicable laws and regulations
- The protocol, protocol amendments, ICF, investigator's brochure, Investigational Directions for Use (IDFU), and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

- Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following, as applicable:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
    - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.

- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies, European Medical Device Regulation 2017/745 for clinical device research, and all other applicable local regulations

This clinical trial will be conducted in accordance with the Clinical Trial Regulation (EU 536/2014), ensuring that all processes and procedures comply with the regulatory requirements set forth by the European Union. The study will adhere to Good Clinical Practice (GCP) guidelines, national laws, and ethical standards to safeguard participant rights, safety, and well-being throughout the trial.

Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Participant Confidentiality

- The investigator or the investigator's representative will explain the nature of the study, including the risks and benefits, to the potential participant and answer all questions regarding the study.
- To prevent compromises to voluntary informed consent, written consent must be obtained from a physician with an independent relationship to the potential participant. This means that if the patient's treating physician is study investigator, he/she cannot carry out the consent procedure with her/his patient.
- Potential participants must be informed that their participation is voluntary. They will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, privacy, and data protection requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be reconsented to the most current version of the ICF(s) during their participation in the study.

Participant Confidentiality

Personal data will be collected on the consent form and as a log of participants. Paper records will be securely stored in a locked cabinet in a secure room. Electronic records will be password protected on institutional computers. Personal records will be help separately from research records.

Research assessments will be completed using electronic and paper schedules, using only study numbers as identifiers. Data and schedules will be securely stored and electronic data will be password protected. All data will be stored using a linked anonymized system so that no personal identifiers are present on the schedules or databases including the collected study data.

Any personal data stored on institutional computers will be subject to appropriate access controls ensuring that access to confidential research information is password protected and restricted to those who need access.

All procedures will be in full compliance with the GDPR.

Data Protection

The documentation of the clinical trial data in adherence to the GCP-guidelines and the trial protocol is the responsibility of the investigator. All essential documents will be kept in the Investigator Site File (ISF), which will be stored at the trial site in accordance with ICH GCP. Original data (source documents) remain in hospital medical records and information in the eCRF must be traceable and consistent with the original data. Source documents are e.g., laboratory results, measurements of clinical scales, vital sign measurements, and questionnaires. No information in source documents about the identity of the patients will be disclosed. Data collected in this clinical trial (see study procedures and trial flow chart) except data for exploratory analysis must be entered in an eCRF which has to be completed by the investigator or authorized trial personnel and signed by the investigator. This also applies for those patients who do not complete the clinical trial. If a patient withdraws from the clinical trial, the reason must be recorded in the eCRF. The principal investigator holds the responsibility for ensuring the accuracy, completeness, and punctuality of all data submitted to the sponsor in the eCRFs and in all mandatory reports.

After database lock, the principal investigator will receive data on an electronic device that includes the investigational site data for archiving in the Investigator Site File (ISF). Data are processed by data management by the study statistician and his team with the support of a study database (eCRF) according to the SOPs. The evaluation of the data takes place by programmed validity and consistency checks. The description of the electronic data capture (EDC) system is detailed in Appendix 5.

Monitoring activities are performed, by NORCRIN, to ensure that the trial is conducted in accordance with the trial protocol, the principles of GCP, and local legislation. A monitoring manual will be applied, describing the scope of the monitoring activities in detail. A monitoring visit report is prepared for each visit describing the progress of the clinical trial and all identified problems. If a pandemic situation will not allow an on-site monitoring, remote monitoring or monitoring by phone is possible if data protection aspects are considered.

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that their personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the

participant who will be required to give consent for their data to be used as described in the informed consent.
The participant must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
The contract between sponsor and study sites specifies responsibilities of the parties related data protection, including handling of data security breaches and respective communication and cooperation of the parties.
Information technology systems used to collect, process, and store study-related data are secured by technical and organizational security measures designed to protect such data against accidental or unlawful loss, alteration, or unauthorized disclosure or access.

Trial management

Professor Clive Ballard is the Chief Investigator and will have academic oversight of the trial and be responsible for reporting to the funder. The Coordinating Investigator for the PATH-1 RCT is Professor Dag Aarsland, who will have overall responsibility for the conduct of the trial and interactions with the sponsor, regulator, and ethics committee, as appropriate and required during the course of the trial. Professor Nigel Greig will be responsible for the analysis of exosomes, the primary outcome measure.

This trial will be managed by a Trial Management Group (TMG), which will include the Principal Investigators, the Trial Manager and a patient representative. The TMG will be co-chaired by the Chief Investigator and the Coordinating Investigator. The group will meet at three-month intervals and additionally as appropriate. The TMG will have direct oversight of and responsibility for the study. The remit of the TMG will also include overall progress of the trial and to monitor progress against milestones. Any discrepancy from milestones will be highlighted and a plan developed to address any difficulties. The day-to-day management of the trial will be conducted by the Trial Manager, who will report to the Coordinating Investigator on a regular basis. The Trial Manager will send a written report to the chairs of the TMG before each TMG meeting, detailing progress. Any non-urgent major decisions will be made by the TMG.

The Trial Steering Committee (TSC), , will meet twice over the course of the study (with additional meetings convened if requested by the Data and Safety Monitoring Board (DSMB) and will be responsible for oversight of trial governance and reporting to the sponsor.

A DSMB chaired by Anne Katrine Bergland (Geriatrician) will provide independent review of unblinded safety data collected during the study. All serious adverse events (SAEs) will be reviewed by the chair within 24 hours, and summary trial reports will be reviewed by the full committee every three months through its completion. The data will be prepared for these reviews by a separate team of unblinded data managers and statisticians who have no involvement in the day-to-day conduct of the study.

The committee will be governed by a charter and will make recommendations to stop, continue or modify the trial based on their ongoing review of the safety data. Emergency meetings will be convened on an ad hoc basis and as warranted by emergent study data.

The DSMB and TSC will monitor recruitment, ethical issues and safety and will have the ultimate responsibility for the continuation or discontinuation of the study.

Dissemination of Clinical Study Data

The clinical trial results will be uploaded in CTIS within 1 year after study end.

Data Quality Assurance

- All participant data relating to the study will be recorded on electronic CRFs unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- Guidance on completion of CRFs will be provided in a SOP.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source documents.
- Monitoring details describing strategy, including definition of study critical data items and processes (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the monitoring plan.
- The sponsor is responsible for the data management of this study, including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (eg, contract research organizations).
- The investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical trial.
- Medical files of subjects shall be archived in accordance with national law.
- No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data and its origin can be found in monitoring guidelines.

- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The sponsor or designee will perform monitoring to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Protocol compliance

Protocol deviations will be reported to the Trial Manager, Coordinating Investigator and Sponsor immediately. An Exception Report will be prepared by the Trial Manager, covering a summary and chronology of events, an assessment of risk and details of the corrective actions to be taken. The Coordinating Investigator will monitor all deviations, ensure that the necessary corrective actions are taken, and identify and address through preventive action any instances where a deviation recurs.

Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the first site open and will be the study start date.

Study/Site Termination

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further study intervention development

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
- Total number of participants included earlier than expected
- Recruitment rate is considered too low

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable

regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Role of Trial Sponsor

Stavanger University Hospital is the sponsor with legal responsibility for the research integrity of the study. This role will be delegated to the Coordinating Investigator.

The sponsor will ensure that the research team has access to resources and support to deliver the research as proposed and that responsibilities for management, monitoring and reporting of the research are in place prior to the study commencing. The sponsor will ensure that there is agreement on recording, reporting and reviewing significant developments as the research proceeds and approve any modifications to design, obtaining requisite regulatory authority.

The sponsor will assume responsibility for operating the management and monitoring systems of the research.

11.1.2. Recruitment strategy

Clinical trial recruitment will be conducted at 6 clinical trial centers. The centers have established track records for successful recruitment. The PIs have worked together on successful clinical cohort and clinical trial studies for more than 15 years and have an excellent record of successful collaboration.

The usual recruitment approach will be supplemented by the PROTECT Norge platform an online cohort study that supports nested clinical trials. It is coordinated as a partnership between the University of Exeter and University Hospital Stavanger. PROTECT Norway currently has more than 5,000 participants over 50 years of age without dementia, including more than 600 participants with stage 2 or stage 3 MCI and is continuing to actively recruit additional participants. Appropriate individuals may be invited to participate as PROTECT has the advantage of recruiting from a population with an established trajectory of cognitive decline and from the specific population on which the power calculations are based.

