**Longitudinal Early-onset Alzheimer's Disease Study (LEADS) Protocol**

**Protocol Short Title: LEADS**

**Protocol Number: ATRI-003**

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| PROTOCOL VERSION | VERSION DATE |
|---|---|
| 10.0 | May 23, 2024 |

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| Aβ | Beta Amyloid |
| AD | Alzheimer's Disease |
| ADAD | Autosomal Dominant Alzheimer's Disease |
| ADAS-Cog | Alzheimer's Disease Assessment Scale – Cognitive |
| ADI | Area Deprivation Index |
| ADNI | Alzheimer's Disease Neuroimaging Initiative |
| ADRC | Alzheimer's Disease Research Center |
| ADSP | Alzheimer Disease Sequencing Project |
| AE | Adverse Event |
| *APOE*/*APOE4* | Apolipoprotein E (*APOE*) epsilon 4 (*APOE4*) |
| *APP* | Amyloid Precursor Protein gene |
| ASL | Arterial Spin Labeling |
| ATRI | Alzheimer's Therapeutic Research Institute |
| C9ORF72 | Chromosome 9 open reading frame 72 |
| CDR | Clinical Dementia Rating |
| CLIA | Clinical Laboratory Improvement Amendments |
| CN | Cognitively normal |
| CSF | Cerebrospinal Fluid |
| CT | Computerized Tomography |
| DIAN | Dominantly Inherited Alzheimer's Network |
| DNA | Deoxyribonucleic Acid |
| DSMB | Data Safety Monitoring Board |
| DSM-IV | Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition |
| DTI | Diffusion Tensor Imaging |
| eCRF | Electronic Case Report Form |
| EDC | Electronic Data Capture System |
| EOAD | Early-onset Alzheimer's Disease |
| EOnonAD | Early-onset non-Alzheimer's Disease |
| FDA | Food and Drug Administration |
| FDG | Fluorodeoxyglucose |
| FLAIR | Fluid Attenuation Inversion Recovery |
| FTD | Frontotemporal Dementia |
| fMRI | Functional Magnetic Resonance Imaging |
| GAAIN | Global Alzheimer's Association Interactive Network |
| GCP | Good Clinical Practice |
| GDS | Geriatric Depression Scale |
| GRN | Progranulin |
| HIPAA | Health Insurance Portability and Accountability Act |
| ICF | Informed consent form |

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|---|---|
| ICH | International Conference on Harmonization |
| IDA | Image Data Archive at LONI |
| INR | International Normalized Ratio |
| iPSCs | Induced pluripotent stem cells |
| IRB | Institutional Review Board |
| LAR | Legally Authorized Representative |
| LOAD | Late-onset Alzheimer's Disease |
| LONI | Laboratory of Neuroimaging at USC |
| LP | Lumbar Puncture |
| lvPPA | Logopenic variant primary progressive aphasia |
| MAPT | Microtubule-associated protein tau |
| MCI | Mild Cognitive Impairment |
| MINT | Multi-lingual Naming Test |
| MMSE | Mini-Mental State Examination |
| MoCA | Montreal Cognitive Assessment |
| MPRAGE | Magnetization Prepared Rapid Gradient Echo |
| MR/MRI | Magnetic Resonance / Magnetic Resonance Imaging |
| MTA | Material Transfer Agreement |
| MTL | Medial temporal lobe |
| NCRAD | National Centralized Repository for AD and Related Dementia |
| NIA | National Institute on Aging, under the NIH |
| NIA-AA | National Institute on Aging – Alzheimer Association |
| NIAGADS | National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site |
| NIH | National Institutes of Health |
| NPI | Neuropsychiatric Inventory |
| OTC | Over-the-Counter |
| PBMC | Peripheral Blood Mononuclear Cell |
| PCA | Posterior cortical atrophy |
| PET | Positron-Emission Tomography |
| PHI | Protected Health Information |
| PI | Principal Investigator |
| PiB | Pittsburgh Compound B |
| PLPH | Post lumbar puncture headache |
| *PSEN1* | Presenilin 1 gene |
| *PSEN2* | Presenilin 2 gene |
| PT | Prothrombin time |
| PTT | Partial thromboplastin time |
| QA / QC | Quality Assurance / Quality Control |
| RDRC | Radioactive Drug Research Committee |
| REB | Research Ethics Board |
| RNA | Ribonucleic Acid |

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|---|---|
| SAE | Serious Adverse Event |
| T | Tesla |
| TIV | Total intracranial volume |
| vMRI | Volumetric Magnetic Resonance Imaging |

## PROTOCOL SYNOPSIS

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|---|---|
| **PROTOCOL TITLE** | Longitudinal Early-onset Alzheimer's Disease Study (LEADS) |
| **PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR** | Liana Apostolova, M.D., M.Sc. |
| **STUDY DESIGN** | Non-randomized, natural history, non-treatment study |
| **STUDY COMPOUNDS** | Florbetaben<br>Flortaucipir<br>Fluorodeoxyglucose (FDG)<br>Pittsburgh Compound B (PiB) (for International sites only)<br>NAV-4694 (Flutafuranol) (for International sites only)<br>Flutemetamol (for International sites only) |
| **RECRUITMENT GOALS AND DURATION OF STUDY** | Up to 850 cognitively impaired participants (in the US) = 3+ Years in duration<br>• Approximately 650 EOAD participants<br>• Approximately 200 EOnonAD participants<br>100 CN participants = 2+ Years in duration<br>40 cognitively impaired participants (across international sites) = 3 + Years in duration<br>10 CN participants (across international sites) = 2+ Years in duration<br>Screening period = < 60 days (including baseline assessments) |
| **SUMMARY OF KEY ELIGIBILITY CRITERIA** | **Cognitively Impaired Cohorts:**<br>• Diagnosis of NIA-AA criteria of MCI or dementia due to probable AD<br>• CDR score ≤ 1.0<br>**Cognitively Normal Cohort:** |

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|---|---|
| | • Meets criteria for cognitively normal, based on an absence of significant impairment in cognitive functions and activities of daily living<br>• Mini-Mental State Exam score between 26-30<br>• CDR score = 0<br>**Both Cohorts:**<br>• 40-64 (inclusive) years of age at the time of consent<br>• Willing and able to undergo study procedures as outlined in the Schedule of Events<br>• Must have study partner (informant) who spends a minimum average of 10 hours per week with the participant. |
| **PRIMARY OBJECTIVES** | • Collect longitudinal assessments and biomarker data for 650 EOAD, and 200 EOnonAD, and 100 CN participants in the US, and an additional 40 CI and 10 CN particpiants internationally.<br>• Compare baseline and longitudinal cognitive and functional characteristics, between EOAD and CN, and EOAD and Late Onset Alzheimer's Diseases (LOAD) from the Alzheimer's Disease Neuroimaging Initiative (ADNI)<br>• Study the associations of longitudinal clinical and cognitive assessments with multimodal imaging and biofluid markers that capture different elements of the AD pathophysiological cascade |
| **OUTCOME MEASURES / STUDY PROCEDURES** | Rate of decline on cognitive, global, and functional tests; rates of change on imaging and fluid biomarkers; Longitudinal extent and rate of brain atrophy, amyloid and tau deposition; Discovery of new AD genetic risk variants |

# 1.0 BACKGROUND AND SIGNIFICANCE

While the risk of AD increases with advancing age, approximately 5% of AD patients develop symptoms before age 65 (~280,000 Americans). Patients with early-onset Alzheimer's disease (EOAD), occurring before the age of 65, are an understudied segment of the patient population with Alzheimer's Disease (AD). Many treatment studies exclude these younger individuals.

Of the two major North American consortia, ADNI includes only a few EOAD cases all with a "typical" amnestic presentation and the Dominantly Inherited Alzheimer Network (DIAN) focuses solely on autosomal dominant AD (ADAD). Clinical and neuroimaging measures that emphasize episodic memory and medial temporal neurodegeneration in

LOAD are insensitive to the baseline deficits and the disease progression in EOAD, which predominantly involve non-memory cognitive domains and posterior cortical neurodegeneration. The overarching aim of this Longitudinal Early-Onset AD Study (LEADS) is to fill this gap in AD research by conducting a clinical and biomarker study in the EOAD population.

LEADS includes key AD research sites across the U.S. and will leverage existing infrastructure and processes applied in ADNI. The over-arching goals are to advance our knowledge about disease mechanisms and develop sensitive composite clinical and biomarker tools that capture disease progression in this unique cohort for implementation in clinical trials. Our secondary goals include: (1) collection of DNA from EOAD participants for exploratory studies applying next generation sequencing; (2) collection and banking of clinical, biofluid and imaging measures for future research and sharing with the larger research community; and (3) establishing a network of EOAD sites that will enable future planning and implementation of clinical trials in EOAD. While the research is focused on EOAD, LEADS will also follow individuals who meet clinical criteria for MCI or dementia due to probable AD but have a negative amyloid PET scan (EOnonAD) with the goal to generate important scientific knowledge about this patient population.

Data collected in this study will address several significant gaps in AD research. In addition to affording this highly motivated population the chance to contribute to AD research, EOAD patients offer the opportunity to study a more "pure" form of AD with fewer age-related brain co-pathologies[1, 2].

The study will also leverage existing data. We plan to compare our EOAD individuals to ADNI LOAD participants. ADNI is a non-randomized, natural history, non-treatment study with up to 2000 longitudinal LOAD participants with clinical, cognitive, MRI, amyloid and tau PET, CSF and peripheral blood data collected across approximately 59 sites in the United States and Canada. ADNI data is freely shared with other researchers.

In addition, all of our sites who are federally funded Alzheimer's Disease Research Centers under the auspices of the National Alzheimer Coordinating Center (NACC) will be encouraged to enroll all subjects in their longitudinal observational clinical cohorts. We will leverage the ADRCs as they are well positioned to follow their research participants indefinitely (until death) and are also funded to perform post mortem examinations of the brain tissue. This partnership will greatly benefit the LEADS program as it will secure a mechanism for longitudinal follow-up. A portion of the standardized NACC assessment will be adopted into the LEADS study.

## 2.0 STUDY RATIONALE

In contrast to the predominant amnestic phenotype of LOAD, 30-64% of EOAD manifest with non-amnestic presentations, including focal cortical syndromes such as posterior cortical atrophy (PCA) and logopenic variant primary progressive aphasia (lvPPA), which can lead to missed or delayed diagnosis. Despite being highly motivated and having fewer age-related comorbidities compared to LOAD, EOAD patients are commonly excluded from clinical research and therapeutic trials due to their young age or non-amnestic deficits, which is increasingly viewed as being marginalizing and unethical [3]. Fewer than 10% of EOAD patients carry a known mutation in *APP* or *PSEN1/2*, and <50% carry the *APOE4+*

risk allele. Still studies suggest high heritability in EOAD in the absence of known mutations or *APOE4+*, signifying that this population may be enriched for novel genetic risk factors [4].

# 3.0 SCIENTIFIC AIMS

## 3.1 Aim 1

To compare the baseline and longitudinal cognitive and functional characteristics of EOAD compared to LOAD and identify optimal outcome measures for clinical trials.

***H1a:*** Controlling for performance on episodic memory tests, EOAD will show greater impairment in executive, language and visuospatial function compared to ADNI LOAD.

***H1b:*** EOAD will show more rapid decline on MMSE, ADAS-Cog and CDR-SB compared to ADNI LOAD.

***H1c:*** A data-driven analysis of functional ratings and psychometric test scores will identify measures more sensitive to change over time in the full spectrum of EOAD (amnestic and non-amnestic subtypes) than existing composite measures (ADAS-Cog and CDR-SB), leading to a new EOAD composite outcome measure for use in future clinical trials and observational studies.

***Rationale for Aim 1:*** Multiple small studies have demonstrated greater impairment in non-memory compared to memory performance[5-7] and more rapid cognitive decline in EOAD than in LOAD[8, 9]. Among these studies the study sample composition (e.g. inclusion of autosomal dominant or focal variants like lvPPA/PCA), assessment and outcome measures vary. Whether clinical trial outcomes employed in LOAD trials (emphasizing episodic memory) should be applied to EOAD or be modified to account for the predominant non-amnestic deficits remains a critical, unanswered question. In this aim we will test both traditional and novel cognitive and functional outcome measures in the full EOAD spectrum defined by current NIA-AA criteria. Our ultimate goal is to empirically reduce our broader battery into a short battery of measures that are especially sensitive in EOAD and feasible for clinical trials.

## 3.2 Aim 2

To compare baseline and longitudinal MRI, amyloid PET, tau PET and CSF and plasma ATN measures between EOAD and LOAD and identify optimal outcome measures for clinical trials.

***H2a:*** EOAD will show greater baseline and longitudinal changes in cortical gray matter atrophy and tau burden than matched ADNI LOAD participants, but no differences in baseline or longitudinal amyloid PET, plasma or CSF measures.

***H2b:*** Baseline and longitudinal change in cortical tau, gray matter atrophy, CSF and plasma measures will correlate with baseline and longitudinal measures of cognitive function.

***H2c:*** Through a data-driven approach we will identify composite biomarker measures that optimally capture change over time, leading to EOAD composite imaging outcomes for use in future clinical trials and observational studies.

***Rationale for Aim 2:*** Neuroimaging outcomes have become critical for subject selection and for gauging target engagement and disease modification in clinical trials. The advent of tau PET ligands such as AV1451 [10] represent a major advance in the field, as they offer both molecular specificity for AD pathophysiology (in contrast with MRI) and temporal correlations with clinical outcomes (in contrast with Aβ PET). While MTL neurodegeneration is considered the imaging hallmark of LOAD, multiple studies have shown more prominent cortical atrophy and hypometabolism and relative sparing of the MTL in EOAD compared to LOAD[11-13]. Early-stage neurodegeneration of the posterior temporo-parietal cortical regions represents a common denominator across all clinical variants of EOAD (amnestic, dysexecutive, lvPPA and PCA)[14-17]. Clinicopathological studies have demonstrated that "hippocampal- sparing AD" is strongly associated with early onset[15]. Current methods do not take into account the spatial topography differences between LOAD and EOAD. Therefore, neurodegenerative biomarkers commonly used to track disease progression and monitor for drug effects will not directly generalize from LOAD to EOAD. The goals in Aim 2 are to characterize the MRI and PET signature of EOAD in comparison to CN, to compare PET, CSF and plasma measures of Aβ and tau with LOAD, to link imaging measures to cognitive outcomes, and to use a data-driven approach to develop summary imaging measures uniquely sensitive to disease progression in EOAD for application in future therapeutic trials.

### 3.3 Aim 3

To investigate the influence of *APOE* genotype on baseline and longitudinal imaging biomarkers and clinical phenotype in EOAD.

***H3a:*** Atypical subtypes of EOAD (PPA, PCA) will show a lower *APOE4+* carrier rate than memory predominant EOAD.

***H3b:*** Relative to non-carriers, *APOE4+* carries will demonstrate greater baseline and longitudinal change in medial temporal atrophy and tau PET signal.

***H3c:*** EOnonAD will show a significantly lower *APOE4* carrier rate than EOAD.

***Rationale for Aim 3:*** *APOE4+,* the most significant genetic risk factor for sporadic AD [18], exerts its maximal effects between ages 65-75[19, 20]. The prevalence of *APOE4+* is substantially lower in EOAD than in LOAD[21]. Furthermore, *APOE4* seems to moderate the phenotype of AD by predisposing to MTL vulnerability[15, 22-29]. Greater memory impairment and MTL atrophy in *APOE4+* AD, and greater non- amnestic presentations and cortical atrophy in *APOE4-* subjects has been reported by some [23, 26-28, 30-33] but not others[34, 35]. Yet since most of these clinical studies lack pathologic or biomarker confirmation of AD pathology, the perceived differences might be driven by increased rates of non-AD pathology in *APOE4-* individuals, necessitating a more definitive study. Furthermore, *APOE* genotype is emerging as a major pharmacogenomic consideration for AD trials. It is therefore critical to stratify EOAD and EOnonAD participants and determine

how rates and patterns of atrophy, Aβ and tau accumulation may differ on the basis of *APOE* genotype.

### 3.4 Exploratory Aim 4

To characterize genetic contributions to EOAD and obtain annually an array of uniformly collected biospecimens for future biomarker development.

***H4a:*** A subset of EOAD will have mutations in genes known to contribute to AD.

***H4b:*** A subset of EOnonAD will have mutations in genes known to contribute to FTLD .

***H4c:*** Next generation sequencing will identify novel genes in EOAD involved in Aβ, tau, inflammation and lipid processing pathways.

***Rationale for Aim 4:*** Pedigree analyses suggest that apparently "sporadic" EOAD is a highly heritable condition, recognized pathogenic mutations in *APP*, *PSEN1* and *PSEN2* are rarely identified in these subjects, and much of the measured heritability of EOAD remains unexplained even after accounting for *APOE4* [36, 37]. Few studies have amassed a large enough sample of sporadic EOAD to be sufficiently powered to identify new genes contributing to disease risk. Next generation sequencing has rarely been applied to this population, yet is well suited to identify rare genes with large effects, recessive or de novo mutations and other more complex heritability patterns. This study's cohort (400 deeply phenotyped sporadic EOAD without a known genetic etiology) will be the focus of this exploratory aim to initiate new gene discovery, which may lead to insights into novel disease mechanisms and pathways. We propose to apply innovative genetic approaches (i.e., whole genome sequencing and epigenetic analyses) to study vulnerability in EOAD. In addition, we will investigate the influence of *APOE* genotype on cognitive, imaging and biofluid biomarkers, which is critical to help plan pharmacogenomic approaches in AD clinical trials.

### 3.5 Aim 5:

To characterize demographic, clinical, neuroimaging, and fluid biomarker measures in EOnonAD cases to explore the possible etiologies of cognitive impairment.

***H5a:*** EOnonAD participants will show MRI and/or FDG PET patterns that are either supportive of a nonAD neurodegenerative disease or suggestive of non-neurodegenerative etiology.

***H5b:*** Frontotemporal atrophy and hypometabolism and elevated plasma neurofilament light (NfL) will predict transition to an FTD spectrum diagnosis over time.

***H5c:*** Data-driven analyses of EOnonAD clinical and biomarker assessments will identify clusters suggestive of potential etiologies, which will be confirmed at autopsy.

