Clinical Trial Protocol: NAV4-04

Study Title:	Beta-Amyloid Imaging With [^{18}F]NAV4694 Positron Emission Tomography (PET) in Predicting Progression to Alzheimer's Disease (AD) in Subjects with Mild Cognitive Impairment (MCI)
Study Number:	NAV4-04
Study Phase:	2
Product Name:	[^{18}F]NAV4694
IND Number:	106,157
EudraCT Number:	2013-002514-12
Indication:	[^{18}F]NAV4694 positron emission tomography (PET) can detect cerebral β-amyloid deposition. The absence of [^{18}F]NAV4694 uptake (and thus β-amyloid deposition) is inconsistent with a diagnosis of Alzheimer's disease at the time of imaging.
Investigators:	Multicenter
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	Date
Original Protocol:	06 February 2013
Amendment 1:	07 June 2013
Amendment 2:	13 May 2014

SYNOPSIS

Study title	Beta-Amyloid Imaging With [^{18}F]NAV4694 Positron Emission Tomography (PET) in Predicting Progression to Alzheimer's Disease (AD) in Subjects with Mild Cognitive Impairment (MCI)
Short title	Detection of β-amyloid in the brain of subjects with MCI
Study phase	Phase 2
Study objective(s)	To investigate whether [^{18}F]NAV4694 positron emission tomography (PET) scan findings have the ability to distinguish subjects with mild cognitive impairment (MCI) who progress to Alzheimer's disease (AD) from those who do not.
Name of radioactive drug substance	[^{18}F]NAV4694
Dose(s)	The administered [^{18}F]NAV4694 radioactive dose will be 8.1 mCi (300 MBq) ± 20%. The tracer mass dose specification will be ≤ 40 μg. The total administered volume will be ≤ 10 mL.
Route of administration	Intravenous (IV) injection
Duration of treatment	One or two injections of a diagnostic agent over 18 months.
Indication	[^{18}F]NAV4694 positron emission tomography (PET) can detect cerebral β-amyloid deposition. The absence of [^{18}F]NAV4694 uptake (and thus β-amyloid deposition) is inconsistent with a diagnosis of Alzheimer's disease at the time of imaging
Study objectives	**Primary Objective** To investigate whether [^{18}F]NAV4694 positron emission tomography (PET) scan findings have the ability to distinguish subjects with mild cognitive impairment (MCI) who progress to AD from those who do not. Assessment of the primary objective will be based on the sensitivity, specificity, PPV, and NPV of [^{18}F]NAV4694 PET scan findings (e.g., majority read findings of baseline scan) in predicting progression from MCI to AD type dementia at 18 months. **Secondary Objectives** • To evaluate the sensitivity, specificity, NPV, and PPV of quantitative findings (e.g., SUVRs) in predicting progression of MCI to AD type dementia in a subset of 20 subjects at 18 months , using a data driven (and a pre-

	specified) threshold for positivity. • To determine the overall changes in amyloid burden over time in subjects with visually positive vs. negative scans (by comparison of baseline and 18-month scan composite SUVR in a subset of 20 subjects). • To determine in a subset of 20 subjects whether changes in quantitative [^{18}F]NAV4694 uptake over time correlate with the rate of cognitive decline in subjects with MCI over 18 months. • To determine the increase in risk of progression to AD for a unit change between subjects in baseline subject level baseline SUVR value. • To evaluate safety and tolerability of [^{18}F]NAV4694 in subjects with MCI.
Main criteria for inclusion	1. Subject has signed informed consent to participate in the study and continues to give willing consent for participation 2. Age ≥ 55 years and diagnosed by the investigator with MCI 3. Educational level of at least 6 years 4. Female subjects will not be of child-bearing potential 5. Availability of a "study partner" who can assist in completing rating scales, for the duration of the study 6. Cognitive complaints reported by the subject and confirmed by the "study partner" 7. Clinical Dementia Rating (CDR) global score = 0.5 8. Mini-mental state examination (MMSE) score of 24-30 9. Diagnostic and Statistical Manual of Mental Disorders, Version 4, Text Revised (DSM-IV-TR) criteria of dementia not fulfilled Ability to complete all procedures and assessments, including neuropsychological testing and have the ability to comply with all requirements of PET and MRI
Study design	Open label, non–randomized, multi-center study in subjects with MCI
Methodology	The proposed study includes: screening, baseline neuro-cognitive testing and PET imaging, neuro-cognitive follow-up examinations (at 6, 12, 18, 24, 30, and 36 months). In addition, a subset of 20 subjects will undergo PET and MR imaging at 18 months. Each subject will be in the study for a fixed period of time (36 months) or until the subject withdraws from the study. **Screening Visit:** The screening visit will include review of

	trial eligibility, consent, collection of subject-specific characteristics, vital signs, physical and neurological exams, neuro-cognitive testing, magnetic resonance imaging, and blood and urine laboratory testing **Baseline testing:** Neuro-cognitive exams and safety laboratory analyses will be performed. **Baseline [^{18}F]NAV4694 injection and PET imaging**: All subjects will receive a single IV injection of the investigational product, and a PET scan will be performed from 50 to 70 minutes post injection. Vital signs will be completed within 10 minutes both before and after injection and again 30 minutes after injection. ECGs will be completed within 10 minutes both before and after injection. Each subject will undergo a follow-up visit approximately 24 hours post injection, and a telephone follow up will occur 7 (+ 3) days after the imaging visit. **Neuro-cognitive follow-up exams at 6, 12, 18, 24, 30 and 36 months:** During these exams, cognitive and functional performance will be evaluated. The investigator will review all clinical and neuro-cognitive data gathered during the trial to determine the diagnostic status (progression to Alzheimer's dementia). DSM-IV-TR criteria will be used to determine whether a subject has dementia and if so, which type. Probable AD will be confirmed using criteria from National Institute of Neurologic, Communicative Disorders, and Stroke – AD and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). **18--month activities (in a subset of 20 subjects):** **MR imaging:** MR imaging will be completed within the 30 days before the 18-month PET imaging visits. **18-month follow-up testing:** Physical and neurological exams and safety laboratory analysis will be performed in the 7 days prior to PET imaging. **PET imaging visits:** PET imaging procedures will be the same as employed at baseline. A 24-hour and 7-day follow-up will be conducted as described for baseline.	
Planned trial dates	**Start of study** March 2013	**End of Study** December 2018
Planned number of trial centers / countries	Approximately 15 sites in US and Germany	

Number of subjects	**Total:** Approximately 120 evaluable subjects. An evaluable subject will be defined as a subject who has a clinician/investigator SoT diagnosis and who has had a baseline [^{18}F]NAV4694 PET scan that can be assessed by the independent blinded readers.
Evaluation of Imaging	[^{18}F]NAV4694 PET will be assessed using visual interpretation and standardized quantitative assessment, both performed at the image core lab. The former will consist of an independent visual assessment by 3 blinded independent readers. Target regions of interests (ROIs) include the frontal, lateral temporal, and parietal cortices and the posterior cingulate/precuneus. The cerebellar cortex will serve as the reference region. The SUVR for each region, as well as for a composite of all regions, will be calculated. The MRI obtained at screening (and before each PET scan, as applicable) will be used for anatomical co-registration with the brain PET scan.
Primary variables	The positivity/negativity of the baseline [^{18}F]NAV4694 PET scan (as determined by the majority read finding of the 3 readers) compared with the final clinical diagnosis at 18 or 36 months
Plan for statistical analysis	This will be an exploratory trial with descriptive statistics only and no hypothesis testing. For the primary efficacy variables, the sensitivity, specificity, PPV, and NPV of [^{18}F]NAV4694 PET scan findings in predicting progression of MCI to AD type dementia (or the lack thereof) after 18 months will be estimated based on the visual assessment of the baseline PET scan. The estimates will be displayed with corresponding 95% confidence intervals (CI). The sensitivity, specificity, PPV, and NPV of the quantitative assessment (SUVRs) will be determined, based both on the pre-determined and a data-driven threshold for positivity. Secondary analyses will consist of (a) assessment of the differences in distribution of baseline subject-level SUVRs for subjects who are diagnosed as negative and positive for transition to AD, (b) correlation of changes in subject-level SUVRs from baseline to the final time point with change from baseline neurocognitive test results assessed at 6, 12, 18, 24, 30, and 36 months, and (c) attempt to model the relationship of baseline subject-level SUVRs to SoT

	diagnosis using logistic regression. Data collected up to and including the 18-month time point will be summarized after completion of the 18-month time point. This summary will not include hypothesis testing and will be conducted only after all subjects remaining on study have completed the 18-month time point and will not involve unblinding of any subject's 24-, 30-, or 36-month data. This 18-month summary will be used for regulatory submission and for decision making by the sponsor.

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LIST OF IN-TEXT TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AD	Alzheimer's disease
ADAS Cog	Alzheimer's Disease Assessment Scale-Cognitive
ADCS-MCI-ADL	Alzheimer's Disease Cooperative Study Scale for Activities of Daily Living in Mild Cognitive Impairment
ADL	activities of daily living
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADR	adverse drug reaction
ADRDA	Alzheimer's Disease and Related Disorders Association
AE	adverse event
ALARA	as low as reasonably achievable
ALT	alanine aminotransferase
A-MCI	amnestic MCI
ApoE4	apolipoprotein E4
CDR	Clinical Dementia Rating
CRA	clinical research assistant / associate
CRF	case report form
CRO	contract research organization
CT	computed tomography
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision
ECG	electrocardiogram
eCRF	electronic case report form
ED	effective dose
FCSRT	Free and Cued Selective Reminding Test
FDA	Food and Drug Administration
FDG	[^{18}F]fluorodeoxyglucose

FOV	field of view
FT3	free triiodthyronine
FT4	free thyroxine
FWHM	full width half maximum
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GT	glutamyl transferase
Hb	hemoglobin
HCT	hematocrit
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IRC	Image Review Charter
IRB	institutional review board
ISF	investigator's site file
ITD	intent-to-diagnose
IV	intravenous
MBq	megabequerel
MCH	mean corpuscular hemoglobin
mCi	millicurie
MCI	mild cognitive impairment
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
min	minute
mL	milliliter
MMSE	Mini Mental State Examination
MNI	Molecular NeuroImaging, New Haven, CT

MRI	magnetic resonance imaging
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke - AD and Related Disorders Association
NPV	negative predictive value
NYU	New York University
OLINDA	Organ Level INternal Dose Assessment
PET	positron emission tomography
PIB	[^{11}C]Pittsburgh compound B
PP	per protocol
PPV	positive predictive value
QA	quality assurance
RAVLT	Rey Auditory Verbal Learning Test
ROC	receiver operating characteristics
ROI	region of interest
RTUS	regional tracer uptake score
SAE	serious adverse event
SAP	statistical analysis plan
SoT	standard of truth
SUSAR	suspected unexpected serious adverse reaction
SUV	standard uptake value
SUVR	standard uptake value ratio
TMF	trial master file
TSH	thyroid stimulating hormone
VOI	volume of interest

TRIAL ADMINISTRATIVE STRUCTURE

The principal investigator must sign the protocol signature sheet before trial participant recruitment may start. Likewise, all protocol amendments must be signed and dated by the principal investigator before coming into effect.

The name and address of the participating center, the investigators, and all required signature documents will be maintained in the trial master file (TMF).

In addition to the principal investigator, there are additional on-site roles that may be performed by other sub-investigators:

- Subject referral to the trial
- Review of subject eligibility and medical records
- Safety assessments
- Cognitive assessments
- Injection and PET imaging
- On-site PET image analysis

Blinded reading of positron emission tomography (PET) images will be done at the core image laboratory listed below.

Trial personnel not listed in this section are identified in a separate personnel list. This list will be updated as needed. The list of personnel will be available in the center's investigator site file (ISF).

LABORATORIES AND OTHER INSTITUTIONS FOR TRIAL CONDUCT

Responsibility	**Name**	**Affiliation / Address**
Statistical analysis and programming	STATKING	STATKING Clinical Services 759 Wessel Drive Fairfield, OH 45014
Central laboratory (clinical laboratory tests)	PPD GCL	PPD Global Central Lab 2 Tesseneer Drive Highland Heights, KY 41076
Image core laboratory	Molecular NeuroImaging	MNI – Molecular NeuroImaging L.L.C. 60 Temple Street, Suite 8A New Haven, CT 06510

1 INTRODUCTION

1.1 Background

"Mild cognitive impairment" (MCI) is a concept that describes the cognitive changes that are more severe than those associated with normal aging and greater than expected for an individual's educational level, but less severe than those associated with dementia and which do not noticeably interfere with the activities of daily living (ADL). The term MCI was first used by Flicker (1991) and described in more detail by Petersen et al. (1999). Recently a working group convened by the Alzheimer's Association and the National Institutes on Aging (Albert et al., 2011) has proposed and drafted updated criteria. The criteria originally published in 1999 include: (1) memory complaint reported by the subject or an informant, (2) objectively measured memory impairment for age and education, (3) generally normal cognition outside of memory, (4) largely preserved ADL, and (5) not demented. The proposed updated criteria for "MCI due to AD" broadened the memory complaint and impairment to include other aspects of cognition such as attention, executive function, language, and visuo-spatial skills.

MCI criteria have been extensively evaluated over the years, and modifications have been suggested by Petersen et al. (Petersen et al., 2001; Petersen, 2004) and other groups with the aim to identify those cognitive deficits that may predict the risk to progress to dementia, particularly to dementia of the Alzheimer's type. Based on various patterns of deficits measured in neuropsychological tests, subtypes of MCI have been identified. Petersen and Negash (2008) developed a classification of MCI subtypes based on the affected cognitive domain (amnestic versus non-amnestic) and the number of (one versus multiple) domains affected. Gauthier et al. (2006) suggested adding an additional subtype for subjects who had only subjective memory or cognitive complaints and found that the risk to progress to dementia was highest in amnestic MCI, lower in non-amnestic MCI and lowest in subjects with "subjective memory complaints." Based on subtle differences in memory and cognitive complaints, Roundtree et al. (2007) subdivided amnestic MCI into 2 subgroups, amnestic MCI (A-MCI) and a more subtle type amnestic-subthreshold MCI (AS-MCI). However, the authors found both types of AMCI to have similar progression rates to AD.

In keeping with discordance across definition of MCI subtype, the results of longitudinal studies of rates of progression from MCI to AD have varied from as low as 5% per year (Feldmann et al, 2007) and 12% to 15% per year (Petersen et al., 1999); (Petersen, 2009); (ADNI)) to 20% to 30% per year (Ritchie et al., 2001); (Larrieu et al., 2002).

Taken together, these data indicate that additional markers/criteria are needed to better identify persons at risk of developing AD. If AD could be diagnosed at an earlier stage of disease progression before clinical dementia has fully developed, successful intervention with current and future treatments could potentially be improved considerably.

Among biomarkers potentially capable of predicting which individuals with MCI will go on to develop AD dementia, targeted PET tracers that permit noninvasive detection of β-amyloid deposition in the brain during life appear to be the most suitable, since:

- Deposition of β-amyloid in the cerebral gray matter is known to be central to the pathogenesis of AD such that, for a definitive diagnosis thereof, β-amyloid plaques (and tau neurofibrillary tangles) must be present in the brain at autopsy (McKhann et al., 1984).
- This pathology is known to occur early in the AD disease process long before (i.e., up to 20 years) development of detectable clinical symptoms (Braak and Braak, 1991).
- Genetic data support the concept that dysregulation of β-amyloid metabolism with formation of insoluble aggregates may be a central causative factor in the neurobiology of both early and late onset AD (Reitz and Mayeux, 2010).
- Finally, β-amyloid is the target for the numerous novel and potentially disease-modifying therapies of AD.