The expected enrolment period is ~3 months from the first patient to last patient randomised. Based on the 8-week treatment period and safety follow-up period for the last patient in, the full

duration of the study is expected to be ~6 months. Recruiting participants from high quality and experienced clinical trial centers will maximize inter-rater reliability. Each center has active memory clinics, research cohorts of people with MCI and active clinical trial programs, further supplemented by PROTECT recruitment as needed.

11.1.3. Participant Confidentiality

Personal data will be collected on the consent form and as a log of participants. Paper records will be securely stored in a locked cabinet in a secure room. Electronic records will be password protected on Hospital or University computers. Personal records will be held separately from research records.

Research assessments will be completed using electronic and paper schedules, using only study numbers as identifiers. Data and schedules will be securely stored, and electronic data will be password protected. All data will be stored using a linked anonymized system so that no personal identifiers are present on the schedules or databases including the collected study data.

Any personal data stored will be subject to appropriate access controls ensuring that access to confidential research information is password protected and restricted to those who need access. All procedures will be in full compliance with the GDPR.

11.2. Appendix 2: Clinical Laboratory Tests

- The safety laboratory will monitor key hematological, biochemical, and urinary parameters throughout the trial period. Complete battery will be performed at screening and Safety follow up, for details refer to the laboratory tests SOP.
- Follow-up visits: Liver and renal function with urinalysis (dipstick) tests will be conducted to monitor participant safety and assess any potential adverse effects throughout the trial duration.
- The tests detailed in Table below will be performed by the local laboratory.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 7: Protocol-required Safety Laboratory Tests

Laboratory Tests	Parameters	
Hematology	• Platelet count	
	• Red blood cell (RBC) count	
	• RBC indices	– Mean corpuscular volume (MCV)
		– Mean corpuscular hemoglobin (MCH)
		– %Reticulocytes
	• White blood cell (WBC) count with differential:	– Neutrophils
		– Lymphocytes
		– Monocytes
		– Eosinophils
		– Basophils
	• Hemoglobin	
	• Hematocrit	
Clinical chemistry[1]	• Blood urea nitrogen (BUN)	• Aspartate aminotransferase (AST)/serum glutamic-oxaloacetic transaminase (SGOT)
	• Potassium	
	• Creatinine	
	• Sodium	• Alanine aminotransferase (ALT)/serum glutamic-pyruvic transaminase (SGPT)
	• Calcium	
	• Glucose (fasting)	• Alkaline phosphatase[2]
	• Vitamin B12 (total and active)	• Total and direct bilirubin
	• CRP	• Total protein

Routine urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, (bilirubin, urobilinogen, nitrite, leukocyte esterase) by dipstick • Microscopic examination (if blood or protein is abnormal)
Pregnancy testing	• Highly sensitive (serum or urine) human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)[3]
	○

NOTES:

1. Liver Safety: Suggested Actions and Follow-up Assessments and Study Intervention Rechallenge Guidelines. All events of ALT or AST ≥3 × upper limit of normal (ULN) and total bilirubin ≥2 × ULN (> 35% direct bilirubin) or ALT or AST ≥3 × ULN and international normalized ratio (INR) > 1.5 (if INR measured), which may indicate severe liver injury (possible Hy's law), must be reported to sponsor in an expedited manner (excluding studies of hepatic impairment or cirrhosis).
2. If alkaline phosphatase is elevated, consider fractionating.
3. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC

Investigators must document their review of each laboratory safety report.

11.3. Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition

- An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Definition of Unsolicited and Solicited AE

- An unsolicited AE is an AE that was not solicited using a participant diary and that is communicated by a participant/participant's legally authorized representative (LAR)(s) who has signed the informed consent. Unsolicited AEs include serious and nonserious AEs.
- Potential unsolicited AEs may be medically attended (ie, symptoms or illnesses requiring a hospitalization, emergency room visit, or visit to/by a healthcare provider). The participants/participant's LAR(s) will be instructed to contact the site as soon as possible to report medically attended event(s), as well as any events that, though not medically attended, are of participant/participant's LAR(s) concern. Detailed information about reported unsolicited AEs will be collected by qualified site personnel and documented in the participant's records.
- Unsolicited AEs that are not medically attended nor perceived as a concern by the participant/participant's LAR(s) will be collected during an interview with the participants/participant's LAR(s) and by review of available medical records at the next visit.
- Solicited AEs are predefined local site and systemic events for which the participant is specifically questioned, and which are noted by the participant in their diary.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease, or more severe than expected for the participant's condition)
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition
- New condition detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an

AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

- Lack of efficacy or failure of expected pharmacological action per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.
- The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as an AE or SAE if they fulfill the definition of an AE or SAE. Lack of efficacy or failure of expected pharmacological action also constitutes an AE or SAE.

Events not Meeting the AE Definition

- Any abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen

Definition of SAE

An SAE is defined as any untoward medical occurrence that, at any dose, meets one or more of the criteria listed:

a. Results in death

b. Is life threatening

The term *life threatening* in the definition of *serious* refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent or significant disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations such as important medical events that that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
    - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, convulsions not resulting in hospitalization, or development of intervention dependency or intervention abuse.

Definition of suspected unexpected serious adverse reaction (SUSAR)
If an event is not an SAE per definition above, then it cannot be a SUSAR
SUSAR Definition
Adverse Reaction: all unwanted and unintended responses to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information.
Suspected Unexpected Serious Adverse Reaction: Unexpected Adverse Reaction that:

- Results in death
- Is immediately life-threatening.
- Requires in-patient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect

Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.
Recording and Follow-Up of AE and/or SAE
AE and SAE Recording

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

- There may be instances when copies of medical records for certain cases are requested by medical monitor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the medical monitor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

- The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories:
- Mild:
  A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate:
  A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe:
  A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship.
- A *reasonable possibility* of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration, will be considered and investigated.
- For causality assessment, the investigator will also consult the IB and/or product information, for marketed products.
- The investigator must review and provide an assessment of causality for each AE/SAE and document this in the medical notes There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the medical monitor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to medical monitor.
- The investigator may change their opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the medical monitor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the medical monitor with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally submitted documents.
- The investigator will submit any updated SAE data to the medical monitor within 24 hours of receipt of the information.

Reporting of SAEs

SAE Reporting to CI and sponsor via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to **CI and sponsor** will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken offline to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken offline, then the site can report this information on a paper SAE form (see next section) or to the **CI and sponsor** by telephone.
- SAE Reporting to Project manager via Paper Data Collection Tool
- E-mail transmission of the SAE paper data collection tool is the preferred method to transmit this information to the medical monitor or project manager.
- In rare circumstances and in the absence of e-mail equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE data collection tool within the designated reporting timeframes.
- Contacts for SAE reporting (medical monitor or project manager) can be found in Appendix 10.

Reporting of SUSAR

The investigator will report SAE to the medical monitor. The medical monitor will evaluate if the SAE also is a SUSAR, if so, the medical monitor will report the SUSAR to the Compentent Authority through Eudravigilance (EV)

Sponsor will ensure that all relevant information about SUSARs that are fatal or life-threatening is recorded and reported as soon as possible and in no case later than seven (7) days after

knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight (8) days.
Other SUSARs will be reported no later than 15 days after the incident.

11.4. Appendix 4: Contraceptive and Barrier Guidance

Definitions

For the purpose of this document, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. For the purpose of this document, a man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

Contraception Guidance

Birth control methods which may be considered as highly effective

For the purpose of this guidance, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation 1:
    - oral
    - intravaginal
    - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation 1:
    - oral
    - injectable
    - implantable 2
- intrauterine device (IUD) 2
- intrauterine hormone-releasing system (IUS) 2
- bilateral tubal occlusion 2
- vasectomised partner 2,3
- sexual abstinence 4

Effective birth control methods which may not be considered as highly effective

Effective birth control methods that result in a failure rate of more than 1% per year include:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide 5
- cap, diaphragm or sponge with spermicide 5

3 A combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods.

Birth control methods which are considered unacceptable in clinical trials

Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together.

