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

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
ADAD	Autosomal Dominant Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale – Cognitive
ADC	Alzheimer's Disease Center
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADSP	Alzheimer Disease Sequencing Project
AE	Adverse Event
APOE/*APOE4*	Apolipoprotein E (*APOE*) epsilon 4 (*APOE4*)
APP	Amyloid Precursor Protein gene
ATRI	Alzheimer's Therapeutic Research Institute
Aβ	Beta Amyloid
ASL	Arterial Spin Labeling
CC	Coordinating Center at ATRI
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
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FLAIR	Fluid Attenuation Inversion Recovery
fMRI	Functional Magnetic Resonance Imaging
GAAIN	Global Alzheimer's Association Interactive Network
GCP	Good Clinical Practice
GDS	Geriatric Depression Scale
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed consent form
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
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 Cell Repository for AD
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
PET	Positron-Emission Tomography
PHI	Protected Health Information
PI	Principal Investigator
PLPH	Post lumbar puncture headache
PCAPCA	Posterior cortical atrophy
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
SAE	Serious Adverse Event
T	Tesla
TIV	Total intracranial volume
vMRI	Volumetric Magnetic Resonance Imaging
WGS	Whole Genome Sequencing

PROTOCOL SYNOPSIS

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 Assessments to be collected at baseline and Year 1 (both Early Onset Alzheimer's Disease (EOAD) and Cognitively Normal (CN) cohorts) and Year 2 (EOAD cohort only)
STUDY COMPOUNDS	Florbetaben Flortaucipir (investigational agent)
PARTICIPANT INFORAMTION AND DURATION OF STUDY	400 EOAD Participants = 2 Years in duration 100 CN Participants = 1 Year in duration Screening period = < 45 days (including baseline assessments)
SUMMARY OF KEY ELIGIBILITY CRITERIA	**EOAD Cohort:** • Diagnosis of NIA-AA criteria of MCI due to AD or probable AD dementia • Amyloid positive status (florbetaben PET scan with evidence of elevated amyloid as determined by a central read) • CDR score ≤ 1.0 **Cognitively Normal Cohort:** • Meets criteria for cognitively normal, based on an absence of significant impairment in cognitive functions and activities of daily living • Mini-Mental State Exam score between 26-30 • CDR score = 0 **Both Cohorts:** • 40-64 (inclusive) years of age at the time of consent • Willing and able to undergo study procedures as outlined in the Schedule of Events

PRIMARY OBJECTIVES	• Collect longitudinal assessments and biomarker data for 400 EOAD and 100 CN participants • 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) • 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.

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 Centers under the auspices of the National Alzheimer Coordinating Center (NACC) will be encouraged to enroll the EOAD and CN individuals in their longitudinal observational clinical cohorts. We will leverage the ADCs 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 measures between EOAD and LOAD and identify optimal imaging 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 or CSF measures.

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

H2c: Through a data-driven approach we will identify composite MRI and tau PET 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 and CSF 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+* rate than memory-predominant EOAD.

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

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 biomarker profiles based on *APOE* status as rates and patterns of atrophy, Aβ and tau accumulation may differ.

3.4 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 autosomal dominant AD.

H4b: 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.

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 400 EOAD participants and 100 CN participants will be enrolled at approximately 14 sites in the United States. Clinical/cognitive, imaging, biomarker, and genetic characteristics will be assessed across the two cohorts: EOAD and CNs.

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 same standardized procedures as in ADNI for multisite florbetaben and flortaucipir PET imaging. The PET Core will be responsible for oversight of site qualification, quality control, standardization, pre-processing and analysis of amyloid and tau 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 chair of the Department of Medical and Molecular Genetics at IU and is the PI of National Cell Repository for Alzheimer's Disease (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. Dr. Fagan's group from Washington University in St. Louis will be responsible for the proposed CSF analyses. Dr. Foroud and Saykin 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 81,000 ADNI MRI and PET images with more than 646,886 images downloaded by approved ADNI data users. Additionally, the clinical and genetic data have been provided to hundreds of users. Finally, subsystems for project management, data user application and review, and a comprehensive website 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.

5.0 STUDY POPULATION

5.1 Inclusion Criteria

EOAD Cohort Only:

1. Meets NIA-AA criteria for MCI due to AD or probable AD dementia
2. Have a global CDR score ≤ 1.0
3. Have capacity to provide informed consent (IC) or has a legal authorized representative or guardian who provides IC
4. Amyloid positive status (PET scan with evidence of elevated amyloid)
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

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

5.2 Exclusion Criteria

EOAD **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* have been excluded
3. Known mutation in an ADAD gene (*APP, PSEN1, PSEN2*) or other autosomal dominant genes associated with other neurodegenerative disorders
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. Medical 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, 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. Must agree not to participate in other clinical studies with neuropsychological measures, with the exception of participants who are co-enrolled in the NACC Uniformed 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. History of torsades de pointes or taking medications known to prolong the QT interval (see "FLORTAUCIPIR LIST OF PROHIBITED MEDICATIONS" supplemental document for a list of prohibited medications)
18. Corrected QT (QTc) interval $\leq$ 458 msec in males or $\leq$ 474 msec in females
19. (*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/μl
        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
20. Deemed ineligible by the Site PI for any other reason

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 Centers (ADCs). 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 EOAD 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.