The first PET tracer used clinically for visual and quantitative assessment of β-amyloid deposition in the brain was the radioactive carbon ([^{11}C])-labeled "thioflavin T" derivative, N-methyl-[^{11}C]2-(4-methylaminophenyl)-6-hydroxybenzothiazole, also known as the [^{11}C] Pittsburg B compound or [^{11}C] PIB (Klunk et al., 2004). Several groups have assessed the rate of progression to AD in [^{11}C]-PIB-positive MCI subjects compared with subjects with a [^{11}C]-PIB-negative PET scan. In all published studies, a higher progression rate to AD was observed in subjects with signs of [^{11}C]-PIB-uptake in their brain, although the yearly progression rates varied considerably in these studies from 30% (Forsberg et al., 2008) to 47% (Forsberg et al., 2008) to over 60% per year (Villemagne et al., 2008).

However, the 20 minute radioactive half-life of [^{11}C] restricts the use of [^{11}C]PIB to PET centers with an on-site cyclotron and extensive radiochemistry infrastructure. Thus, an [^{18}F]-labeled molecule with a radioactive half-life of 110 minutes would be preferable since it allows widespread distribution of the tracer to more than one PET center with a single production run, as is currently the case with [^{18}F]-fluorodeoxyglucose (FDG).

2-(2-fluoro-6-methylaminopyridin-3-yl)-1-benzofuran-5-ol ([^{18}F]NAV4694; previously referred to as [^{18}F]AZD4694) is an [^{18}F]-labeled radiotracer that has demonstrated high affinity and specificity for β-amyloid in nonclinical and clinical studies. In a recent Phase 2 study (D2750N00006) involving 24 subjects (10 AD, 10 older healthy volunteers, and 4 young healthy volunteers), subjects with AD could be differentiated from healthy volunteers (young and older) on the basis of both visual and quantitative analysis (e.g., using standard value uptake ratio [SUVR] calculation of various pre-specified ROIs) of the scans. For visual assessment, the test-retest reliability was excellent (3% to 5%), and for the SUVRs the intraclass correlation coefficient was 0.96 to 1.00 for most regions. The tracer was well tolerated, and no adverse events (AEs) were considered to be related to [^{18}F]NAV4694. It should also be noted that, in contrast to other [^{18}F] β-amyloid-targeted neuro-PET ligands [^{18}F]NAV4694 exhibits very little white matter uptake. Non-specific white matter uptake

can make differentiation between a positive and negative PET scan difficult and can potentially hinder detection of low levels of β-amyloid present, for example, in the early stages of AD such as MCI.

The present study will be a prospective open-label, 5-center study administering [^{18}F]NAV4694 as a diagnostic agent to detect the presence or absence or β-amyloid in the cerebral gray matter. The primary statistical approach will entail a comparison of outcome (e.g., progression to dementia of the Alzheimer's type, cognitive decline) between subjects with a positive or negative β-amyloid PET scan who have MCI. It is expected that the assessment of the [^{18}F]NAV4694 PET image will enable accurate classification of MCI subjects into those at risk and those not at risk of developing dementia of the Alzheimer's type before the appearance of any clinical symptoms of dementia.

1.2 Benefit-Risk Assessment

There is an unmet clinical need for a biomarker indicating the presence of AD pathology before symptoms become clinically apparent. If preventive measures are to be efficacious in AD, they have to be initiated in patients early in the preclinical phase of the disease or early in life for those at high risk to develop the disease. The present study aims to identify persons with MCI progressing to AD by means of in vivo imaging of β-amyloid deposition in the brain using [^{18}F]NAV4694 PET performed at baseline and at 18 and 36 months. All subjects participating will be ≥ 55 years of age. Therefore, pregnant or potentially pregnant women will not be included.

Safety and Tolerability of the Active Substance Administered

The mass dose of the active substance will be up to 40 µg per subject. It is expected that a single intravenous (IV) injection of [^{18}F]NAV4694 with this microdose of ligand will not produce any pharmacological effects.

Excellent safety of [^{18}F]NAV4694 has been indicated by clinical results from three completed trials. In study (D2750C0001), 22 subjects (10 AD and 12 HVs) were administered [^{18}F]NAV4694. Seven AEs were reported for 5 subjects, including 1 serious adverse event (SAE); none of the AEs were considered related to the investigational product. In study (D2750N00006), 27 subjects (11 AD and 16 HVs) were administered [^{18}F]NAV4694. Fifteen AEs were reported for 9 subjects; none of the AEs were considered related to the investigational product. In study (D2750C00002), 16 subjects (8 ADs and 8 HVs) were administered [^{18}F]NAV4694. Five AEs were reported by 4 subjects; one adverse reaction (cough) was considered by the investigator to be related to [^{18}F]NAV4694. In these 3 trials, the clinical results obtained for routine safety parameters demonstrated no trends and were not indicative of a safety concern, thus confirming nonclinical findings.

Total Effective Dose of the Required PET Imaging Procedure

Initial dosimetry data were determined in study D2750C0001, in which 5 HVs were administered ~210 MBq of [^{18}F]NAV4694 and underwent serial, whole body PET scanning

over approximately 6 hours post injection. For all subjects taken together, the mean urinary bladder wall radiation absorbed dose was the largest; 0.11 ± 0.017 mSv/MBq (mean ± SD), with a range of 0.097 to 0.14 mSv/MBq (bladder void interval 4.8 hours). There were no other mean organ radiation doses exceeding 0.10 mSv/MBq. The effective dose (ED) of [^{18}F]NAV4694 was 0.023 mSv/MBq (4.8 hour bladder void interval), which is comparable to that of ^{18}F-FDG (effective dose equivalent [EDE] = 0.027 mSv/MBq).

High quality PET imaging relies on attenuation correction (AC), which is usually achieved with a transmission scan acquired immediately before or after emission scanning. In the present study, AC should be performed before emission scanning and before any required repositioning of the subject. In addition to dedicated PET scanners, in the present study, state of the art PET/computed tomography (CT) machines will also be permitted. For dedicated PET scanners AC is performed using an external radionuclide source such as germanium-68 with the resulting ED of the transmission scan < 0.05 mSv. The low dose CT for a PET/CT scanner should not exceed 0.25 mSv.

Risk and Benefit

Clinical results derived with other tracers to present have indicated the clinical value of PET scanning for detection of the presence or absence of β-amyloid during life (Rowe et al., 2008; Vandenberghe et al., 2010; Barthel et al., 2011). In particular the absence of this protein in the brain has been considered particularly useful in that it makes the presence of AD highly unlikely. Although the diagnostic value of PET imaging with [^{18}F]NAV4694 has not yet been confirmed and the tracer is not yet approved for detection of β-amyloid deposition in the brain, clinical studies with this "second generation" PET tracer will generate valuable prospective clinical safety and efficacy data contributing to the current knowledge base. In addition, knowledge of the [^{18}F]NAV4694 scan results can provide increased diagnostic certainty and resulting increased quality of life, for both subjects and their families.

Aside from the radiation exposure from tracer administration and transmission scanning (described above), the risks to subjects are mainly related to the intravenous (IV) injection. IV injection is known to carry a small risk of infection, injection site pain, and hematoma.

This information on risk and benefit will be conveyed to all participating subjects verbally and in writing as part of the informed consent process prior to inclusion in the trial. In view of an excellent safety profile of the drug substance and the clear medical need for a tracer of this type, the discomfort of the subjects and the low risk resulting from the radiation dose appear justified.

2 STUDY OBJECTIVES

2.1 Primary Objective

To investigate whether [^{18}F]NAV4694 positron emission tomography (PET) scan findings have the ability to distinguish subjects with mild cognitive impairment (MCI) who progress to AD from those who do not.

Assessment of the primary objective will be based on the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of [^{18}F]NAV4694 PET scan findings (e.g., majority read findings of baseline scan) in predicting progression from MCI to AD type dementia at 18 months.

2.2 Secondary Objectives

- To evaluate the sensitivity, specificity, NPV, and PPV of quantitative findings (e.g., SUVRs) in predicting progression of MCI to AD type dementia at 18 months, using a data driven (and a pre-specified) threshold for positivity.
- To determine the overall changes in amyloid burden over time in subjects with visually positive vs. negative scans (by comparison of baseline and 18-month scan in a subset of 20 subjects).
- To determine in a subset of 20 subjects whether changes in quantitative [^{18}F]NAV4694 uptake over time correlate with the rate of cognitive decline in subjects with MCI over 18 months.
- To determine the increase in risk of progression to AD for a unit change between subjects in subject level SUVR value.
- To evaluate safety and tolerability of [^{18}F]NAV4694 in subjects with MCI.

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Study Design

This is an open label, non–randomized, multi-center study in subjects with MCI. This will be an exploratory study with descriptive statistics only and no hypothesis testing. The standard of truth (SoT) will be the final clinical classification at 18 months, established by the investigator who will also be responsible for the neuro-cognitive testing and who will be kept blinded to the results of the PET scan.

The proposed study includes: screening, baseline neuro-cognitive testing and PET imaging, neuro-cognitive follow-up examinations (at 6, 12, 18, 24, 30, and 36 months). In addition, a subset of 20 subjects will undergo PET and MR imaging at 18 months. Each subject will be in the study for a fixed period of time (36 months) or until the subject withdraws from the study.

Screening: At screening, medical history will be recorded from the subject and verified and/or supplemented by the "study partner" (see Section 3.2.1). Physical, neurological, and neuro-cogntive assessments will be performed; only subjects who are not demented will be enrolled in the study. All subjects will have a magnetic resonance imaging (MRI) scan of the brain and laboratory tests, unless these have been performed within 6 months of screening.

In addition to providing a 3D T1-weighted volumetric image to be used for co-registration with the PET scan and subsequent SUVR generation, the brain MRI scanning (both 3D T1- and T2-weighted images) is required for visualization of structural abnormalities that assist in ruling out other causes of impaired cognition and/or constitute pre-specified exclusion criteria. The images will also be evaluated by the core image laboratory for the presence and degree of general cerebral and/or medial temporal lobe (MTL) atrophy and the presence or absence of peri-ventricular white matter.

Baseline PET Imaging: All subjects will receive a single IV injection of the investigational product, and a PET scan will be performed from 50 to 70 minutes post injection to assess the uptake pattern of [^{18}F]NAV4694 in the brain. For assessment of the primary efficacy endpoint, the PET images will be visually evaluated by 3 independent blinded readers at the core image laboratory and will be categorized as β-amyloid positive (yes or no) according to a pre-specified scoring system.

An additional unblinded read will be performed by the on-site radiologist or nuclear medicine physician using a provided Visual Image Training Manual.

PET image acquisition, processing, display and visual/quantitative analysis methods are discussed briefly in Section 8.1.8 and in detail in the Image Review Charter (IRC).

Each subject will be asked to return to the site for a follow-up visit (20 to 28 hours after investigational product administration), and a telephone follow-up will occur 7 (+ 3) days thereafter. Safety will be assessed during both follow-up visits.

Neuro-cognitive follow-up exams and diagnostic status (6, 12, 18, 24, 30, and 36 months) and 18-month MR: During these exams, cognitive and functional performance will be evaluated every 6 months ± 30 days at scheduled visits. The Clinical Dementia Rating (CDR), Alzheimer's Disease Cooperative Study Scale for Activities of Daily Living in Mild Cognitive Impairment (ADCS-MCI-ADL), and Mini-Mental Status Examination (MMSE), Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog), the Rey Auditory Verbal Learning Test (RAVLT) and Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision (DSM-IV-TR) criteria for major depressive disorder, recurrent will be used. Diagnostic status is the documentation of the subject's current diagnosis of MCI or dementia. Unscheduled (interim) visits and evaluations are allowed if a study subject, a "study partner," or the subject's personal physician believes this is necessary, e.g., if there is a marked cognitive decline. The 18-month MR image will be completed within the 30 days before the 18-month PET imaging visits and will be performed only for the subset of 20 subjects that is imaged at 18 months.

18-month PET: PET imaging will follow the 18-month clinical and neuro-cognitive visits for a subset of 20 subjects. Subjects will receive a single IV injection of the investigational product, and a PET scan will be performed from 50 to 70 minutes post injection to assess [^{18}F]NAV4694 uptake pattern in the brain. Diagnostic status is the documentation of the subject's current diagnosis of MCI or dementia.

Note: The end of study may also be due to subject's discontinuation or termination from the study.

The Schedule of Study Events (see Appendix 1) contains a detailed description of all study procedures and time points.

Assessment of Alzheimer's disease

DSM-IV-TR criteria will be used to determine whether a subject has dementia and if so, which type. Probable AD will be confirmed using criteria from National Institute of Neurologic, Communicative Disorders, and Stroke – AD and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984).

Here, the MRI will be performed to rule out non-AD type brain pathology, e.g., cortical or subcortical stroke, brain tumor, normal pressure hydrocephalus.

3.2 Justification for Study Design and Population

This study is designed to establish imaging with the PET tracer [^{18}F]NAV4694 as a diagnostic method to assist in identifying subjects at increased risk of progression to AD dementia. If a positive β-amyloid PET scan is indeed predictive of increased risk of progression from MCI to AD, this prognostic test could be used in conjunction with clinical data to select subjects for preventive treatment prior to the emergence of clinical signs of dementia. It could also be used for subject selection and/or enrichment in clinical studies evaluating new disease modifying therapies. Information regarding the change in amyloid

load over time (between the baseline and 18-month PET scan) may be used to determine the relationship between cognitive decline and β-amyloid concentration and will be examined as a secondary outcome.

3.2.1 Study Partner

Each subject in the study must have a "study partner." A "study partner" for this study is defined as a friend or family member who is in close contact with the study subject (at least 10 hours per week), who can assist in completing rating scales, participate in interviews, and answer questions during phone calls, etc., for the duration of the study. The study partner should accompany the study subject to the clinical appointments throughout the study and supply information as needed. If the study partner is unable to accompany the subject, he/she should be available via telephone to complete the scales, interviews, and questions.

The study partner will be asked to judge changes in behavior or cognitive and memory performance of the subject.

3.2.2 Rational for Subject Selection

The selection criteria as described in the inclusion criteria (see Section 4.1.1) are based on the following considerations:

Although it is generally accepted that subjects with MCI have a higher risk of developing AD than subjects without MCI (average annual progression rate of up to 15%), for a considerable percentage of subjects, MCI may be a stable or reversible condition that does not progress to dementia.

There is a lack of agreement on the criteria that should be used to determine whether an individual does or does not have MCI (Dubois, 2000).

However, the risk to progress to AD seems to be highest in A-MCI with a lower risk in non-A-MCI, and the lowest risk in subjects with "subjective memory complaints" (Gauthier et al., 2006).

When A-MCI was further sub-divided into 2 subgroups, A-MCI (as defined by Petersen's operational criteria, 2001) and a more subtle type AS-MCI, both types had similar progression rates to AD (Rountree et al., 2007).

To meet the objectives of this study, study subjects should meet criteria for MCI as originally described by Petersen et al. (1999), i.e., the memory domain must be affected, but should also include those subjects with subtle memory impairment, as described by Rountree (2007) and Albert, et al. (2010)

3.2.3 Justification for Radiation Exposure With Proposed [^{18}F]NAV4694 PET Radioactive Dose

In nuclear medicine imaging, the diagnostic quality of an image is dependent upon the number of radioactive events (or counts) detected by the camera in the regions of interest (ROIs). The number of recorded events (counts) increases with the duration of the acquisition period and, for a given acquisition period, with the radioactivity administered. Although the injected radioactivity should then be high, the higher the applied radioactivity dose, the higher the radiation exposure to the subject. Thus, in nuclear medicine, the optimal radioactive dose for a given radiopharmaceutical is usually defined as the lowest radioactive dose that renders an image of sufficient quality to provide high diagnostic confidence, commonly referred to as ALARA (as low as reasonably achievable) practices for radiation exposure.