11.5. Appendix 5: Electronic data capture (EDC)

Viedoc is a web-based solution for collection of data which satisfies all regulatory requirements for clinical trials, for example GDPR and IHC GCP. The Viedoc platform includes functionality for randomization of patients, drug logistics including advanced allocation of drugs, and coding of medical terms. Access is controlled by the study team with 2-factor identification, and all actions are logged including data edits. Main functionalities provided by Viedoc are subject screening, online data entry, data validation, review and signing. Data is easily exported, and there is an online reports portal for quick overview of study progress and statistics. Viedoc's platform is designed to streamline and enhance various aspects of clinical trial management, including:

- Randomization Algorithms: EDC systems like Viedoc allow researchers to define randomization algorithms that determine how participants are assigned to different treatment groups. Common methods include simple randomization, block randomization, and stratified randomization, among others.
- Centralized Randomization: In multicenter clinical trials, central randomization ensures that the random assignment is done independently of the site and is managed centrally to maintain the integrity of the randomization process.
- Data Collection: Viedoc's platform allows for the creation of electronic case report forms (eCRFs), which replace traditional paper-based data collection methods. This digital approach can improve data accuracy and reduce the time and effort required for data entry.
- Data Management: The system helps with the secure storage and management of clinical trial data. It often includes features for data validation, discrepancy management, and audit trail creation to ensure data quality and compliance with regulatory requirements.
- Remote Monitoring: Viedoc's EDC system may support remote monitoring of clinical trial data, making it easier for sponsors and monitors to review and verify data without the need for physical site visits.
- Regulatory Compliance: The system is typically designed to meet regulatory standards and guidelines, such as those set by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA).
- Real-time Reporting: Researchers can often generate real-time reports and analytics from the collected data, enabling quicker decision-making and analysis of trial progress.

11.6. Appendix 6: User involvement

User involvement is important to ensure the study design is feasible and acceptable for participants, that the research questions are relevant and important for society, for dissemination and spread of the study to enhance recruitment and for dissemination of the study results to help implementation of relevant changes. A dedicated user representative, Jorunn Nilsen, has been allocated to the study. We will ensure user involvement in all phases and all levels of the study, through regular national meetings with a structure that ensures user involvement, to involve and consult end-users throughout the program, from protocol design, informed consent/information leaflets, to dissemination and implementation support. The user involvement will capitalize on SESAM's platform for user and community involvement, WiseAge, which was established in 2015. The WiseAge platform includes a User Panel and User Advisory Board, and all members can give advice and recommendations concerning ongoing and planned research The NORADD-TP plan was presented to the User involvement reference group on Nov 25th, 2021, and received positive feedback. It was noted that participating patients should receive treatment as usual, that the need for lumbar puncture is well explained, that any gastrointestinal side-effects from phenserine are adequately monitored and to ensure that the user involvement is nation-wide. These elements have been implemented and relevant changes to the study plan have been made. SESAM's user involvement strategy will be implemented at all participating centres and a dedicated user representative will be hired at each participating centre. The Norwegian Health Association, as the national patient organization for persons with dementia and their relatives, will contribute regarding PPI, and one of their members will represent the stakeholders in the project organization.

11.7. Appendix 7: Subject diary

Sample of the subject diary for participants allocated to treatment with phenserine

HELSE STAVANGER
Stavanger University Hospital

SESAM
Regionalt kompetansesenter for eldremedisin og samhandling

Deltaker- ID:_____ **Navn på studielege:**_____

Studiemedisin: fenserin **Sykehus:**_____

Telefon:_____

PATH-1 Studien

Behandlingsstudie for tidlig Alzheimer`s sykdom

Behandling med fenserin

MEDISINDAGBOK

Deltaker-ID: _____

Formålet med denne medisindagboken er å hjelpe deg med daglig loggføring og oversikt over ditt inntak av studiemedisinen fenserin, som er en medisin under utprøving.

I tillegg kan du legge inn eventuelle bivirkninger eller andre hendelser som oppstår i løpet av behandlingsperioden. Vi vil også gjerne at du oppgir eventuelle endringer i den faste medisineringsplanen din hvis du tar andre medisiner i tillegg til fenserin.

Du vil få en instruksjon på hvordan du fører medisindagbok av studiepersonell på sykehuset.

I tillegg anbefaler vi deg følgende hjelpemidler:

- Dosett/pilleeske
- Sett inn en påminnelse på mobiltelefonen din
- Be din nærmeste pårørende om å minne deg på å ta medisinen din

I tekstboksen på neste side finner du doseringsplanen for behandling med fenserin, samt forholdsregler i tilfelle du glemmer en dose eller du tar for mye.

HELSE STAVANGER
Stavanger University Hospital

SESAM
Regionalt kompetansesenter for eldremedisin og samhandling

Deltaker- ID:_____ **Navn på studielege:**_____

Studiemedisin: fenserin **Sykehus:**_____

Telefon:_____

Inntak av fenserin:
Kapselen skal svelges med et glass vann og bør tas sammen med mat. Kapselen skal ikke tygges eller knuses.

Doseopptrappingsplan for fenserin

1. Startdose
   2 kapsler 5 mg daglig (1 kapsel morgen og kveld) i 2 uker
2. Dosering fra uke 3:
   2 kapsler 10 mg daglig (1 kapsel morgen og kveld) i 2 uker
3. Dosering fra uke 5:
   3 kapsler 10 mg daglig (1 kapsel morgen, ettermiddag og kveld) i 4 uker

Eller behandling etter avtale med din studielege

Dersom du har glemt å ta en kapsel

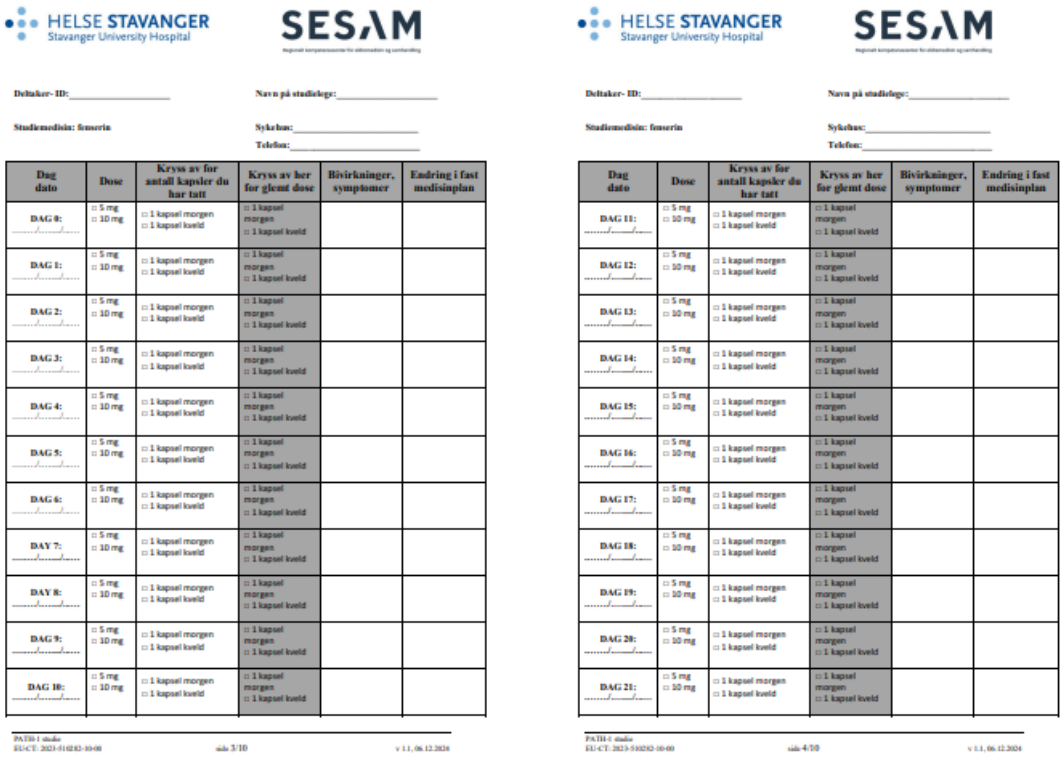
- Dersom du har glemt en dose og det er mindre enn 2 timer siden den skulle ha blitt tatt, kan du ta den glemte dosen.
- Hvis det har gått mer enn 2 timer siden den planlagte dosen, skal du vente til neste dose. Husk å kryss av for glemt dose i dagboken.

Tidsrom mellom dosene:

- 2 kapsler daglig: Ta dosene med et tidsrom på omtrent 12 timer (2 timer tidligere eller senere er akseptabelt).
- 3 kapsler daglig: Ta dosene med et tidsrom på omtrent 8 timer (2 timer tidligere eller senere er akseptabelt).

Dersom du tar for mye av fenserin
Ved overdosering eller symptomer på overdosering (f. eks. alvorlig kvalme, oppkast, svetting, blodtrykksfall, pustesvikt, kollaps og/ eller krampeanfall) må du kontakte din studielege (telefonnr.: _____) eller sykehus (telefonnr.:_____) øyeblikkelig.