***Rationale for Aim 5:*** To date, no studies have systematically investigated EOnonAD subjects. Amyloid-negative (A-) tau-negative (T-) late onset amnestic cases show focal medial temporal and posterior cingulate FDG PET hypometabolism relative to their amyloid

positive counterparts. In ADNI 36% A- MCI individuals show neurodegenerative changes on both FDG PET and MRI, 41% on MRI only, and 23% on FDG only.
Essentially all studies of A- LO MCI to date have shown higher conversion rates ranging between 18-56% in A- neurodegeneration-positive (N+) compared to subjects without neurodegeneration but with brain amyloidosis (A+N+) and to biomarker-negative (A-N-) subjects. Whether the same holds true for early onset subjects remains unknown.
Some researchers have argued that A-N+ subjects might be a neurodegeneration-first AD variant. There is some evidence to suggest that this could be the case for some of these subjects. Mormino et al. showed that these cases are more likely to have subthreshold mean amyloid PET SUVR compared to biomarker-negative controls. The rates of conversion of A-N+ to A+N+ in the literature are variable. Caroli et al. reported that 64% of A-N+ cases converted to A+N+ in their series, yet the rate reported by Gordon et al. for the WashU cohort was only 14-17% at 3 years.

### 3.6 Aim 6:

To follow all patients to autopsy and perform neuropathologic analyses of postmortem brain tissue to identify the pathophysiologic substrate and comorbid pathologies in EOAD and EOnonAD cases.

***H6a:*** Neuroimaging and fluid biomarkers collected during life in EOAD/EOnonAD cases will correlate with postmortem neuropathologic changes, which will be the basis for prediction models.

***Rationale for Aim 6:*** EOAD and LOAD share a comparable neuropathologic substrate, however, there are notable differences in their clinical and biological phenotypes. Neuropathologically, EOAD is reported to have a more severe pathology (particularly neurofibrillary tangles) than LOAD; however, at this time, there is no standardized neuropathology research protocol for analyzing brains of non-autosomal dominant EOAD individuals. A major goal of the NPC will be to correlate clinical and imaging data with the presence of neuropathologic lesions in different brain areas. Histologic examination is also the ultimate confirmation of AD diagnosis and it is fundamental to determine the presence of comorbidities co-occurring with AD. Although new diagnostic biomarkers hold promise for increasing the clinical diagnostic accuracy for AD, it is expected that there will continue to be overlap in these measures between AD subjects, subjects with non-AD dementias and cognitively intact individuals. Importantly, enabling correlations between antemortem biomarkers and neuropathology and creating a tissue repository for in-depth state-of-the-art neuropathological and biological/omic studies of LEADS cases (EOAD, EOnonAD and controls) will be invaluable. The neuropathologic examination of well-characterized brain tissue from non-autosomal dominant EOAD individuals may lead to the identification of novel AD phenotypes. Novel technology may be applicable to interrogate the tissue in order to determine the stages in the evolution of the biochemical, molecular and, possibly, structural changes that take place in the trajectory of formation of neuropathologic lesions. By expanding our knowledge of the structural and genetic properties associated with pathologic CNS tissue of LEADS subjects, each brain we study will become an invaluable resource for the AD research community at-large.

# 4.0 STUDY DESIGN AND INVESTIGATIONAL PLAN

The LEADS design builds on the success of two major natural history/biomarker initiatives in AD: ADNI and DIAN. Like those initiatives, the LEADS approach links longitudinal clinical and cognitive assessments with multiple imaging and biofluid markers that capture different elements of the AD pathophysiological cascade. Its alignment to ADNI will allow us to use ADNI LOAD participants as a disease comparison group.

LEADS is a non-randomized, natural history, non-treatment study. Approximately 850 cognitively impaired (EOAD and EOnonAD) participants and 100 CN participants will be enrolled at approximately 20 sites in the United States. Approximately 40 CI participants and 10 CN participants will be enrolled in sites outside of the U.S. as a part of iLEADS, the international expansion of LEADS. Clinical/cognitive, imaging, biomarker, and genetic characteristics will be assessed across the three cohorts: EOAD, EOnonAD, and CN.

## 4.1 LEADS Consortium Structure

LEADS is modeled after ADNI and many ADNI leaders are involved in the LEADS study.

**4.1.1 Administrative Core** The Administrative Core, with headquarters at Indiana University (IU), has the overall responsibility for the entire project. The core will oversee the activities of all sites and cores and facilitate collaboration with ADNI, DIAN, and other relevant projects. This core will be responsible for approving the protocol and any amendments, reviewing and approving study budgets, reviewing interim data and amending the methodology as appropriate, reviewing and approving significant changes to study timelines, overseeing publication planning and reviewing and approving joint publications.

**4.1.2 Clinical Core** The Clinical Core at IU and the Clinical Coordinating Center (ATRI at USC) will be responsible for managing the day-to-day clinical operations. ATRI is the Coordinating Center for ADNI. The Clinical Core/Coordinating Center will be responsible for oversight of clinical activities, contracting with all sites, performance oversight, data management, tracking and quality control, recruitment and retention of participants, and regulatory oversight. Clinical Monitors, under the supervision of the ATRI, will regularly visit all LEADS sites to ensure compliance with regulatory requirements and protocol procedures, and accurate data entry. As leader of the Clinical Core, Dr. Apostolova will have final responsibility for all aspects of data acquisition.

**4.1.3 MRI Core** The LEADS MRI Core Components at Massachusetts General Hospital (MGH)/Harvard and Mayo Clinic Rochester will perform standardization of data acquisition and quality control, including creation and distribution of protocols to each site, qualifying each scanner and re-qualifying after every upgrade, performing quality control assessments of every exam. It will also perform quantitative MR measurements for each MR modality. ADNI Imaging protocols will be used to ensure our ability to conduct combined analyses of LEADS and ADNI MRI data. Collaborations with other ADNI-affiliated and non-affiliated investigators will be developed to perform data analyses. The core will work with the Administrative Core to assure that all regulatory compliance is in place and with the Laboratory of Neuroimaging (LONI) to ensure the protocols and procedures for data uploading are in place. As leader of the MRI Core, Dr. Dickerson will have final responsibility for all aspects of MRI data acquisition.

**4.1.4 PET Core** The PET Core at the University of California San Francisco (UCSF) and University of Michigan will implement the standardized procedures for multisite

florbetaben, PiB, NAV-4694, flutemetamol, flortaucipir, and FDG PET imaging. The PET Core will be responsible for oversight of site qualification, quality control, standardization, pre-processing and analysis of florbetaben, PiB, NAV-4694, flutemetamol, flortaucipir, and FDG PET images acquired in the study. The PET Core will work with the Administrative Core to assure that all regulatory compliance is in place and with LONI to ensure the protocols and procedures for data uploading are in place. As leader of the PET Core, Dr. Rabinovici will have final responsibility for all aspects of PET data acquisition.

**4.1.5 Genetics and Biorepository Core** This core will be led by Dr. Foroud who is the PI of National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD). NCRAD is a national resource funded by the National Institute on Aging (NIA) where clinical information and biological material (such as DNA, plasma, serum, RNA, CSF, cell lines, and brain tissue) from individuals with AD, related dementias and normal controls can be stored and requested. The Core will prepare all protocols and materials to be used with participants when discussing the decision to receive information about pathogenic mutations in ADAD genes, if identified. The Core will coordinate confirmatory CLIA laboratory genetic testing of pathogenic mutations, ensure that confirmed pathogenic mutations are communicated to the site where the participant was seen, and if needed, assist sites in providing genetic counseling. This core will be responsible for receiving, processing and banking genetic, peripheral blood and CSF material. As Genetics & Biorepository Core Co-Leader, Dr. Dage will oversee all fluid biomarkers analyses. Dr. Foroud will ensure that genetics and related data is comprehensively analyzed and reported using state-of-the-art approach including candidate pathway and genome-wide analyses. As leader of the Genetics and Biorepository Core, Dr. Foroud will have final responsibility for all aspects of genetic and fluid biomarker processing, banking and analyses.

**4.1.6 Biostatistics Core** The Biostatistics Core at Brown University will have the overall responsibility for meeting the analytic goals of this project and will provide expertise in longitudinal modeling of clinical and biomarker data, in modeling multivariate trajectories, statistical applications in consideration for future clinical trial design, as well as exploratory and discovery studies. The core will also provide design and analysis support for validation and calibration studies as necessary. New strategies will be considered. As leader of the Biostatistics Core, Dr. Eloyan will have final responsibility for all aspects of the statistical analyses.

**4.1.7 Informatics Core** The Informatics Core located at LONI at USC will also follow ADNI's model. The Core will provide a secure and reliable environment for storing and sharing neuroimaging and related data and a supportive, responsive team dedicated to meeting the evolving needs of the community. As it has done for ADNI and many other studies, the Informatics Core will provide a reliable, long-term repository for imaging, clinical and related data storage and distribution. To date, ADNI Informatics Core has stored more than 87,000 ADNI MRI and PET images with more than 21 million images downloaded by approved ADNI data users. Additionally, the clinical and genetic data have been provided to thousands of users. Finally, subsystems for image quarantine and release, data management, and data user application and review have been implemented and the same set of operations will be provided for LEADS. The Informatics Core will work closely with ATRI and NACC to assure that all clinical and cognitive data is safely transferred and stored in LONI and is accessible as appropriate to approved investigators. The Informatics Core will provide support to sites uploading MRI and PET scans.

### 4.1.8 Neuropathology Core

The LEADS Neuropathology Core comprises three hub sites that will oversee organization of participating clinical sites. The three hub sites are located at Indiana University, University of California, San Francisco, and Mayo Clinic Jacksonville. The three hub sites coordinate autopsies at participating sites and perform clinicopathologic analyses on EOAD and EOnonAD brain tissue. The Neuropathology Core leaders will support the clinical sites, collaborate with LEADS investigators, organize and participate in consensus-building activities, and lead tissue-sharing efforts. The Neuropathology Core will fulfill the following goals: 1) Facilitate and secure brain procurement at the time of death from all LEADS participants; 2) Conduct thorough, uniform postmortem neuropathologic examinations and assign all appropriate diagnoses for each case and contribute neuropathologic data to the NACC database (including diagnoses, accurate staging, and semiquantitative assessments of distribution and density of neuropathologic lesions); 3) Maintain a resource of formalin-fixed brain tissue blocks for LEADS investigators and outside investigators with an updated database of frozen tissue location and availability; and, 4) Review tissue requests by committee and provide samples and data to qualified investigators.

## 4.2 End of Study Definition

Given the overarching goal of the study to advance our knowledge and facilitate implementation in clinical trials with this unique cohort, there is no specific end point. Study activities are anticipated to continue until funding is no longer available.

# 5.0 STUDY POPULATION

## 5.1 Inclusion Criteria

Cognitively impaired (EOAD and EOnonAD) Cohorts Only:

1. Meets NIA-AA criteria for MCI due to AD or probable AD dementia
2. Have a global CDR score $\leq 1.0$
3. Have capacity to provide informed consent (IC) or has a legal authorized representative or guardian who provides IC
4. Age between 40-64 years (inclusive) at the time of consent
5. Must have a study partner (informant) who spends a minimum average of 10 hours per week with the participant (e.g., family member, significant other, friend, caregiver) who is generally aware of the participants' daily activities and can provide information about the participant's cognitive and functional performance. If the participant does not have a study partner who spends at least 10 face-to-face hours per week, other arrangements for identifying a viable study partner will be granted on a case-by-case basis by the Site PI
6. Willing and able to complete longitudinal study procedures aside from LP which is an optional procedure
7. Not pregnant or lactating. Women must be two years post-menopausal, be surgically sterile, or have a negative pregnancy test prior to each PET scan
8. Fluent in English or Spanish if enrolled in the U.S.
9. Fluent in English, Spanish, Dutch or Swedish for sites outside the U.S., according to site's spoken language(s).

Cognitively Normal (CN) Cohort Only:

1. Meets criteria for cognitively normal, based on an absence of significant impairment in cognitive functions or activities of daily living
2. Have a global CDR score = 0
3. Have capacity to provide informed consent
4. Have a Mini-Mental State Exam score between 26-30 (inclusive). Exceptions may be made for participant with less than 8 years of education at the discretion of the Site PI
5. Age between 40-64 years (inclusive) at the time of consent
6. Must have a study partner (informant) who spends a minimum average of 10 hours per week with the participant (e.g., family member, significant other, friend, caregiver) who is generally aware of the participants' daily activities and can provide information about the participant's cognitive and functional performance. If the participant does not have a study partner who spends 10 face-to-face hours per week, other arrangements for identifying a viable study partner will be granted on a case-by-case basis by the Site PI
7. Willing and able to complete longitudinal study procedures aside from LP which is an optional procedure
8. Not pregnant or lactating. Women must be two years post-menopausal, be surgically sterile, or have a negative pregnancy test prior to each PET scan
9. Fluent in English or Spanish if enrolled in the U.S.
10. Fluent in English, Spanish, Dutch or Swedish for sites outside the U.S., according to site's spoken language(s).

Returning Participant (RP) Cohort Only:

1. Must be a previous participant of LEADS that met initial eligibility criteria and completed a baseline visit
2. Have capacity to provide informed consent (IC) or has a legal authorized representative or guardian who provides IC
3. Must have a study partner (informant) who spends a minimum average of 10 hours per week with the participant (e.g., family member, significant other, friend, caregiver) who is generally aware of the participants' daily activities and can provide information about the participant's cognitive and functional performance. If the participant does not have a study partner who spends at least 10 face-to-face hours per week, other arrangements for identifying a viable study partner will be granted on a case-by-case basis by the Site PI

See section 8.8 for description of Returning Participants.

## 5.2 Exclusion Criteria

EOAD, EOnonAD and CN Cohorts:

1. Meets core clinical criteria for non-AD dementia
2. Two or more first degree relatives with a history of early-onset dementia suggestive of autosomal dominant transmission, unless known pathogenic mutations in *APP, PSEN1, PSEN2, MAPT, GRN and C9ORF72* have been excluded
3. Known CLIA certified mutation in an ADAD gene (*APP, PSEN1, PSEN2*), or other autosomal dominant genes associated with other neurodegenerative disorders (MAPT, GRN, C9ORF72)

4. Contraindications to 3T MRI (e.g., claustrophobia, pacemaker, select aneurismal clip, artificial heart valve, select ear implants, select stents incompatible with 3T MRI, metal fragments or foreign objects in the eyes, skin or body, etc.)
5. Lifetime medical history of a brain disorder other than the disorder causing dementia except for headache (exceptions are allowed at the discretion of the Site PI - e.g., seizure disorder thought to be due to EOAD).
6. MRI scan with evidence of infection or focal lesions, cortical strokes, multiple lacunes (single lacune is allowable unless it meets criteria for strategic lacune affecting cognition)
7. Any significant systemic illness or unstable medical condition, which could lead to difficulty complying with the protocol (at the discretion of the Site PI)
8. Research radiation exposure will be assessed by the study physician. If the candidate participant has had more than one nuclear medicine study in the prior 12 months for research-related purposes, study inclusion will require approval from the PET Core
9. Investigational agents are prohibited 30 days prior to entry
10. Previous enrollment in a therapeutic trial targeting amyloid or tau.
11. Participation in other clinical studies with neuropsychological measures, with the exception of participants who are co-enrolled in the NACC Uniform Data Set (UDS) protocol (Note: This criterion is intended to reduce repeat measures effects during neuropsychological testing. Exceptions are allowed at the discretion of the Site PI)
12. Lifetime history of schizophrenia spectrum disorders (DSM-5 criteria)
13. Current history (in previous 12 months) of DSM-5 diagnosis of mania, bipolar disorder with or without psychotic features
14. Current history (in previous 6 months) of moderate or severe substance abuse (nicotine or caffeine is allowed)
15. Suicidal behaviors in the past 12 months or active suicidal ideations
16. Residing in a 24-hour care skilled nursing facility (at the time of screening)
17. (*For optional lumbar puncture procedure only*):
    a. Clinical laboratory values must be within normal limits or, if abnormal, must be judged to be not clinically significant by the Site PI
        i. Platelet count <100,000/ml
        ii. INR>1.2
        iii. Abnormal PT or PTT at screening
    b. Contraindications to the procedure, including but not limited to severe degenerative joint disease, deformity of the spine, history of a bleeding disorder
    c. Suspected elevated intracranial pressure, Arnold Chiari malformation or mass lesion
    d. Use of the anticoagulant medications such as but not limited to warfarin, rivaroxaban, dabigatran
18. Deemed ineligible by the Site PI for any other reason

<u>RP Cohort:</u>

1. Investigational agents are prohibited for five half-lives prior to study re-entry.

NOTE: Deviations from the inclusion/exclusion criteria will be considered with prior review and approval by the Site PI, the Clinical Core, and the IRB. Contact the ATRI Coordinating Center to request a review.

# 6.0 RECRUITMENT

Participants will be recruited from the diagnostic and treatment clinics or longitudinal research cohorts at each study site, most of which are Clinical Cores of the federally funded Alzheimer's Disease Research Centers (ADRCs). Study-wide recruitment efforts will be overseen by a recruitment team at the Alzheimer's Association. The Alzheimer's Association (AA) has developed a coordinated recruitment plan to ensure enrollment occurs in a timely fashion. The overall goals of the plan are to raise awareness of the trial among the targeted population and to ensure adequate enrollment. The AA's recruitment and retention team developed materials specific to the LEADS for use by sites and will provide ongoing assistance and support.

In addition, the AA will work both through its local field offices and through the TrialMatch database to help identify EO cognitively impaired participants and connect them with the enrolling sites. The AA will reach out directly to individuals who may be eligible for the LEADS study and who live near study sites to inform them of the study and how they can participate.

# 7.0 STUDY PROCEDURES

See Schedule of Events (Appendix 1).

All assessments will be completed by study personnel trained to administer the instruments and will be based on interviews with and examination or testing of the participant, interviews with the study informant, and/or questionnaires completed by the participant and the informant.

LEADS participants that are co-enrolled in other AD research (e.g., local NACC protocols) may have their data shared between the studies.

International sites may share pre-existing data with LEADS from their standard clinical assessments to reduce repeat measure effects and participant burden. Data collected within 6 months of the LEADS consent or visit date may be used to fulfill the required assessments within the Schedule of Events. See relevant study manual for further details.

## 7.1 Description of Study Visits and Procedures

### 7.1.1 Prescreen phase (not an actual visit)

During the prescreen phase, sites will assess existing (i.e., outpatient clinics, other observational studies) and referred potential participants for eligibility criteria, such as age, disease history, comorbidities and ability to tolerate procedures.

### 7.1.2 Screening

The purpose of the Screening Visit is to further determine eligibility and to complete the informed consent process. The screening procedures will be conducted as outlined in the Schedule of Events (Appendix 1).