Human radiation dosimetry of [^{18}F]NAV4694 PET was investigated in 6 healthy male volunteers who received approximately 210 MBq in study (D2750C0001). The OLINDA (for Organ Level INternal Dose Assessment) software (Stabin et al., 2005) was used to calculate radiation exposure. The ED per applied radioactivity was 0.023 mSv/MBq (4.8 hour urinary void interval). With the injected activity of 210 MBq, this results in an ED of 4.83 mSv, extrapolating to a proposed injected activity of 300 MBq, this results in an ED of 6.9 mSv.

For a listing of the respective radiation absorbed doses to body organs, see the Investigator's Brochure.

The ED upon administration of [^{18}F]NAV4694 PET can be compared with that resulting from a routine thoracic or abdominal computed tomography (CT) scan or from administration of [^{18}F]-FDG, the only PET radiopharmaceutical currently in widespread clinical use. With [^{18}F]-FDG, the ED per administered radioactivity is 0.019 mSv/MBq. The radioactive dose typically injected for cancer imaging is 370 MBq of [^{18}F]-FDG which corresponds to an ED of 7 mSv. In brain imaging with [^{18}F]-FDG, the dose recommended by the European Association of Nuclear Medicine is 125 to 250 MBq. However, the [^{18}F]-FDG package insert recommends a dose of 185 to 370 MBq for adults, resulting in an administered ED of approximately 3.5 to 7 mSv. The ED value resulting from the recommended [^{18}F]NAV4694 dose (approximately 6.9 mSv/300 MBq) is within this range.

3.3 Justification of Safety Plan

Safety pharmacology testing in animals up to present have demonstrated a favorable safety profile with no relevant neuroreceptor binding of [^{18}F]NAV4694 and no effect on central nervous or cardiovascular system. In an extended single-dose toxicity study in rats and 1-month repeated-dose toxicity studies in rats and dogs, [^{18}F]NAV4694 was well tolerated at doses greater than 25 times the maximum intended human dose. Details of the nonclinical safety studies can be found in the Investigator's Brochure.

A favorable safety profile has also been demonstrated in humans in a Phase 1 trial (D2750C0001) that evaluated safety, pharmacokinetics, and dosimetry in 20 subjects (10 AD and 10 healthy volunteers), a Phase 1 trial (D2750C00002) that evaluated safety and efficacy at a low and high mass dose in 16 subjects (8 AD and 8 HVs), and a Phase 2 trial (D2750N00006) that evaluated safety, test-retest reliability, and efficacy of different scanning parameters in 24 subjects (10 AD, 10 older healthy volunteers, and 4 young healthy volunteers). There were no safety or tolerability concerns identified in these trials. Only one AE (cough) occurred that was judged by the investigator to be related to the investigational product (trial D2750C00002). In the present trial, the design of the safety plan permits an appropriate and adequate evaluation of the safety response to [^{18}F]NAV4694 under baseline and post- investigational product administration conditions and allows a comparison of this study's safety data set with that of the previous studies. The measures used to assess safety are well defined and reliable within the context of the PET imaging environment, and the proposed safety analyses are adequate to assess the effects of study drug injection. The safety plan aims to restrict the collection of data up to and including 20 to 28 hours post injection based on the pharmacokinetic characteristics of [^{18}F]NAV4694 and the absence of any study procedures beyond this time point aside from a 7 day telephone follow-up.

3.4 Protocol Adherence

Strict adherence to all specifications laid down in this protocol is required for all aspects of the study conduct; the investigator may not modify or alter the procedures described in this protocol. If protocol modifications are necessary, all alterations that are not solely of an administrative nature require a formal protocol amendment (see Section 11.1 for the involvement of International Ethics Committee(s) IEC(s)/ International Review Board(s) IRB(s)).

If an investigator has deviated from the protocol in order to eliminate an immediate hazard to subjects or for other inevitable medical reasons, the investigator shall document all such deviations, including the reasons thereof, and submit the document to the sponsor and the head of the medical institution as applicable.

3.5 Study Duration

Subjects will be enrolled for approximately 36 to 38 months depending on the duration of the screening window (potentially 8 weeks).

4 STUDY POPULATION

4.1 Eligibility

Subjects with MCI, as established by a comprehensive neuro-cognitive evaluation (for details see Section 8.1.4) and diagnostic criteria, will be eligible to participate.

Subjects who fulfill all respective inclusion and none of the exclusion criteria will be eligible for enrollment into the study. All inclusion/exclusion criteria must be verified before a subject may be considered eligible for enrollment into the study. A subject will be considered enrolled in the study on the morning of study day 1 when they arrive at the study site. Written, dated (with time noted) informed consent will be obtained from all subjects.

4.1.1 Inclusion Criteria

1. Subject has signed informed consent to participate in the study and continues to give willing consent for participation
2. Age ≥ 55 years with a diagnosis of MCI
3. Educational level of at least 6 years
4. Female subjects will not be of child-bearing potential (> 1 year post-menopausal or surgically sterile)
5. Availability of a "study partner" who can assist in completing rating scales for the duration of the study
6. Cognitive complaints reported by the subject and confirmed by the "study partner"
7. Clinical Dementia Rating (CDR) global score = 0.5
8. Mini-mental state examination (MMSE) score of 24-30
9. Diagnostic and Statistical Manual of Mental Disorders, Version 4, Text Revised (DSM-IV-TR) criteria of dementia not fulfilled

4.1.2 Exclusion Criteria

1. Has been previously enrolled in this study and received the investigational product
2. Has received an investigational product within 30 days prior to screening
3. Has received disease-modifying therapy that could have changed amyloid brain deposition
4. Has exceeded yearly radioactive dose of 30 mSv
5. Has a known allergy to the study drug or any of its constituents
6. Has a history of alcohol abuse or alcohol dependency in the 3 years prior to study entry, or is an alcoholic or drug addict, as determined by the investigator
7. Has ongoing clinically significant (as judged by the investigator), metabolic or any other disease that could currently cause impaired memory (e.g., untreated thyroid disease, vitamin or other nutritional deficiencies, chronic kidney, or liver disease)
8. Memory impairment that can be attributed to a disease or condition other than an early phase neurodegenerative syndrome
9. Has a parkinsonian movement disorder

10. Use of psychoactive medications that would affect the subject's ability to reliably perform neurocognitive testing or create uncertainty in distinguishing between the effects of the psychoactive medication and the subject's underlying cognitive impairment (e.g., benzodiazepines, sedatives, antipsychotics)
11. Has received any contrast material (X-ray, MRI) or radiopharmaceutical within 48 hours prior to, or a therapeutic radiopharmaceutical (e.g., ^{131}I) within 10 days prior to, or any radiopharmaceutical administration within 10 radioactive half-lives prior to the administration of the investigational product or for whom administration of such substances is planned within 7 days after investigational product administration
12. History of major recurrent depressive disorder (per DSM-IV-TR) within the last 5 years prior to screening
13. Has a brain tumor or other intracranial lesion, a disturbance of cerebral spinal fluid circulation (e.g., normal pressure hydrocephalus), and/or a significant history of head trauma or brain surgery
14. Has signs of major cerebrovascular disease, as verified by medical history and/or brain MRI
15. Is scheduled for surgery and/or another invasive procedure within the 7 days following investigational product administration
16. Has any contraindication to MRI examination, e.g., metal implants, phobia, or cannot undergo an MRI for other reasons such as the inability to lie flat

4.1.3 Justification of Selection Criteria

The selection of participants reflects a balance between including subjects with a higher likelihood of progressing to dementia of the Alzheimer's type and those with a lower likelihood of progressing in a way that ensures that the study is feasible but includes a broad range of different types of MCI subjects with impaired memory. In addition, the exclusion criteria were defined to exclude subjects with impaired memory caused by other diseases, such as metabolic diseases.

4.2 Recruitment

Subjects will be recruited in accordance with the inclusion and exclusion criteria listed above from neurology or geriatric practices. Potentially suitable subjects will be asked by their treating specialist about their willingness to participate in this study.

4.3 Withdrawal

In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time and without providing a reason.

Should a subject withdraw after administration of the investigational product all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. All 36 month visit procedures should be completed at the time of the subject's withdrawal for those subjects withdrawing at or after 6 months post baseline injection as determined by the willingness of the subject. Priority for 36 month visit procedures should be

safety and then neuro-cognitive assessments. An explanation should be given of why the subject is withdrawing or being withdrawn from the study.

Subjects that begin regular use of concomitant medication that impairs cognition i.e., large doses of hypnotics, anxiolytics, tricyclic antidepressants and/or antipsychotics should be discussed with the medical monitor. Subjects taking cognition impairing medication may need to be withdrawn from the trial if the concomitant medication interferes with the investigator's ability to detect change in the subject's underlying cognitive impairment.

The investigator may withdraw a subject from the study at any time at the discretion of the investigator for any of the following reasons:

- A protocol violation occurs
- A serious or intolerable AE occurs
- A clinically significant change in a laboratory parameter occurs
- At the investigator's/sponsor's discretion as long as it is in the best interest of the subject
- The sponsor or investigator terminates the study
- The subject requests to be discontinued from the study

4.4 Replacement

Subjects will be replaced under the following conditions:

- Subjects who prematurely discontinue participation before completion of the 6-month visit will be replaced.
- Subjects who receive the study medication, but for whom the acquisition of PET imaging data is not adequately possible, e.g., due to inability to support the head immobilization regimen, an intercurrent adverse event, requiring priority treatment, or withdrawal of consent during imaging will be replaced by an additional subject.
- Subjects who did not receive the study medication due to withdrawal of consent, any intercurrent medical reason that is not merely transient in manner, or the presence of an exclusion criterion will be replaced by an additional subject.

4.5 Subject Identification

After the subject provides written informed consent, the site will assign the subject a 7-digit subject number. Subject numbers are to be assigned in a sequential manner using the following format:

Digits 1 to 2: Trial number "04"
Digits 3 to 4: Site number (e.g., "03")
Digits 5 to 7: Sequential subject number (e.g., "001", "002", "003", etc.)

For example, the first subject consented at Site 03 is subject number "04-03-001".
Subjects will maintain the same number given at screening for the entire study. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Identification of Investigational Product

The investigational product is 2-(2-[^{18}F]-fluoro-6-(methylamino)pyridin-3-yl)benzofuran-5-ol ([^{18}F]-NAV4694). [^{18}F]-NAV4694 will be prepared by a qualified PET Manufacturing Facility (PMF) from precursor supplied by Navidea Biopharmaceuticals.

The nuclear medicine physician or designee is responsible for ensuring that deliveries of investigational product and other materials from the sponsor and/or the PMF are correctly received, recorded, handled, and stored safely and properly in accordance with all applicable regulatory guidelines, and used in accordance with this protocol.

Unless otherwise agreed, investigational product transport containers (e.g., "lead pigs") must be returned to the PET production center and investigational product and other materials disposed of (via decay or otherwise) on-site after use. A list of investigational product(s) or other materials that were returned or disposed of must be prepared and signed by the principal investigator or designee. If there are any discrepancies, an explanation for these should also be provided.

The current Investigator's Brochure will be supplied to the investigative center.

5.2 Identity and Production

Investigational product will be manufactured and handled according to the applicable Good Manufacturing Practice (GMP) at a qualified PMF using an automated process that includes radio-labeling of the non-radioactive precursor (NAV4614) with fluorine-18 (the radioactive source of fluoride produced by an on-site cyclotron) followed by purification. Before release, each batch of investigational product will be assessed by a number of quality control tests according to methods approved by the sponsor. The investigational product must meet pre-specified criteria for appearance (clarity, color, particulate content), identity, purity, specific activity, pH, and bacterial endotoxin level; although tested for sterility, the results will not be available until after administration. The investigational product will be formulated as a sterile solution for intravenous injection at the pre-specified radioactivity dose of 300 MBq (8.1 mCi) ± 20%. All manufacturing, quality control testing, and documentation will be controlled and documented by the responsible person at the production site.

The subject dose is prepared from a production batch in a manner such that at the timepoint of administration, the volume is between 1 and 10 mL and the activity is 300 MBq (8.1 mCi) of [^{18}F]NAV4694.

A complete record of batch numbers and expiry dates of all study medication will be maintained in the Trial Master File (TMF).

The PMF will assign individual batch numbers to all investigational product production runs. A complete batch documentation in which manufacturing, quality control, analytical results, and the batch release for human use are detailed will be generated. These documents will be archived at the respective production site, with copies provided to the sponsor. Copies of the

master batch documentation and of the individual manufacturing batch documentations will also be made available to the sponsor for the Trial Master File (TMF).

5.3 Dosage and Administration

Study participants will be administered [^{18}F]NAV4694 under the direct supervision of a nuclear medicine physician or designee. For administration of [^{18}F]NAV4694, access into a large vein (e.g., antecubital vein) should be established using a suitable indwelling catheter (e.g., Venflow). To avoid extravasation of [^{18}F]NAV4694, correct localization of the catheter must be ensured by a test injection of normal saline prior to the injection of [^{18}F]NAV4694.

Each study participant will receive a single IV injection of [^{18}F]NAV4694 at baseline, and a subset of 20 subjects will receive a second single IV injection of [^{18}F]NAV4694 at 18 months with a total radioactivity dose amounting to 300 MBq (8.1 mCi) ± 20% on each occasion. This single IV injection will contain a maximum mass dose of NAV4694 (total: ^{18}F containing material plus ^{19}F containing material) of no more than 40 µg and a total volume of up to 10 mL. [^{18}F]NAV4694 must be administered manually via slow IV injection followed by a 10 mL saline flush. **Note**: The use of a motorized power injection system is not permitted.

The exact radioactive dose administered must be determined by calculating the difference between the radioactivity in the syringe immediately before injection and in the syringe and injection system immediately after injection using a suitable counter. Measured radioactivity values and times of measurement will be recorded in the source documentation, along with the total injected volume. Injected radioactivity values outside the above stated range, i.e., values lower than 240 MBq/6.48 mCi or higher than 360 MBq/9.72 mCi will be considered as protocol deviations.

5.4 Treatment Assignment

In this open-label non-randomized pivotal study, all subjects will receive the same treatment.

5.5 Blinding

This is an open-label study as the [^{18}F]NAV4694 will be injected in an open-label manner.

The overall assessment as to cognitive state (e.g., cognitive decline and/or progression to dementia) will be performed by the investigator who will be blinded to the results of [^{18}F]NAV4694 PET findings (the baseline and 18-month scans) until the clinical diagnostic status for the subject has been determined at 36 months. PET scan results can be shared with the subject at the end of their participation at the discretion of the investigator and preference of the subject. Results of the subject's genetic testing can be shared with the subject at the discretion of the investigator and the preference of the subject.

The onsite PET image analysis will be performed by an onsite nuclear medicine physician or radiologist who is not otherwise participating in the diagnostic workup or diagnostic

categorization of the subject. This physician will not be blinded to the subject's clinical information or diagnosis.

The PET images of all subjects (baseline and 18-month visits) will be forwarded in DICOM 3.0 format to the image core lab (Molecular NeuroImaging [MNI], New Haven, CT). Here, the PET images will be freed of all subject identifiers and independently assessed by 3 blinded readers.

For the 6-, 12-, 24-, 30-, and 36- month follow-up visits the investigator will conduct the neuro-cognitive examination on all subjects who have returned and will compare results to those of the previous visits for the purpose of assessing and documenting the subject's current diagnosis and progression status.

For the 18- month follow-up visit the investigator will again conduct the neuro-cognitive examination, in addition to a comprehensive clinical and neurological assessment of all returning subjects and compare the results to those of the previous visits for the purpose of assessing and documenting the subject's current diagnosis and progression status.

All Sponsor personnel and all service providers involved in the data analysis for the trial will remain blinded to all trial efficacy results until such time that the clinical database for the study has been locked.

5.6 Packaging and Labeling

The investigational product will be provided in a vial. The vial (or syringe) will be shielded by a secondary container for radiation protection and product integrity purposes, and with the intent that the vial and secondary container remain together.