This is just a sample of the first 4 pages of the diary. The diary will cover the entire treatment period.



HELSE STAVANGER
Stavanger University Hospital

SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: finserin Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av for antall kapsler du har tatt	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 0:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 1:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 2:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 3:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 4:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 5:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 6:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAY 7:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAY 8:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 9:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 10:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		

PATH-1 studie
EU-CT: 2023-510282-10-00 side 3/10 v 1.1, 06.12.2024

HELSE STAVANGER
Stavanger University Hospital

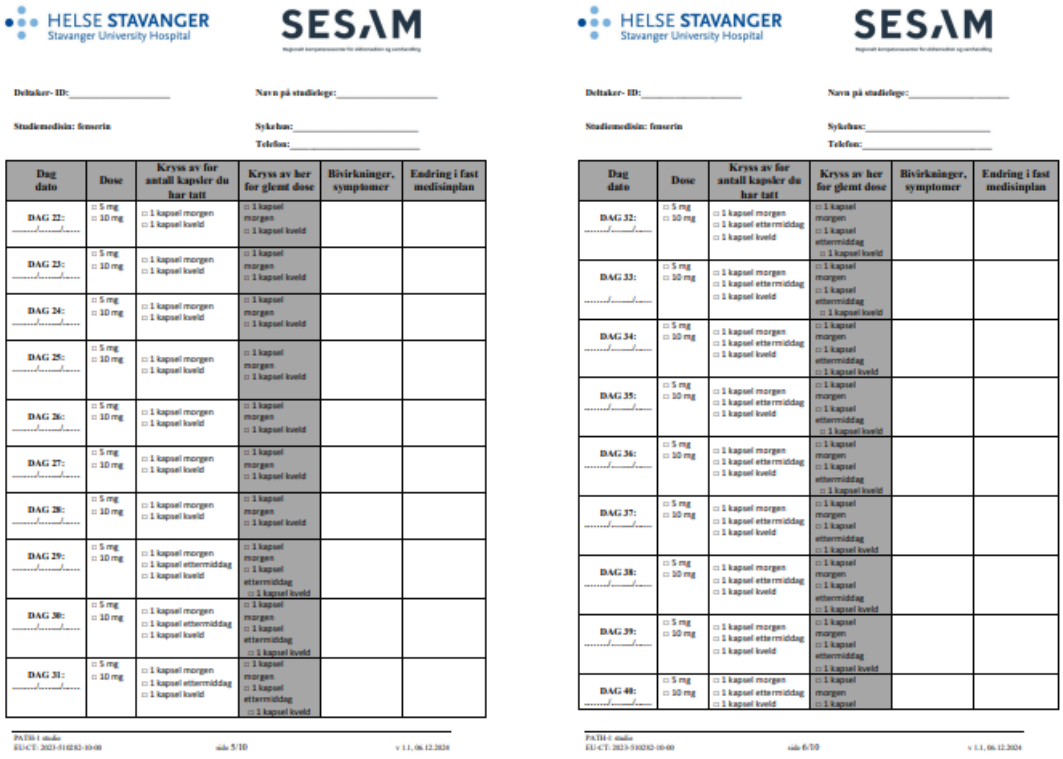
SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: finserin Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av for antall kapsler du har tatt	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 11:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 12:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 13:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 14:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 15:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 16:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 17:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 18:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 19:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 20:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 21:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		

PATH-1 studie
EU-CT: 2023-510282-10-00 side 4/10 v 1.1, 06.12.2024



HELSE STAVANGER
Stavanger University Hospital

SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: finserin Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av for antall kapsler du har tatt	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 22:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 23:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 24:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 25:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 26:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 27:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 28:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel kveld		
DAG 29:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 30:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 31:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		

PATH-1 studie
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HELSE STAVANGER
Stavanger University Hospital

SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: finserin Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av for antall kapsler du har tatt	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 32:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 33:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 34:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 35:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 36:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 37:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 38:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 39:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld		
DAG 40:	□ 5 mg □ 10 mg	□ 1 kapsel morgen □ 1 kapsel ettermiddag □ 1 kapsel kveld	□ 1 kapsel morgen □ 1 kapsel		

PATH-1 studie
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Sample of the subject diary for participants allocated treatment with donepezil

HELSE STAVANGER
Stavanger University Hospital

SESAM
Regionalt kompetansesenter for eldremedisin og samhandling

Deltaker- ID:_____ **Navn på studielege:**_____

Studiemedisin: donepezil **Sykehus:**_____

Telefon:_____

PATH-1 Studien

Behandlingsstudie for tidlig Alzheimer`s sykdom

Behandling med donepezil

MEDISINDAGBOK

Deltaker-ID: _____

Formålet med denne medisindagboken er å hjelpe deg med daglig loggføring og oversikt over ditt inntak av medisinen donepezil, som er en godkjent medisin mot mild og moderat grad av Alzheimers sykdom.

I tillegg kan du legge inn eventuelle bivirkninger eller andre hendelser som oppstår i løpet av behandlingsperioden. Vi vil også gjerne at du oppgir eventuelle endringer i den faste medisineringsplanen din hvis du tar andre medisiner i tillegg til donepezil.

Du vil få en instruksjon på hvordan du fører medisindagbok av studiepersonell på sykehuset.

Inntak av donepezil:
Kan tas med eller uten mat. Knusing/deling anbefales ikke.

Dosering

1. Startdose
   5 mg en gang daglig. Donepezil tas oralt om kvelden, like før sengetid.
2. Dosering fra uke 5:
   10 mg en gang daglig. Donepezil tas oralt om kvelden, like før sengetid.

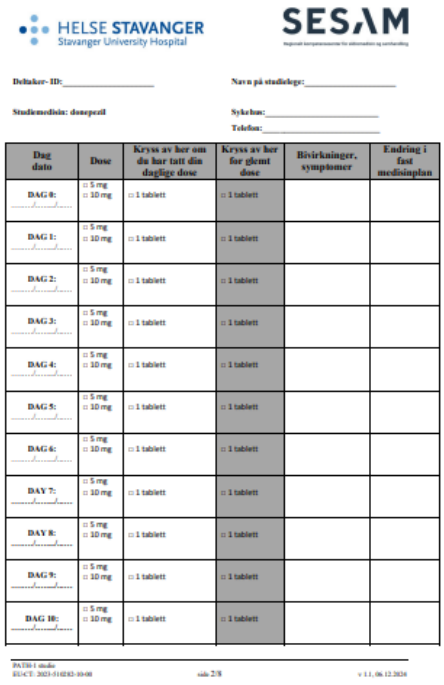
Eller behandling etter avtale med din studielege.

Dersom du glemmer å ta en tablett, tar du en tablett neste dag til vanlig tid. Du skal ikke ta dobbel dose som erstatning for en glemt dose.

Dersom du tar for mye av donepezil, ber vi deg ta kontakt med din studielege (telefonnr.:_____), fastlege eller sykehus så raskt som mulig.

Se pakningsvedlegg for mer informasjon om bivirkninger og kontraindikasjoner for donepezil.

This is just a sample of the first 4 pages of the diary. The diary will cover the entire treatment period.



HELSE STAVANGER
Stavanger University Hospital

SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: donepezil Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av her om du har tatt din daglige dose	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 0:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 1:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 2:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 3:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 4:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 5:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 6:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAY 7:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAY 8:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 9:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 10:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		

PATH-1 studie
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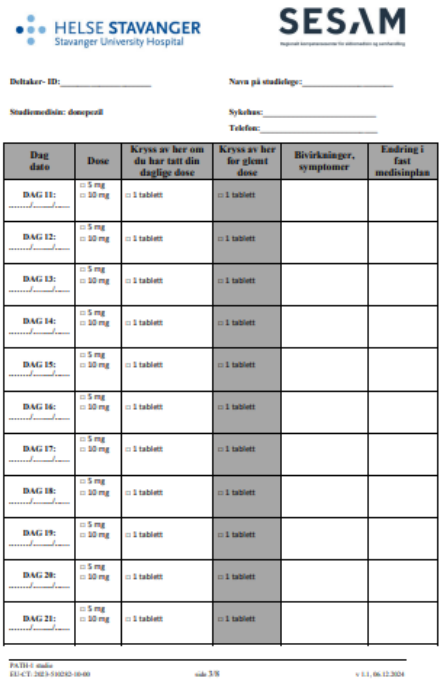