If previous NACC data exists within 6 months of consent date, it may be used to assess eligibility at Screening. If participant is consented, this previous existing NACC data, within 6 months of consent date, can be used to fulfill the NACC UDS/FTLD modules and neuropsychological battery required at Screening or Baseline. See Schedule of Events Appendix 1.

The participant's capacity to consent will be assessed whenever consent is collected. Guidelines for assessing capacity to consent will be left at the local level to adhere to institutional-specific standard operating procedures (SOPs). Clinical monitors will review that capacity to consent was assessed during their interim monitoring visits against the local SOPs. The relying sites' plan for assessing participants' capacity to consent is to be provided to the reviewing IRB through completion of a relying site form. If the local process for assessing capacity to consent changes at the relying site during the study, the relying site must submit an amendment with an updated site form to the reviewing IRB along with the corresponding SOP, as applicable.

### 7.1.3 Active Study Phase (Baseline to Endpoint Assessments)

The baseline visit will occur within 60 days of screening. The remaining study procedures will be conducted as outlined in Appendix 1.

### 7.1.4 Wellness Check

Telephone calls will be made to participants within 48 hours post lumbar puncture procedure to ascertain if adverse events occurred post procedure. Additional telephone calls will be made to follow up on any adverse events reported that are not already resolved. Additional calls may be conducted at the discretion of the Site PI.

Additionally, a telephone call will be made to cognitively impaired participants within two weeks post amyloid results disclosure to assess the impact of having amyloid PET status results disclosed. If applicable, the impact of having tau PET and FDG disclosure may also be discussed during the telephone call. If there are any concerns about the impact of results disclosure on the participant or family, a follow-up plan will be developed.

### 7.1.5 PET Scan Results Disclosure

Cognitively impaired individuals will meet with the study clinician to discuss the results of the amyloid, tau, and FDG PET scans. Amyloid PET results must be disclosed within the 60 day visit window. Tau and/or FDG PET results may be disclosed outside of the 60 day visit window.

### 7.1.6 Genetic Counseling Sessions and Genetic Testing Disclosure

Cognitively impaired participants will be asked if they wish to receive results if a known pathogenic mutation in an ADAD or other neurodegenerative disease gene (*APP*, *PSEN1*, *PSEN2, C9ORF72, MAPT* and *GRN*) is identified. If a known pathogenic mutation is found in one of these genes and confirmed in a CLIA laboratory and the participant has opted to receive pathogenic mutation results, the participant will be referred to the site's genetic counselor for result disclosure and appropriate counseling. At international sites only, the Site PI or clinical investigator with acceptable credentials may disclose results, if a genetic counselor is not available. Acceptable credentials for a clinical investigator are those who have clinical training in genetic counseling and have experience disclosing diagnostic genetic testing results. Unless required by local site-specific practice, when a positive

mutation is identified, these results may be discussed and the CLIA report may be placed in their medical record if the participant or his or her surrogate decision-maker chooses.

Cognitively impaired participants who have opted to receive pathogenic mutation results, but a known pathogenic mutation is not found, will also be informed of the results. Negative results (when no pathogenic mutation is found) will not be confirmed in the CLIA laboratory. Participants should be instructed that these results are from research screening only and not verified in a CLIA laboratory.

See section 7.2 for more detail.

### 7.1.7 Neuropathology

A member of the study team will discuss autopsy brain donation with each participant at every visit unless the participant has indicated refusal. For those participants who agree to participate in autopsy brain donation, consent will be collected for LEADS even if there is already an existing autopsy brain donation consent for the site's local ADRC. There are 3 objectives of the discussion: 1) to convey information about the value of brain autopsy in confirming the clinical diagnosis and advancing knowledge regarding EOAD; 2) to initiate consideration of the individual's wishes concerning an autopsy; and 3) to answer questions, misconceptions, or concerns about autopsy. This repeated dialogue will be offered at every visit, even after the participant has agreed/consented to autopsy brain donation, unless the participant has clearly refused autopsy. Ongoing discussions can be helpful to ensure that the study participant conveys their wishes to their family.

This decision is voluntary and designed not to involve pressure; participants are encouraged to involve family members, clergy, physicians, or any other appropriate persons in their decision-making. Participants are assured that refusing autopsy in no way jeopardizes their research participation or any other patient rights. It is important to note that the autopsy will not interfere with funerary arrangements (e.g., an open-casket funeral is acceptable as there will be no visual disfigurement) nor will it be a financial burden to the participant's family. As a supplement to this discussion, the LEADS Neuropathology Core will develop an Autopsy Brochure to dispel some of the common myths and concerns regarding the autopsy procedure and a Brain Donation letter, which will explain the importance of autopsy and brain donation for research in layperson language. We will encourage the study team to use these tools when discussing autopsy with participants.

Regular contact (e.g., at each in-clinic visit, phone calls) with individuals and their family who have provisionally consented, can help ensure that autopsy arrangements are made in advance, which aids successful brain donation. This includes annual phone checks after completion of the LEADS study and after all LEADS study visits have been completed, until the death of the participant. Periodically revisiting the procedure to follow at the time of death and reiterating the participant's wish for donation to family members strengthens the likelihood of a completed brain donation at time of death.

Participation in the autopsy component of the study is optional for international sites. Therefore, international sites may refrain from approaching their participants for autopsy brain donation. If international sites choose to take part, they may leverage existing brain banks in order to store the brain tissue locally and share tissue and/or data with LEADS as regulations allow.

#### *After Obtaining Provisional Consent*

When voluntary autopsy consent is granted, more detailed information should be provided to the participant about procedures to follow at time of death, including telephone numbers to call and other guidelines. The LEADS Neuropathology Core has developed autopsy notification materials such as wallet cards that list contact information for the person to be notified at the time of death and letters to primary care physicians that communicate the participant's wishes regarding autopsy. Participants are strongly encouraged to share this information with the next-of-kin, legally authorized representatives (e.g., Durable Power of Attorney (DPOA)), and private physicians. In many states, final legal authorization by the DPOA or next-of-kin must be obtained at the time of death. LEADS is a multi-center study; sites must follow state and local laws regarding autopsy consent procedures.

#### *At the Time of Expiration*

Sites will follow standard autopsy procedures as outlined in the LEADS Neuropathology Manual upon notification of the death of a LEADS participant.

### 7.1.8 Study Participation Report

Study participants can be optionally provided a report of study procedure results. The report will summarize key results from relevant procedures (cognitive testing, diagnosis, and MRI results) and be used to aid in study retention. Amyloid and tau PET scan results will be provided to cognitively impaired participants only. FDG PET scan results will be provided to EOnonAD participants only.

Clinically significant results will be relayed in a timely manner by the Site clinician. Appropriate follow-up with a treating physician will be arranged as indicated.

### 7.1.9 Participant Travel

In certain situations (e.g., imaging machine is down at home site, tracer is unavailable, etc.) it is permissible for participants participating at one clinical site to complete some procedures at another clinical site. Contact the Coordinating Center for prior approval and guidance.

## 7.2 Clinical Assessments and Procedures

#### *Demographics*

Participants and study informants will provide basic demographic information throughout the study.

#### *Family History*

Detailed family history will be collected from all participants. Participants with two or more first degree family members with EOAD (defined as AD with onset < 64 years) will be ineligible for LEADS, unless known pathogenic mutations in *APP, PSEN1, PSEN2, MAPT, GRN and C9ORF72* have been excluded.

#### *Early Developmental History Questionnaire*

Studies have shown that neurodevelopmental differences (non-right-handedness, learning disabilities, etc.) may be overrepresented in atypical early-onset neurodegenerative diseases. [38, 39] Preliminary data suggests that neurodevelopmental differences might contribute towards disease susceptibility. A

brief questionnaire will be used to assess handedness and previous neurodevelopmental disorders in the research participant or participant's family. In symptomatic participants, the questionnaire should be completed by the study informant on behalf of or together with the participant. In asymptomatic participants, the questionnaire can be completed independently. This questionnaire may be administered by study staff if need be.

### *Autoimmune History Questionnaire*

Studies have shown that non-thyroid autoimmune disorders may be overrepresented in atypical early-onset neurodegenerative diseases – including conditions due to underlying Alzheimer's disease. [40, 41] Preliminary data suggests that select autoimmune diseases might contribute individually towards disease susceptibility. Brief autoimmune history will be collected for the research participant and participant's family. In symptomatic participants, the questionnaire should be completed by the study informant on behalf of or together with the participant. In asymptomatic participants, the questionnaire can be completed independently. This questionnaire may be administered by study staff if need be.

### *Sex and Reproductive Health Questionnaire*

The Sex and Reproductive Health is a self-report questionnaire that will be administered to all participants. The questionnaire should be administered in-person but may be administered over the phone when in-person administration is not feasible.

### *Genetic Counseling and Genetic Testing – cognitively impaired participants only*

At the Screening Visit, participants will view a video that outlines the implications of mutations in known ADAD loci (e.g., in *APP*, *PSEN1*, *PSEN2, C9ORF72, MAPT* and *GRN*) and be provided a genetics handout summarizing the information reviewed in the video. The video outlines the implications positive results might have for participants and their families. The video will describe how receipt of the genetic testing results and identification of pathogenic mutations will provide information regarding the likelihood that close relatives of the participant may also develop EOAD. Additionally, results of genetic testing may reveal incorrect assumptions in family relationships (such as learning that a child is adopted or has a different father). The participant will also be asked if they would like to speak with a genetic counselor at baseline either in person or via a telephone/video bridge to verify their understanding of the implications of genetic testing and answer any questions the participant may have. Some international sites may not have access to a genetic counselor. In these cases, participants will be offered a session with the site PI or an appropriately trained clinical investigator. Participants will be asked if they wish to receive results if a known pathogenic mutation is identified in an ADAD gene and will provide written consent.

A 6 ml blood sample will be collected at the Baseline Visit from all cognitively impaired participants. The sample will be used for CLIA confirmation of a known pathogenic mutation, if one is found. The availability of this sample for all cognitively impaired participants will also allow a participant who initially did not want to learn their mutation status to change his or her mind later. This tube will be sent to and stored at NCRAD.

Research genetic screening will be performed in a research laboratory as part of the LEADS study and the data will be used to screen for mutations in *APP*, *PSEN1*, *PSEN2*, *MAPT, C9ORF72,* and *GRN*, and for research analyses. If a known pathogenic mutation is found in one of these genes and the participant has opted to receive pathogenic mutation results, the CLIA laboratory will utilize the stored blood sample to confirm the mutation identified through the research studies. If the participant did not request the pathogenic mutation results, NCRAD will destroy the stored blood sample at the time of study completion.

If the mutation is confirmed in the CLIA laboratory, the participant will meet with the site's genetic counselor (or Site PI/clinician for international sites) for result disclosure and appropriate counseling and be discontinued from the LEADS study and given a referral to research studies focused on familial neurodegenerative disorders, such as the Dominantly Inherited Alzheimer's Network (DIAN) research study. Whenever feasible, results should be disclosed in person. If an in-person disclosure is not feasible due to geographic distance, other participant-related factors, or in special circumstances a videobridge genetic counseling session will be considered a viable substitute with approval from the Genetics Core.

### Prior neurodegenerative disease genetic testing

Participants with previous negative genetic testing results for mutations in *APP, PSEN1, PSEN2, MAPT, C9ORF72* and *GRN* performed in a CLIA laboratory may not require research testing. Participants enrolled at international sites may have previous genetic testing that was performed in a non-CLIA laboratory, but with similar standards. All previous genetic testing reports must be submitted to the Genetics Core to determine if research testing under the LEADS protocol is required. Submitted reports must be: 1) de-identified and labeled with the participant study ID, 2) in English or translated into English, 3) include the genes tested and the result in standard protein or DNA nomenclature, 4) include the date of testing, 5) include the name of the laboratory or laboratory director and any certifications of quality assurance, 6) include description of methodology. If after review, the previous testing meets the requirements of the LEADS study, genetic screening for mutations in the six genes will not be performed and participants will not be required to view the video, receive the genetics handout, complete the separate genetic testing consent, provide a 6 ml blood sample, or meet with the genetic counselor.

#### *Physical and Neurological Examination*

A medically qualified professional will perform a physical examination of the major body systems. Neurological examination will include an assessment of cranial nerves, motor function, coordination, reflexes, sensation and gait.

#### *Medical History*

The participant's lifetime medical history will be collected as listed in Appendix 1. Medical history includes previous and current diseases, psychiatric history, and substance use history.

#### *Medication List*

The participant's current medications will be collected at each visit as indicated in Appendix 1.

### *Vital Signs*

Vital signs will include height, weight, systolic and diastolic blood pressure, and pulse.

### *Clinical Dementia Rating (CDR)*

The CDR is a semi-structured interview of the informant and participant that assesses for impairment in 8 areas of functioning - memory, orientation, judgment and problem solving, community affairs, home and hobbies, personal care, behavior, personality, and language [42].

### *Functional Assessment Questionnaire (FAQ)*

Based on an interview with the informant, a participant is rated on his/her ability to carry out ten complex activities of daily living [43].

### *Amsterdam Instrumental Activity of Daily Living Questionnaire*

The Amsterdam IADL Questionnaire (A-IADL-Q) is a questionnaire designed to assess impairments in instrumental activities of daily living (IADL) in (early) dementia. The questionnaire is completed by a caregiver, such as a spouse, relative or friend. The A-IADL-Q covers a broad range of IADL, including household activities, household appliances, finances, work, computer, and leisure activities. It was demonstrated to be a reliable and valid instrument in the evaluation of dementia [64, 65], with good diagnostic value [64] and sensitivity to change over time [66].

### *Neuropsychiatric Inventory (NPI-Q)*

The NPI-Q is a well-validated, reliable, multi-item instrument to assess the neuropsychiatric features in AD based on an interview with the informant [44]. It evaluates severity of delusions, hallucinations, dysphoria, anxiety, agitation/aggression, euphoria, disinhibition, irritability/lability, apathy, and aberrant motor behavior.

### *Geriatric Depression Scale (GDS) Short Form*

The GDS Short Form is a self-report scale designed to screen for symptoms of depression in the elderly. [45].

### *Social Norms Questionnaire*

The intent of the questionnaire is to determine how well participants can understand and identify social boundaries that are part of mainstream culture in the United States [46]. The participant is asked to check the most accurate response to “Is it socially acceptable to” questions. Example: Is it socially acceptable to eat pasta with your fingers?

### *Social Behavior Observer Checklist*

The intent of this checklist is to aid clinicians with the recognition of distinct patterns of social behaviors such as self-consciousness, anxiety, embarrassment, failure to adapt, etc.

### *Clinical Symptom Assessment*

A clinical determination of the participant’s current symptomatology and onset of symptoms will be collected by a trained clinician. Information will be obtained through the participant, study partner, medical records, and/or observations.

#### *Clinical Diagnosis*

At each site, a formal consensus panel will assess the participant's diagnosis within 30 days of each study visit.

#### *Area Deprivation Index (ADI)*

The ADI is a composite measure of socioeconomic disadvantage for the United States. Using United States Census indicators of poverty, education, housing, and employment, neighborhood socioeconomic status is ranked by disadvantage at the state and national level. Each census block/neighborhood is split into state deciles and national percentiles, with lower percentile scores indicating less socioeconomic disadvantage [67, 68]. This measure will only be collected for U.S. sites.

## 7.3 Cognitive Testing

### 7.3.1 NACC Uniform Data Set (UDS) and Frontotemporal Lobar Degeneration (FTLD) Neuropsychological Batteries

#### *Montreal Cognitive Assessment (MoCA)*

The MoCA is a rapid screening instrument designed to help health professionals detect cognitive dysfunction. It assesses numerous cognitive domains: attention and concentration, executive function, memory, language, visuospatial skills, conceptual thinking, calculations and orientation[47].

Note: The MoCA cannot be administered on the same day as the MMSE. MMSE should be done at the screening visit. MoCA should be administered at a subsequent visit.

#### *Craft Stories*

This test assesses one's ability to recall a short story[48]. Hearing acuity must be established prior to the test. The participant is read a short story and asked to recall it. The participant is asked to repeat the test again after 20 minutes to assess delayed recall (episodic memory).

#### *Benson Complex Figure Copy and Recall*

The purpose of this test is to assess a participant's visuospatial skills and visual memory. It is a simplified version of the Rey-Osterrieth Complex Figure[49, 50]. The participant is asked to first copy and after 10-15 minutes to draw the figure again from memory.

#### *Number Span Forward and Backward*

This is a test of working memory, and it taps two different constructs. The first, Forward Number Span, measures the capacity for briefly holding information and repeating it exactly. The second, Backward Number Span, measures the ability not only to hold the information but also to manipulate the numbers by reversing the sequence. Sequences of 2 to 9 numbers (two trials at each sequence length) are presented for both Forward and Backward Number Span[48].

### *Category Fluency*

This is a widely used measure of verbal fluency. The participant is asked to name different exemplars of a given semantic category (e.g., animals), and the number of unique exemplars named is scored[51].

### *Trail Making Tests (A and B)*

These are tests of processing speed and executive function. Both Parts A and B depend on visuomotor and perceptual-scanning skills. Part B also requires cognitive flexibility in shifting from number to letter sets under time pressure. The participant's performance is judged in terms of time[52].

### *The Multilingual Naming Test (MINT)*

The MINT is a language test that examines one's ability for visual object naming [48, 69]. Line drawings are presented to the participant with the instruction to say the name of the object.

### *Phonemic Fluency*

This is a widely used measure of word generation that may be sensitive to dysfunction in the dominant frontal lobe [51]. In this version, the participant is asked to say as many words as possible that being with the letter "F" in 60 seconds, and then as many words that being with the letter "L" in 60 seconds.

### *Word Reading*

This is a test of word reading that includes regularly spelled and irregularly spelled words. The participant is asked to read out loud from the regular and irregularly spelled word lists. Accurate word reading is scored.

### *Semantic Associates Test*

This is a test of knowledge of the meaning of objects [53]. In this test, a participant reviews pairs of pictures and is instructed to select those that depict related objects. Correct associations are scored.

### *Semantic Word Picture Matching*

This test evaluates spoken word recognition and assesses for semantic errors in word comprehension. The stimuli consist of four-picture displays including pictures of four objects that are semantically related. One of the objects is named by the examiner and the participant is asked to point to that object.

### *Northwestern Anagram*

This is a test of grammatical knowledge[54]. In this test, the participant is shown pictures and is then asked to assemble a sentence describing the pictures using printed words that are provided.

### *Sentence Repetition*

This is a test of oral repetition of sentence-length utterances. A sentence is read out loud to the participant. The participant then repeats the sentence verbatim. Correct sentences, omitted words and semantic errors are recorded for scoring purposes.