The vial (or syringe) along with the secondary container will be labeled according to the local standards and the specifications of the local drug law in the local language. As both are intended to remain together, reduced information is given on the vial (or syringe) label.

All labels will be in accordance with the requirements of Good Clinical Practice (GCP) and FDA. The label will include the following:

- Name and address of sponsor
- Name/identifier and strength of the preparation (including formulation)
- Batch number, Lot Number, or Rx Number
- Pharmaceutical dosage form
- Route of administration
- Date and time the total activity in the vial was measured

Guidance to the investigator regarding dosage is provided in a separate document provided by the respective PMF site upon delivery of the investigational product ("Dosing Guide").

This guidance is intended for the person administering the investigational product and provides a list of recommended volumes for multiple 5-minute increments (related to end of synthesis time) corresponding to approximately 300 MBq/8.1 mCi ± 20%. The volumes listed in this dosing guide are only recommendations, and different volumes for a particular timepoint may be interpolated on-site to achieve the target radioactivity dependent upon infusion systems and/or other factors.

A sample copy of the label will be provided to the sponsor for use in the TMF.

5.7 Drug Logistics, Storage, and Accountability

[^{18}F]NAV4694 should be kept in a secure place under appropriate storage conditions for radioactive agents. For all drug products, a system of medication numbering in accordance with all requirements of GMP will be used. This will ensure that for each subject, any dose of [^{18}F]NAV4694 can be identified and traced back to the production run. If imaging is not performed at the PET production center, the investigational product will be delivered to the PET imaging site by an approved courier. The final quality control processes for the investigational product will occur and be documented before the substance is administered to the subject at the PET imaging center. The investigational product will not be administered to the subject without release for human use.

The investigator (or designated personnel) at the PET imaging site will ensure that the correct volume (containing 300 MBq/8.1 mCi ± 20%) will be filled from the vial into the syringe and that the administration of the investigational product does not exceed the expiry date and time as given on the appropriate documentation. The investigator (or designated personnel) will manage the transfer of the syringe containing the investigational product to the person at the PET imaging site responsible for administering to the subject.

If the location of syringe filling and study administration to the subject differs, then the investigator (or designated personnel) must ensure that the syringe is labeled appropriately to avoid any ambiguity.

5.8 Supply, Dispensing, and Return

The study drug will be manufactured by a qualified PET production center and will be delivered to the study site on request of the investigator.

The investigator must not order any investigational product before approvals from the IRB/IEC and competent authorities are available in written form.

The shelf life of the investigational product is up to 8 hours from the time of calibration. It should be noted that the investigator orders the investigational product for a certain time point of administration. The PET production center will then provide an investigational product containing sufficient activity for the treatment at time of administration. The investigational product should be prepared in a way that there will be enough activity for an administration at least up to 60 minutes later than the planned time of injection. The

information on required volume at time of administration is provided to the nuclear medicine physician (or designee) by the PMF.

All non-radioactive transport containers (opened, unopened, or empty) must be returned to the PET production center. Containers (vials) that are radioactive or contained radioactive materials must be disposed of (after decay) at the PET imaging site after the study according to the local standard operating procedures/policies. The disposal needs to be documented and filed in the investigator site file.

5.9 Drug Accountability

The investigator (or designated personnel) will confirm receipt of the investigational product in writing and will use the investigational product only within the framework of this clinical study and in accordance with this study protocol. For each subject he/she will keep a record of the investigational product dispensed including a printout providing relevant parameters of synthesis and formulation of the investigational product, and all other accompanying forms to the investigational product. These documents are to be filed in the investigator site file.

Overall drug accountability and reconciliation will be completed by the sponsor or its representative. A list of investigational product vials and other materials that were returned, or disposed of, must be prepared and signed by the principal investigator or an appropriately qualified designee as documented in the study site responsibility sheet. An overall accountability and reconciliation form of the investigational product will also be prepared and completed. If there are any discrepancies, an explanation for these must be provided.

Receipt, distribution, and return of the investigational product must be properly documented on the forms provided by the sponsor giving the following information: study protocol number, sender, receiver, date, quantity, batch number, expiration date, and retest date, if applicable.

6 THERAPIES OTHER THAN INVESTIGATIONAL PRODUCT

6.1 Prior and Concomitant Therapy

Subjects will be excluded from the study if they are taking regular medication that impairs cognition (as determined by the investigator), e.g., subjects who have received large doses of hypnotics, anxiolytics, tricyclic antidepressants, and/or antipsychotics.

Subjects must not have any (even) short-term medication of large doses of hypnotics, anxiolytics, tricyclic antidepressants, and/or antipsychotics within 2 days before neuro-cognitive testing. In these cases, neuro-cognitive testing should be postponed.

Subjects will be withdrawn from the study if they subsequently commence regular medication that impairs cognition (as determined by the investigator) during the study, i.e., large doses of hypnotics, anxiolytics, tricyclic antidepressants and/or antipsychotics.

Subjects already being treated with acetylcholinesterase inhibitors and/or memantine should be on stable doses of these medications for 30 days prior to screening.

If treatment with an acetylcholine esterase inhibitor and/ or memantine is initiated during the trial, the doses of these medications should be stable for 30 days before the next neurocognitive assessment.

After a subject has progressed to dementia, exposure to disease-modifying therapy would not require withdrawal from participation. Subjects initiating disease-modifying therapy before progression to dementia would need to be withdrawn from participation.

6.2 Post-Study Therapy

Not applicable.

7 SCHEDULE OF EVALUATIONS AND VISIT DESCRIPTION

7.1 Schedule of Evaluations

Evaluations will be performed during a period of 36 months, in addition to a screening period of maximum 8 weeks. A schedule of evaluations is provided in the Schedule of Study Events (see Appendix 1).

7.2 Visit Description

7.2.1 Screening Visit

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the eCRF and enrollment log
- Interview including the following subject-specific characteristics
  - Demographic data
  - Postmenopausal status
  - Medical/surgical history (including concomitant diseases)
  - History of MCI
  - Family history of dementia
  - Documentation of diagnostic tests outside this study related to MCI diagnosis
  - Concomitant medications (medication history, see Section 6.1).
- A battery of cognitive and functional performance evaluations will be conducted at the screening visit as described in Section 8.1.4
- Vital signs (heart rate and blood pressure after at least 3 minutes in resting position)
- Physical examination including height and weight and a review of major body systems. Any clinically relevant finding is to be documented as baseline finding.
- Neurological examination
- Urine collection for routine urinalysis
- Blood draw, see Section 8.4.3
  - for routine blood hematology and chemistry parameters
  - for genetic analysis
  - for special laboratory tests related to cognitive impairment
- Brain MRI (unless scan with adequate acquisition sequences is available from previous 6 months)
- Final check of inclusion/exclusion criteria

7.2.2 Baseline Testing Visit (within 7 days before [^{18}F]NAV4694 injection)

- A battery of cognitive and functional performance evaluations will be conducted at the baseline visit as described in Section 8.1.5
- Physical examination
- Safety laboratory samples will be collected, including samples for routine hematology, chemistry and urinalysis as described in Section 8.4.1
- Concomitant medication review, see Section 6.1
- Assessment of adverse events, see Section 8.3.1

7.2.3 Baseline [^{18}F]NAV4694 Injection and PET Imaging Visit

7.2.3.1 Before Injection

The baseline visit will take place on the PET imaging day prior to investigational product injection and will comprise the following procedures/assessments:

- Body weight measurement
- Insert intravenous catheter and check localization of indwelling catheter by test injection of normal saline
- Vital signs (within 10 minutes prior to investigational product injection, subject in resting position for at least 3 minutes), see Section 8.3.2.2
- Electrocardiogram (within 10 minutes prior to investigational product injection, subject in resting position), see Section 8.3.2.2)
- Assessment of adverse events, see Section 8.3.1

7.2.3.2 Administration of [^{18}F]NAV4694 (≤ 56 days after the screening visit)

Investigational product injection will comprise activities described in see Section 5.3.

- Measurement of the [^{18}F] radioactivity in the syringe immediately prior to injection
- [^{18}F]NAV4694 injection via slow IV injection followed by 10 mL saline flush (**Note**: The use of a motorized power injection system is not permitted)
- Remove injection system
- Measure residual [^{18}F] radioactivity in the syringe and injection system immediately after injection
- Assessment of adverse events, see Section 8.3.1

7.2.3.3 Post [^{18}F]NAV4694 Injection

- Vital signs (within 10 minutes and 30 ± 5 minutes post injection), see Section 8.3.2.2
- Electrocardiogram (within 10 minutes post injection), see Section 8.3.2.3
- PET Imaging Session (scanning from 50 to 70 minutes post injection)
- Assessment of adverse events, see Section 8.3.1

7.2.4 Safety Follow-Up Visit (24 ± 4 hours after PET imaging visit)

This visit will comprise the following procedures/assessments:

- Vital signs
- Physical examination
- Electrocardiogram
- Blood draw for routine blood hematology and chemistry parameters, see Section 8.4.3
- Urine collection for routine urinalysis
- AE assessment
- Concomitant medication assessment

7.2.5 Telephone Follow-up 7 (+ 3) days post injection

A telephone follow-up will be performed with the subject 7 days post injection after study administration. Focus of the interview is to determine any AEs that may have occurred subsequent to the previous post-imaging safety assessment by questioning the subject or study partner about any changes in the subject's health or concomitant medications.

7.2.6 Neuro-Cognitive Follow-up Examinations at 6-, 12-, 18-, 24-, 30-, and 36-Months (± 30) days post injection

- A battery of cognitive and functional performance evaluations will be conducted at the 6-, 12-, 18, 24-, 30-, and 36-month follow-up visits as described in Section 8.1.6. Neuro-cogntive examinations at 18-months should be completed in the 30 days before PET imaging.
- DSM-IV-TR criteria will be used to determine whether a subject has dementia and if so, which type. Probable AD will be confirmed using criteria from NINCDS-ADRDA (McKhann et al., 1984).

7.2.7 Additional 18-Month Activities (in a subset of 20 subjects)

7.2.7.1 Within 30 Days Before 18-month PET Imaging

- MRI of the brain
- A battery of cognitive and functional performance evaluations will be conducted as described in Section 8.1.5.

7.2.7.2 Within 7 Days Before 18-month PET Imaging

- Neurological exam
- Physical examination
- The investigator will review all of the clinical and neuro-cognitive data gathered for each subject over the course of the trial to determine the diagnostic status (progression to Alzheimer's dementia).
- DSM-IV-TR criteria will be used to determine whether a subject has dementia and if so, which type. Probable AD will be confirmed using criteria from NINCDS-ADRDA (McKhann et al., 1984).
- Safety laboratory samples will be collected include samples for routine hematology, chemistry and urinalysis as described in Section 8.4.1
- AE assessment
- Concomitant medication assessment

7.2.7.3 PET Imaging and Follow Up

- PET imaging - the same procedures will be performed as in the Baseline [^{18}F]NAV4694 Injection and PET Imaging Visit described in Section 7.2.3
- Follow-up safety visit (24 ± 4 hours after PET imaging visit) - the same procedures will be performed as described in Section 7.2.4.
- Follow-up telephone contact (7 days [+3 days] after PET imaging visit) - the same procedures will be performed as described in Section 7.2.5.

7.2.8 Neuro-Cognitive Testing Schedule (screening and 6-, 12-, 18-, 24-, 30-, and 36-month visits)

Neuro-cognitive assessments will be conducted according to the schedule shown in Table 1.

Table 1. Neuro-Cognitive Testing and Assessment of Progression to Dementia

	Visit		
Assessments[a]	**Screening**	**Baseline**	**6-, 12-,18-, 24-, 30-, and 36-month visits**
CDR	X		X
ADCS-MCI-ADL		X	X
MMSE	X		X
DSM-IV-TR criteria for dementia	X		X
DSM-IV-TR criteria for major depressive disorder, recurrent	X		X
RAVLT		X	X
ADAS-cog		X	X

ADAS-cog = Alzheimer's Disease Assessment Scale-Cognitive; ADCS-MCI-ADL = Alzheimer's Disease Cooperative Study Scale for Activities of Daily Living in Mild Cognitive Impairment CDR = Clinical Dementia Rating; DSM-IV-TR = Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision; RAVLT = Rey Auditory Verbal Learning Test

[a] See Sections 8.1.4, 8.1.5, and 8.1.6 for descriptions

The investigators or nominated designee(s) responsible for conducting the neuro-cognitive tests will be blind to the results of the [^{18}F]NAV4694 PET scans.

7.2.9 End of Study

For the entire study, end of study is defined as last subject last visit.

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Approximately 120 evaluable male or female subjects with MCI aged ≥ 55 years will be included. Subjects must have ≥ 6 years of education. Additional prerequisites for participation are adequate visual and auditory acuity to complete neuro-cognitive testing, availability of reliable caregiver who is capable to provide correct information about the subject's clinical symptoms and completion of the clinical and neuro-cognitive evaluation as well as assignment of a diagnosis of MCI. Subjects must have an MMSE score of 24-30 and a CDR score of 0.5.

8.1.2 Medical and Surgical History

Medical and surgical histories are to be recorded for the following body systems: head, ear, eye, nose, throat, respiratory, cardiovascular, gastrointestinal, genitourinary, musculoskeletal, endocrine, neurological, and psychiatric and skin/dermatological.

As part of the medical history, the date of the last spontaneous menstruation will be recorded, if childbearing potential is not excluded by surgical sterilization and/or hysterectomy.

8.1.3 Prior and Concomitant Medication

All prior medication used within the last 30 days before the first screening examination and concomitant medications will be documented.

For instructions regarding prior and concomitant medications, please refer to Section 6.

8.1.4 Inclusion Criteria Assessments at Screening to Confirm Mild Cognitive Impairment

To determine whether a subject has MCI and no dementia, the following assessments will be performed by the on-site clinician during screening.

Clinical Dementia Rating scale (see Section 8.1.4.1)

Mini-Mental State Examination (see Section 8.1.4.2)

In addition, the on-site clinician will determine that the subject does not meet the following criteria at screening.

- Diagnostic and Statistical Manual of Mental Disorders, Version 4, Text Revised criteria for dementia (see Section 8.1.4.3)

If the result of any of these assessments does not meet the requirements specified in inclusion criteria (see Section 4.1.1), the subject will not be included in the study.

If the subject meets all of the requirements specified in inclusion criteria, the following assessment will be made to determine whether depression may be responsible for or contributing to the clinical diagnosis of MCI.

- Diagnostic and Statistical Manual of Mental Disorders, Version 4, Text Revised criteria for a major depressive disorder, recurrent (see Section 8.1.4.4)

If the subject is suffering from major depressive disorder, recurrent, he/she will not be included in the study (see exclusion criterion 9, Section 4.1.2).

8.1.4.1 Clinical Dementia Rating Scale

The CDR scale (Hughes et al., 1982; Morris, 1993) has become the gold standard for global rating of primary degenerative dementia and has been extensively validated (Morris et al., 1988; Berg et al., 1992; Galasko et al., 1995). Its advantage is that it incorporates the clinician's assessment of subjects' varying educational, cultural, socio-economic, and other types of bias in its staging.

The CDR is a dementia rating instrument that uses a 5-point scale to characterize 6 domains of cognitive and functional performance applicable to AD and related dementias: Memory, Orientation, Judgment & Problem Solving, Community Affairs, Home & Hobbies, and Personal Care. The necessary information to make each rating is obtained through a semi-structured interview of the subject and the subject's "study partner."

In addition to ratings for each domain, a global CDR score may be calculated through the use of the following algorithm. Memory is considered the primary category and all others are secondary. The global CDR score is equal to the memory score if at least three secondary categories are given the same score as memory. When the memory score is 0.5, the global CDR score equals 1 if at least three of the other categories are scored 1 or greater. If the memory score is 0.5, the global CDR score cannot be 0; it can only be 0.5 or 1. If the memory score is 0, the global CDR score equals 0 unless there is impairment (0.5 or greater) in two or more secondary categories, in which case the global CDR score equals 0.5. Whenever three or more secondary categories are given a score greater or less than the memory score, the global CDR score equals the score of the majority of the secondary categories from the side of the memory score that has the greater number of secondary categories. If three secondary categories are scored on one side of the memory score and two secondary categories are scored on the other side of the memory score, the global CDR score equals the memory score.