HELSE STAVANGER
Stavanger University Hospital

SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: donepezil Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av her om du har tatt din daglige dose	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 11:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 12:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 13:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 14:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 15:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 16:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 17:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 18:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 19:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 20:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 21:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		

PATH-1 studie
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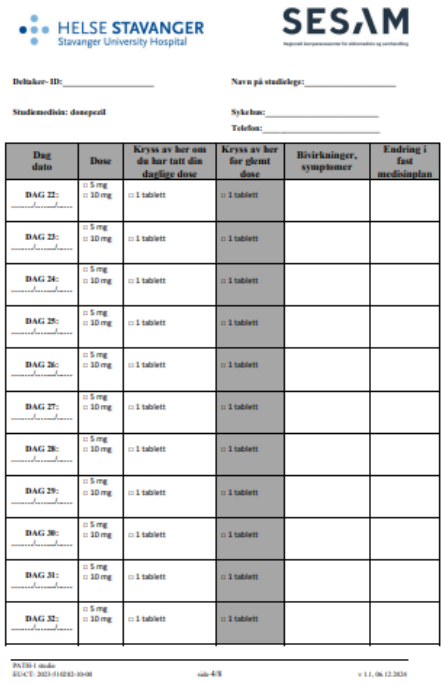


HELSE STAVANGER
Stavanger University Hospital

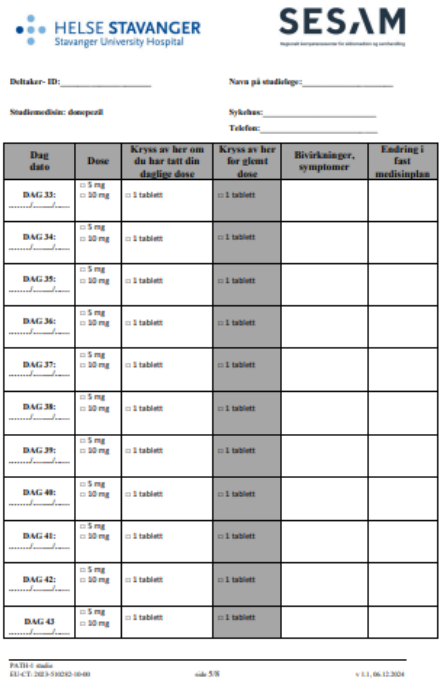
SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: donepezil Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av her om du har tatt din daglige dose	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 22:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 23:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 24:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 25:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 26:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 27:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 28:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 29:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 30:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 31:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 32:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		

PATH-1 studie
EU-CT: 2023-510282-10-00 side 4/8 v 1.1, 06.12.2024



HELSE STAVANGER
Stavanger University Hospital

SESAM

Deltaker- ID:_____ Navn på studielege:_____

Studiemedisin: donepezil Sykehus:_____
Telefon:_____

Dag dato	Dose	Kryss av her om du har tatt din daglige dose	Kryss av her for glemt dose	Bivirkninger, symptomer	Endring i fast medisinplan
DAG 33:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 34:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 35:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 36:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 37:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 38:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 39:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 40:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 41:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 42:	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		
DAG 43	□ 5 mg □ 10 mg	□ 1 tablett	□ 1 tablett		

PATH-1 studie
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11.8. Appendix 8: Procedure and prescription template for dispensing donepezil

Procedure for dispensing donepezil to study subjects in PATH-1 study:

- The study investigator at the coordinating site Stavanger University Hospital will submits an e-prescription on white prescription for treatment with donepezil for 4 participants (prescription per participant: 1x28 pack of 5 mg and 1x 50 pack of 10 mg).
- When a participant is randomized to treatment with donepezil at one of the hospitals, the study nurse will obtain a letter of authorization from the participant to be able to collect the prescription (which is in the participant's name) at the local hospital pharmacy.
- The prescription template (see text box below) will be handed out with the medicine package for donepezil. Dosing is carried out in accordance with the package insert.
- The contents of the medicine package (28 pieces for 5 mg and 50 pieces for 10 mg) will be recorded on a drug accountability log before it is dispensed to the participant. Due to frequent study visits (every 2 weeks), the participant will only receive one pack of medicine at a time.
- For each study visit, the participant is encouraged to bring the entire medication package, including empty blisters, for counting, record and compliance check. Dose titration of donepezil according to the package leaflet and instruction on the subject diary from week 5. The participant will return leftovers from 5 mg tablets, as well as the medicine package and receive a new pack of 10 mg. Delivery and return will be recorded on the drug accountability log.
- Leftover medicine is destroyed in accordance with local hospital routines.
- In addition, various tests and examinations will be taken during the study visits. Should there be side effects or contraindications with the treatment, the study investigator will re-evaluate the treatment dose (as in normal clinical practice).

Text template for dispensing a prescription for donepezil in the PATH-1 study:

TIL KLINISK UTPRØVNING
EU-CT nummer: 2023-510282-10-00
Studienavn: PATH-1
Utprøver: Dag Årsland
Adresse: SESAM, Postboks 8100,
4068 Stavanger
Telefonnummer: 94 86 83 04

Deltaker-ID: _____

BRUKSANVISNING:
1 tablett 5 mg daglig i 4 uker, deretter 1 tablett 10 mg i 6 uker, eller etter avtale med studielege.

11.9. Appendix 9: Information sheet for participants

Sample of information sheet for participants allocated treatment with phenserine

HELSE STAVANGER
Stavanger universitetssjukehus

SESAM
Regionalt kompetansesenter for eldremedisin og samhandling

Kjære deltaker,

Du har blitt inkludert i PATH-1-studien og har fått tildelt behandling med den utprøvende studiemedisinen fenserin.

Ved ditt første studiebesøk (også kjent som baseline) vil du få utlevert en boks med studiemedisin som vil inneholde 50 kapsler med 5 mg fenserin. Den første kapselen med fenserin vil du ta på sykehuset under ditt studiebesøk. Resten av medisinen tar du selv hjemme. Vi anbefale deg å bruke en dosett (pilleeske). Sammen med medisinen får du utlevert en medisindagbok som vi ønsker at du skal føre under behandlingsperioden. Studiesykepleieren vil gi deg instruksjon på hvordan du skal bruke medisindagboken og hvordan du skal krysse av for medisinen du har tatt.

For behandling med fenserin vil du følge den planlagte doseopptrappingsplanen beskrevet i tekstboksen nedenfor. Studielegen vil gå gjennom din behandling med deg under hvert studiebesøk på sykehuset:

Doseopptrappingsplan for fenserin

1. Startdose:
   2 kapsler 5 mg daglig (1 kapsel morgen og kveld) i 2 uker
2. Dosering fra uke 3:
   2 kapsler 10 mg daglig (1 kapsel morgen og kveld) i 2 uker
3. Dosering fra uke 5:
   3 kapsler 10 mg daglig (1 kapsel morgen, ettermiddag og kveld) i 4 uker

Eller behandling etter avtale med din studielege

Du vil møte opp på sykehuset annenhver uke, og her vil du gjennomgå undersøkelser og det vil bli tatt prøver av deg. Basert på samtalen, undersøkelsene og prøvene vil studielegen din vurdere om du skal øke dosen av studiemedisinen i forhold til doseringsplanen eller ikke. Studielegen og studiesykepleier vil gå gjennom din behandlingsplan under hvert studiebesøk og du vil få utlevert ny boks med studiemedisin.