### *Noun and Verb Naming*

This is a test of confrontation naming of objects and actions. In this test, the participant is shown pictures of objects or things, as well as pictures of people doing various actions. The participant is then asked to name each picture as quickly and as accurately as possible. The primary measure of performance is the noun-to-verb ratio[55].

#### *Sentence Reading*

In this test, the participant is given a sheet of paper with five short sentences and is asked to read the sentences out loud. The primary measure of performance is the number of accurately read sentences.

### 7.3.2 Additional Cognitive Tests:

#### *Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog13)*

The ADAS-Cog13 [56] is an in-person examiner-administered, structured scale that evaluates memory, reasoning, language, orientation, ideational praxis and constructional praxis. Ratings of spoken language, language comprehension, word finding difficulty, and ability to remember test instructions are also obtained.

#### *Digit Symbol Substitution Test*

The Digit Symbol Substitution test [57] measures complex attention. The numbers 1-9 are paired with different nonsense symbols. A string of these numbers is randomly printed directly above a row of blank squares. Following a short series of practice trials, the participant must use the key to fill in the blank squares with the correct nonsense symbol in order working from left to right across the rows. This test engages multiple cognitive abilities including attention, psychomotor speed, complex scanning, visual tracking, and immediate memory.

#### *Mini-Mental State Examinations (MMSE)*

The MMSE is a brief, frequently used screening instrument for Alzheimer's disease drug studies [58]. The MMSE scale evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two overlapping pentagons. The MMSE will only be administered once during the screening visit.
Note: The MMSE cannot be administered on the same day as the MoCA. MMSE should be done at the screening visit. MoCA should be administered at a subsequent visit.

#### *Rey Auditory Verbal Learning Test (AVLT)*

The AVLT is a list-learning task, which assesses multiple cognitive parameters associated with learning and memory[49, 50].

#### *Tablet-Based Cognitive Assessment Tools (TabCat)*

Tablet-Based Cognitive Assessment Tools (TabCat) is a tablet application that is composed of a growing suite of measures assessing cognitive and behavioral functions. Software packages can be installed on an iPad or Android-based tablet. The following four separate TabCat tasks will be administered: Flanker, Line Length, Line Orientation, and Match (Digit Symbol) [59].

### 7.3.3 Additional Cognitive Tests to be Used at Select International Sites:

#### *Logical Memory*

The logical memory test may be used in place of the Craft Stories in order to administer in different languages. The logical memory test is a measure of memory (declarative/episodic) in which a brief story is read to the subject, who is then asked to retell it from memory immediately. The primary measure of performance is the

number of story units recalled. Alternate paragraphs for the Logical Memory stories are not available, so as not to introduce more variability. The participant is asked to repeat the story again after 20 minutes to assess delayed recall (episodic memory) [69].

#### *Boston Naming Test*

The Boston Naming Test may be used in place of the MINT in order to administer in different languages. The Boston Naming Test is a measure of the ability to orally label (name) line drawings of objects. This test is sensitive to aphasia and also to object recognition deficits.

Results from the NACC Uniform Data Set Neuropsychological Battery Crosswalk Study will be used to allow scores to be converted to equivalent scores on the Craft Stories and MINT tests, respectively [69].

A summary of the neuropsychological testing may be entered in a participant's medical record for clinical purposes.

### 7.3.4 Audio Recording and Transcription

The administration of select language and memory tests will be audio recorded for every participant, at every visit in the U.S.. These tests will not be audio recorded for participants at international sites due to language differences. This data will be collected to allow for additional analyses. A recording device, such as Olympus VN-722PC digital voice recorder or similar digital recording device, will be used. Audio recordings will be securely transmitted to a contracted cloud-based transcription service for automatic transcription.

## 7.4 Imaging

Use of short-to medium-acting benzodiazepines specifically for treatment of claustrophobia or anxiety related to imaging is allowed. The use of sedatives or hypnotics should be avoided for 8 hours before the administration of cognitive tests.

### 7.4.1 Magnetic Resonance Imaging (MRI)

All participants will be scanned on a 3T MR instrument with a protocol that conforms to FDA safety standards. The MRIs will be conducted as outlined in the Schedule of Events (Appendix 1). The MRI protocol includes: scout, structural T1-weighted MRI, FLAIR, T2-weighted, diffusion tensor imaging, ASL perfusion MRI, and task free resting state functional MRI. The total scan time is approximately one hour but may be longer depending on technical factors. If the participant becomes uncomfortable they can ask to be removed from the scanner at any time. MRI scan findings of clinical significance, determined by the local neurologist or radiologist, will be shared with the participant and the participant's local physician (if necessary – i.e., in the case of actionable findings).

The MRI report or summary of MRI findings may be entered in participant's medical record for clinical purposes.

### 7.4.2 PET scanning. Amyloid, Tau, and FDG PET Imaging

All participants will complete PET scanning on a PET instrument according to the protocol. The PET scans will be conducted as outlined in the Schedule of Events (Appendix 1). There must be at least 365 days between the first PET scan of the previous visit and the first PET

scan of the next yearly visit. There must also be at least 365 days from second-to-second PET, so that there are no more than two study-related PET scans in a year. FDG PET is not subject to this consideration; it can be the third study-related PET in 365 days. Consult the ATRI Coordinating Center for guidance on scheduling participant visits.

Note: PET scans must be done on separate days and at least 12 hours apart.

#### *7.4.2.1 Amyloid PET scanning*

Cognitively impaired and CN participants will undergo an amyloid PET scan as outlined in Appendix 1. The study staff will evaluate each participant prior to administration of tracer to determine if they are still suitable to undergo the scan (e.g., previous research PET scan exposure in the previous 12 months). For women of childbearing potential, a urine pregnancy test will be obtained and negative result confirmed on the day of scanning.

The screening scans for cognitively impaired participants only will be interpreted by the PET Core to assess amyloid status. The read will be based on a consensus between visual and quantitative assessments. Dichotomous visual interpretations of summed images will be performed by a qualified rater using validated criteria. Standardized Uptake Value Ratio (SUVR) images will be created using whole cerebellum as a reference region and a threshold SUVR will be used to define scan positivity. Scans in which visual and quantitative classifications that are incongruent will be arbitrated by consensus by at least two members of the PET Working Group.

Screening amyloid PET results for all cognitively impaired participants and M36 amyloid PET results, as applicable, for all EOnonAD participants, positive and negative, will be provided to sites. The Site PI or an affiliated study physician will be responsible for disclosing the results to the study participant and their family. The clinician should follow best practices in disclosing amyloid PET results. Whenever feasible, results should be disclosed in person. If an in-person disclosure is not feasible due to geographic distance or other factors, a telemedicine appointment could be considered a viable substitute. Disclosure of results by phone call only is discouraged but allowable at the discretion of the Site PI.

Longitudinal amyloid PET scans for the EOAD and EOnonAD cohorts will not receive an official read, however trans-axial images may be provided to the participant at the discretion of the site PI. See sections 7.4.2.2-7.4.2.5 below, as not all participants will complete longitudinal amyloid PET scans.

Amyloid PET scans from the CN cohort will not receive an official read and results will not be disclosed.

#### *7.4.2.2 Florbetaben (Neuraceq)*

Florbetaben scanning entails 8.1 mCi +/- (300MBq) 10% injection of tracer through an intravenous line and a 90 minute (+/- 5 minutes) uptake followed by a 20-minute image acquisition scan. Every effort should be made to scan at exactly 90 minutes

post injection. A low dose CT scan will be acquired on all PET/CT scanners for attenuation correction prior to beginning acquisition. The injection site will be observed for evidence of inflammation or damage to the surrounding tissue where the dose was injected and adverse events will be monitored during the imaging session. Participants will be asked to void completely after completion of the scan.

Additional information regarding florbetaben (i.e., production, delivery, administration, safety) is provided in section 9.0 Study Compounds.

#### *7.4.2.3 C-Pittsburgh Compound B ($^{11}$C-PiB) (for international sites only)*

Select sites may use PiB in place of Florbetaben with a prior agreement in their site's contract with IU. International sites using PiB will follow Good Manufacturing Practice (GMP) pharmaceutical regulations.

PiB scans will only be completed at screening for all subjects for cohort assignment. Sites that use PiB in place of florbetaben will not complete longitudinal amyloid PET scans.

PiB scanning entails 10 mCi (370 MBq) +/- 10% injection of tracer through an intravenous line and a 40-minute (+/- 5 minutes) uptake followed by a 30-minute image acquisition scan. Every effort should be made to scan at exactly 40 minutes post injection. A low dose CT scan will be acquired on all PET/CT scanners for attenuation correction prior to beginning acquisition. The injection site will be observed for evidence of inflammation or damage to the surrounding tissue where the dose was injected and adverse events will be monitored during the imaging session. Participants will be asked to void completely after completion of the scan.

Additional information regarding PiB (i.e., production, delivery, administration, safety) is provided in section 9.0 Study Compounds.

#### *7.4.2.4 [$^{18}$F]NAV-4694 (for international sites only)*

Select sites may use NAV-4694 in place of Florbetaben with a prior agreement in their site's contract with IU. International sites using NAV-4694 will follow Good Manufacturing Practice (GMP) pharmaceutical regulations.

NAV-4694 will only be used for cohort assignment at screening. Sites that use NAV-4694 in place of florbetaben will not complete longitudinal amyloid PET scans.

NAV-4694 scanning entails 8.1 mCi (300 MBq) +/- 10% injection of tracer through an intravenous line and a 50 minute (+/- 5 minutes) uptake followed by a 20-minute image acquisition scan. Every effort should be made to scan at exactly 50 minutes post injection. A low dose CT scan will be acquired on all PET/CT scanners for attenuation correction prior to beginning acquisition. The injection site will be observed for evidence of inflammation or damage to the surrounding tissue where the dose was injected and adverse events will be monitored during the imaging session. Participants will be asked to void completely after completion of the scan.

Additional information regarding NAV-4694 (i.e., production, delivery, administration, safety) is provided in section 9.0 Study Compounds.

#### *7.4.2.5 Flutemetamol (for international sites only)*

Select sites may use Flutemetamol in place of Florbetaben with a prior agreement in their site's contract with IU. International sites using Flutemetamol will follow Good Manufacturing Practice (GMP) pharmaceutical regulations.

Flutemetamol will only be used for cohort assignment at screening. Sites that use Flutemetamol in place of florbetaben will not complete longitudinal amyloid PET scans.

Flutemetamol scanning entails 5mCi (185 MBq) +/- 10% injection of tracer through an intravenous line and a 90 minute (+/- 5 minutes) uptake followed by a 20-minute image acquisition scan. Every effort should be made to scan at exactly 90 minutes post injection. A low dose CT scan will be acquired on all PET/CT scanners for attenuation correction prior to beginning acquisition. The injection site will be observed for evidence of inflammation or damage to the surrounding tissue where the dose was injected and adverse events will be monitored during the imaging session. Participants will be asked to void completely after completion of the scan.

Additional information regarding Flutemetamol (i.e., production, delivery, administration, safety) is provided in section 9.0 Study Compounds.

#### *7.4.2.6 Flortaucipir*

Cognitively impaired and CN participants will undergo a flortaucipir PET as outlined in Appendix 1. Participants from select international sites will not complete Tau PET scans. The site study physicians will evaluate each participant prior to administration of flortaucipir to determine if they are still suitable to undergo the scan (e.g., previous research PET scan exposure in the previous 12 months). For women of childbearing potential, a urine pregnancy test will be obtained and negative result confirmed on the day of scanning.

Baseline tau PET results for all newly recruited cognitively impaired participants, positive and negative, will be provided to sites. Participants from select international sites will not complete tau PET scans and therefore will not receive tau PET results. For sites with participants past their baseline timepoint at the time when protocol version 8.0 was implemented, tau PET scan results related to their next study visit timepoint will be provided. The Site PI or an affiliated study physician will be responsible for disclosing the results to the study participant and their family. The clinician should follow best practices in disclosing tau PET results. At Baseline, tau PET scan results and amyloid PET scan results should be disclosed at the same time. If this is not possible due to scheduling conflicts, the study clinician will provide amyloid PET scan results prior to disclosing tau PET scan results. Whenever feasible, results should be disclosed in person. If an in-person disclosure is not feasible due to geographic distance or other factors, a telemedicine appointment could be considered a viable substitute. Disclosure of results by phone call only is

discouraged but allowable at the discretion of the Site PI.

Longitudinal tau PET scans for the EOAD and EOnonAD cohorts will not receive an official read, however trans-axial images may be provided to the participant at the discretion of the site PI.

Tau PET scans from the CN cohort will not receive an official read and results will not be disclosed.

Flortaucipir scans will be acquired on the PET scanner at each site using standard procedures. Flortaucipir scanning entails 10 mCi (370 MBq) +/- 10%injection of tracer through an intravenous line and a 75-80 minute uptake followed by 30-minute image acquisition scan. Every effort should be made to scan at exactly 75 minutes post injection. After approximately 75-minute-long uptake period, participants will undergo a 30-minute dynamic, 3D PET scan consisting of six 5-minute frames (i.e., from 75-105 minutes post-injection). A low dose CT scan will be acquired on all PET/CT scanners for attenuation correction prior to beginning acquisition. The injection site will be observed for evidence of inflammation or damage to the surrounding tissue where the dose was injected and adverse events will be monitored during the imaging session. Participants will be asked to void completely after completion of the scan.

Study personnel, will consult with study physicians as needed and will assess the participant prior to discharge from the imaging center to determine well-being. Participants who experience an adverse event will not be discharged until the event has resolved or stabilized.

Additional information regarding flortaucipir (i.e., production, delivery, administration, safety) is provided in section 9.0 Study Compounds.

#### *7.4.2.7 FDG PET*

EOnonAD and CN participants will undergo a FDG PET scan as outlined in Appendix 1. The study staff will evaluate each participant prior to administration of fluorodeoxyglucose to determine if they are still suitable to undergo the scan (e.g., previous research PET scan exposure in the previous 12 months). For women of childbearing potential, a urine pregnancy test will be obtained and negative result confirmed on the day of scanning.

Clinical reads for EOnonAD participants will be provided to sites. The Site PI or an affiliated study physician will be responsible for disclosing the results to the study participant and their family. The clinician should follow best practices in disclosing FDG PET results. Whenever feasible, results should be disclosed in person. If an in-person disclosure is not feasible due to geographic distance or other factors, a telemedicine appointment could be considered a viable substitute. Disclosure of results by phone call only is discouraged but allowable at the discretion of the Site PI.

FDG PET scans from the CN cohort will not receive an official read and results will not be disclosed.

FDG PET scans for all participants will be reviewed locally for safety purposes and clinically significant findings (e.g., hypermetabolic lesion concerning for malignancy) will be disclosed to sites. Clinically significant incidental findings should be disclosed to participants by the site PI.

Fluorodeoxyglucose scans will be acquired on the PET scanner at each site using standard procedures. Participants must be fasting for 4 hours or overnight; prior to the scan a blood glucose measurement will be made by fingerstick and scans will be postponed or rescheduled if the reading is > 180 mg/dL. Fluorodeoxyglucose scanning entails a 5 mCi (185MBq) +/- 10% injection of tracer through an intravenous line and a 30-minute uptake followed by a 30-minute image acquisition scan. A low dose CT scan will be acquired on all PET/CT scanners for attenuation correction prior to beginning acquisition. The injection site will be observed for evidence of inflammation or damage to the surrounding tissue where the dose was injected and adverse events will be monitored during the imaging session. Participants will be asked to void completely after completion of the scan.

Study personnel will consult with study physicians as needed and will assess the participant prior to discharge from the imaging center to determine well-being. Participants who experience an adverse event will not be discharged until the event has resolved or stabilized.

Additional information regarding fluorodeoxyglucose (i.e., production, delivery, administration, safety) is provided in section 9.0 Study Compounds.

## 7.5 Fluid Biomarker Collection

Biofluids will be collected at time points specified in the Schedule of Events (Appendix 1). Samples will be collected to accommodate the assay of the broadest range of the best antecedent biomarkers/analytes.

Peripheral blood for biomarker analyses and CSF should be collected after a minimum 6-hour fast, preferably in the morning. When fasting, only water (no food) will be permitted until the blood draw and the (optional) LP procedure are completed. Every effort to gather the samples fasting will be done. In special circumstances and on a case-by-case basis, non-fasting collection will be allowed by the Site PI or their designee and not considered a protocol deviation.

### 7.5.1 Peripheral Blood Collection for Fluid Biomarker Analyses

Up to 80 mL of blood will be collected. This sample will be used to extract DNA, RNA, plasma, serum and PBMC. For sample processing please see section 7.7.

### 7.5.2 Peripheral Blood Collection for Genetic Testing

All cognitively impaired individuals will have a 6 ml blood sample collected at the Baseline Visit. This sample will be sent to and stored in NCRAD and will be used for CLIA

confirmation of a known pathogenic mutation, if it is found. If a known pathogenic mutation is found, the CLIA laboratory will utilize the stored blood sample to confirm the mutation identified through the research studies. The availability of this sample will also allow a participant to consent to genetic testing results at any time during the study.

### 7.5.3 Lumbar Puncture and CSF (optional)

Lumbar puncture (LP) is a technique to sample cerebrospinal fluid (CSF). The procedure involves introducing a needle into the subarachnoid space of the lumbar sac, at a level safely below the spinal cord. Lumbar puncture will be completed using standard collection procedures. CSF collection will be performed using a small caliber atraumatic needle. CSF should be obtained via gravity flow using the 22 gauge Sprotte needle, although aspiration through this or smaller needles is allowable. Prior approval from the Clinical Core is required before the aspiration method or use of other sized needles can be utilized. Sites must designate the method of CSF collection for data tracking purposes.

Participants will be instructed to lie down for 30-60 min after the procedure, to drink plenty of fluids before and after the procedure and take OTC pain medications to alleviate any post-procedure headache or back ache (see Section 10.0 Side Effects for more details).

Approximately 2 ml of CSF will be sent to the site's clinical laboratory for routine cell count, protein and glucose analyses. The remaining (up to 18 ml) CSF will be aliquoted at the site and shipped to NCRAD. iLEADS sites must send a minimum of 10 mL CSF; exceptions may be made at the discretion of the Genetics and Biorepository Core.

International sites may have stored CSF that was collected previously for other EOAD studies or routine assessments in clinic. In order to avoid repeat lumbar puncture procedures and reduce participant burden, international sites may send these pre-existing CSF samples to LEADS if collected up to 12 months prior to a participant's consent date and approval is received by the Genetics and Biorepository Core. These sites must contain applicable language in their consent form. If approval is obtained for LEADS, these samples will be shipped to NCRAD.