The above rules do not cover all possible scoring combinations. Unusual circumstances are scored as follows.

- With ties in the secondary categories on one side of the memory score, choose the tied scores closest to the memory score for the global CDR score. For example, if the memory score and another secondary category equal 3, two secondary categories equal 2, and two secondary categories equal 1, then the global CDR score equals 2.
- When only one or two secondary categories are given the same score as the memory score, the global CDR score equals the memory score as long as no more than two secondary categories are on either side of the memory score.
- When the memory score is 1 or greater, the global CDR score cannot be 0; in this circumstance, the global CDR score equals 0.5 if the majority of secondary categories are 0.

This global CDR score is useful for characterizing and tracking a subject's level of impairment/dementia (Morris, 1993).

0 = Normal (no dementia)
0.5 = Uncertain or deferred diagnosis
1 = Mild dementia
2 = Moderate dementia
3 = Severe dementia

The subjects to be included in this study must have a global rating score of 0.5 on the CDR and a diagnosis of MCI. In addition scores in each of the six CDR domains will be included in the source documentation.

8.1.4.2 Mini-Mental State Examination

The MMSE (Folstein et al., 1983) is the most widely used and studied screening measure of cognitive impairment. It has the advantage of brevity, ease of administration (approximately 10 minutes), and high test/retest and inter-rater reliability. The MMSE is rated on a scale ranging from 0 (worst possible outcome) to 30 (best possible outcome, no measureable cognitive deficits). Scores below 24 are traditionally taken as indicative of dementia, although the MMSE score alone will not be decisive in making a dementia diagnosis.

The most sensitive items on the MMSE for dementia of the Alzheimer's type are delayed recall of 3 items and orientation.

8.1.4.3 Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision Criteria for Dementia

The DSM-IV-TR defines the diagnostic criteria for dementia as follows.

A. The development of multiple cognitive deficits manifested by both

   1. memory impairment (impaired ability to learn new information or to recall previously learned information) and

2. one (or more) of the following cognitive disturbances:
    a. aphasia (language disturbance), or
    b. apraxia (impaired ability to carry out motor activities despite intact motor function), or
    c. agnosia (failure to recognize or identify objects despite intact sensory function), or
    d. disturbance of executive functioning (i.e., planning, organizing, sequencing, abstracting)

B. The cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from previous level of functioning.

C. The course is characterized by gradual onset and continuing decline.

D. The cognitive deficits in criteria A1 and A2 are not due to any of the following.

1. other central nervous system conditions that cause progressive deficits in memory and cognition (e.g., cerebrovascular disease, Parkinson's disease, Huntington's disease, subdural hematoma, normal-pressure hydrocephalus, brain tumor)
2. systemic conditions that are known to cause dementia (e.g., hypothyroidism, vitamin B12 or folic acid deficiency, niacin deficiency, hypocalcemia, neurosyphilis, human immunodeficiency virus infection)
3. substance-induced conditions

E. The deficits do not occur exclusively during the course of a delirium.

F. The disturbance is not better accounted for by another Axis I disorder (e.g., major depressive disorder, schizophrenia).

8.1.4.4 Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision Criteria for Major Depressive Disorder, Recurrent

Depression is a frequent accompanying symptom of dementia of the Alzheimer's type (and other dementias), and severe, late-onset depression can lead to a similar degree of cognitive impairment. In the present study, the DSM-IV-TR will be used to assist the on-site clinician in objectifying depressive symptoms in detecting/ruling out major depressive disorder, recurrent diagnosed more than 5 years prior to participation in this study as a cause for symptoms of cognitive impairment. Results of the assessment will be recorded in the source documentation.

The DSM-IV-TR defines the criteria for Major Depressive Disorder, Recurrent as follows:

A. Presence of two or more Major Depressive Episodes (**Note:** To be considered separate episodes, there must be an interval of at least 2 consecutive months in which criteria are not met for a Major Depressive Episode.)
B. The Major Depressive Episodes are not better accounted for by Schizoaffective Disorder and are not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.
C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode. (**Note:** This exclusion does not apply if all of the manic-like, mixed-like, or hypomanic-like episodes are substance or treatment-induced, or are due to the direct physiological effects of a general medical condition.)

Criteria for Major Depressive Episode:

A. Five or more of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. (**Note:** Do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations.)

    1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful). (**Note:** In children and adolescents, can be irritable mood.)
    2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
    3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (**Note:** In children, consider failure to make expected weight gains.)
    4. Insomnia or hypersomnia nearly every day
    5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
    6. Fatigue or loss of energy nearly every day
    7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
    8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
    9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. The symptoms do not meet criteria for a Mixed Episode.

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).

E. The symptoms are not better accounted for by Bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

8.1.5 Assessment of Cognitive Performance at Baseline

The following three tests will be performed:

- Alzheimer's Disease Cooperative Study Scale for Activities of Daily Living in Mild Cognitive Impairment (see 8.1.5.3)
- Rey Auditory Verbal Learning Test (see Section 8.1.5.1)
- Alzheimer's Disease Assessment Scale-Cognitive (see 8.1.5.3)

8.1.5.1 Alzheimer's Disease Cooperative Study Scale for Activities of Daily Living in Mild Cognitive Impairment

This interview was developed to assess impairment of everyday tasks in non-demented subjects. It is highly sensitive and covers 18 areas. The overall score varies between 0 (worst performance) and 78 (best performance).

Subjects with minor limitations on complex ADL should not be excluded from the study. However, to ensure that subjects with significant functional impairment, including the loss of basic ADL and who might already have dementia, are not included, the ADCS-MCI-ADL will be used to assess deficits in more complex everyday tasks.

This cut-off is based on results from the German group in an MCI study in which the study population was defined as having "No impairment on basic activities of daily living. More complex activities of daily living may be slightly impaired" (Perneczky et al., 2006). Using this definition, a significant difference between healthy normal volunteers and non-demented subjects with MCI was found in more complex ADL.

8.1.5.2 Rey Auditory Verbal Learning Test

The Rey Auditory Verbal Learning Test (RAVLT) was developed in the 1940s as a measure of verbal learning and memory, including proactive inhibition, retroactive inhibition, retention, encoding versus retrieval, and subjective organization (Andersson et al., 2006).

Testing procedures can be administered in approximately 15 minutes and begin with a list of 15 words, which are read aloud by an examiner at the rate of one per second. The subject is asked to repeat all remembered words, in any order. This sequence is repeated five times. A second list of 15 words is presented with only one recall attempt allowed. Finally, the subject is asked to recall all words possible from the original list (Schmidt, 2010).

The RAVLT is scored by using the RAVLT Record Sheet and Score Summary which accompanies the RAVLT Handbook (Schmidt, 2010).

8.1.5.3 Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog)

The Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog; (Rosen et al., 1984)) will be used to assess cognitive performance.

The 13 items in the ADAS-Cog (Mohs et al., 1997) will be used to assess the general cognitive performance of subjects in the study. This is a well-accepted assessment instrument that has been used in large number of investigational studies and clinical trials. The ADAS-Cog is a brief cognitive test battery that assesses learning and memory, language production, language comprehension, constructional praxis, ideational praxis, and orientation.

The 13 test items should be given in the order indicated. The Word Recall task is given first; the Delayed Word Recall task should be done approximately 5 minutes later.

The 13 items are scored in the ranges given below. The total ADAS-Cog 13-item score can range from 0 to 85; higher scores reflect more severe cognitive performance.

- Word Recall 0 to 10
- Commands 0 to 5
- Constructional Praxis 0 to 5
- Executive Function 0 to 10
- Naming 0 to 5
- Ideational praxis 0 to 5
- Orientation 0 to 8
- Word Recognition 0 to 12
- Remembering Test Instructions 0 to 5
- Comprehension 0 to 5
- Word Finding 0 to 5
- Spoken Language Ability 0 to 5

  Number Cancellation 0 to 5

For the 6-, 12-, 18-, 24-, 30-, and 36-month visits the principal investigator will conduct the clinical and neurological assessment on all subjects who have returned and will compare results with those of the previous neuro-cognitive examination.

Finally, the assessment of progression from MCI to dementia will be conducted by the principal investigator or investigator under the close consultation with the principle

investigator. These physicians will be blind to the results of the investigational PET scan. The final decision will be based on the neuro-cognitive test results over time as well as on an interview of the subject together with the study partner or spouse.

8.1.6 Assessment of Cognitive Performance at the 6-, 12-, 18-, 24-, 30-, and 36-Month Assessments

The following assessments will be performed by the on-site clinician at the 6-, 12-, 18-, 24-, 30-, and 36-month visits

- The CDR scale (see Section 8.1.4.1)
- The ADCS-MCI-ADL (see Section 8.1.5.1)
- The MMSE (see Section 8.1.4.2)
- RAVLT (see Section 8.1.5.1)
- ADAS-Cog (see Section 8.1.5.3)
- DSM-IV-TR criteria of dementia not fulfilled (see Section 8.1.4.3)
- DSM-IV-TR criteria for a major depressive disorder, recurrent (see Section 8.1.4.4)

8.1.7 MRI Examination of the Brain

Brain MRI scanning (both 3D volumetric T1- and T2-weighted images) enables detailed visualization of structural abnormalities that assist, either in substantiating the diagnosis of probable AD (i.e., mesial temporal lobe [MTL] atrophy), or in identifying cerebral lesions that constitute pre-specified exclusion criteria. In the present study, a non-contrast enhanced, T2 weighted brain MRI using a 1.5 or a 3 Tesla scanner and a non-contrast enhanced 3D volumetric T1-weighted brain MRI, along with the FLAIR sequence, must be performed during screening. In addition to facilitating the above diagnostic tasks, the digital 3D T1-weighted images are required by the sponsor for co-alignment with the PET image data to create the anatomy-based regions of interest (ROIs) required for subsequent subject level visual and quantitative analysis of [^{18}F]NAV4694 tracer uptake.

T1-weighted acquisition procedure

A T1-weighted, 3D sequence (e.g., MPRAGE or SPGR) is required, and adherence to the manufacturer specific recommendations stated in this protocol (below) are mandatory. The total scan time is expected to be in the range of 20 to 30 minutes. The field of view (FOV) must include the cerebellum and pons. The recommended acquisition parameters for the T1 3D sequence are displayed in Table 2. In addition, approximately 6 oblique slices (slice thickness 5 mm, interslice gap 1 mm, in plane resolution 0.8 x 1.0 mm) parallel to the brainstem axis should be performed from a midsagittal scout image.

T2 acquisition procedure

For detailed assessment of cerebral vasculature, a T2-weighted double echo sequence should also be performed. The manufacturer specific recommended acquisition parameters for the T2 sequence are displayed in Table 2.

Both the T1 and T2 weighted MRI scans are to be evaluated by a qualified on-site radiologist for structural pathology, including changes indicative of major vascular disease/ischemia, normal pressure hydrocephalus, and/or, MTL atrophy. Medial temporal atrophy and ventricular volume assessment will be performed at MNI (New Haven, CT).

FLAIR procedure

The FLAIR procedure is also displayed within Table 2. Navidea has provided general guidance in acquiring the FLAIR sequence which should be performed along with the T1, and T2 sequences, and assessed to determine eligibility of the subject.

Optional DWI procedure

An optional DWI sequence can be used at the discretion of the investigator. General guidance on this sequence is also displayed in Table 2.

Table 2. Manufacturer Specific Recommended Acquisition Parameters for Volumetric Required T1 3D MRI, T2, FLAIR and Optional DWI Images.

Recommended Acquisition Parameters for Volumetric T1 3D MRI				Other Recommended Settings		
Manufacture/ Sequence Type	**General Electric** 3-D Fast SPGR {IRSPGR}	**Philips** 3D TFE	**Siemens** 3D MP-RAGE	**ALL/ T2**	**ALL/ FLAIR**	**ALL/ DWI**
Orientation	Sagittal	Sagittal	Sagittal	Axial	Axial	Axial
TR(ms)	N/A	Shortest	2400 (1.5T) 2300 (3.0T)	750 {750-900}	9000 {6000-10000}	min {4500-10000}
TE(ms)	min full	4 (1.5T) shortest (3.0T)	3.5(1.5T) 2.9 (3.0T)	35 (1.5T) 25 (3.0T)	145 {140-155}	min {65-120}
TI(ms)	Prep Time = 600 (1.5T) = 400 (3.0T)	See TFE prepulse delay	1000 (1.5T) 900 (3.0T)	N/A	2200 {2000-2500}	N/A
Flip Angle (degree)	8	8	8 (1.5T) 9 (3.0T)	20	90 {GE /Phillips} 180 {Siemens}	90
Num. of Slices	170	170	160	≥28	≥28	≥28
Slice thickness (MM)	1.2	1.2	1.2	4	4	4
Slice Gap (mm)	0	0	0	0.8	0.8	0.8
FOV (mm)	240 (1.5T) 260 (3.0T)	240	240	240	240	240
Rect. FOV (%)	100% (1.5T) 94% (3.0T)	100% (1.5T) 94% (3.0T)	100% (1.5T) 94% (3.0T)	≥75%	≥75%	100%
Phase Foldover Direction	AP	AP	AP	RL	RL	RL
Acquisition Matrix (1.5 T)	192x192	192x192	192x192	224x256	224x256	128x128
Acquisition Matrix (3.0T)	256x256	256x256	256x256	192x320	256x512	128x192
Num. of Acquisitions (NSA/NEX)	1	1	1	1	1	1
Bandwidth	16 kHz (1.5T) 31 kHz (3.0T)	165 (1.5T) 240 (3.0T)	180 Hz/Px (1.5T) 240 Hz/Px (3.0T)	Default	Default	Default
Specific Settings	SCIC OFF/ PURE ON (if option available)	TFE prepulse Delay = 1000 (1.5T) shortest (3.0T) TFE shot interval= 2300 (1.5T) 3000 (3.0T) CLEAR ON	N/A	ETL=1 For compatible scanners, Susceptibility Weighted Imaging will be used in addition the GRE T2*	ETL=1	N/A

FLAIR = Fluid Attenuated Inversion Recovery; DWI = DiffusionWeighted Imaging; ETL = Echo Train Length; SPGR = Spoiled Gradient Recalled; TFE = Turbo Field Echo; MP-RAGE = Magnetization-Prepared Rapid Acquisition with Gradient Echo; T = Tesla; GRE = Gradient Echo = AP = Anterior-Posterior, RL-Right-Left,

At a minimum, the T1-weighted, 3D volumetric MRI images must be forwarded in Digital Imaging and Communications in Medicine (DICOM) 3.0 format to the imaging core laboratory, which is responsible for quality control, image data preparation, archiving, and subsequent performance of the independent blinded read (at MNI). The details of performing the image transfer will be discussed with the study team on-site radiologist during the technical site set-up visit meeting with MNI. .

Assessment as to whether the brain MR images are normal (age-appropriate) or abnormal will be determined by an investigator or designee.

8.1.8 PET Image Acquisition

Preparation of study participants

Preparation of study participants consists of inserting an IV catheter (e.g., Venflow) into a vein, preferably in the study subject's non-dominant arm. Correct localization of the indwelling cannula must be ensured by test injection of normal saline prior to injection of the investigational product.

Injection of the investigational product

[^{18}F] radioactivity in the syringe will be measured immediately prior to injection using a suitable counter.

A tracer dose of 300 MBq (8.1 mCi) ± 20% [^{18}F]NAV4694 with a substance dose not exceeding a mass of 40 µg should be administered as a slow IV injection. The injection volume will be ≤ 10 mL.

After injection of the investigational product, the cannula must be flushed with 10 mL saline.