Av hensyn til din sikkerhet og for gjennomføringen av studien har vi laget en sjekkliste for hvert studiebesøk, som vi ønsker at du går gjennom (gjerne sammen med din pårørende) dagen før du møter opp på sykehuset. Vi har oppsummert en oversikt over punktene du vil finne i sjekklistene som er vedlagt dette brevet:

- Du vil få medisiner av studiepersonalet under studiebesøket på sykehuset i henhold til doseringsplanen som er beskrevet i tekstboksen på side 1. Studiepersonalet må føre legemiddelregnskap og telle gjennom alle kapslene ved hvert studiebesøk, så det er svært viktig at du tar med deg pakningsemballasjen (fulle, åpne og tomme) du har fått utlevert ved tidligere besøk. Hvis du bruker dosett, er det også viktig at du tar med denne. **Viktig!** Tom pakningsemballasje eller pakningsemballasje med rester av medisin skal alltid leveres tilbake til studiepersonell og må ikke kastes! Det er krav til legemiddelregnskap i kliniske studier og det er viktig at alle tomme og ubrukte pakninger eller bokser returneres til sykehuset.
- Du vil få utlevert en medisindagbok sammen med studiemedisinen. Vi vil be deg om å føre opp daglig i medisindagboken når du har tatt medisinene dine. Det er også viktig at du dokumenterer hvis du glemmer å ta en dose en dag, eller hvis du ved et uhell skulle komme til å ta en dobbel dose. Studiepersonalet vil kunne gi deg instruksjoner om hvordan du skal føre medisindagboken.
- Vi vil be deg ta med en urinprøve til hvert studiebesøk. Du vil få utlevert uringlass på sykehuset. Dato og klokkeslett for når prøven ble tatt føres på vedlagt sjekkliste for det aktuelle studiebesøket.
- Skulle det forekomme endringer i medisinplan din for dine faste medisiner, er det viktig at du informerer studielegen din om disse endringene, ettersom det kan ha innvirkning på din behandling og sikkerhet.
- Skulle du oppleve bivirkninger under medisinbehandling (f. eks hodepine, svimmelhet, oppkast, fall, sykehusinnleggelse) er det viktig at du informerer din studielege eller studiesykepleier om dette. I slike tilfeller ønsker vi at du fører opp hendelsen i medisindagboken, eller du kan også føre dette opp på sjekkliste som er vedlagt.
- Dersom du skulle bli utsatt for en alvorlig og/eller livstruende hendelse (som medfører sykehusinnleggelse eller forlenget sykehusopphold), er det viktig at du eller din pårørende eller behandlende lege tar kontakt med din studielege eller studiesykepleier så snart som mulig.

Ta gjerne kontakt om det er noe som er uklart. Studiesykepleier vil også kunne gå gjennom sjekklisten med deg på telefon før besøket om du ønsker det.

Med vennlig hilsen

SESAM studieteam

Studiebesøk nr.1 (Baseline): _____ (dato og klokkeslett)

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

_____

Studiebesøk nr.2 (Uke 2): _____ (dato og klokkeslett)

☐ **Viktig!** Det er viktig at du tar den siste dosen av medisinen din ca. 90 minutter før studiebesøket, da det vil bli tatt en blodprøve som gir oss viktig informasjon om effekten av behandlingen din. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste kapsel med fenserin før studiebesøket.

_____
Dato og klokkeslett for inntak av siste tablett med fenserin

☐ **Viktig!**
Det er viktig at du tar med pakningsemballasje for studiemedisin (fulle, åpne og tomme) for fenserin, som du fikk utlevert ved forrige besøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.3 (Uke 4): _____ (dato og klokkeslett)

☐ **Viktig!** Det er viktig at du tar den siste dosen av medisinen din ca. 90 minutter før studiebesøket, da det vil bli tatt en blodprøve som gir oss viktig informasjon om effekten av behandlingen din. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste kapsel med fenserin før studiebesøket.

_____
Dato og klokkeslett for inntak av siste tablett med fenserin

☐ **Viktig!** Det er viktig at du tar med pakningsemballasje for studiemedisin (fulle, åpne og tomme) for fenserin, som du fikk utlevert ved forrige besøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.4 (Uke 6): _____ (dato og klokkeslett)

☐ **Viktig!** Det er viktig at du tar den siste dosen av medisinen din ca. 90 minutter før studiebesøket, da det vil bli tatt en blodprøve som gir oss viktig informasjon om effekten av behandlingen din. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste kapsel med fenserin før studiebesøket.

_____
Dato og klokkeslett for inntak av siste tablett med fenserin

☐ **Viktig!** Det er viktig at du tar med pakningsemballasje for studiemedisin (fulle, åpne og tomme) for fenserin, som du fikk utlevert ved forrige besøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.5 (Uke 8): _____ (dato og klokkeslett)

☐ **Viktig!** Til dette (avsluttende) studiebesøket på sykehuset ønsker vi at du kommer medisinfastende. Det betyr at du ikke skal ta medisinen din den dagen du skal møte opp til studiebesøk på sykehuset. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok den siste kapselen dagen før:

_____
Dato og klokkeslett for inntak av siste tablett med fenserin

☐ **Viktig!** Det er viktig at du tar med pakningsemballasje for studiemedisin (fulle, åpne og tomme) for fenserin, som du fikk utlevert ved forrige besøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.6 (Oppfølgingsbesøk etter avsluttet behandling): _____ (dato og klokkeslett)

☐ **Viktig!**
Hvis du ikke har fått levert fra deg rester eller pakningsemballasje med fenserin, er det viktig at du tar dette med på besøket og leverer det tilbake til studiepersonal.

☐ **Viktig!**
Husk å ta med medisindagboken din, om du ikke allerede har levert den fra deg under sist besøk.

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

Sample of the information sheet for participants allocated treatment with donepezil

HELSE STAVANGER
Stavanger universitetssjukehus

SESAM
Regionalt kompetansesenter for eldremedisin og samhandling

Kjære deltaker,

Du har blitt inkludert i PATH-1-studien og har fått tildelt behandling med donepezil, som er kjent under produktnavnet Aricept og som er en godkjent medisin mot demens i Norge og Europa.

Av praktiske årsaker vil medisinen donepezil bli skrevet ut som en e-resept i ditt navn. Studiesykepleieren vil innhente en fullmakterklæring fra deg for å kunne hente ut denne resepten for deg på sykehusapoteket. Medisinen er gratis for deg, og du trenger ikke å betale noe for den.

Ved ditt første studiebesøk (også kjent som baseline) vil du få utlevert en pakke med 5 mg donepezil (Aricept) som inneholder 28 tabletter. Du vil ta den første dosen med 5 mg donepezil på sykehuset under studiebesøket. Resten av medisinen tar du selv hjemme. Vi anbefaler at du bruker en dosett.

Ved ditt tredje studiebesøk (besøksuke 4) vil du få utlevert en ny pakke med 50 tabletter à 10 mg donepezil. Hvis du opplever bivirkninger eller annet ubehag under behandlingen med donepezil, kan studielegen justere doseringsplanen slik at den avviker fra den som er beskrevet nedenfor. I dette tilfellet vil studielegen gi deg og din pårørende skriftlig informasjon om endringen.

For behandling med donepezil skal du følge følgende doseringsplan:

- 1 tablett 5 mg daglig i 4 uker,
- deretter 1 tablett 10 mg daglig i 6 uker
- eller etter avtale med studielege

Av hensyn til din sikkerhet og for gjennomføringen av studien har vi laget en sjekkliste til hvert besøk, som vi ønsker at du går gjennom (gjerne sammen med din pårørende) dagen før du møter opp på sykehuset. Vi har oppsummert en oversikt over punktene du vil finne i sjekklistene som er vedlagt:

- Du vil få medisiner av studiepersonalet under studiebesøket på sykehuset i henhold til doseringsplanen som er beskrevet i boksen ovenfor.
  Studiepersonalet må føre legemiddelregnskap og telle gjennom alle tablettene

ved hvert studiebesøk, så det er svært viktig at du tar med deg pakningsemballasjen og blisterpakningene (fulle, åpne og tomme) du har fått utlevert ved tidligere besøk. Hvis du bruker dosett, er det også viktig at du tar med denne.
Viktig! Tom pakningsemballasje og tomme blistere eller blistere med rester av medisin skal alltid leveres tilbake til studiepersonell og må ikke kastes! Det er krav til legemiddelregnskap i kliniske studier og det er viktig at alle tomme og ubrukte pakninger returneres til sykehuset.

- Du vil få utlevert en medisindagbok sammen med medisinene dine. Vi vil be deg om å føre opp daglig når du har tatt medisinene dine. Det er også viktig at du dokumenterer hvis du glemmer å ta en daglig dose en dag, eller hvis du ved et uhell skulle komme til å ta en dobbel dose en dag. Studiepersonalet vil kunne gi deg instruksjoner om hvordan du skal føre medisindagboken.
- Vi vil be deg ta med en urinprøve til hvert studiebesøk. Du vil få utlevert uringlass på sykehuset. Dato og klokkeslett for når prøven ble tatt føres på vedlagt sjekkliste for det aktuelle studiebesøket.
- Skulle det forekomme endringer i medisinplan din for dine faste medisiner, er det viktig at du informerer studielegen din om disse endringene, ettersom det kan ha innvirkning på din behandling og sikkerhet.
- Skulle du oppleve bivirkninger under medisinbehandling (f. eks hodepine, svimmelhet, oppkast, fall, sykehusinnleggelse) er det viktig at du informerer din studielege eller studiesykepleier om dette. I slike tilfeller ønsker vi at du fører opp slike uønskede hendelser i medisindagboken, eller du kan også føre dette opp på sjekkliste som er vedlagt.
- Dersom du skulle bli utsatt for en alvorlig og/eller livstruende hendelse (som medfører sykehusinnleggelse eller forlenget opphold), er det viktig at du, dine pårørende eller behandlende lege tar kontakt med din studentlege eller studiesykepleier så snart som mulig, da slike hendelser er meldepliktige til norske og europeiske myndigheter.