For information about sample processing, see section 7.7.

## 7.6 Safety Assessments

### 7.6.1 Concomitant Medication Review

Concomitant medications will be reviewed per SOE.

### 7.6.2 Safety Laboratory Assessments

Prior to lumbar puncture all participants will be subjected to the following safety labs. Safety labs will be conducted locally. However, if previous safety labs exist from a CLIA or appropriately certified laboratory within 60 days of LP, they can be used to review clinical significance and determine LP eligibility.

- complete blood count with differential (CBC w/diff)
- basic coagulation panel

The laboratory reports must be reviewed and signed and dated by the Site PI (or a medically-qualified individual delegated by the PI) prior to conducting the study LP.

### 7.6.3 Urine Pregnancy Test

On the day of each PET scan, prior to the scan, all females of childbearing potential will complete a urine pregnancy test. Participants with positive pregnancy test will be ineligible for amyloid, tau, and FDG PET scanning.

### 7.6.4 Brain Imaging

A brain image documenting absence of space occupying lesion, another reason for increased intracranial pressure and Arnold Chiari malformation should be performed prior to the baseline lumbar puncture, or if missed at baseline, prior to the first lumbar puncture and to assess overall eligibility (see exclusion #6). A previously existing MRI within 12 months of consent can be used to expedite eligibility determination. However, the site is still required to complete a study MRI scan at screening or baseline per the schedule of events. If no previous imaging from the past 12 months exists, the study MRI scan will need to be completed and reviewed locally by a neurologist or radiologist prior to the lumbar puncture procedure.

## 7.7 Biofluid Sample Processing

### 7.7.1 Site processing:

1. Peripheral blood sample – up to 80mL of blood will be collected. The following will be extracted from this sample:
    i. DNA – buffy coat collected from a blood sample will be shipped to NCRAD where DNA will be extracted and used for a range of genomic analyses including whole genome sequencing and epigenetic analyses, and to enable future genomic analyses.
    ii. RNA - blood sample will be shipped to NCRAD where RNA will be extracted and used for expression analyses including RNA sequencing.
    iii. Peripheral blood mononuclear cells (PBMC) – blood sample will be shipped to NCRAD where PBMCs will be isolated and used for the development of induced pluripotent stem cells (iPSCs). These cells can also support other functional genomic studies. Some cells may be derived into new materials. PBMCs are optional for international sites and if collected, will be stored locally.
    iv. Plasma – the blood sample will be processed at the site. The plasma will be subaliquoted at the site and frozen. Frozen aliquots will be shipped to NCRAD for use in biomarker assay development and validation.
    v. Serum - the blood sample will be processed at the site. The serum will be subaliquoted at the site and frozen. Frozen aliquots will be shipped to NCRAD for use in biomarker assay development and validation.
2. CSF (optional) - 2 mL will be sent to the site's clinical laboratory for routine labs - cell count, protein and glucose analyses. The remaining (up to 18 ml) CSF will be aliquoted at the site and frozen as soon as possible, but at maximum, within 24 hours at -80$^{o}$C. Frozen aliquots will be shipped to NCRAD for use in biomarker assay development and validation.
3. 6 mL of blood from cognitively impaired participants will be shipped to NCRAD for CLIA genetic testing, as applicable. See section 7.2 Prior neurodegenerative disease genetic testing section for more information.

All sample collection and shipment from international sites to the U.S. will follow local regulations. If regulation does not allow international sites to ship samples to the U.S., samples will be stored locally until plans for analyses and shipment comply with the regulations.

### 7.7.2 National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) Sample Processing

Biofluids will be sent to NCRAD where samples will be processed, stored, and distributed to approved researchers for analysis. Most samples will be sent frozen; however, the blood sample for PBMC isolation will be sent the same day it is drawn to ensure maximal utility for subsequent analyses. NCRAD will request a re-sampling, if the condition of the sample on arrival prevents processing. All other blood samples, the plasma and serum aliquots and CSF aliquot will be shipped frozen in batches.

The identity of participants will not be shared with NCRAD or with any investigators. A unique bar-code number will be affixed to all specimen tubes as well as affixed to the Sample Form/Draw Sheet. All transfer tubes, vessels and storage vials will be pre-labeled prior to sample processing.

# 8.0 CRITERIA FOR REPEAT ASSESSMENTS, INCOMPLETE VISIT PROCEDURES, RESCREENING, EARLY TERMINATION, DISCONTINUATION, REMOTE PROCEDURES, AND RETURNING PARTICIPANTS.

## 8.1 Re-screens

A participant may be re-screened once after an initial screen failure. The re-screen should occur at least 3 months after the original screen failure date. Exceptions are allowed at the discretion of the Site PI.

## 8.2 Repeat Assessments

In the event of unforeseen circumstances or if the data quality is unsatisfactory, participants may be asked to return to the clinic for repeat assessments. At the discretion of the Site PI, data collection may be repeated. Please contact the ATRI Coordinating Center for guidance regarding repeat assessments.

## 8.3 Incomplete Visit Procedures and Protocol Deviations

All attempts should be made to complete visit procedures as outlined in Appendix 1 within the allowable visit window period. On occasion, a participant might not be able to complete some of the required procedures (i.e., too impaired to complete the full cognitive assessment battery, unable to tolerate the full 60 minute MRI scan protocol, etc.) If a participant is unable to complete the full visit procedure as outlined in Appendix 1, the study team will document the reason for non-completion in the source documents. Participants will be followed as long as they are willing, and every attempt should be made to retain participants for longitudinal follow-up as long as possible. If participants are not willing or able to complete the full schedule of assessments at any visit, those assessments or procedures they are willing to complete should be conducted. It should be emphasized that the major priority

is to keep participants in the study, even if data collection is limited. If participants are no longer willing or able to travel to the clinic for annual visits, as much information should be collected via telephone as long as is possible, see section 8.7. Unless a participant has clearly declined brain autopsy, the neuropathology program should be discussed at each visit. Whenever a study procedure cannot be completed, sites will properly document the reason and review with the study team or IU IRB to determine if a protocol deviation is required.

A protocol deviation is an alteration/modification to the IRB-approved protocol that is not approved by the IRB prior to its initiation or implementation. The IRB-approved protocol includes the detailed protocol, informed consent document(s), recruitment materials, and any other information relating to the research study. A *minor* protocol deviation does not impact participant safety and/or affect the integrity of study data. Examples: Failure to follow the approved study procedure that, in the opinion of the Site PI, does not affect participant safety or data integrity (e.g., study procedure conducted out of sequence; omitting an approved portion of the protocol; missing lab results). A *major* protocol deviation is a deviation to the IRB-approved protocol that may place subjects at greater risk of harm, cause actual harm to subjects or others, or compromise the integrity of study data such that the subject's data can no longer be used in analysis of study outcomes. Examples: Enrollment of a participant who did not meet all inclusion/exclusion criteria; performing a study procedure not approved by the IRB; study compound dispensing or dosing error; failure to perform a required lab test or any other events that, in the opinion of the Site PI or Study PIs, may affect subject safety and/or data integrity. For U.S. sites, if a protocol deviation is major, or prompt reportable, it needs to be reported to the IU IRB right away or within 5 days of becoming aware of the event. See the IU HRPP Policy on reportable events here: https://research.iu.edu/compliance/human-subjects/guidance/reportable.html. See the relevant study manuals for more information about reporting expectations and mechanisms.

NOTE: Contact IU, ATRI Regulatory Affairs, or the IU IRB with questions regarding protocol deviations.

## 8.4 Early Termination Visit

If a participant wishes to exit the study, an early termination visit will be scheduled (if the participant is willing). This should include as many evaluations as possible, with the exception of PET imaging (see Appendix 1 for visit procedure details). Please contact ATRI Coordinating Center for guidance on what specific procedures should be conducted at an early termination visit.

## 8.5 Discontinuation

Participants with known pathogenic mutation who have opted to receive results will be discontinued from the study after genetic counseling. A referral to research studies focused on familial neurodegenerative disorders, such as the DIAN study will be provided.

## 8.6 Participant Re-Contact after Study Withdraw

If a participant wishes to withdraw from the study, the participant will be asked to provide consent for the LEADS study team to contact them to request additional information or biospecimens if needed. If the participant is enrolled in brain donation, this re-contact will

include annual phone checks after completion of the LEADS study visits until death of the participant.

### 8.7 Remote Procedures

Approved visit procedures depicted within the Schedule of Events (Appendix 1) can occur remotely any time it is not possible to conduct the visit in-person due to participant barriers. Remote activities can be conducted via telephone or video bridge. Neuropsychological batteries must be completed via video bridge following the virtual testing procedures manual. Examples of obstacles preventing participants from coming in-person to their visits may include disease progression, or inability to travel for any reason. When possible, participants should still come in-person to complete blood draw, LP, MRI and PET scans as applicable. A combination of remote and in-person data collection is allowed for the same visit. The study team will reassess participant ability to attend in person visits annually. If participants can resume in person visits, they will do so. If not, they will continue remote visit procedures. Prior approval from the IU team is required to conduct fully remote visits and confirmation of method of remote data collection, i.e. via telephone or video must be approved by IU and local regulatory body.

### 8.8 LEADS Participants Returning After Therapeutic Trial (Returning Participants (RPs))

RPs may return to LEADS after completing their participation on a therapeutic trial if they were enrolled in LEADS prior to entering the therapeutic trial and completed a LEADS baseline visit. Upon their return to LEADS, RPs will be reconsented to the study. Once reconsented, RPs will resume their study activities based on last completed study visit, continue to follow schedule of events, and be asked questions regarding their trial involvement. RPs will complete any tasks that they're willing and able to complete. If there are missed activities, coordinating center review will determine if a protocol deviation is required.

## 9.0 STUDY COMPOUNDS

### 9.1 Florbetaben

Florbetaben will be provided by Life Molecular Imaging Ltd. One mL (1 mL) of the solution for each injection/vial contains 300 MBq of florbetaben. The other ingredients are ascorbic acid, ethanol anhydrous, macrogol 400, sodium ascorbate, and water for injections.

Florbetaben will be prepared at the site's contracted radiopharmacy and delivered to each site's PET facility by courier on the day of administration. The dose will be received by a trained nuclear medicine technologist at each site. The dose will be administered in its entirety according to the Schedule of Events (Appendix 1). Florbetaben dose of 8 mCi +/- 10% will be used in this study. Post administration, the used syringe will be placed in the sharps disposal container for radioactive materials. If a dose is not administered, it will be destroyed and placed in the sharps disposal container for radioactive materials.

### 9.2 C-Pittsburgh Compound B (11C-PiB) (for International sites only)

C-Pittsburgh Compound B, also known as PiB, will be made available to select sites on the day of administration.

The dose will be administered in its entirety according to the Schedule of Events (Appendix 1). PiB dose of 10 mCi (370 MBq) +/- 10% will be used in this study. Post administration, the used syringe will be placed in the sharps disposal container for radioactive materials. If a dose is not administered, it will be destroyed and placed in the sharps disposal container for radioactive materials.

### 9.3 [18F]NAV-4694 (for international sites only)

[18F]NAV-4694, also known as NAV, will be made available to select sites on the day of administration.

The dose will be administered in its entirety according to the Schedule of Events (Appendix 1). NAV-4694 dose of 8.1 mCi (300 MBq) +/- 10% will be used in this study. Post administration, the used syringe will be placed in the sharps disposal container for radioactive materials. If a dose is not administered, it will be destroyed and placed in the sharps disposal container for radioactive materials.

### 9.4 Flutemetamol (for international sites only)

Flutemetamol will be manufactured on site, at select international sites only.

The dose will be administered in its entirety according to the Schedule of Events (Appendix 1). Flutemetamol dose of 5mCi (185MBq) +/- 10% will be used in this study. Post administration, the used syringe will be placed in the sharps disposal container for radioactive materials. If a dose is not administered, it will be destroyed and placed in the sharps disposal container for radioactive materials.

### 9.5 Flortaucipir

Flortaucipir also known as [$^{18}$F]AV-1451, or Tauvid, will be provided by Avid Radiopharmaceuticals, Inc. (Avid) or PETNET Solutions, Inc. Avid and PETNET will provide oversight of the dose deliveries from contract manufacturing organizations/radiopharmacies.

Flortaucipir will be utilized in accordance with the Avid Investigator's Brochure: "Flortaucipir ($^{18}$F; 18F-AV-1451 ([F-18]T807) Injection for Brain Tau Imaging" for more information.

Tauvid will be utilized in accordance with the Tauvid Package Insert.

Flortaucipir will be prepared at the site's contracted radiopharmacy and delivered to each site's PET facility by courier on the day of administration following the standard procedures of acceptance and disposal of radioactive tracers. The dose will be received by a trained nuclear medicine technologist at each site. Some sites will manufacture their own batches of AV-1451 Injection, in accordance with a material transfer agreement with Avid and prior agreement in their site's contract with IU. One dose, 10mCi (370MBq)+/- 10%, will be administered intravenously to each participant. The dose will be administered in its entirety according to the Schedule of Events (Appendix 1). Post administration, the used syringe will be placed in the sharps disposal container for radioactive materials. If a dose is not

administered, it will be destroyed and placed in the sharps disposal container for radioactive materials.

### 9.6 FDG

FDG will be prepared at the site's contracted radiopharmacy and delivered to each site's PET facility by courier on the day of administration following the standard procedures of acceptance and disposal of radioactive tracers. The dose will be received by a trained nuclear medicine technologist at each site. One dose, 5 mCi (185MBq) +/- 10%, will be administered intravenously to each participant. The dose will be administered in its entirety according to the Schedule of Events (Appendix 1). Post administration, the used syringe will be placed in the sharps disposal container for radioactive materials. If a dose is not administered, it will be destroyed and placed in the sharps disposal container for radioactive materials.

## 10.0 SIDE EFFECTS

### 10.1 Lumbar Puncture

The most common complications of LP consist of post-LP back pain and post-LP headache (PLPH). PLPH typically begins within three days after the procedure in most participants. If a participant develops typical PLPH, bed rest, adequate hydration, and simple analgesics should be started.

In a large multicenter LP study, 31% of participants reported post-LP complaints; however, these were mostly mild in nature. Severe complications were very rare [60].
Common side effects included:

- Back pain (17%)
- Headache (19%)
- Typical post-LP headache (PLPH) (9%)

Lower rates of post-LP headache were noted when atraumatic (Sprotte) needles were used. [61, 62] Sprotte needles will be used in this study.

Very rare (prevalence of <0.01%) but potential serious complications consist of post-LP infections, spinal and subdural cerebral hematoma, and cerebral venous thrombosis. In an effort to mitigate these risks, a trained clinician must perform the LP.

### 10.2 PET Imaging

#### 10.2.1. Radiation Exposure

The primary risk related to PET is that of radiation exposure associated with the injected radiotracers and accompanying CT (if a PET/CT scanner is used). There is also minor risk associated with the venipuncture, placement of an intravenous catheter, and radioisotope injection (pain and bruising or painful infiltration of a failed injection).

The radiation doses for each PET scan are not themselves expected to produce any harmful effects, although there is no known minimum level of radiation exposure considered to be

totally free of the risk of causing genetic defects or cancer. The risk associated with the amount of radiation exposure participants receive in this study is considered low and comparable to everyday risks. If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed. Participants who may have other sources of radiation exposure (thallium testing, radiation therapy) should be evaluated by the study physician.

There must be at least 365 days between the first PET scan of the previous visit and the first PET scan of the next yearly visit. There must also be at least 365 days from second-to-second PET, so that there are no more than two PET scans in a year. FDG PET is not subject to this consideration; it can be the third PET in 365 days.

### 10.2.2 Other Side Effects Associated with Amyloid Tracers

Specific risk information can be found in the tracer's Package Insert or Investigator's Brochure.

### 10.2.3 Other Side Effects Associated with Flortaucipir

A review of the safety information for flortaucipir showed that the product is generally well-tolerated with a low incidence of mild and transient adverse events. More information about the known and expected benefits, risks, and reasonably anticipated adverse events (AEs) of flortaucipir may be found in the Investigator's Brochure (IB) and the Tauvid Package Insert. Details on the clinical information to date regarding flortaucipir exposure and risks will be provided to participants in the informed consent form (ICF).

## 10.3 Psychological Risks from Genetic Testing

There may be a psychological impact from receiving genetic test results. Identifying a pathogenic mutation can solidify a participant's diagnosis and increases the likelihood that close relatives of the participant will also develop a similar condition. It is also possible that genetic testing may reveal incorrect assumptions in family relationships (such as learning that a child is adopted or has a different father). These risks will be discussed with the participant either in person with a genetic counselor or with a video that is viewed by the participant and anyone who accompanies them to the visit (study partner, caregiver, family members, etc.). Participants viewing the video will have the opportunity to speak with a genetic counselor if they have additional questions. Any pathogenic mutations identified in this testing will also be communicated to the participant in a genetic counseling session. Participants at international sites may speak with the site PI or appropriately trained clinical investigator.

## 10.4 Psychological Risks from PET Results

It is possible that some participants may be upset by learning the results of their amyloid, tau, or FDG PET scan. Depending on the results, a participant may learn they are at increased or decreased likelihood that AD is causing their symptoms. It is possible that both positive and negative results could be psychologically upsetting. However, in a recent randomized clinical trial, there was no deleterious effect of learning amyloid status on depression or anxiety scales [63]. The Imaging Dementia – Evidence for Amyloid Scanning (IDEAS) study, led by Dr. Rabinovici, has scanned over 18,000 cognitively impaired

patients with amyloid PET. The study is monitoring all known deaths following amyloid PET disclosure, and thus far no suicides have been reported. The disclosure of results from a tau scan has not been rigorously studied; however, this study will monitor data collected throughout the disclosure process for any significant effects a participant may exhibit.

### 10.5 Loss of Privacy

In this study, a great deal of information about participant health status is collected. Study staff at the clinic sites will be collecting personal protected health information such as name, date of birth, social security number, address, phone number, and emails. All participants will be given a participant code number and all data will be associated with the code number. The clinic site will maintain the personal protected health information (such as name, date of birth, social security number, address, phone number, and emails) in a secure and locked location. The data, associated with the code number, will be distributed widely, but it will not be possible to identify an individual participant from the data. However, there is a very unlikely possibility that there will be a security failure, and that somehow the protected health information will be no longer protected. This is an extremely unlikely but possible occurrence and is a risk of this study.