Measurement and documentation of the injected radioactivity

[^{18}F] radioactivity must be measured in the syringe and injection system immediately after injection using a suitable counter.

The following data will be documented by the investigator in the source documentation:

- Batch number
- Date and time of end of calibration of the system used to measure radioactivity before and after injection
- Date and time of expiration
- Tracer mass per volume (µg per mL)
- Radioactivity in syringe prior to injection (MBq, measured) and time of measurement
- Time of injection
- Volume of injection (mL)

- Radioactivity in syringe and injection system after injection (MBq, measured) and time of measurement

For a fraction of the above data, the tear-off part of the label is the source document. It will be stored in the ISF.

Note: The data documented by the investigator will be used by the sponsor to calculate the following parameters: Tracer mass injected (μg, calculated as the product of tracer mass per volume and volume of injection) and the injected radioactivity (MBq, calculated from the radioactivity measured prior to and after injection, the time point of injection, and the time points of radioactivity measurement). If the counter used for measurement of radioactivity is calibrated for the time of injection, the injected radioactivity will be obtained by simple subtraction of the radioactivity measured in the syringe and injection system after injection from the radioactivity measured in the syringe prior to injection. Furthermore, the time difference between the end of synthesis and the time of injection will be recorded.

Placement of subject and head immobilization

Images will be acquired using dedicated PET or PET-CT scanner capable of 3D PET image acquisition. Study participants will be placed on the table of the PET or PET-CT scanner. To minimize head motion, the subject's head will be immobilized using the institution's head holder/fixation equipment (e.g., thermoplastic mask, paper tape). The head of the subject will be positioned in the scanner with the total brain within the FOV. Special attention must be paid to include the entire cerebellum in the image as this region serves as a reference region for subsequent quantification.

Measurement of attenuation correction and emission scan acquisition

After the subject is lying comfortably with the head immobilized, attenuation correction will be measured. Measurement of attenuation correction must be started early enough to allow the first emission scan to proceed at the predefined time point (50 $\pm$ 5 minutes after injection). Attenuation correction must be performed via low dose CT or isotope transmission brain scan per camera protocol before start of the emission scan. A scout scan to determine proper patient positioning is suggested to occur prior to the transmission scan.

The 3D PET brain emission scanning should be performed in four 5 minute frames from 50 to 70 minutes post injection using the following general guidelines:

- Resolution of at least 4 to 5 mm full width half maximum (FWHM) value of the scanner.
- 3D mode, zoom = 2.0
- Use of a Neuroshield or similar scatter protector (optional)
- Matrix size 128 x 128 x 64
- Voxel size 2.5 x 2.6 x 2.4 mm (matched to the FWHM of the scanner)
- Standardized correction for radioactive decay and scatter

- Data acquisition in 4 successive 5 minutes frames (4 x 5)
- Iterative OSEM reconstruction algorithm (e.g., 4 iterations, 16 subsets), axial post filtering (Gaussian, FWHM 5.0 mm)

Note: Individualized, site-specific acquisition parameters will be determined during the technical site visit.

PET image optimization, quality control, image data transfer

The sponsor and/or image core laboratory (MNI) will provide the on-site radiologist physician with any technical support necessary to ensure optimal PET image acquisition and rapid transfer to the image core laboratory. To optimize parameters for PET acquisition for multi-center and multi-camera pooling of image data, a technical site set up visit will be performed by the image core laboratory (MNI) at each PET center before the study begins. The visit will include an overall evaluation of the center's camera and related quality assurance (QA) procedures, processes, and controls over imaging data and the camera-specific acquisition protocol developed, tested using a 3D Hoffman brain phantom, and saved. Finally, image reconstruction and filtering, anonymization and DICOM conversion, as well as the logistics of image data transfer will be discussed. All centers must agree to comply with the image acquisition methods, processing, and raw image data storage and transfer discussed during this meeting. Each center will be provided with a technical site binder containing core laboratory contact details, a technical operations manual, and image data transfer documents, as well as a technical/correspondence log. Recruitment of subjects into the study cannot proceed at a center until this visit has been performed.

The investigator will document the manufacturer and type of the PET or PET/CT scanner used in both the subject's file and eCRF. At each center, the same PET or PET/CT scanner should be used during the entire course of the study, and any updates to hardware or software should be avoided. If any changes are anticipated to occur, they must be reported to the image core laboratory and sponsor prior to being made, if at all possible.

To facilitate rapid image QA and preparation of the images for the independent blinded read, each PET center must provide the [^{18}F]NAV4694 PET image data set for each participant to the image core laboratory in DICOM 3.0 format – as close to within 24 hours of image acquisition as possible. Transfer to the image core laboratory will be performed via secure file transfer protocol (sFTP) connection (preferred) or CD-ROM.

PET Image QA, preparation, and storage; independent blinded read

Upon receipt of the image data set by the image core laboratory, the anonymized [^{18}F]NAV4694 PET images will be technically and scientifically quality assured (i.e., evaluated for proper conversion, interpretability, and diagnostic quality) and prepared for an independent blinded read. All study participants who have a complete set of [^{18}F]NAV4694 PET image data (including all time frames for the imaging period) acquired according to the study and camera specific protocol, irrespective of whether they are

regarded as interpretable by the on-site investigator, will be included in the blinded read. Feedback will be given to the respective center in case of technical and/or image quality issues arising. In case of poor image quality, a pre-specified escalation plan between the image core laboratory and the sponsor will be implemented and recruitment stopped at that particular center until the quality issue is resolved. All images will be stored in DICOM format, and a complete audit trail documenting any changes to the DICOM file headers or other manipulation of the data will be maintained.

The efficacy evaluation of the [^{18}F]NAV4694 PET images will be based on an independent blinded read, hosted by MNI (in New Haven, CT) and performed by 3 independent radiologists/nuclear medicine physicians experienced in the field of PET neuroimaging. The readers will be blinded to all clinical data. The readers will not be affiliated with the on-site evaluation of subjects at any of the study centers. The blinded read will be conducted in accordance with GCP/ICH guidelines and the sponsor's global and imaging core laboratory's clinical standard operating procedures. An electronic CRF (eCRF) will be used to ensure that the images and the diagnostic findings are properly aligned and to ensure that all questions are answered completely by the blinded readers. The details of the independent blinded read will be presented in the IRC.

8.1.8.1 Subject Level Visual Assessment (Primary Efficacy Endpoint)

The subject level visual assessment will be performed both by the independent blinded readers and by the on-site radiologist/nuclear medicine physician. The visual assessment procedure of the PET scans specified below (based on the method originally described by Rowe et al. (2008) and modified by Barthel et al. (2011) for the PET tracer florbetaben and adapted for [^{18}F]NAV4694) must be followed and the results recorded in the source documentation.

Briefly, for each subject with a complete image data set, the independent blinded readers and the on-site investigators are to visually assess the regional tracer uptake score (RTUS) for the 4 pre-specified brain regions (frontal cortex, posterior cingulate/precuneus, lateral temporal cortex, and the parietal cortex; see Table 3 and Table 4).

As mentioned above, the on-site radiologist/nuclear medicine physician as well as the independent blinded readers (both) will assess the subject level score based on whether an RTUS score of 1 is obtained in all of the 4 pre-specified brain regions (subject level score of 1) or if an RTUS score of 2 is obtained for any or all of the 4 pre-specified brain regions (subject level score of 2). No deviations from this rule will be allowed.

The above is to be performed for the imaging window 50 to 70 minutes post injection, both the onsite radiologist/nuclear medicine physician and the independent blinded reader will perform the visual assessments on the basis of the PET images alone, i.e., without co-registration of the MRI brain scans.

Gray scale only is permitted as the display mode for the subject level assessment by the independent blinded readers. The readers should select the mid-sagittal and transverse slices

at the level of the cerebellar white matter in which this region is best visualized. This region will be chosen as the reference region for visual interpretation.

The typical appearance of images is then as follows:

Negative images (no cortical tracer uptake): Gray matter in the respective pre-specified region appears of lower intensity when compared with the maximum white matter intensity in that region. The best way of determining this so called "target" white matter intensity is to compare with that of the white matter intensity in the cerebellum.

Positive images (cortical tracer uptake): Gray matter region in the respective pre-specified region is of similar or higher intensity as that of the respective "target" white matter region.

Per gray matter region, an RTUS **score** of 1 or 2 will be assigned, as described in Table 3:

Table 3. Definitions of Regional Cortical Tracer Uptake Scores

Regional Tracer Uptake Score (RTUS)		Image Features
1	No cortical tracer uptake	Tracer uptake in gray matter in the region is lower than in cerebellar or cortical white matter
2	Cortical tracer uptake observed	Area of tracer uptake in some part of the region is equal to or higher than that present in white matter and higher than the cerebellar gray matter uptake − the area extends beyond the white matter rim to the outer cortical margin and − is seen in the majority of the axial slices in the respective region

The on-site radiologist/nuclear medicine physician and the independent blinded readers will document the RTUS score as follows:

Table 4. Documentation of Regional RTUS Score

Gray matter structures (cortical areas)	Enter 1 or 2 In case of side differences, a score of 2 will be documented
Frontal cortex	
Posterior cingulate/precuneus	
Lateral temporal cortex	
Parietal cortex	

An overall subject level score will be assigned by "collapsing" scores of the pre-specified regions of putative relevance in the pathophysiology of AD into a single score of 1 or 2, as a means to assess β-amyloid burden of the brain globally.

This subject score will be assigned by the 3 independent blinded readers (for the imaging window 50 to 70 minutes post injection) as follows:

1 = Scan without β-amyloid deposition: Regional cortical tracer binding score of 1 (no cortical tracer uptake) in each of the 4 brain regions listed in Table 4.
2 = Scan with β-amyloid deposition: Regional cortical tracer binding score of 2 (cortical tracer uptake) in at least 1 of the 4 brain regions listed in Table 4.

The on-site radiologist/nuclear medicine physician as well as the independent blinded reader **must** classify the whole brain assessment score as 1 or 2 on the basis of the individual RTUS scores in the 4 brain regions.

8.1.8.2 Subject Level Quantitative Assessment (Secondary Efficacy Endpoints)

Quantitative assessment of the [^{18}F]NAV4694 PET image data will be performed by one experienced nuclear medicine expert at the image core laboratory for the image data sets acquired from 50 to 70 minutes post injection. Brain regions to be used for ROI sampling are listed in Table 5. The expert will align the T1 weighted MRI scan and each PET frame using a standard mutual information algorithm. An ROI template will then be applied to the aligned MRI for standardized regional brain sampling and will be adjusted on the MRI for optimal fit to the respective individual neuroanatomy. After transfer of the adjusted ROIs to the PET study, for each ROI the Bq/cc brain tissue will be extracted for calculation of the standard uptake values (SUVs). For each region, the SUV will be derived from activity in a volume of interest (VOI) determined as described above by taking into consideration study participant weight and dose injected. For the subject level assessment, acquired SUV data

will then be normalized to the cerebellar cortex resulting in a "region to cerebellar" ratio termed the SUVR.

Table 5. The Regions of the Brain Used for ROI Sampling for Subject Level Quantitative Analysis

frontal cortex	orbitofrontal cortex
temporal cortex	lateral temporal cortex
mesial temporal cortex	parietal cortex
pons	cerebellar cortex
occipital cortex	anterior cingulate
posterior cingulate	gyrus rectus
putamen	caudate nucleus
cerebellar white matter	white matter (subcortical white matter)
thalamus	

A more detailed description of the various quantification methods and templates used is provided in the IRC.

8.2 Pharmacokinetics

No pharmacokinetic investigation will be performed in this study.

8.3 Safety

8.3.1 Adverse Events

8.3.1.1 Definition of Adverse Event

The definitions below follow International Conference on Harmonization (ICH) – Good Clinical Practice (GCP) (see also ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Adverse Event (AE)

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

By definition for this study, all untoward medical occurrences beginning on the day of injection through the assessment 7 days post injection are to be reported as AEs.

Additionally, untoward medical events occurring prior to the day of injection and after the 7 day post injection follow up will only be captured as AEs if they are related to a study procedure. SAEs will be reported from the time of consent through the end of participation.

8.3.1.2 Categories for Adverse Event Assessment

All AEs will be assessed and documented by the investigator according to the categories detailed below.

Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 8.3.1.5.

Severity

The intensity of an AE is classified according to the following categories, taking into account the possible range of the intensity of the event:

- Mild The adverse event is transient and easily tolerated by the subject.
- Moderate The adverse event causes the subject discomfort and interrupts the subject's usual activities
- Severe The adverse even causes considerable interference with the subject's usual activities and may be incapacitating or life-threatening.

Investigational product action

Any potential investigational product action to resolve the AEs is to be documented as "Drug discontinued."

Specific drug treatment

Any specific drug treatment will be documented.

Causal relationship to investigational product

The investigator will use the following definitions to assess the relationship of the adverse event to the use of investigational product:

Definitely related: Event can be fully explained by administration of the investigational product.

Probably related: Event is most likely to be explained by administration of the investigational product rather than the subject's clinical state or other agents/therapies.

Possibly related: Event may be explained by administration of the investigational product or by the subject's clinical state or other agents/therapies.

Probably not related: Event is most likely to be explained by the subject's clinical state or other agents/therapies, rather than the investigational product.

Definitely not related: Event can be fully explained by the subject's clinical state or other agents/therapies.

For causality assessments, events meeting the categories of definitely, probably, or possibly related will be considered to be related to investigational product.

Outcome

The outcome of the AE is to be documented as follows:

- Resolved
- Resolved with sequelae
- Ongoing
- Unknown
- Lost to Follow-up
- Death

8.3.1.3 Assessments and Documentation of Adverse Events

Attention shall be paid to the occurrence of AEs at all stages of the PET examination. Thus, the subject should be closely observed by the investigator both during and after the examination.

Any AE (observed, volunteered, or elicited) should be recorded in detail in the source documenation.

The following information is required:

- The **date** and **time of onset** of any AE.
- The **duration** (the entire duration of an event or symptom, calculated from date of onset to date of end, if not recorded directly).
- The **seriousness** of the AE will be assessed by the investigator. If the investigator deems that an AE qualifies as an SAE, a special form provided by the sponsor should be completed and the event must be immediately reported to the sponsor. A definition of serious adverse events is provided below.
- The maximum **intensity** (mild, moderate, or severe).

- Whether investigational product was discontinued
- Specific drug treatment
- The **relationship** of the AE to the investigational product and to study conduct (for definitions, see above).
- The **outcome** of the AE (recovered/resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with residual effects, fatal, unknown).

AEs will be coded according to an internationally recognized dictionary (Medical Dictionary for Regulatory Activities or MedDRA).

8.3.1.4 Expected Adverse Events

Expected Disease-Related AEs

A worsening of the subject's cognitive impairment and potential diagnosis of dementia will not be considered an adverse event.

Expected Conduct-Related AEs

The use of an indwelling venous cannula for the purpose of blood sampling and administration of investigational product may be accompanied by mild bruising and also, in rare cases, by transient inflammation of the vessel wall. After initial irritation, the presence of an indwelling cannula is usually painless and hardly noticeable. The same applies to single vein punctures for blood sampling.

Expected Adverse Drug Reactions

The definition below follows ICH-GCP (see also ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting):

Adverse Drug Reaction (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established, all noxious and unintended responses to a medicinal product related to any dose should be considered as ADR. The phrase 'responses to a medicinal product' means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility (definitely, probably, possibly), i.e., the relationship cannot be ruled out.

Investigational Product-Related Risks

No adverse effects are expected from the administration of [^{18}F]NAV4694.

Adverse events from the radioactive dose are not expected, since the applied radiation doses are far below doses that can cause acute effects in human tissues.

Precautionary Measures

Special precautionary measures are not considered to be necessary for this study. In case of emergency, standard emergency procedures will be employed.

Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction is defined as an adverse reaction that in nature and severity is not consistent with the applicable product information (e.g., Investigator's Brochure).