Ta gjerne kontakt om det er noe som er uklart. Studiesykepleier vil også kunne gå gjennom sjekklisten med deg på telefon før besøket om du ønsker det.

Med vennlig hilsen

SESAM

Studiebesøk nr.1 (Baseline): _____ (dato og klokkeslett)

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

_____

Studiebesøk nr.2 (Uke 2): _____ (dato og klokkeslett)

☐ **Viktig!** Det er viktig at du tar den siste dosen av medisinen din ca. 90 minutter før studiebesøket, da det vil bli tatt en blodprøve som gir oss viktig informasjon om effekten av behandlingen din. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste tablett med donepezil før studiebesøket. Vennligst dokumenter her:

_____
Dato og klokkeslett for inntak av siste tablett med donepezil

☐ **Viktig!**
Det er viktig at du tar med medisinpakning samt alle blistere (fulle, åpne og tomme) for donepezil, som du har fått utlevert på tidligere studiebesøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.3 (Uke 4): _____ (dato og klokkeslett)

☐ **Viktig!** Det er viktig at du tar den siste dosen av medisinen din ca. 90 minutter før studiebesøket, da det vil bli tatt en blodprøve som gir oss viktig informasjon om effekten av behandlingen din. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste tablett med donepezil før studiebesøket. Vennligst dokumenter her:

_____
Dato og klokkeslett for inntak av siste tablett med donepezil

☐ **Viktig!**
Det er viktig at du tar med medisinpakning samt alle blistere (fulle, åpne og tomme) for donepezil, som du har fått utlevert på tidligere studiebesøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.4 (Uke 6): _____ (dato og klokkeslett)

☐ **Viktig!** Det er viktig at du tar den siste dosen av medisinen din ca. 90 minutter før studiebesøket, da det vil bli tatt en blodprøve som gir oss viktig informasjon om effekten av behandlingen din. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste tablett med donepezil før studiebesøket. Vennligst dokumenter her:

_____
Dato og klokkeslett for inntak av siste tablett med donepezil

☐ **Viktig!**
Det er viktig at du tar med medisinpakning samt alle blistere (fulle, åpne og tomme) for donepezil, som du har fått utlevert på tidligere studiebesøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.5 (Uke 8): _____ (dato og klokkeslett)

☐ **Viktig!** Til dette (avsluttende) studiebesøket på sykehuset ønsker vi at du kommer medisinfastende. Det betyr at du ikke skal ta medisinen den dagen du skal møte opp til studiebesøk på sykehuset. Vi vil derfor be deg om å dokumentere dato og klokkeslett for når du tok din siste tablett dagen før:

_____
Dato og klokkeslett for inntak av siste tablett med donepezil

☐ **Viktig!**
Det er viktig at du tar med medisinpakning samt alle blistere (fulle, åpne og tomme) for donepezil, som du har fått utlevert på tidligere studiebesøk. Ta også med dosett om nødvendig.

☐ **Viktig!**
Husk å ta med medisindagboken din

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

☐ Er det endring i medisinplan for faste medisiner siden sist besøk? Hvis ja, kan du beskrive endringene her:

_____

☐ Har du opplevd bivirkninger fra studiemedisinen

_____

☐ Annen informasjon som kan være relevant for din deltakelse:

_____

Studiebesøk nr.6 (Oppfølgingsbesøk etter avsluttet behandling): _____ (dato og klokkeslett)

☐ **Viktig!**
Hvis du ikke har fått levert fra deg pakning, blisterpakning eller tabletter med medisin, er det viktig at du tar med og leverer fra deg på dette besøket.

☐ **Viktig!**
Husk å ta med medisindagboken din, om du ikke allerede har levert den fra deg under sist besøk.

☐ Ta med urinprøve
Dato og klokkeslett når prøven ble tatt:

_____

11.10. Appendix 10: Protocol for Acetylcholinesterase (ACHE) assay

V0.4

Checklist (before starting experiment)

- ☐ Biotek Synergy H1m is free to use. Start a new experiment with existing protocol and chose the AchE protocol. Adjust the plate layout according to today's experiment
- ☐ Plate layout for today is set up (on paper or in PipettePilot)
- ☐ Work bench is tidy, washing buffer reservoir cleaned, pipettes working properly
- ☐ Lab timer is ready
- ☐ Necessary buffers and chemicals are available

Important things to remember

- ☐ Work quickly but carefully! Avoid delays! Try not to exceed intervals by more than 5 min.
- ☐ Mix all reagents gently and avoid foaming! Do not heat reagents to quicken thawing!
- ☐ Use gloves and change them regularly! Because of high sample dilutions any contamination will have critical impact on the result!
- ☐ Reconstitute DTNB (Ellman's reagent) and ATCh-iodide (Acetylthiocholine-iodide) in assay buffer and prepare dilution series of recombinant AchE standard **when ready to start the assay right away**! Have samples ready before preparing the standard series!
- ☐ Briefly spin or centrifuge vial of recombinant AchE standard before opening
- ☐ Use electronic pipettes (be familiar with working principle)
- ☐ Use the pipette that is best suited for the desired volume: blue tips: above 200µl; green tips: 20-300µl; yellow tips: 10-200µl; and red tips: below 10µl.

Date: ___ / ___ / ____ PlateNr. _____ Operator(s): _____
_____
_____
_____

Please note lot nr for every reagent used in today's assay:

Item	Supplier	PartNr	LotNr
Clear 96 non-binding plate	greiner	655101	
rec. AchE standard	abcam	ab138871 Reconstituted on:	
DTNB	Merck/Sigma	A14331.03	
ATCh-iodide	Merck/Sigma	A5751-1G	
Sample diluent			Preparation date:
a. 0.9% normal saline (NaCl)	Self-made	N/A	__ . __ . ____

b. assay buffer (see below)			__ . __ . ____
Assay buffer			Preparation date:
50mM TRIS-HCl pH 8.2	Self-made	N/A	__ . __ . ____
PBS pH 7.2	ThermoFisher	28372	
Phenserine tartrate	Kragerø Tablett	N/A	

Please check chapter on preanalytical sample handling before starting an assay!

Reagent preparation

TRIS-HCl (0.05M pH 8.2): *2-Amino-2-hydroxymethyl-propane-1,3-diol (MW 121,14)*

Weigh in 6.06g TRIS and dissolve in 950ml ddH_2O
Adjust pH with 1N HCl to 8.2 (tolerance ± 0.1)
Fill up to 1000ml with ddH_2O
Store at RT

Perform the following preparations while samples are thawing on ice

DTNB: *5,5'-Dithio-bis(2-nitrobenzoic acid) [CAS 69-78-3], MW = 396.35;*
10mM = 3.9635mg/ml .

Weigh in about 4-5mg (a bit less than a peppercorn size) per plate. Dissolve in assay buffer to achieve a 10mM working solution. You will need at least 800µl of this per plate. You can use a 2ml Sarstedt screw cap tube for this.

☐ *weighed in: ___ mg → dissolve in x/3.9635 = ____ ml assay buffer (fx1)*

NB! If not readily dissolved, heat to 37°C. This is usually not necessary.

ATCh: (*2-Mercaptoethyl)trimethylammonium iodide acetate [CAS 1866-15-5], MW = 289.18;*
15mM = 4.3377mg/ml .

Weigh in about 10mg (peppercorn size) per plate. Dissolve in assay buffer to achieve a 15mM working solution. You will need at least 2.4ml of this per plate. You can use a 2ml Sarstedt screw cap tube for this. However, first add only half of the required buffer (gaining a 30mM solution) and then combine it with the same volume of assay buffer in a 15ml tube to achieve the final 15mM.