## 11.0 ADVERSE EVENTS/UNANTICIPATED PROBLEMS

An adverse event (AE) is defined as any untoward medical occurrence. Adverse events, including but not limited to those deemed related to the study compounds or procedures by the Site PI will be tracked during the study.

The following events are considered AEs:

(1) worsening or change in nature, severity, or frequency of conditions or symptoms present at the start of the study
(2) participant deterioration due to primary illness
(3) intercurrent illness
(4) drug interaction

An abnormal laboratory result, imaging finding, and change to baseline medical conditions will only be reported as an AE if the Site PI or medical designee considers it to be clinically significant.

The Site PI is obliged to follow participants with AEs until the events have subsided, the conditions are considered medically stable, or the participants are no longer available for follow-up. Participants who discontinue due to adverse experiences will be treated and followed according to established medical practice. All pertinent information will be entered into the eCRF.

The Site PI should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs or symptoms. Symptoms and conditions present at the beginning of the study will be characterized, so that AEs can be defined as any new symptom, or any increase in frequency or severity of an existing symptom. Adverse events should be described with medical terminology so that the event can be matched against a

medical coding dictionary, such as Medical Dictionary for Regulatory Activities (MedDRA).

Site PIs should report their assessment of the potential relatedness of each AE to the protocol procedure(s). Following questioning and evaluation, all AEs, whether determined to be related or unrelated to the protocol procedure(s) by a medically qualified Site PI or medical designee must be documented in the participant's records, in accordance with the Site PI's normal clinical practice, and on the AE eCRF.

For more detail, refer to the Code of Federal Regulation Title 21 Part 312:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32

ATRI Coordinating Center staff will monitor the unanticipated problems for overall safety and scientific relevance on an ongoing basis and provide information to the Data Safety Monitoring Board.

## 12.0 SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event is defined as an adverse event or suspected adverse reaction that results in any of the following outcomes:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalization or prolongation of existing hospitalization (see below for more information regarding hospitalization)
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Hospitalizations that fulfill one of the following conditions will not have to be reported as SAE:

- Admission for treatment of a pre-existing condition that is not associated with the development of a new AE or with a worsening of the pre-existing condition (i.e., work-up for persistent lab abnormality that occurred prior to the study)
- Social admission (i.e., participant has no place to sleep)
- Administrative admission (i.e., yearly physical exam)
- Protocol-specified admission (i.e., for a procedure required by the study protocol)
- Optional admission not associated with a precipitating clinical AE (i.e., pre-planned treatments, elective cosmetic surgery)

For more detail, refer to the Code of Federal Regulation Title 21 Part 312:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32

### 12.1 Reporting of SAEs

Serious adverse events will be reported to the Project Director/IU **and** the ATRI Coordinating Center within **24 hours of learning of the event**. See relevant study manuals for SAE reporting and expectations. The principal Site PIs and Coordinating Center staff will monitor the study procedures for overall safety and scientific relevance on an ongoing basis and provide information to the Data Safety Monitoring Board.

## 13.0 SITE QUALIFICATIONS

Participants will be enrolled at approximately 25 clinical sites in the United States and abroad. All of these sites have significant experience with diagnosing and treating patients with EOAD and have agreed to participate in the study.

### 13.1 Study Personnel

The Site PI is responsible for the overall conduct of the study at the site. The Site PI is to supervise project personnel and ensure that clinical raters are trained and maintain a high level of skill and accuracy in conducting assessments. Additionally, the Site PI, to the extent possible, will personally perform or supervise clinical evaluation of all participants and ensure protocol adherence.

### 13.2 MRI Instrument

Each site must be qualified for MRI using the ADNI3 MRI protocol. If the site is planning to use a scanner that has already been ADNI3 qualified, the qualification process will be expedited by requesting that the site conducts only phantom and not human qualification (see below). Site scanners that are not ADNI3 qualified will need to undergo the full site qualification protocol.

The procedures for MRI scanner qualification consist of two parts, phantom and human scanning. In terms of human scanning, each site will image a volunteer participant with the protocol and send the images to LONI. The MRI Core will check each parameter in each of the pulse sequences in the protocol. In the event that the scan has not been performed according to protocol, the site will be asked to perform another human volunteer scan. This will be repeated as many times as necessary until the site has demonstrated exact execution of the MR protocol in a volunteer participant, at which point they will have passed the human scanning portion of MR site qualification. The volunteers do not need to be elderly controls; in fact, scanning for site qualification may be more easily performed with normal younger volunteers. In the event that repeat attempts are needed, repeat scans need not be on the same volunteer participant. Once a site has demonstrated perfect execution of the protocol, the protocol will be stored permanently on the scanner at that site that will be used in the study.

### 13.3 PET Instrument

Each site must be qualified for PET. If the PET scanner being used has already been certified by the ADNI PET Core and has not experienced any major software or hardware upgrades, re-qualification will not be required. The PET scanner must be able to perform site's applicable amyloid PET, tau PET, and FDG PET imaging protocols. Each scanner requires qualification only once for each tracer. Qualification will employ the same methods utilized for site qualification in ADNI3.

Sites will use a Hoffman brain phantom (if scanner not yet qualified for ADNI3) and a technical manual for the data acquisition using the PET tracers in the LEADS protocol and site's contract. The phantom must be scanned on two sequential days using the protocol identical to that required for human imaging. This enables the PET Core to ascertain the characteristics of the scanner (particularly resolution and uniformity) and assure that sites are capable of performing the protocol for acquisition and image reconstruction. All phantom images will be forwarded to PET Core QC group for review and qualification.

For all PET scans, either a PET transmission (PET-only scanners) or x-ray CT (PET/CT scanners) will be obtained for attenuation correction. PET/MRI scanners will not be qualified to perform PET scans in LEADS.

# 14.0 DATA COLLECTION

## 14.1 Data Summary

The LEADS database will consist of data collected from three participant cohorts (EOAD, EOnonAD, and CN).

The data collected in this study will be compared to ADNI LOAD participants (data freely available through the ADNI data sharing agreements and regulations). The LEADS data will include demographic information, clinical test results, MRI summary measures, amyloid, tau, and FDG PET summary measures, blood biomarker and selected genomic data. The actual imaging files will be stored at LONI. The genetic data will be stored in two locations, NCRAD and LONI. The cognitive tests will capture changes in the following cognitive domains – processing speed/attention, episodic memory, language, visuo-spatial, working memory/executive, and global cognition. Results will be obtained from several separate instruments within each category, which will be used to build domain-specific composite scores. MRI summary measures derived using Freesurfer will include estimates of subcortical gray matter (GM) volumes, cortical GM morphometrics (volume, thickness, surface area, and curvature measures), total hippocampal volume and hippocampal subfield measures. Amyloid and tau PET summary metrics (SUVR values) will be extracted after normalization to whole cerebellum or cerebellum gray matter regions to quantify amyloid and tau PET data, respectively. Fluid biomarker measurements will be included in the database.

## 14.2 Case Report Form

The Site PI or designee will record all original source data collected (either written or electronic record of data). Written or electronic data of record must be entered on the electronic Case Report Form (eCRF) provided for that purpose. The site will be suitably trained on the use of the eCRF and appropriate site personnel will be authorized to provide electronic signatures. The Site PI is responsible to verify the integrity of the data and acknowledge as such by signature.

All site entries will be made in a secured web site and the Site PI will review the record for completeness. If corrections are necessary to the eCRFs, the Site PI or designee will update the eCRF and provide the reason for change.

# 15.0 DATA ENTRY AND STORAGE

## 15.1 Clinical Data Storage and Sharing

The official clinical data repository will be housed in the Informatics Core in LONI. This database will be frequently updated. Standard LEADS data acquired by the ATRI Coordinating Center and NACC will be provided to LONI. Only minimally necessary protected health information (PHI) and clinical data required for data analysis will be included in the LEADS database, and Site PIs will take reasonable steps to limit the use or disclosure of, and requests for, protected health information.

There is a slight risk that there could be a breach in the security of the database system resulting in the access of information. However, safeguards are in place to minimize this risk. All participants will be assigned a participant ID code, that will be used for all data storage and communication with sites. Protected Health Information (PHI) will be recorded and kept under the need to know principle (i.e. only when necessary) at the enrolling site. The data key linking the participant personal information and participant study code numbers will only be available to a limited number of authorized study staff at the enrolling sites. The ATRI Coordinating Center, NACC and LONI will not have access to these keys. Hard copies of data will be stored in locked file cabinets at the study sites, while electronic data will be password protected and maintained on a secure network. PHI that the study team at ATRI and NACC UDS have access to in the EDC system will be limited to the minimum necessary for authorized oversight of the research study.

### 15.1.1 National Alzheimer's Disease Coordinating Center (NACC) Database

NACC was selected to leverage their pre-existing infrastructure for data collection. The NACC Uniform Data Set (UDS) and Frontotemporal Lobar Degeneration (FTLD) module protocols will be utilized in LEADS. The electronic data capture through NACC's available infrastructure is already available for ADC and non-ADC studies. Please note that a complete NACC UDS packet is required for submission to the NACC Data Repository. Partial visit procedure packets will not be accepted by NACC. There are some NACC forms required for LEADS that are not explicitly required for NACC. These include the A3, B5-B7, and C4F-C6F.

### 15.1.2 Unique LEADS data

Completed LEADS database eCRFs will be submitted to the LEADS Data Portal according to ATRI Coordinating Center instructions. All personal identifying data will be kept in a secure location at the enrolling site.

If necessary, data correction requests will be generated for resolution by the study site. Data will be transmitted securely via the Internet to ATRI at USC by enrolling sites. Database access will be granted to study team members based on role. Each user of the system has an individual account with a password which is required to be reset at set intervals to comply with USC password requirements. Users will be logged out of the system after a period of

inactivity. All communication to and from the data system will be encrypted. Data transmission will occur through a secure internet connection-https (hypertext transfer protocol secured) at 128 bit SSL. The ATRI Coordinating Center will provide web-based reporting on data flow and assure optimal data security and redundant data backups.

Unique LEADS data will be stored and maintained on servers hosted on Amazon Web Services under an Enterprise Agreement with USC. All communication with the servers is encrypted. Access is controlled on a per-user basis and access logs are kept and monitored on an ongoing basis to ensure data security and integrity, keeping data protected from improper use and disclosure.

### 15.2 MRI and PET Imaging Data Storage

LONI at the University of Southern California (USC) was selected to leverage the pre-existing infrastructure for imaging and clinical data storage. MRI and PET data will be transmitted by enrolling sites directly to LONI. All scans will be labeled using LEADS participant identifiers and scanner specific series descriptions. All scans will undergo a de-identification process, which is embedded within the LONI upload process to ensure that no direct participant identifier information is present in the image files.

- **MRI Images:** Images will be uploaded by sites directly to LONI. PHI will be limited to the minimum necessary for authorized oversight and placed into quarantine until they pass quality assurance evaluation conducted by the MRI Core. The MRI Core will perform a quality control review on each MRI scan. Quality control for MRI will result in failure of some scans, which may need to be repeated. Repeat scans must be scheduled as soon as possible and no later than four weeks of the visit date. Repeat scans will not be considered protocol deviations.

- **PET Images:** Images are uploaded by site users to LONI. PHI will be limited to the minimum necessary for authorized oversight and placed into quarantine until they pass quality assurance evaluation conducted by the PET Core. The aim of this work is not only to make sure that all PET scans are acquired and reconstructed using the appropriate protocols and that image quality is good, but to standardize the images from the different sites (and hence the different PET scanner vendors and models) as much as possible in order to reduce inter-site differences. Quality control of scans could necessitate salvage with reprocessing of the raw imaging data. All sites are required to save original PET data for the duration of the study.

### 15.3 Biospecimen Storage – NCRAD and NIH databases

Samples including PBMCs, DNA, RNA, plasma, serum and CSF aliquots and their derivatives will be processed and stored indefinitely at NCRAD. PHI will be limited to the minimum necessary for authorized oversight. All samples will be stored in secure freezers within a secure facility at Indiana University. Since NCRAD is a NIH dedicated specimen repository designed for sample sharing, a general protocol has been approved by the IRB at Indiana University that covers all sample receipt, processing and distribution. The protection of participant confidentiality and the use of stored genetic specimens will be in accordance with the rules and procedures established by the Indiana University IRB. The protection of

participant confidentiality and the use of stored genetic specimens provided by international sites will comply with applicable local regulation, including GDPR.

NCRAD will maintain a secure database for tracking all incoming LEADS samples. Information that will be maintained in this database may include the LEADS unique participant identifier, kit number (assigned to all tubes that come in a single shipment for an individual), specimen number (barcode #), type of sample received, date drawn, date received, initial volume collected for each tube type, time of draw, year of birth and gender. Other data related to the processing of the specimens will also be recorded in the database.

Genomic and all other data can be linked to other de-identified clinical research data for purposes of scientific analyses. The only linkage of genetic test results to participant identity will be possible at the specific clinical site where they were enrolled.

### 15.4 Audio Recording Transcription and Storage

The audio files acquired in this study will be securely transmitted and stored in databases being used for the study. The audio recorded during the administration of select assessments should not contain PHI. Sites will be responsible for ensuring that no PHI is included in the recordings prior to upload. Audio files that are transmitted to contracted cloud-based remote servers for automatic transcription will not be stored on these servers and will be destroyed after the transcription is complete.

### 15.5 Global Unique Identifiers (GUIDs)

Global Unique Identifiers (GUIDs) for all LEADS participants will be acquired. Each GUID unambiguously identifies a research study participant across different research studies without exposing PHI. When investigators pool data together from multiple studies, GUIDs provide the means to detect participants who participate in more than one study.

## 16.0 DATA SHARING

Data from this research will be shared with other researchers pursuant to the 02/26/2003 *"NIH Final Statement on Sharing Research Data".* The NIH policy on data sharing can be found online at: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html. Data sharing is essential for further translation of research results into knowledge, products, and procedures to improve human health. The NIH endorses the sharing of final research data to serve these and other important scientific goals. To protect participants' rights and confidentiality, PHI will be limited to the minimum necessary for authorized oversight before the data are shared.

Instructions concerning how to access these data will be available on the LEADS website. Access to the LEADS study data will be facilitated in collaboration with Global Alzheimer's Association Interactive Network (GAAIN) run by LONI. As a GAAIN Data Partner, data from this study will be shared in aggregate through the GAAIN portal (gaain.org). GAAIN provides a global infrastructure for cooperative research by linking data repositories that have collected information from thousands of participants who are at risk for or have been diagnosed with Alzheimer's disease. Working with the GAAIN technical team, client software will be used to respond to the GAAIN server data requests. The data connection

will be updated to allow global researchers to visualize the available data, and in aggregate to evaluate the LEADS data in context of the 30+ other studies and nearly 500,000 clinical participants. GAAIN will allow for the global scientific community to visualize the metadata available through the LEADS study, while allowing the LEADS scientific team the opportunity to control access to the raw data from GAAIN-directed and other potential users.

Metadata will be accessible through GAAIN as described above. The actual data files will be available for download from the LEADS Image and Data Archive overseen by the Informatics Core Leader. LONI has developed Data sharing environment for multiple other studies including but not limited to ADNI, Neuroimaging in Frontotemporal Dementia (NIFD), the 4-repeat Tau Neuroimaging Initiative (4RTNI), and the Parkinson's Progression Markers initiatives (PPMI). MRI and PET data collected in the LEADS study will also be available for download from the LEADS Image and Data Archive (IDA) website. Both raw and processed scans will be available for download.

Genetics, genomics, and related data will be shared with other researchers pursuant to the NIA Alzheimer's Disease Genetics sharing Policy: http://www.nia.nih.gov/research/dn/alzheimers-disease-genetics-sharing-plan.
National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS), along with other NIA-approved sites, will make genetic, genomic and related data and associated phenotypic data available to qualified investigators in the scientific community for secondary analysis in accordance with standards established by NIA. These data repositories are under strict security provisions, including multiple firewalls, separate servers, and data encryption protocols. Investigators and their sponsoring institutions seeking access to data from the NIA-approved data repository must submit a data access request that specifies both the data to which access is sought and the planned research use, and agree to the terms of access set forth in the Data Use Certification. Investigators are approved by a Data Access Committee for access to specific datasets for a specific use(s). In addition, the Data Use Certifications include a provision that approved users and their institutions agree to store the requested data securely and to not share the requested data with third parties.

# 17.0 REQUESTS FOR LEADS DATA

In order to receive access to the LEADS data, investigators will be asked to provide a written request via a form available through the LEADS website (www.leads-study.org), which includes information about the identity of the investigator(s), data requested and plans for data analysis. Applications for data use will be reviewed by the LEADS Data Sharing and Publications Committee and as outlined in the LEADS Data Sharing and Publication Policy protocol.

## 17.1 Biospecimen Data Requests

Investigators and their sponsoring institutions seeking access to specimens from NCRAD must submit a sample request that specifies both the samples to which access is sought and the planned research use. Investigators will be approved by the LEADS Data Sharing Committee and when applicable, the NCRAD Biospecimen Review Committee for access to

specific samples for a specific use(s). All samples will be distributed by NCRAD with a Material Transfer Agreement (MTA). The MTA will include a provision that approved recipients and their institutions agree to store the requested samples securely and to not share the samples with third parties. Investigators must also agree to upload the results of their experimental analyses into a NIA-approved data repository.

### 17.2 Terms & Obligations of Data Usage

Acceptance of LEADS data obligates the recipient to reference the grant in any presentation or publication that may result from this research.

Should publications result from the use of LEADS resources now or in the future, the recipient agrees to notify the LEADS Administrative Core with details (reference, PubMed and PubMedCentral ID#) and provide a copy of the publication so productivity derived from the LEADS resources can be reported to the funding agency (the NIA). Such publications require compliance with NIH public access policies and LEADS data sharing/publication policies.

Should new funding result from research using LEADS data now or in the future, the recipient will be required to notify the LEADS Administrative Core within 30 days of the award and provide details (grant title, sponsor, number, dollar total, dates) so productivity derived from the LEADS resources can be reported to NIA.

No sharing of data with a third party is allowed without written permission from the LEADS Administrative Core.

## 18.0 DATA AND SAFETY MONITORING BOARD (DSMB)

The Data Safety Monitoring Board (DSMB) is an independent group providing recommendations to the LEADS study leadership, and the NIA. The DSMB will be responsible for monitoring enrollment, participant progress, drop-out rates, ongoing conduct of the research, protocol deviations, and safety monitoring (review of AEs and safety mailings (if applicable). The DSMB members can ask questions and make comments and/or recommendations to the Site PIs. Data on study progress and safety will be reviewed by the Board at least semi-annually and more frequently if needed. Based on the review of these data, the DSMB will provide recommendations to continue, modify or terminate the study, and will communicate other recommendations or concerns as appropriate. The DSMB chair will communicate recommendations or findings by way of a DSMB letter that is issued after each meeting of the board. These letters are provided to the Project Director and the ATRI Coordinating Center for submission to the IRB based on IRB reporting requirements.