Any adverse experience that is not listed in the current Investigator's Brochure or which is, with regard to the specificity or severity, not consistent with the risk information shall be regarded as unexpected.

Examples would be (a) acute renal failure listed in the Investigator's Brochure with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis. "Unexpected" as used in this definition refers to an adverse drug experience that has not been previously observed and included in the product information, rather than from the perspective of such experience not being anticipated from the pharmacological properties of the investigational product.

8.3.1.5 Serious Adverse Events

Definition of Serious Adverse Events

Definition

The following SAE definition is based on ICH guidelines and the final rule issued by the Food and Drug Administration (FDA) and effective 06 Apr 1998. It is to be applied to AEs (defined in Section 8.3.1.1).

An SAE is classified as any untoward medical occurrence that at any dose

- results in death, or
- is life threatening, or
- requires inpatient hospitalization or prolongation of existing hospitalization, or
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect, or
- is an important medical event (see paragraphs below).

The term 'life threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether it is appropriate to report an AE as serious also in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm or blood dyscrasias or convulsions that do not result in subject hospitalization.

Actions and reporting obligations in case of serious adverse events

The investigator should take appropriate diagnostic and therapeutic measures to minimize the risk to the subject.

If any SAE occurs over the course of the study, investigators or other site personnel will inform Navidea Biopharmaceutical representatives within one day (i.e., within 24 hours) of becoming aware of the SAE. Written notification of the SAE will be faxed to Navidea Biopharmaceuticals at (855) 793-7501 or email to Pharmacovigilance@navidea.com. For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately.

All SAEs must also be recorded on the Adverse Event eCRFs.

Notification of the IECs/IRBs

The sponsor and/or the investigator will notify the IECs/IRBs about all relevant events (e.g., Serious Adverse Events [SAEs] and Suspected, Unexpected, Serious Adverse Reactions [SUSARs]) according to all applicable regulations.

Notification of the authorities

The sponsor will process and report all relevant events (e.g., SAEs, SUSARs) to the authorities according to all applicable regulations.

Sponsor's notification of the investigators

The sponsor will inform all investigators about reported relevant events (e.g., SAEs, SUSARs) according to all applicable regulations.

8.3.2 Further Safety Assessments

8.3.2.1 Physical Examination

Complete physical examinations will be conducted according to the Schedule of Study Events (see Appendix 1).

Physical examination will be performed for the following body systems:

- General appearance
- Skin/dermatological
- Eyes, ears, nose, throat
- Head and neck (including thyroid)
- Lungs
- Heart
- Abdomen (liver, kidney, spleen, gastrointestinal)
- Lymph nodes
- Musculoskeletal
- Psychiatric
- Neurologic

8.3.2.2 Vital Signs

Vital signs comprise the measurement of systolic and diastolic blood pressure and heart rate. All vital signs will be measured after the subject has been in a resting position for at least 3 minutes. Heart rate should be measured immediately before or immediately after blood pressure measurement.

Blood pressure and heart rate will be measured at baseline (10 minutes before investigational product injection), 10 and 30 minutes post injection, and at the 24 hour follow-up visit.

Any clinically significant change from baseline that results in a change in subject management will be considered an AE.

8.3.2.3 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be obtained 10 minutes before investigational product injection), as well as within 10 minutes after investigational product injection, and at the 24 hour follow-up visit. The ECG will be measured with the subject in a resting position for at least 3 minutes. No continuous ECG monitoring will be required. ECG clocks should be synchronized with the time point for injection used in the eCRF.

On-site investigator's responsibilities

The immediate cardiac safety of the subject will be ensured by the on-site qualified physician. Any 12-lead ECG intervals, waveform abnormalities, and rhythm changes that are clinically significant in that they result in a change in subject management will be considered an AE. In the case of an SAE, once SAE notification is decided upon, investigators are required to follow the procedure described for SAE notification and document abnormal ECG findings (intervals and waveforms). Any interval data or abnormal waveform finding that resulted in an AE (i.e., change of patient management) must be followed for the duration of the subject's participation in the study until normalization or return to baseline takes place.

Each 12-lead ECG tracing must be signed and dated and stored in the ISF.

Independent cardiologist responsibilities

A dedicated ECG core laboratory will be contracted by the sponsor to perform the independent assessment of the ECGs.

The original ECG from all time points will be electronically transferred and independently read within 14 days of the ECG examination by an independent board-certified cardiologist. The independent cardiologist will review, interpret, and provide a written assessment of the ECGs for each subject in consecutive order, determining all intervals (e.g., PR, QRS, QT, and RR) and waveform changes. The reports will be signed and dated. A copy of the ECGs at each time point will be retained in the investigator's study record for each subject (identified with the subject's initials, study number, date, and time of recording).

The QT interval will be calculated by the core laboratory cardiologist using the correction formulae according to Bazett ($QTc = QT/RR^{0.5}$) and Fridericia ($QTc = QT/RR^{0.33}$).

8.4 Other Procedures and Variables

8.4.1 Blood Sampling

8.4.2 Laboratory Data Related to Screening and Safety

Clinical laboratory tests to be evaluated in this study include hematology, serum chemistry (including vitamin B12, folic acid, and thyroid function at screening), and urinalysis. Blood and urine samples for safety will be obtained according to the Schedule of Study Events (see Appendix 1). Table 6 shows the parameters to be assessed:

Table 6. Clinical Laboratory Parameters

Hematology	Leukocytes, erythrocytes, hemoglobin (Hb), hematocrit (HCT), red blood distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) platelets, mean platelet volume (MPV), neutrophils, eosinophils, basophils, lymphocytes, monocytes
Serum chemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (gamma-GT), alkaline phosphatase, total bilirubin, creatinine, chloride, potassium, sodium, total protein, albumin, globulin, carbon dioxide (CO2), blood urea nitrogen (BUN)
Serum chemistry (screening only)	Vitamin B12, folic acid, FT3/FT4, TSH, glucose (non-fasting)
Serology (screening only)	Syphilis (treponama pallidum particle agglutination assay)

A central laboratory will be used for all blood and urine samples to be determined in this trial.

The central laboratory will provide the necessary kits to collect the blood and urine samples and will also provide appropriate information regarding shipping of the samples.

Care is to be taken not to potentially produce an erroneous value, e.g., by inappropriate use of the tourniquet or forceful withdrawal of blood. Each original laboratory report will be retained in the subject files.

All laboratory reports must be promptly reviewed by the investigator, and upon review, initialed and dated by the investigator. Change(s) in post-dose test values considered clinically significant, which would require either additional control or therapy and, in case of disturbing or influencing factor(s) on values/samples, details of the appropriate value(s) and the source of disturbance or influence (e.g., quality of sample, co-medication etc.) are to be recorded. Good clinical practice would suggest that a copy of the safety laboratory results be available to the subject and to the subject's referring physician.

The following are general guidelines and are provided in order to assist the investigator in evaluating changes in laboratory values after the administration of investigational product. Since from previous clinical experience, these changes are unexpected, in all instances laboratory error must be considered and ruled out. One common cause of error is dilution of the blood sample, which occurs when blood is drawn through a fluid-filled catheter.

Changes in most individual laboratory values cannot be interpreted without concomitant/concordant changes in related parameters. For example, a decrease in hematocrit of approximately 5 percentage points (49% to 44%) at 24 hours post injection can be considered clinically significant, but without a similar change in hemoglobin and RBC,

laboratory error must be ruled out. Shifts in hematocrit may also occur with changes in subject's hydration status. With comparable decreases in hematocrit, hemoglobin and RBC, the investigator must evaluate the subject for blood loss/hemorrhage, especially at the site of venepuncture. If coagulopathy or hemolysis is suspected appropriate follow-up, including diagnostic tests and treatment, should be undertaken. Decreases in platelet count by greater than 30% of baseline value or an absolute value of less than 50,000/μL after a normal baseline value should also be followed up to rule out laboratory error or an underlying problem, such as a coagulopathy.

Increases in 2 or more liver function and related parameters (alkaline phosphatase, AST, ALT, and bilirubin) that result in values twice that of the upper limit of the normal range must be followed up to rule out any acute liver and/or cardiac illness. The investigator must obtain the appropriate tests as indicated by the subject's clinical status. Changes in sodium, potassium and chloride that are greater than 20% of baseline value must be followed up.

In all instances of changes in multiple parameters, regardless of the magnitude, the investigator must evaluate the subject in light of the clinical signs and symptoms and institute appropriate treatment. There may be other abnormal laboratory changes not specifically noted above that the investigator may want to follow up as appropriate for a particular subject.

Any change in laboratory value which results in a change in subject management (additional controls or treatment required) will be reported as a clinically significant change. Clinically significant changes in laboratory parameters which are not the result of laboratory error are to be recorded as AEs.

Any clinically significant changes in laboratory values are to be followed up with repeated tests at appropriate intervals (as determined by the investigator and the medical monitor) until the values return to baseline level or until the abnormality is explained by the investigator. Such additional tests should also be done at the central laboratory and will be entered into the database.

The amount of blood to be withdrawn is shown in Table 7.

Table 7. Amount of Blood Withdrawn

	Safety
Laboratory examination (screening)	24 mL
Laboratory examination (baseline, baseline follow-up, 18 month[1], 18 month follow-up[1])	4 x 8 mL
Archival Sample (optional)	6 mL
Total	62 mL

[1] Only for the subset of 20 subjects that is imaged at 18 months

Blood and urine specimens will be sent to a central vendor for analysis. Specimen collection, packaging, and shipping instructions are provided in a separate laboratory manual.

8.4.3 Blood Sampling and Processing for Genetic Analyses

Providing a sample for archival is optional. Providing blood samples for all other laboratory examinations is required, including samples for genetic tests. Results of the genetic tests will be shared with the subject at the discretion of the investigator and the preference of the subject.

Handling of Blood Samples

A blood specimen collected at the screening visit will be sent to a central vendor for ApoE genotype and other potential genetic determination. This result will be used for purposes of analysis for exploratory objectives in this study.

Evaluation of Data

Currently apolipoprotein E4 (Apo-E4) genotyping is envisaged. Further genes known or suspected to confer an increased risk of AD (e.g., APP, PSEN1, PSEN2) may be included as well.

ApoE4 will be classified according to:

- homozygous non-E4/non-E4
- heterozygous non-E4/E4
- heterozygous non-E4/E4 or homozygous E4/E4
- homozygous E4/E4

9 STATISTICAL METHODS

9.1 Introduction

This study is an open-label, non-randomized, multi-center study to investigate whether [^{18}F]NAV4694 PET scan findings have the ability to distinguish subjects with MCI who progress to AD from those who do not. The study will be conducted at approximately 15 study centers in the US and Germany. Subjects will be enrolled in the trial for a period of approximately 36 months. The objectives of the statistical analyses are to investigate the ability of the study procedure (PET scan findings using [^{18}F]NAV4694) to distinguish between subjects who progress to AD from MCI and those who do not and to establish the safety and tolerability of [^{18}F]NAV4694 in subjects with MCI.

A study center is defined as a treatment administration site under the control and supervision of the same Principal Investigator.

9.2 Randomization Methods

This is a non-randomized clinical trial where each subject will undergo PET imaging using [^{18}F]NAV4694 and a battery of neurocognitive testing.

9.3 Pooling of Study Centers

Study centers will be pooled for all statistical analyses.

9.4 Efficacy Variables

9.4.1 Primary Efficacy Variables

The primary efficacy variables of this study are the diagnostic result from the PET imaging after administration of [^{18}F]NAV4694 at baseline and the SoT diagnosis of transition from MCI to AD made by the investigator at the study site at the 18- and 36-month time points. The diagnostic results for both entities are that the subject has/has not transitioned to AD.

Each subject will undergo brain imaging that will be read by 3 independent readers blinded to clinical information. Each reader will score each region of the brain and then follow the scoring algorithm described in Section 8.1.8.1 of the protocol to arrive at a final classification (AD vs. no AD) for the subject. The final overall subject level classification (AD vs. no AD) will be made by a majority rule of the individual reader classifications.

At the 18- and 36-month time points, the investigator will determine the SoT diagnosis of transition from MCI to AD based on a review all of the clinical and neuro-cognitive data gathered for each subject over the course of the trial.

9.4.2 Secondary Efficacy Variables

The secondary efficacy variables for this study are:

- the diagnostic result from the PET imaging after administration of [^{18}F]NAV4694 at 18 months
- neuro-cognitive test battery scores at screening and/or baseline, 6, 12, 18, 24, 30, and 36 months (CDR Domains (Memory, Orientation, Judgment & Problem Solving, Community Affairs, Home & Hobbies, and Personal Care), CDR-Global, ADCS-MCI-ADL, MMSE, RAVLT,ADAS-Cog)
- subject-level neocortical SUVR at baseline and 18 months (computed as the average of the SUVRs from the frontal, lateral temporal, parietal, occipital and posterior and anterior cingulate cortices)

9.5 Pharmacokinetic Variables

Not applicable.

9.6 Safety Variables

9.6.1 Primary Safety Variables

The primary safety variables for this study are:

- Physical exams (screening, pre and 24 hours post injection at baseline, day 1, and pre and post 24 hours post injection for all subjects, and at month 18 for a subset of 20 subjects)
- Vital signs (screening, pre- and post-injection at baseline, pre- and post-injection for all subjects, and at month 18 for a subset of 20 subjects)
- Laboratory parameters (screening, pre and 24 hours post injection at baseline, day 1 and pre and 24 hours post injection for all subjects, and at 18 months for a subset of 20 subjects)
- Adverse events
- ECG parameters (baseline, pre, post and 24 hours post injection at baseline and pre, post and 24 hours post injection for all subjects, and at 18 months for a subset of 20 subjects)

9.7 Sample Size Justification

Approximately 120 evaluable subjects will be enrolled in the study. It is expected that 40% of the patients will progress to AD over the 36 month study period. Taking a drop-out rate of 30% into account, the study will provide 34 MCI patients progressing to AD vs. 50 who will not progress at the 36 month timepoint. As no preliminary data exists for this compound in MCI, accurate sample size calculations are not possible. The sample size is consistent with that employed in most early phase PET studies.

9.8 Handling of Missing Data

In the statistical analysis of the primary efficacy endpoints of the study, only subjects with evaluable endpoint values for both the blinded read and the SoT diagnosis will be used in the statistical analysis, i.e., a complete case analysis. The handling of missing values for all other study endpoints will be described in the Statistical Analysis Plan (SAP) for the study.

9.9 Statistical Analysis

9.9.1 Analysis Populations

The following analysis populations will be defined:

Intent-to-Diagnose (ITD) Population – All subjects in whom any PET imaging with [^{18}F]NAV4694 was performed, with valid standard of truth for the subject-level brain assessment, will be included in the ITD population.

Per Protocol (PP) Population – All subjects belonging to the ITD population who do not have protocol deviations predefined as leading to non-evaluability of the subject for the primary efficacy variable. Such predefined protocol deviations may include, but are not restricted to:

- no PET acquisition during the 50 to 70 minute imaging window

Protocol deviations leading to non-evaluability will be listed in a separate document that will be finalized before the first image is read.

Safety Population – All subjects who received any amount of [^{18}F]NAV4694 will be included in the safety analysis set.

The analysis of the primary efficacy endpoints will be conducted on ITD and PP populations. The primary analysis set will be the PP analysis set. All safety analyses will be conducted on the safety population.

9.9.2 Analysis of Baseline and Demographic Characteristics

Baseline and demographic characteristics, including clinically relevant covariates, will be summarized for all subjects in the safety population. Continuous variables will be displayed via summary statistics (mean, median, sample size, standard deviation, minimum, and maximum). Categorical variables will be summarized via counts and percentages.