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).

7.1.3 Active Study Phase (Baseline to Endpoint Assessments)

The baseline visit will occur within 45 days of screening. The remaining study procedures will be conducted as outlined in Appendix 1.

7.1.4 Wellness Telephone Check

Telephone calls will be made to participants within 48 hours post flortaucipir administration and lumbar puncture procedure to ascertain if adverse events occurred post procedure. Additional telephone visits may be conducted at the discretion of the Site PI.

7.1.5 Amyloid PET Disclosure Visit

EOAD individuals will meet with the study doctor to discuss the results of the amyloid PET scan. Participants who do not have evidence of brain amyloid (negative results) will not be eligible for the study.

7.1.6 Genetic Counseling Sessions and Genetic Testing Disclosure

EOAD individuals will be asked if they wish to receive results if a known pathogenic mutation is identified in an ADAD gene (*APP*, *PSEN1* and *PSEN2*). If a known pathogenic mutation is found in one of these genes and confirmed in 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. See section 7.2.5 for more detail.

7.1.7 Nursing Home Placement

Transfers to a skilled nursing home will be recorded in the clinical database. All assessments will be completed to the extent possible according to the Schedule of Events (Appendix 1).

7.1.8 Study Participation Report

EOAD participants will be provided a study participant report within 60 days of receipt of study procedure results. The report will summarize key results from relevant procedures (cognitive testing, diagnosis, and amyloid status) and be used to aid in study retention. Clinically significant results will be relayed in a timely manner by the Site PI. Appropriate follow-up with a treating physician will be arranged as indicated.

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, PSEN* 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 EOAD 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.

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 EOAD 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.

Genetic Counseling and Genetic Testing – EOAD 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*). 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 also provide information regarding the likelihood that close relatives of the participant will 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). If a video is used, 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. 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

An additional 6 ml blood sample will be collected at the Baseline Visit from all EOAD participants. This sample will be sent to and stored by the study's CLIA laboratory. The sample will be used for CLIA confirmation of a known pathogenic mutation, if one is found. The availability of this sample for all EOAD 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.

Whole genome sequencing (WGS) 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*, 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, the CLIA laboratory 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 for result disclosure and appropriate counseling and be discontinued from the LEADS study and given a referral to 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.

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 Scale (FAS)

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].

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.

7.3 Cognitive Testing

7.3.1 NACC - Uniformed Data Set (UDS), Frontotemporal Lobar Degeneration (FTLD) Module, and Neuropsychological Battery:

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].

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 name 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]. 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.

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].

7.4 Imaging

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. The total scan time is approximately 1 hour. 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 radiologist, will be shared with the participant and the participant's local physician (if necessary – i.e., in the case of actionable findings).

7.4.2 PET scanning. Amyloid (florbetaben) and Tau (flortaucipir) 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).

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

7.4.2.1 Florbetaben (Neuraceq)

EOAD and CN participants will undergo a florbetaben PET as outlined in Appendix 1. The study staff will evaluate each participant prior to administration of florbetaben to determine if they are still suitable to undergo the scan (e.g., previous 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 the EOAD cohort only will be interpreted by the PET Core to confirm amyloid-positivity, which is required for study inclusion. The read will be based on a consensus between visual and quantitative assessments. Dichotomous visual interpretations of florbetaben summed images will be performed by a qualified rater using validated criteria. Florbetaben 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.

Amyloid PET results for all EOAD 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.

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

Florbetaben scanning entails 8 mCi $\pm$ 0.8 mCi injection of tracer through an intravenous line and a 90-minute (+/- 10 minutes) uptake followed by a 20-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.

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

7.4.2.2 Flortaucipir (Investigational Agent)

EOAD and CN participants will undergo a flortaucipir PET as outlined in Appendix 1. 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 PET scan exposure in the previous 12 months, torsades de pointes, prolonged QT interval, see supplemental document titled, "FLORTAUCIPIR LIST OF PROHIBITED MEDICATIONS " for a list of exclusionary medications). Participants will not be scanned if they have initiated a medication known to prolong QT interval. For women of childbearing potential, a urine pregnancy test will be obtained and negative result confirmed on the day of scanning.