Any reportable events will be immediately directed to the ATRI Coordinating Center to follow the reporting procedures for the Project Director and IRB (see section 12.1 for SAE reporting information).

# 19.0 STUDY MONITORING

Ongoing study monitoring at U.S. sites will be completed by the ATRI coordinating center. Ongoing study monitoring for international sites will be completed by IU, in close collaboration with the ATRI coordinating center. The LEADS clinical monitors will be responsible for inspecting the electronic case report forms and source documentation at regular intervals at each participating site throughout the study to verify adherence to the protocol, completeness and accuracy of the data, and adherence to local regulations on the conduct of clinical research. The monitoring visits must be conducted according to the applicable ICH, GCP, and local guidelines to ensure protocol adherence, quality of data, study compound accountability, compliance with regulatory requirements and continued adequacy of the investigational site and its facilities. The Site PI will cooperate in the monitoring process by ensuring the availability of the eCRFs, source documents and other necessary documents at the time of the monitoring visits. The Site PI will promptly address any matters brought to his/her attention by the monitor. The Site PI may also be asked to meet in-person with the site monitor.

# 20.0 ETHICS AND REGULATORY CONSIDERATIONS

## 20.1 Good Clinical Practice

This study will be conducted in compliance with the protocol, in accordance with GCP guidelines, and in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46 – Protection of Human Subjects, 21 CFR Part 50 – Protection of Human Subjects, 21 CFR Part 56 - IRBs, and/or the ICH E6, HIPAA, State and Federal regulations and all other applicable local regulatory requirements and laws.

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

No study document shall be destroyed without prior written agreement between the Coordinating Center and the Site PI. Should the Site PI wish to assign study records to another party or move them to another location, he/she may do so only with the prior written consent of the Coordinating Center.

Institutions must hold a current US Federal-Wide Assurance (FWA) issued by OHRP to participate. Refer to: http://www.hhs.gov/ohrp/assurances/.

## 20.2 Informed Consent

Informed consent will be obtained in accordance with 45 CFR 46, 21 CFR 50, 21 CFR Part 56 and in adherence to ICH GCP. Informed consent and HIPAA authorization for research will be obtained from all participants, their legally authorized representative (LAR), or their court-appointed guardian prior to starting study procedures.

The participant's capacity to consent will be assessed whenever consent is collected. Guidelines for assessing capacity to consent will be left at the local level to adhere to institutional-specific standard operating procedures (SOPs). Clinical monitors will review

that capacity to consent was assessed during their interim monitoring visits against the local SOPs. The relying sites' plan for assessing participants' capacity to consent is to be provided to the reviewing IRB through completion of a relying site form. If the local process for assessing capacity to consent changes at the relying site during the study, the relying site must submit an amendment with an updated site form to the reviewing IRB along with the corresponding SOP, as applicable.

### 20.3 Confidentiality and HIPAA/GDPR Compliance

Participant confidentiality is strictly held in trust by the Site PIs and study personnel. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Some of the data collected as part of this protocol is of potential medical relevance including cognitive and neuropsychological testing, brain MRI, PET, and genetic testing. These are all tests that can be obtained as part of routine medical care for patients with symptoms suspected as being due to Alzheimer's disease, and are being collected in this study using clinically standard methods. The results of these tests may be disclosed to our study participants and their study partners.

Due to the clinical relevance, the MRI report (or summary of the findings), summary of the cognitive testing, clinical diagnosis and summary of PET results (CI cohorts only) can be placed in medical records by the study team or the participant. Participants will be informed of this in the informed consent. Since genetic testing results are sensitive information, participants will be explicitly asked for permission to place genetic results from LEADS in their medical record. In the rare occasion where the institutional policies require that all genetic testing results are part of the medical record, the participant will be informed and have the option to opt out of participation in LEADS.

Authorized representatives of the sponsoring institution may inspect all research documents and records required to be maintained by the Site PI, as well as medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The enrolling study site will permit access to such records. Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB/IEC, the FDA, the NIA, and the OHRP.

Information about U.S. study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed participant HIPAA Authorization informing the participant of the following:

- What PHI will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research participant to revoke their authorization for use of their PHI.

In the event that a U.S. participant revokes authorization to collect or use PHI, the Site PI, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. Each Site PI, under the guidance of the IRB, is responsible for

ensuring that all applicable HIPAA regulations and State laws are met. International Site PIs, under the guidance of their IRB are responsible for ensuring that all applicable local regulations are met.

Information about international study participants will be kept confidential and managed according to the requirements of the General Data Protection Regulation (GDPR), or their local regulations.

In the event that an international participant revokes authorization to collect or use PHI, the Site PI, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. Each international Site PI, under the guidance of their local regulatory body is responsible for ensuring that all applicable GDPR regulations and/or local laws are met.

### 20.4 Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality has been automatically issued by the NIH to protect identifiable research information from forced disclosure. It allows the Site PI and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

## 21.0 STATISTICAL CONSIDERATIONS

### 21.1 Analysis Goals and Strategies

The Biostatistics Core will conduct interim and final analyses of the clinical, imaging and genomic data for the hypotheses and aims of the study. The goals of the analyses are briefly described below.

#### 21.1.2 Statistical analysis

The analysis of the data will focus on comparing the cognitive and biomarker measures between LEADS EOAD and LEADS CN and LEADS EOAD and ADNI LOAD participants. We will also compare *APOE4*+ and non-carriers within and between diagnostic groups (EOAD, CN, and LOAD). We will study the associations between our cognitive composite scores and imaging and other biomarkers. Longitudinal analyses will be conducted to identify differences of longitudinal changes in cognitive, imaging and fluid biomarker measures between LEADS EOAD and ADNI LOAD participants and between *APOE4*+ and non-carriers within and between diagnostic groups (EOAD and LOAD). Finally, we will perform exploratory analysis of EOnonAD patient data.

##### *Aim 1*

Baseline composite cognitive scores will be developed using clustering techniques such as kNN clustering. The cognitive data will be collected in integer scales for each test. Differences of the composite scores within cognitive domains between

LEADS EOAD and ADNI LOAD participants will be assessed using multivariate linear regression controlling for the effects of candidate confounders such as education and sex. The results will be presented using graphical methods and tables of statistical summary estimates and test results. Longitudinal changes in cognitive scores will be assessed using linear mixed models and machine learning approaches.

### *Aim 2*

MRI and PET based biomarker volumes computed at the $mm^3$ scale, will be used to assess baseline and longitudinal associations of composite cognitive scores and imaging metrics and identify differences between LEADS EOAD and ADNI LOAD participants. Specifically, multiple linear regression analysis will be used to identify differences between imaging-based metrics in LEADS EOAD vs. ADNI LOAD at baseline and to assess correlations between composite cognitive and imaging measures, while longitudinal data will be analyzed using mixed effects models when data for more than 2 visits per participant is collected. Relevant confounders (e.g. sex, education) will be included as covariates. In addition, summary MRI and PET measures will be analyzed using machine learning techniques to identify a composite metric capturing change across LEADS EOAD participants.

### *Aim 3*

Memory sparing will be compared between *APOE4*+ and *APOE4*- participants using Fisher's exact tests to compare the proportion of *APOE4*+ and *APOE4*- participants between the LEADS participants in each atypical subtype of EOAD. Differences between MRI/PET measures of *APOE*+ and non-carriers will be assessed at baseline and longitudinally using multiple linear models and linear mixed models after controlling for potential confounders (e.g. education and sex). The results will be presented using graphical representations. The rates of APOE4+ and APOE4- participants will be compared between EOAD and EOnonAD participants will be compared using Fisher's exact tests.

### *Aim 4*

Research-based genetic screening will be performed in a research laboratory. Established pipelines will be used for processing and annotating sequencing variants. Known pathogenic mutations in the six screened genes will be confirmed by a CLIA-certified laboratory. Sequence data will be generated in a research laboratory and combined with publicly available sequence data from the Alzheimer Disease Sequencing Project (ADSP; phs000572.v1.p1). Gene-based case-control tests will be used to identify genes and pathways with a greater burden of rare variants in cognitively impaired as compared with CN individuals.

### *Aim 5*

t-tests, non-parametric tests, and Analysis of Variance (ANOVA) models will be used to compare clinical domain scores between CN and EOnonAD participants. Multiple linear regression models will be used to investigate whether FDG PET is more sensitive than MRI when identifying changes in neurodegeneration in EOnonAD cohort. Unsupervised clustering methods will be utilized to find groups of participants in the EOnonAD cohort with similar imaging and clinical characteristics and identify potential etiologies in EOnonAD.

### 21.1.3 Missing Data

Data collection will be monitored on an ongoing basis to ensure that the scores and imaging biomarkers are within the expected ranges and scales and missing data is rare. Multiple imputation will be used for missing data. Although we do not anticipate extensive missing data for a large proportion of participants in the study, participants with such extensive missing data (e.g. ≥50% cognitive scores) will be removed from analyses.

### 21.1.4 Sample Size

Sample size considerations were developed for each aim and are presented below. The computations of sample size assumed a target power of 90% at significance level of 0.05. A total sample size of 650 EOAD participants is envisioned for the study. The specific effect sizes for each of the aims are presented below. In general, these effect sizes are smaller or equal to those observed in our preliminary data.

#### *Aim 1*

We will have 90% power to identify a -0.26 group difference in mean composite cognitive Z-scores between EOAD and LOAD participants after controlling for episodic memory performance. We will have 90% power to detect an average difference of 0.7 in decline in CDR-SB between EOAD and ADNI LOAD. Similarly, we will have 90% power to identify an effect size of -1.5 for decline in MMSE.

#### *Aim 2*

We will have 90% power to detect cross-sectional differences in cortical gray matter volumes of at least -0.2. We will have 90% power to detect a cross-sectional difference of 0.2 $mm^3$ in flortaucipir SUVR. For longitudinal data, we will have 90% power to detect a difference of -0.001 in annualized Jacobian values between EOAD and LOAD in the region-of-interest. Preliminary longitudinal flortaucipir data in EOAD were too sparse to estimate effect size or power. We will have power of 90% to observe correlations of 0.1 or greater between MRI/ flortaucipir and clinical composite scores - these correlations are considerably lower than the smallest flortaucipir-cognitive correlations observed in our preliminary data.

#### *Aim 3*

Assuming ~50% *APOE4+,* we will have 90% power to detect a difference of 20% in the proportion of non-amnestic (lvPPA/PCA) participants in *APOE4+* vs. *APOE4-* participants. We will have 90% power to detect a cross-sectional mean difference in TIV-adjusted MTL volume of -4.4 ml between carriers and non-carriers and an average difference in flortaucipir MTL SUVR 0.1 between the two groups.

#### *Aim 4:*

When testing 171 genes in AD pathways, we have 80% power to detect genes contributing to disease risk having an odds ratio of 1.5-3.0 if we assume 50% of the variants retained following filtering contribute to disease risk/resilience (alpha = 2.9 x 10-4).

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# APPENDIX 1 SCHEDULE OF EVENTS

## All (EOAD, EOnonAD, and CN) Participants– Screening and Baseline

| Procedure \ Visit | Screening[a] | Baseline[a] | Genetics Status Disclosure |
|---|---|---|---|
| Window Period (days) | ≤ 60 | | See footnote **b** |
| Informed Consent[u] | X | | |
| Brain donation discussion/provisional consent[c,u] | | X | |
| Vital Signs | X | | |
| Neurological and Physical Exams | X | | |
| Demographics & Medical History[u] | X | | |
| Clinical Diagnosis and Clinical Symptom Assessment | X | | |
| Family History[u] | X | | |
| Early Developmental History Questionnaire[u] | | X | |
| Autoimmune History Questionnaire[u] | | X | |
| Sex and Reproductive Health Questionnaire[u] | | X | |
| Area Deprivation Index[u,w] | | X | |
| Concomitant Medications[u] | X | | |
| NACC UDS and FTLD Modules, and Neuropsychological Battery[d,u] | X[e,f] | | |
| MMSE[u] | X[e] | | |
| Digit Symbol | X[f] | | |
| Amsterdam IADL Questionnaire[u] | X[f] | | |
| ADAS-Cog13[u] | X[f] | | |
| Rey Auditory Verbal Learning Test (RAVLT)[u] | X[f] | | |
| TabCat: Flanker, Line Length, Line Orientation, and Match | | X | |
| CDR[u] | X | | |
| Pregnancy Test[g] | X | X | |
| 3T MRI/fMRI[i] | X[h] | | |
| Amyloid PET Scan | | X[j] | |
| Tau PET Scan[v] | | X[j] | |
| Adverse Event[u] | | X | |
| Genetic Counseling[o,t, u] | X[k] | | |
| Amyloid & Tau Status Disclosure[u,v] | | X[l] | |
| PET Scan: FDG[r] | | X[m] | |
| FDG Status Disclosure[u] | | X[ml] | |
| Wellness Check[n,u] | | X | |
| Study Participation Report[o] | | X | |
| Blood for Genetic testing[p] | | X | |
| Blood for AD blood biomarkersr[r], DNA, and RNA, PBMC[q] | | X | |



(continued on next page)

| | | | |
|---|---|---|---|
| LP Safety Labs (CBC, coagulation profile) | X | | |
| Lumbar Puncture (optional)[r,s] | | X | |

a) Visit procedures may be split over multiple days without constituting a protocol deviation.
b) Timing for result disclosure is not set and dependent on the availability of the genetic screening results.
c) Post mortem brain donation discussion to confirm consent/interest. If participant refuses to participate, no further inquiry should take place. Undecided participants will be approached at each study visit until they consent or refuse to participate.
d) The NACC UDS and FTLD modules include demographics, clinical, cognitive, functional, and behavioral assessments.
    - Version C2 the NACC UDS Neuropsychological Battery will be used in this study
    - UDS forms A1, A3, A5, B4-B9, C2, D1, and D2 are required for LEADS
    - UDS forms A5/D2, D1a, and D1b will take the place of A5, D2, and D1 if using UDSv4
    - FTLD forms B3F, B9F, C1F-C6F, E2F, and E3F are required for LEADS
e) The MMSE and MoCA cannot be administered on the same day. MMSE is required at screening to confirm CN eligibility. MMSE can be done anytime between screening and baseline for CI participants.
f) Can be conducted anytime between screening and baseline for CI participants if sufficient testing has been completed to meet NIA-AA criteria for MCI due to AD or probable AD. These activities must occur at Screening for CN participants.
g) If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed prior to amyloid, tau, and FDG PET. Only participants with a negative result will be eligible for PET scanning.
h) The first MRI scan should be done at screening unless a previously existing MRI within 12 months can be used to assess overall eligibility and safety prior to LP procedure. If a previous scan is not available, the study MRI must be completed at the Screening Visit to facilitate eligibility determination. A study MRI must be completed within the 60 day visit window regardless of a pre-existing MRI being used to assess study eligibility. See section 7.6.4 for more detail.
i) All attempts should be made to acquire the full 60 minute MRI protocol; however the MPRAGE and 3D FLAIR should be prioritized. The MPRAGE and 3D FLAIR are the mandatory sequences for enrollment. If needed, the other sequences may be skipped for any reason without constituting a protocol deviation. Note the reason for non-completion in the source documents.
j) The amyloid and tau PET scans can be conducted anytime between screening and baseline following MRI. Inclusion/exclusion must be confirmed prior to both scans. The amyloid and tau PET scans must be conducted at least 12 hours apart.
k) At the Screening Visit, EOAD and EOnonAD participants will view a video that will discuss the implications of mutations in known ADAD loci. The participant will also be asked if they would like to speak with a genetic counselor to verify their understanding of the implications of genetic testing and answer any questions the participant may have. These participants at international sites may meet with a genetic counselor or investigator with appropriate clinical training in genetic counseling as opposed to watching the video. Written informed consent will be obtained on all participants prior to genetic testing (see section 7.2 Genetic Counseling and Testing section for more detail). Disclosure will be performed as outlined in section 7.1.6.
l) Amyloid and tau disclosure is required for EOAD and EOnonAD participants only and should be conducted between screening and baseline. Amyloid disclosure should be conducted within the 60 day visit window. Tau disclosure may be conducted outside of the 60 day visit window due to the timing of the scan and availability of the PET read. FDG PET disclosure is required for EOnonAD participants only. FDG disclosure may be conducted outside of the 60 day visit window due to the timing of the scan and availability of the PET read.
m) The FDG PET scan at Baseline applies to CN and EOnonAD participants only. EOAD participants do not receive an FDG PET scan. The FDG PET scan for EOnonAD participants cannot be scheduled until after amyloid disclosure. For sites that have radiation safety committees (RSC), ability to conduct FDG PET scans will also be contingent on local RSC approval.
n) To be completed within 48 hours of lumbar puncture. Additionally, a telephone call will be made within two weeks post amyloid results disclosure. See section 7.1.4 for more detail.
o) May be provided to participants at the discretion of the PI. See section 7.1.8 for more detail.
p) Blood for genetic testing will be drawn for EOAD and EOnonAD participants only; CN participants will not have blood drawn for genetic testing.
q) PBMC samples must be collected and shipped the same day. Fasting is not required. PBMC sample collection is optional for international sites. If collected, international sites will store PBMC samples locally. See NCRAD Biospecimen, Collection, and Shipping manual for more details.
r) LP should be collected after a minimum 6-hour fast (preferably in the morning after an overnight fast). FDG-PET should be collected after an overnight fast for scans conducted in the morning, or a minimum 4-hour fast for scans collected later in the day; prior to the scan, a blood glucose measurement will be made by finger stick and scans will be postponed or rescheduled if the reading is > 180 mg/dL.
s) Can be conducted anytime between screening and baseline. Completion of safety lab report and MRI review with PI signature are required prior to LP. See section 7.6.2 for more detail.

t) The CN cohort does not receive their genetic results and therefore do not require genetic counseling. Positive genetic results require genetic couseling for the CI cohort if they have agreed to receive their genetic results. Participants at international sites may speak with the site PI or appropriately trained clinical investigator.
u) Visit procedures noted with "u" can be completed remotely. See section 8.7 for more information. See the procedures manual for more information on collecting the NACC UDS and FTLD modules remotely.
v) Tau PET scan is not required for select international sites. Participants from select international sites will not complete tau PET scans and therefore will not receive tau PET results.
w) The Area Deprivation Index is only collected at U.S. sites.