9.9.3 Analysis of Primary Variables

The analysis of the primary efficacy variables will consist of calculating sensitivity, specificity, false negative rate (FNR), false positive rate (FPR), positive predictive value (PPV), negative predictive value (NPV) and accuracy of the baseline [^{18}F]NAV4694 PET scan reader evaluation findings relative to the 18- and 36-month SoT clinician diagnosis of

transition from MCI to AD. Exact 95% binomial confidence intervals will be constructed for all computed parameters.

9.9.4 Analysis of Secondary Variables

In order to compare the results obtained using blinded readers to results obtained from the imaging device (specifically, subject level SUVRs), a receiver operating characteristics (ROC) curve analysis will be conducted using the subject level SUVRs from the baseline [^{18}F]NAV4694 PET scan and the 18- and 36-month SoT clinician diagnosis of transition from MCI to AD. An empirical ROC curve will be constructed and graphically displayed using each SUVR in the dataset as the cut point to compute sensitivity and specificity of the [^{18}F]NAV4694 PET scan relative to the SoT clinician diagnosis. A nonparametric estimate of the area under the ROC curve (ROC) will be computed along with the DeLong variance estimate and a 95% confidence interval on the AUC. The optimal cut point will be obtained by visual inspection of the ROC curve. Once the optimal cut point has been determined, all diagnostic parameters (sensitivity, specificity, FNR, FPR, PPV, NPV and accuracy) will be calculated using the optimal cut point for SUVRs and the 18- and 36-month SoT diagnosis. The 95% exact binomial confidence intervals for each parameter will also be calculated. These same parameters (sensitivity, specificity, FNR, FPR, PPV, NPV and accuracy) will also be computed using a pre-determined cut point based on historical data.

In order to assess changes in amyloid burden over time in subjects with reader evaluated positive or negative scans, summary statistics (n, mean, median, standard deviation, minimum and maximum) will be computed on the subject level SUVRs change from baseline to 18 month data (for a subset of 20 subjects) by baseline reader evaluation status (positive or negative for AD) and overall.

To assess correlation of change over time in the subject level SUVRs to neurocognitive test results, Pearson correlation coefficients will be computed on the change from baseline subject level SUVRs at 18 months (for a subset of 20 subjects) and all change from baseline neurocognitive test results at the 6-, 12-, 18-, 24-, 30-, and 36-month time points.

In order to predict the probability of transition from MCI to AD, a logistic regression model will be fit using the subject level SUVRs at baseline as the independent variable and the SoT clinician diagnosis as the dependent variable. Model fit parameters will be obtained as well as the predicted probability of transition from MCI to AD for each subject. The odds ratio of transition from MCI to AD based on the logistic regression model will be computed in two ways:

- For a one unit difference in subject level SUVR
- For a difference in the median subject level SUVR for each quintile of the observed subject level SUVR distribution (these results will be displayed in a two way tabular form)

Other exploratory statistical analyses may be conducted on the primary and secondary efficacy variables and will be described in the SAP for the study.

9.9.5 Safety Analyses

All AEs will be observed for each subject from enrollment until termination from the study.

Prior to analysis, all AEs will be coded using MedDRA. Based on these coded terms, AEs will be summarized using system organ class and preferred terms. All AEs will be listed.

The analysis of all other safety variables for this study will be described in the Statistical Analysis Plan document.

9.10 Interim Analyses

No formal interim analyses will be conducted during this study. In order to make a regulatory submission for this product that will include results from this study, an analysis of the data collected up to and including the 18-month time point will be conducted. This will not be considered a statistically-based interim analysis and will not need alpha level adjustment for the following reasons:

- The summary will not be conducted until all subjects who remain in the study have reached the 18-month time point.
- The database will be "soft locked" so that no 36-month data is unblinded at the time of the 18-month analysis.
- This study is exploratory and there will be no hypothesis testing.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

The investigator will document in the file of the study participant at least the items listed in Section 10.2

Data required according to this protocol are to be entered into the eCRFs (provided by the sponsor) as soon as possible.

10.1.1 Electronic CRF design

Electronic data capture with the sponsor's eCRF will be used for collecting all data generated during the study. The eCRF application has a built-in plausibility check, forcing the investigators to answer the questions in the appropriate manner, and auto-checks for missing data. The system type and eCRF details will be documented in a separate document that will be provided by the sponsor and maintained in the TMF.

10.2 Monitoring

This study will be monitored regularly by a clinical research associate (CRA) from the sponsor or a contract research organization (CRO). Monitoring procedures include one or more visits designed to clarify all prerequisites before the study starts. Interim monitoring visits will take place on a regular basis according to a schedule fixed by mutual agreement. During these visits, the CRA will check for completion of the entries on the eCRFs, their compliance with the protocol and with GCP, and will compare the eCRF entries with the source data.

All data recorded in the eCRF will be captured in the source documentation.

The CRA will verify the correct use of the investigational product. The investigational product will not be supplied to the investigator site prior to a favorable opinion from the IRB/IEC and the regulatory authority and, if appropriate, from the radiation protection authorities.

In addition, the CRA will determine whether all AEs and SAEs have been appropriately reported (including adherence to the time periods required for SAEs).

10.3 Data Processing

Study data documentation will be maintained specifying all relevant aspects of data processing for the study (including data validation, cleaning, correcting, releasing). This documentation will be stored in the TMF.

For data coding (e.g., AEs, medication, medical/surgical history), internationally recognized and accepted dictionaries will be used. These and the processes used for coding will be specified in the SAP.

10.4 Auditing

A member of the sponsor's (or a designated CRO) quality assurance unit may arrange to visit the investigator in order to audit the performance of the study at the study site and the study documents originating there. The auditor(s) will usually be accompanied by a CRA or the study team lead. The investigator will be informed about the outcome of the audit.

In addition, inspections by health authority representatives and IEC(s)/IRB(s) are possible at any time. The investigator is to notify the sponsor of any such inspection immediately.

10.5 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution, or private practice. Where the archiving procedures do not meet the minimum timelines required by the sponsor, alternative arrangements must be made to ensure the availability of the source documents for the required period.

The investigator/institution notifies the sponsor if the archival arrangements change (e.g., relocation or transfer of ownership).

The investigator site file is not to be destroyed without the sponsor's approval.

The investigator's contract will contain all regulations relevant for the study center.

10.6 Premature Termination of the Study

Termination by the Sponsor

The Sponsor may terminate the study at any time for any of the following reasons:

1. Failure to enroll subjects
2. Protocol violations
3. Inaccurate or incomplete data
4. Unsafe or unethical practices
5. Questionable safety of the investigational product
6. Suspected lack of efficacy of the investigational product
7. Administrative decision

Termination by the Investigator

If the Investigator terminates the study prematurely, the Investigator must do the following:

- Return all unused investigational products and related study materials to the Sponsor.
- Provide the IRB/IEC and the Sponsor with a written statement describing why the study was terminated prematurely. Prompt compliance with this requirement is essential so that the Sponsor may comply with its regulatory obligations.

10.6.1 Study as a Whole

The sponsor retains the right to prematurely terminate the study as a whole at any time. Reasons for such premature termination may include the following:

At the discretion of the sponsor, the entire study may be canceled for medical reasons. In addition, the sponsor retains the right to end the study at any time if the study cannot be carried out as agreed upon in the protocol.

In case of premature termination or suspension of the study, the principal investigator/sponsor will promptly inform the investigator/institutions, regulatory authorities, and IRB/IEC of the termination or suspension and the reason for that.

10.6.2 Center

At any time, the trial may be terminated at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.6.3 Study Participant

Individual subjects may be withdrawn from the study according to the criteria specified in Section 4.3.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

The planning and conduct of this clinical study are subject to national laws. Only when all of the requirements of the appropriate regulatory authority have been fulfilled will the study begin. The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and the ICH-GCP Guidelines of 17 Jan 1997. At the discretion of the investigator, the entire study may be canceled for medical reasons. In addition, the sponsor retains the right to end the study for medical-scientific or GCP-relevant reasons. In case of premature termination the investigators, IRB/IECs and Regulatory Authorities will be informed by the Study Manager. As required by local law, current safety-relevant information will be provided to the IEC / IRB and the regulatory authorities by the sponsor. The sponsor will also inform all investigators about relevant safety events according to the applicable regulations.

11.2 Subject Information and Consent

All relevant information on the study will be summarized in the subject consent form and additionally as required by the investigator's institution in an integrated subject information and consent sheet. A sample informed consent form is provided as a document separate to this protocol.

Based on this subject informed consent form, the investigator will explain all relevant aspects of the study to each subject, before his/her entry into the study (i.e., before examinations and procedures associated with selection for the study are performed).

The investigator will also mention that written approval of the IRB/IEC has been obtained.

Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Following this informative discussion, the subject will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the subject voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator or his nominated designee will personally sign and date the form, too. The subject will receive a duplicate of the signed and dated form.

The investigator will record in the source documentation the time and date of obtaining informed consent.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

The informed consent form and any other written information provided to subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol which necessitates a change to the content of the subject information and/or the written informed consent form. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IRB/IEC's approval/favorable opinion in advance of use.

11.3 Financing/Financial Disclosure

Each investigator (including principal and/or any subinvestigators; as well as their spouses and dependent children) who is directly involved in the treatment or evaluation of research subjects has to provide a financial disclosure according to all applicable legal requirements. All relevant documentation will be filed in the TMF and/or ISF, as appropriate.

11.4 Publication Policy

The Sponsor will be responsible for determining when any trial results should be published. The Sponsor will work jointly with the investigator(s) to publish information in a timely manner. The investigator(s) shall not submit any information gleaned under the direct support or sponsorship of the Sponsor to journals or professional societies without the prior written approval of the Sponsor. A "publication" is meant to include any abstract, letter, manuscript or public announcement in any form or length that contains information gleaned under the direct support or sponsorship of the Sponsor.

11.5 Subject Injury

In general, if a subject is injured as a direct result of the investigational product but not due to medical negligence on the part of the Principal Investigator or study staff, the Sponsor will pay for reasonable and necessary medical treatment for the injury, to the extent the expenses are not covered by the subject's medical insurance, a government program, or other responsible third party. If laws or regulations of the locality in which the trial is taking place require additional payment of expenses, the sponsor shall comply with such law or regulation. Where applicable, the Sponsor has taken specific national insurance.

12 REFERENCE LIST

(D2750C0001). Open label Positron Emission Tomography Study with [18F]AZD4694 and [11C]AZD2184, Candidate Radioligands for Aβ Amyloid, to Determine and Compare in vivo Brain Uptake and Distribution in Healthy Volunteers and Patients with Alzheimer's Disease.

(D2750C00002). Open-Label Positron Emission Tomography Study with the Selective β-Amyloid Radioligand [18F]AZD4694 to assess the Effects of Varying Mass of AZD4694 on Radioligand Binding Parameters in Healthy Volunteers and Patients with Alzheimer's Disease

(D2750N00006). A Phase II, Open Label, Non-Randomized Study of [18F]AZD4694 to Compare PET Measurements of Fibrillar Amyloid Burden Obtained Using Different Scanning Parameters, Reference Region Procedures and to Assess Test-retest Reliability in Patients with Alzheimer's Disease and Healthy Adult Volunteers.

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Appendix 1 Schedule of Events

	Screening	**Baseline**	**Baseline Day 1**						**24 hr follow-up**	**Telephone follow-up**
	Day -56 to 0	Day -7 to 0	Prior to injection	10 min before injection	Injection	Within 10 min post- injection	30 ± 5 min post injection	50-70 min post injection	Day 2 (24 ± 4 hr) post- injection	Day 8 (7 [+3]) days post injection
Consent	X									
Interview (subject specific characteristics)	X									
Vital signs	X			X		X	X		X	
Physical exam[1]	X	X							X	
Body weight	X		X							
Neurological exam	X									
Screening neuro-cog battery[2]	X									
Baseline neuro-cog battery[3]		X								
MRI brain scan[4]	X									
Urine collection for urinalysis	X	X							X	
Blood draw	X[5]	X[6]							X[6]	
Electrocardiogram				X		X			X	
Insertion and test of catheter			X							
Measure [^{18}F] activity in syringe					X					
Investigational product injection					X					
Measure [^{18}F] activity					X					
PET scan imaging procedure								X		
On-site PET image analysis								X		
Adverse events	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X							X	X
Telephone contact										X

[1] Document height and weight of subject at screening
[2] Screening assessments: CDR, MMSE, DSM-IV-TR criteria for dementia, DSM-IV-TR criteria for major depressive disorder, recurrent
[3] Baseline assessments: RAVLT, ADAS-cog, ADCS-MCI-ADL
[4] MRI is required unless scan with adequate acquisition sequences is available from previous 6 months
[5] Screening blood draw: routine blood hematology and chemistry parameters, genetic analysis, laboratory tests related to cognitive impairment; optional archival sample
[6] Blood draw: routine blood hematology and chemistry parameters

	6, 12, 18[1], 24, 30, and 36 Month Visits	**18[2] Month or Early Withdraw Assessments**									
	Neuro-cog exam (± 30 days)	Day - 30 to 0	Day -7 to 0 prior PET imaging	Prior to injection	10 min before injection	Injection	Within 10 min post injection	30 ± 5 min post injection	50-70 min post injection	24 hr (± 4 hr) follow-up	Telephone follow-up
Consent											
Interview (subject specific characteristics)											
Vital signs					X		X	X		X	
Physical exam			X							X	
Body weight				X							
Neurological exam			X								
6, 12, 18, 24, 30, 36 m neuro-cog battery[3]	X	X									
MRI brain scan		X									
Urine collection for urinalysis			X							X	
Blood draw[4]			X							X	
Electrocardiogram					X		X			X	
Insertion and test of catheter				X							
Measure [^{18}F] activity in syringe						X					
Investigational product injection						X					
Measure [^{18}F] activity						X					
PET scan imaging procedure									X		
On-site PET image analysis									X		
Documentation of subject diagnosis and progression status[5]	X		X								
Adverse events			X	X	X	X	X	X	X	X	X
Concomitant medication			X							X	X
Telephone contact											X

[1] Excluding the subset of 20 subjects imaged at 18 months
[2] 18 month assessments for the subset 20 subjects imaged at 18 months
[3] All cognitive assessments to be performed at 6, 12, 18, 24, 30 and 36 month follow up visit
[4] Blood draw: routine blood hematology and chemistry parameters
[5] DSM-IV-TR criteria will be used to determine whether a subject has dementia and if so, which type. Probable AD will be confirmed using criteria from NINCDS-ADRDA.

Appendix 2 Sponsor Signatures

Study Title:	Beta-Amyloid Imaging With [^{18}F]NAV4694 Positron Emission Tomography (PET) in Predicting Progression to Alzheimer's Disease (AD) in Subjects with Mild Cognitive Impairment (MCI)
Study Number:	NAV4-04
Original Protocol Date:	06 February 2013
Amendment 1 Date:	07 June 2013
Amendment 2 Date:	13 May 2014

This clinical study protocol was subject to critical review and has been approved by the sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: _____ Date: 14 May 2014
Fredrick O. Cope, PhD, FACN, CNS
Senior VP and Chief Science Officer
Navidea Biopharmaceuticals

Signed: _____ Date: 14 MAY 2014
Cornelia Reininger, MD, PhD
Senior VP and Chief Medical Officer
Navidea Biopharmaceuticals

Signed: _____ Date: 14 MAY 2014
William Regan
Senior VP, Global Regulatory Strategy
Navidea Biopharmaceuticals

Appendix 3 Investigator's Signature

Study Title:	Beta-Amyloid Imaging With [^{18}F]NAV4694 Positron Emission Tomography (PET) in Predicting Progression to Alzheimer's Disease (AD) in Subjects with Mild Cognitive Impairment (MCI)
Study Number:	NAV4-04
Original Protocol Date:	06 February 2013
Amendment 1 Date:	07 June 2013
Amendment 2 Date:	13 May 2014

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed:_____ Date:_____

<enter name and credentials>
<enter title>
<enter affiliation>
<enter address>
<enter phone number>