☐ *weighed in: ___ mg → dissolve in x/(2*4.3377) = ____ ml assay buffer (fx2)*
☐ *combine ____ ml of 30mM solution (fx2) with ____ ml assay buffer to achieve 15mM*

Phenserine tartrate: (MW 487.5); *10mM = 4.875mg/ml .*

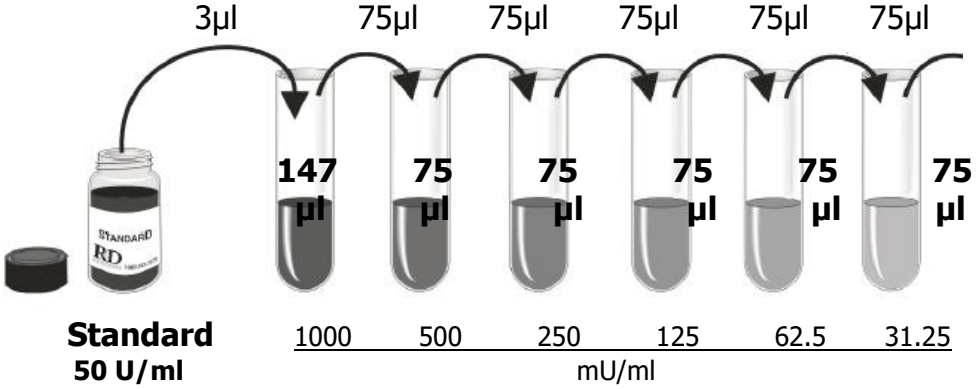
Weigh in about 1mg per plate. Dissolve in assay buffer to achieve a 10mM working solution. You can use a 2ml Sarstedt screw cap tube for this. The 10mM phenserine tartrate stock is diluted 1:200 to achieve a 50nM working concentration.

- ☐ *weighed in: ___ mg → dissolve in x/4.875 = ____ ml assay buffer* *(f_x3)*
- ☐ prepare a 1:200 dilution in assay buffer, e.g. add 100µl 10mM stock + 8.9ml assay buffer

AchE standard: Component D of the ab138871 abcam AchE activity assay (colorimetric)

Component D has been reconstituted in 0.1% BSA (50 U/ml) and was frozen in 10µl portions.

Make a serial dilution as described below in sample buffer.



NB! Diluted AchE standard is stable for about 4 hours!

Sample preparation

1. ☐ Thaw samples on ice
2. ☐ **Serum** (AchE) and **plasma** (BchE) are to be diluted **1:50** to **1:100** in assay buffer
3. ☐ **Whole blood** samples are to be diluted **1:100** in assay buffer
4. ☐ Normal saline stabilized whole blood has a 1:100 dilution (99 vol normal saline + 1vol whole blood)
5. ☐ Run samples in triplicates or more replicates

Recommendation: 1:50 → Add 5µl sample to 245µl assay buffer (enough for 20 replicates)

1:100 → Add 5µl sample to 495µl assay buffer

Include at least 4 wells with buffer instead of sample as blank

Include same sample type QC control on plate. QC measurements must be logged in ACHE CONTROL TRACKING SHEET over time.

Phenserine treatment (50nM) of samples should result in an activity decrase to 20-40% of normal levels.

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	BL	BL	QC	QC	QC	QC					
B	BL	BL	BL	QC	QC	QC	QC					
C	BL	BL	BL	SPL1 1:100	SPL1 1:100	SPL1 1:100	SPL1 1:100					
D	31.2	31.2	31.2	SPL1 1:100	SPL1 1:100	SPL1 1:100	SPL1 1:100					
E	62.5	62.5	62.5	SPL2 1:100	SPL2 1:100	SPL2 1:100	SPL2 1:100					
F	125	125	125	SPL2 1:100	SPL2 1:100	SPL2 1:100	SPL2 1:100					
G	250	250	250	QC	QC	QC	QC					
H	500	500	500	QC	QC	QC	QC					

1-3 = AchE standard, X = Assay buffer with 50nM phenserine tartrate;

QC = “Blood 2” for whole blood AchE; = “Serum 2” for serum AchE;

Plate ID: _____

Assay procedure

☐ Confirm that samples and reagents have been prepared, Synergy H1m is pre-heated to 37C

1) *Make assay working solution (example is for one whole 96well plate) by mixing*
   - ☐ 750µl of 10mM DTNB in assay buffer with
   - ☐ 33ml assay buffer

   → final concentration will be 0.2mM (in the 300µl assay volume)

2) Make 50nM phenserine tartrate assay working solution (example is for 16 wells) by mixing
   - ☐ 150µl of 10mM DTNB in assay buffer with
   - ☐ 6.6ml assay buffer with 50nM phenserine

3) *Fill each well of the 96well plate with assay working solution*
   - ☐ 270 µl assay working solution w or w/o phenserine tartrate according to plate layout

4) *Add sample(s) to each well*
   - ☐ 10µl sample, standard, or assay buffer (blank) per well

5) *Load plate to Synergy H1m*
   - ☐ Start read procedure. The reader will mix the plate, perform a pre-substrate read and start incubation of the plate. You will be promted to continue the read. Wait until 10mins have passed (see next step).
   - ☐ Incubate for 10mins inside the plate reader

6) *Add ATCh (substrate)*

NB! The reactions will start at soon as the substrate is added. Avoid longer delays between fist and last well

- ☐ Add 20µl 15mM ATCh solution to each well with multichannel electronic pipette. Change tips between columns

→ final concentration will be 1mM

The solution will turn pale yellow upon addition of the substrate

7) *Continue read protocol*
   - ☐ Place plate back to the Synergy H1m reader and continue run by acknowledging the prompt on the screen and clicking on “RUN” (green circle with play symbol) and then chosing “Resume read”

The Synergy will mix the plate, perform one initial read, then a read after 15mins, and a final read after 30mins.

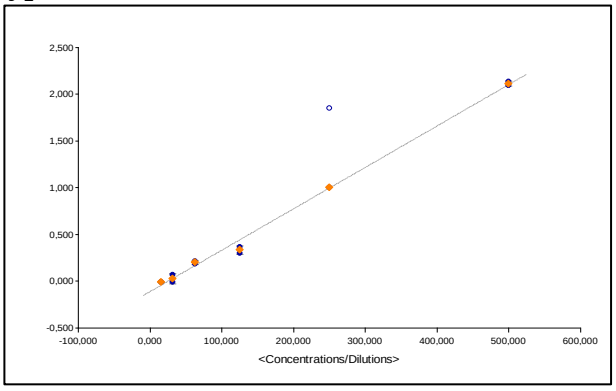
Synergy H1m read protocol

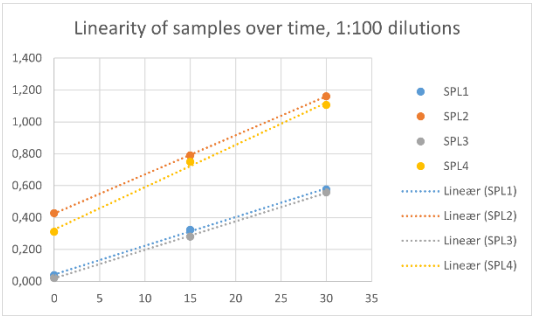
Temperature:	Setpoint 37°C (preheat before cont. with next step)
Shake:	Double Orbital for 30sec (282cpm (3mm))
Read:	DTNB pre-substrate (A) 412 (Absorbance, endpoint, 412nm)
Delay:	10min
Temperature:	Setpoint 37°C (preheat before cont. with next step)
Stop/Resume:	Add substrate and reload (Eject plate before Stop)
Shake:	Double Orbital for 30sec (282cpm (3mm))
Read:	DTNB 0min (A) 412 (Absorbance, endpoint, 412nm)
Temperature:	Setpoint 37°C (preheat before cont. with next step)
Delay:	15min
Shake:	Double Orbital for 10sec (282cpm (3mm))
Read:	DTNB 15min (A) 412 (Absorbance, endpoint, 412nm)
Delay:	15min
Shake:	Double Orbital for 10sec (282cpm (3mm))
Read:	DTNB 30min (A) 412 (Absorbance, endpoint, 412nm)
STOP	

Gen5 data analysis

Data Reduction:	Blanked Data 30m = DTNB 30min (A) 412 – DTNB 0min (A) 412
Calibration Curve:	Linear, input: Blanked Data 30m, concentrations
Alternative[1]:	Activity of AChE (Units/L) = (Blanked Data 30m / 30) × 56520

Typical data





SPL1 = SERUM, -80C; SPL2 = SERUM, +4C

SPL3 = BLOD, -80C; SPL4 = BLOD, +4C

[1] https://doi.org/10.18332/pht/172229, use only if assay buffer has pH 7.2!

12. References

1. Alzheimer's Disease International. World Alzheimer Report 2023: Reducing Dementia Risk: Never too early, never too late [Internet]. 2023 [cited 2024 Aug 18]. Available from: https://www.alzint.org/resource/world-alzheimer-report-2023/
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