Flortaucipir scans will be acquired on the PET scanners at each site using standard procedures. Participants will receive a single bolus intravenous

injection of approximately 10 mCi (+/- 10%, 20µg mass dose) followed by a saline flush. The maximum mass dose will not exceed 20µg. The participant will then sit quietly for 75 minutes. 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, 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 even has resolved or stabilized. A follow-up Wellness Check telephone call will be conducted within 48 hours after the imaging to confirm well-being and ask about any new or additional adverse events.

Detailed information regarding flortaucipir (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 60 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 EOAD individuals will have an additional 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 (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 can be utilized. Sites must designate the method of CSF collection for data tracking purposes. Participants will be instructed 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. For sample processing please see section 7.7.

7.6 Safety Assessments

7.6.1 Electrocardiograph (ECG)

A single supine, 12 lead ECG will be performed according to the Schedule of Events (Appendix 1). Potentially clinically significant ECG abnormalities will be interpreted by a local cardiologist at the discretion of the Site PI. Participants with torsades de pointes or prolonged QT (QTc) interval ($\leq$ 458 msec in males or $\leq$ 474 msec in females) will be ineligible for flortaucipir PET.

7.6.2 Concomitant Medication Review

Concomitant medications will be reviewed at screening and prior to each flortaucipir PET scan. See supplemental document titled, "FLORTAUCIPIR LIST OF PROHIBITED MEDICATIONS" for more details.

7.6.3 Safety Laboratory Assessments

Prior to lumbar puncture all participants will be subjected to the following safety labs. Safety labs will be conducted locally.

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

7.6.4 Urine Pregnancy Test

On the day of the 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 florbetaben and flortaucipir PET scanning.

7.6.5 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 lumbar puncture. Any previously existing clinical CT or MRI from the past 12 months will be acceptable. If no previous imaging from the past 12 months exists, the MRI scan will need to be completed and reviewed by the local neuroradiologist prior to the lumbar puncture procedure.

The laboratory reports and brain scan must be reviewed, signed and dated by the Site PI (or a medically-qualified individual delegated by the PI) prior to LP and PET scanning respectively.

7.7 Biofluid Sample Processing

7.7.1 Site processing:

1. Peripheral blood sample – up to 50mL of blood will be collected. The following will be extracted from this sample:
    i. DNA – 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.
    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 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. An additional 6 mL of blood will be collected from all EOAD participants for CLIA testing

7.7.2 National Cell Repository for Alzheimer Disease (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 within 2 weeks of collection.

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, RESCREENING, EARLY TERMINATION, AND DISCONTINUATION

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. EOAD participants who fail screening due to negative amyloid PET scan may not be re-screened.

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 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.

8.4 Discontinuation

Participants with known pathogenic mutation who have opted to receive results will be discontinued from the study after genetic counseling. A referral to the DIAN study will be provided.

9.0 STUDY COMPOUNDS

9.1 Florbetaben

Florbetaben will be provided by Piramal 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. Each site will keep accountability logs for all doses received, administered, and destroyed.

9.2 Flortaucipir (Investigational Agent)

Flortaucipir also known as [^{18}F]AV-1451 will be provided by Avid Radiopharmaceuticals, Inc. (Avid) Avid will manage the ordering of doses from contract manufacturing organizations/radiopharmacy and will provide oversight of the dose deliveries via a third-party vendor upon receipt of a Dose Request Form provided by enrolling sites.

Flortaucipir is not FDA-approved and 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. (IND #119,863).

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. One dose (10mCi +/- 10%, 20µg mass dose) 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. Each site will keep accountability logs for all doses received, administered, and destroyed.

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, a 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 and when atraumatic (Sprotte) needles are 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.

Assuming that a participant has all of the PET scans described in this study protocol, an individual undergoing florbetaben and flortaucipir would have up to an annual effective dose of 15.12 mSv. Together, these doses are roughly equivalent to 5 years of background radiation. The organ that receives the maximum exposure is the gallbladder, which receives an annual dose of 5,466 rem for the combination of florbetaben and flortaucipir. These doses are well below the 21 CFR 361.1 guidelines for RDRC approved studies. Participants who may have other sources of radiation exposure (thallium testing, radiation therapy) should be evaluated by the study physician.

Effective dose for a participant receiving 8 mCi florbetaben and 10mCi flortaucipir

	microSv/ MBq	mSv/ study	mSv/ Scan with CT	mrem/ Scan with CT
Florbetaben	19	5.62	6.02	602
Flortaucipir	24	8.70	9.10	910

(continued on next page)

Organ dosimetry for a participant receiving 8 mCi florbetaben and 10 mCi flortaucipir