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## EOAD Participants – Longitudinal Visits

| Procedure \ Visit | Genetics Status Disclosure | Month 12[a] | Month 24[a] | Month 36[a] | Month 48 / Ongoing Annual Visits[a] | Annual Neuropath. Phone Check[c] | Early Termination[d] |
|---|---|---|---|---|---|---|---|
| Window Period (days) | See footnote **e** | +/- 60[b] | | | | | |
| Brain donation discussion/provisional consent[c,r] | | X | X | X | X | X | X |
| Vital Signs | | X | X | X | X | | X |
| Neurological and Physical Exams | | X | X | X | X | | X |
| Demographics & Medical History[r] | | X | X | X | X | | X |
| Clinical Diagnosis and Clinical Symptom Assessment | | X | X | X | X | | X |
| Sex and Reproductive Health Questionnaire[r] | | X[o] | | | | | |
| Area Deprivation Index Score[r] | | X[o] | | | | | |
| Concomitant Medications[r] | | X | X | X | X | | X |
| NACC UDS and FTLD Modules, and Neuropsychological Battery[f,r] | | X | X | X | X | | X |
| Amsterdam IADL Questionnaire[r] | | X | X | X | X | | X |
| ADAS-Cog13[r] | | X | X | X | X | | X |
| Rey Auditory Verbal Learning Test (RAVLT)[r] | | X | X | X | X | | X |
| TabCat: Flanker, Line Length, Line Orientation, and Match | | X | X | X | X | | X |
| CDR[r] | | X | X | X | X | | X |
| Pregnancy Test[g] | | X | X | X | | | |
| 3T MRI/fMRI[h] | | X | X | X | | | X |
| Amyloid PET Scan[i] | | X | X | X | | | |
| Tau PET Scan[i,p,q] | | X | X | X | | | |
| Adverse Event[r] | | X | X | X | X | | X |
| Tau Status Disclosure[p,q,r] | | X[p] | | | | | |
| Genetic Counseling[r,s,t] | X | | | | | | |
| Study Participation Report[j] | | X | X | X | X | | X |
| Blood for AD blood biomarkers[m], DNA, and RNA, PBMC[k] | | X | X | X | X | | X |
| LP Safety Labs (CBC, coagulation profile) | | X | X | X | | | X |
| Lumbar Puncture (optional) [l,m] | | X | X | X | | | X |
| Wellness Check[n,r] | | X | X | X | | | X |

a) Visit procedures may be split over multiple days without constituting a protocol deviation.
b) Longitudinal visits should commence within 365 days **+/- 60 days** from the previous visit. However, there must be at least 365 days between the first PET scan of the previous visit and the first PET scan of the next yearly visit. There must also be at least 365 days from second-to-second PET, so that there are no more than two PET scans in a year. FDG PET is not subject to this consideration; it can be the third PET in 365 days. Every attempt should be made to complete longitudinal visits within 60 days.
c) Those who have consented to brain donation will be contacted annually by phone and until death once unable to come into the clinic. Post mortem brain donation discussion should occur to confirm/review consent even if participant has already agreed. Follow up continues after all LEADS study visits have been completed until

participant death. If participant has refused to participate, no further inquiry should take place. Undecided participants will be approached at each study visit until they consent or refuse to participate. See section 7.1.7 for more detail.

d) Contact ATRI Coordinating Center for guidance on what specific procedures should be conducted at this visit.
e) Timing for result disclosure is not set and dependent on the availability of the genetic screening results.
f) The NACC UDS and FTLD modules include demographics, clinical, cognitive, functional, behavioral assessments.
    - Version C2 the NACC UDS Neuropsychological Battery will be used in this study
    - UDS forms A1, A3, A5, B4-B9, C2, D1, and D2 are required for LEADS
    - UDS forms A5/D2, D1a, and D1b will take the place of A5, D2, and D1 if using UDSv4
    - FTLD forms B3F, B9F, C1F-C6F, E2F and E3F are required for LEADS
g) If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed prior to amyloid and tau PET scans. Only participants with a negative result will be eligible for PET scanning.
h) All attempts should be made to acquire the full 60 minute MRI protocol; however the MPRAGE and 3D FLAIR should be prioritized. The MPRAGE and 3D FLAIR are the mandatory sequences. If needed, the other sequences may be skipped for any reason without constituting a protocol deviation. Note the reason for non-completion in the source documents.
i) For sites that have radiation safety committees (RSC), ability to conduct the PET scans will be contingent on local RSC approval.
j) May be provided to participants at the discretion of the PI. See section 7.1.8 for more detail.
k) PBMC samples must be collected and shipped the same day. Fasting is not required. PBMC sample collection is optional for international sites. If collected, international sites will store PBMC samples locally. See NCRAD Biospecimen, Collection, and Shipping manual for more details.
l) Review of LP safety lab report with PI signature prior to LP is mandatory for participants who consented to the lumbar procedure. See section 7.6.2 for more detail.
m) LP should be collected after a minimum 6-hour fast (preferably in the morning after an overnight fast).
n) To be completed within 48 hours of lumbar puncture.
o) The Sex and Reproductive Health Questionnaire and the Area Deprivation Index are only to be collected one time and should be completed at the next in-clinic visit. Participants who completed Sex and Reproductive Health Questionnaire at Baseline will not complete the Sex and Reproductive Health Questionnaire at any longitudinal visits. Participants who completed the Area Deprivation Index at Baseline will not complete the Area Deprivation Index at any longitudinal visits. The Area Deprivation Index is only collected at U.S. sites.
p) If tau PET results were not disclosed at Baseline, results of the most recent or to-be-conducted tau PET scan should be disclosed at the next in-person visit. Only one tau PET scan is read per participant.
q) Tau PET scan is not required for select international sites. Participants from select international sites will not complete tau PET scans and therefore will not receive tau PET results.
r) Visit procedure noted with "r" can be completed remotely. See section 8.7 for more information.
s) The CN cohort does not receive their genetic results and therefore do not require genetic counseling. Positive genetic results require genetic cousenling for the CI cohort if they have agreed to receive their genetic results. Participants at international sites may speak with the site PI or appropriately trained clinical investigator.

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## EOnonAD Participants – Longitudinal Visits

| Procedure \ Visit | Genetics Status Disclosure | Month 12[a] | Month 24[a] | Month 36[a] | Month 48 / Annual Visit[a] | Anuual Neuropath. Phone Check[c] | Early Termination[d] |
|---|---|---|---|---|---|---|---|
| Window Period (days) | See footnote **e** | +/- 60[b] | | | | | |
| Brain donation discussion/provisional consent[c,s] | | X | X | X | X | X | X |
| Vital Signs | | X | X | X | X | | X |
| Neurological and Physical Exams | | X | X | X | X | | X |
| Demographics & Medical History[s] | | X | X | X | X | | X |
| Clinical Diagnosis and Clinical Symptom Assessment | | X | X | X | X | | X |
| Sex and Reproductive Health Questionnaire[s] | | X[p] | | | | | |
| Area Deprivation Index[s] | | X[p] | | | | | |
| Concomitant Medications[s] | | X | X | X | X | | X |
| NACC UDS and FTLD Modules, and Neuropsychological Battery[f, s] | | X | X | X | X | | X |
| Amsterdam IADL Questionnaire[s] | | X | X | X | X | | X |
| ADAS-Cog13[s] | | X | X | X | X | | X |
| Rey Auditory Verbal Learning Test (RAVLT)[s] | | X | X | X | X | | X |
| TabCat: Flanker, Line Length, Line Orientation, and Match | | X | X | X | X | | X |
| CDR[s] | | X | X | X | X | | X |
| Pregnancy Test[g] | | X | X | X | | | |
| 3T MRI/fMRI[h] | | X | X | X | | | X |
| Amyloid PET Scan | | | | X | | | |
| Tau PET Scan[r] | | | | X[i] | | | |
| FDG PET Scan[n] | | | X[j] | | | | |
| Adverse Event[s] | | X | X | X | X | | X |
| Amyloid Status Disclosure[s] | | | | X | | | |
| Tau Status Disclosure[r,s] | | | X[q] | | | | |
| FDG Status Disclosure[s] | | | X[j] | | | | |
| Genetic Counseling[s,t] | X | | | | | | |
| Study Participation Report[k] | | X | X | X | X | | X |
| Blood for AD blood biomarkers[n], DNA, and RNA, PBMC[l] | | X | X | X | X | | X |
| LP Safety Labs (CBC, coagulation profile) | | X | X | X | | | X |
| Lumbar Puncture (optional) [m,n] | | X | X | X | | | X |
| Wellness Check[o,s] | | X | X | X | | | X |

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a) Visit procedures may be split over multiple days without constituting a protocol deviation.
b) Longitudinal visits should commence within 365 days **+/- 60 days** from the previous visit. However, there must be at least 365 days between the first PET scan of the previous visit and the first PET scan of the next yearly visit. There must also be at least 365 days from second-to-second PET, so that there are no more than two PET scans in a year. FDG PET is not subject to this consideration; it can be the third PET in 365 days. Every attempt should be made to complete longitudinal visits within 60 days.
c) Those who have consented to brain donation will be contacted annually by phone and until death once unable to come into the clinic. Post mortem brain donation discussion should occur to confirm/review consent even if participant has already agreed. Follow up continues after all LEADS study visits have been completed until participant death. If participant has refused to participate, no further inquiry should take place. Undecided participants will be approached at each study visit until they consent or refuse to participate. See section 7.1.7 for more detail.
d) Contact ATRI Coordinating Center for guidance on what specific procedures should be conducted at this visit.
e) Timing for result disclosure is not set and dependent on the availability of the genetic screening results.
f) The NACC UDS and FTLD modules include demographics, clinical, cognitive, functional, behavioral assessments.
    - Version C2 the NACC UDS Neuropsychological Battery will be used in this study
    - UDS forms A1, A3, A5, B4-B9, C2, D1, and D2 are required for LEADS
    - UDS forms A5/D2, D1a, and D1b will take the place of A5, D2, and D1 if using UDSv4
    - FTLD forms B3F, B9F, C1F-C6F, E2F and E3F are required for LEADS
g) If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed prior to amyloid, tau, and FDG PET scans. Only participants with a negative result will be eligible for PET scanning.
h) All attempts should be made to acquire the full 60 minute MRI protocol; however the MPRAGE and 3D FLAIR should be prioritized. The MPRAGE and 3D FLAIR are the mandatory sequences for enrollment. If needed, the other sequences may be skipped for any reason without constituting a protocol deviation. Note the reason for non-completion in the source documents.
i) The tau PET scan applies only to EOnonAD participants that become amyloid positive and cannot be scheduled until after Month 36 amyloid disclosure. EOnonAD participants (who remain amyloid negative) will not receive the Month 36 tau PET scan and the associated disclosure and pregnancy test.
j) The FDG PET scan and FDG PET status disclosure are only to be conducted one time and should be completed at the next in-clinic visit. Participants who completed FDG PET scan at Baseline will not complete an FDG PET scan and the associated FDG status disclosure at any longitudinal visits. For sites that have radiation safety committees (RSC), ability to conduct FDG PET scans will also be contingent on local RSC approval.
k) May be provided to participants at the discretion of the PI. See section 7.1.8 for more detail.
l) PBMC samples must be collected and shipped the same day. Fasting is not required. PBMC sample collection is optional for international sites. If collected, international sites will store PBMC samples locally. See NCRAD Biospecimen, Collection, and Shipping manual for more details.
m) Review of LP safety lab report with PI signature prior to LP is mandatory for participants who consented to the lumbar procedure. See section 7.6.2 for more detail.
n) LP should be collected after a minimum 6-hour fast (preferably in the morning after an overnight fast). FDG-PET should be collected after an overnight fast for scans conducted in the morning, or a minimum 4-hour fast for scans collected later in the day; prior to the scan, a blood glucose measurement will be made by finger stick and scans will be postponed or rescheduled if the reading is > 180 mg/dL.
o) To be completed within 48 hours of lumbar puncture. Additionally, a telephone call will be made within two weeks post amyloid results disclosure. See section 7.1.4 for more detail.
p) The Sex and Reproductive Health Questionnaire and the Area Deprivation Index are only to be collected one time and should be completed at the next in-clinic visit. Participants who completed the Sex and Reproductive Health Questionnaire at Baseline will not complete the Sex and Reproductive Health Questionnaire at any longitudinal visits. Participants who completed the Area Deprivation Index at Baseline will not complete the Area Deprivation Index at any longitudinal visits. The Area Deprivation Index is only collected at U.S. sites.
q) If tau PET results were not disclosed at Baseline, results of the most recently conducted tau PET scan should be disclosed at the next in-person visit.
r) Tau PET scan is not required for select international sites. Participants from select international sites will not complete tau PET scans and therefore will not receive tau PET results.
s) Visit procedure noted with "s" can be completed remotely. See section 8.7 for more information.
t) The CN cohort does not receive their genetic results and therefore do not require genetic counseling. Positive genetic results require genetic cousenling for the CI cohort if they have agreed to receive their genetic results. Participants at international sites may speak with the site PI or appropriately trained clinical investigator.

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## Cognitively Normal Participants – Longitudinal Visits

| Procedure \ Visit | Month 12[a] | Month 24[a] | Annual Neuropath. Phone Check[c] | Early Termination[d] |
|---|---|---|---|---|
| Window Period (days) | +/- 60[b] | | | |
| Neuropathology discussion/ provisional consent[e,q] | X | X | X | |
| Vital Signs | X | X | | X |
| Neurological and Physical Exams | X | X | | X |
| Demographics and Medical History[q] | X | X | | X |
| Clinical Diagnosis and Clinical Symptom Assessment | X | X | | X |
| Sex and Reproductive Health Questionnaire[q] | X[o] | | | |
| Area Deprivation Index[q] | X[o] | | | |
| Concomitant Medications[q] | X | X | | X |
| NACC UDS and FTLD Modules and Neuropsychological Battery[e,q] | X | X | | X |
| Amsterdam IADL Questionnaire[q] | X | X | | X |
| ADAS-Cog13[q] | X | X | | X |
| Rey Auditory Verbal Learning Test (RAVLT)[q] | X | X | | X |
| TabCat: Flanker, Line Length, Line Orientation, and Match | X | X | | X |
| CDR[q] | X | X | | X |
| Pregnancy Test[f] | X | | | |
| 3T MRI/fMRI[g] | X[p] | X | | |
| FDG PET Scan | X[i] | | | |
| Adverse Events[q] | X | X | | X |
| Study Participation Report[j] | X | X | | X |
| Blood for AD Biomarkers[k], DNA, and RNA, and PBMC[l] | X | X | | X |
| LP Safety Labs (CBC, coagulation profile) | | X | | |
| Lumbar Puncture (optional)[k, m, n] | | X | | |
| Wellness Check[h, q] | | X | | |

a) Visit procedures may be split over multiple days without constituting a protocol deviation.
b) Month 12 and 24 visits should commence within 365 days **+/- 60 days** from the previous visit. However, there must be at least 365 days between the first PET scan of the previous visit and the first PET scan of the next yearly visit. There must also be at least 365 days from second-to-second PET, so that there are no more than two PET scans in a year. FDG PET is not subject to this consideration; it can be the third PET in 365 days. Every attempt should be made to complete longitudinal visits within 60 days.
c) Those who have consented to brain donation will be contacted annually by phone and until death once unable to come into the clinic. Post mortem brain donation discussion should occur to confirm/review consent even if participant has already agreed. Follow up continues after all LEADS study visits have been completed until participant death. If participant has refused to participate, no further inquiry should take place. Undecided participants will be approached at each study visit until they consent or refuse to participate. See section 7.1.7 for more detail.
d) Contact ATRI Coordinating Center for guidance on what specific procedures should be conducted at this visit.
e) The NACC UDS and FTLD battery includes clinical, cognitive, functional, behavioral assessments.

- Version C2 the NACC UDS Neuropsychological Battery will be used in this study
- UDS forms A1, A3, A5, B4-B9, C2, D1, and D2 are required for LEADS
- UDS forms A5/D2, D1a, and D1b will take the place of A5, D2, and D1 if using UDSv4
- FTLD forms B3F, B9F, C1F-C6F, E2F and E3F are required for LEADS

f) If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed prior to the FDG PET scan. Only participants with a negative result will be eligible for scanning.

g) All attempts should be made to acquire the full 60 minute MRI protocol; however the MPRAGE and 3D FLAIR should be prioritized. The MPRAGE and 3D FLAIR are the mandatory sequences for enrollment. Other sequences be skipped for any reason without constituting a protocol deviation. Note the reason for non-completion in the source documents.

h) To be completed within 48 hours post lumbar puncture. See section 7.1.4 for more detail.

i) The FDG PET scan is only to be conducted one time and should be completed at the next in-clinic visit. Participants who completed FDG PET scan at Baseline will not complete an FDG PET scan at any longitudinal visits. For sites that have radiation safety committees (RSC), ability to conduct FDG PET contingent on local RSC approval.

j) May be provided to participants at the discretion of the PI. See section 7.1.8 for more detail.

k) LP should be collected after a minimum 6-hour fast (preferably in the morning after an overnight fast). FDG PET should be collected after an overnight fast for scans conducted in the morning, or a minimum 4-hour fast for scans collected later in the day; prior to the scan, a blood glucose measurement will be made by finger stick and scans will be postponed or rescheduled if the reading is > 180 mg/dL.

l) PBMC samples must be collected and shipped the same day. Fasting is not required. PBMC sample collection is optional for international sites. If collected, international sites will store PBMC samples locally. See NCRAD Biospecimen, Collection, and Shipping manual for more details.

m) Review of LP safety lab report with PI signature prior to LP is mandatory for participants who consented to the lumbar procedure. See section 7.6.2 for more detail.

n) If LP was unsuccessful at baseline, participants can be reapproached and LP collected at Month 12.

o) The Sex and Reproductive Health Questionnaire and the Area Deprivation Index are only to be collected one time and should be completed at the next in-clinic visit. Participants who completed the Sex and Reproductive Health Questionnaire at Baseline will not complete the Sex and Reproductive Health Questionnaire at any longitudinal visits. Participants who completed the Area Deprivation Index at Baseline will not complete the Area Deprivation Index at any longitudinal visits. The Area Deprivation Index is only collected at U.S. sites.

p) MRI at M12 will only be conducted for CN participants who are undergoing an FDG PET scan at their M12 visit. If the participant is not undergoing an FDG PET at M12, no MRI scan will be conducted at M12.

q) Visit procedure noted with "q" can be completed remotely. See section 8.7 for more information.