Organ	mrad/ 8 mCi Florbetaben	mrad/10 mCi Flortaucipir	Total dose
Adrenals	385	526	911
Brain	385	311	696
Breasts	207	264	471
Gallbladder wall	4059	1407	5466
Lower large intestine wall	1037	1289	2326
Small Intestine	919	3130	4049
Stomach	356	467	823
Upper large intestine wall	1126	3537	4663
Heart wall	415	1100	1515
Kidneys	711	1478	2189
Liver	1156	2119	3275
Lungs	444	1256	1700
Muscle	296	333	629
Ovaries	474	767	1241
Pancreas	415	533	948
Red marrow	356	374	730
Osteogenic cells	444	426	870
Skin	207	221	428
Spleen	296	378	674
Testes	267	257	524
Thymus	267	318	585
Thyroid	237	249	486
Urinary bladder wall	2074	1393	3467
Uterus	474	674	1148
Total Body	326	441	767

10.2.2 Other Side Effects Associated with Florbetaben

In addition to radiation risks listed above, safety data was extracted from the Neuraceq prescribing information, dated April 2016. The possible side effects include:

Common (may affect up to 1 in 10 people):

- Injection site reactions: injection site irritation, injection site pain, redness of the skin at injection site (injection site erythema)

Uncommon (may affect up to 1 in 100 people):

- Burning sensation, headache, neuralgia (intense, typically intermittent pain along the course of a nerve), tremor (an involuntary quivering movement)
- Vessels: flushing (sudden reddening of the face and/or neck), hematoma (a bruise, a black and blue mark), hypotension (low blood pressure)
- Stomach: diarrhea, nausea (feeling sick)
- Liver: abnormal liver function
- Skin: hyperhidrosis (excessive sweat), rash, toxic skin eruption (acute skin affections with measles-type erythema of the skin, potentially including blisters and ulcerations)
- Muscles and bones: limb discomfort, pain in extremity
- Injection site conditions: pain and discomfort around the injection site, injection site hematoma (a bruise, a black and blue mark at injection site), injection site warmth, tiredness, feeling hot, pyrexia (raised body temperature, a fever) Abnormal blood test: increased blood creatinine levels (reduced kidney function)

10.2.3 Other Side Effects Associated with Flortaucipir

In addition to radiation risks listed above, the tau ligand flortaucipir is an experimental compound undergoing clinical evaluation, and risks from the agent are not fully known. Details on the clinical information to date regarding flortaucipir exposure and risks will be provided to participants in the informed consent form (ICF).

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. Site PIs should be aware of the following risk information related to flortaucipir and the PET procedures:

- In completed studies with a total of 59 subjects diarrhea, headache and dysgeusia were reported in at least 1% of subjects. Only dysgeusia was thought by the investigator to be related to study procedures.

Adverse Events Observed in Sponsored Studies (Participant N=59)

Adverse Event	N (% of patients)		
	TEAE (%)	Drug-related TEAE (%)	Protocol/Procedure Related TEAE (%)
Diarrhea	**2 (3.4%)**	**0 (0%)**	**0 (0%)**
Headache	**2 (3.4%)**	**0 (0%)**	**0 (0%)**
Hypertension	**2 (3.4%)**	**0 (0%)**	**2 (3.4%)**
Musculoskeletal discomfort	**1 (1.7%)**	**0 (0%)**	**1 (1.7%)**
Dysgeusia	**1 (1.7%)**	**1 (1.7%)**	**0 (0%)**

TEAE=treatment-emergent adverse event

All reported events were mild in severity, and all participants recovered. One event (dysgeusia) was considered related to flortaucipir administration by the investigator. The events of hypertension (blood pressure increased) and musculoskeletal pain were considered related to protocol procedures, and not to flortaucipir administration by

the investigator. No consistent or clinically relevant changes in vital signs, laboratory values, or ECG results have been observed in completed studies.

Flortaucipir injection is currently being administered in ongoing Avid-sponsored studies and in studies being conducted by other sponsors where flortaucipir is being used as a biomarker. These include studies of AD therapeutics being conducted by Eli Lilly (Avid Radiopharmaceuticals is a wholly-owned subsidiary of Eli Lilly and Company). Lilly Global Patient Safety maintains the global safety database for flortaucipir.

Although serious adverse events have been reported by participants receiving flortaucipir, none has been assessed as related to the compound. Flortaucipir administration is not anticipated to cause serious adverse events. However, in the disease population of MCI and AD, the occurrence of dementia/cognitive symptoms, malignancies, major cardiovascular events (including MI, stroke, cerebral hemorrhage, etc.), and infections are reasonably anticipated due to the age of the population and associated comorbid conditions.

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).

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 EOAD. 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.

10.4 Psychological Risks from Amyloid PET Results

It is possible that some participants may be upset by learning the results of their amyloid PET scan, which increases (if positive) or reduces (if negative) the 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.

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 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) and also to the investigational product. Following questioning and evaluation, all AEs, whether determined to be related or unrelated to the protocol procedure(s) or investigational product 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**. 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 14 clinical sites in the United States. 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 both the florbetaben and flortaucipir 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 both PET tracers in the LEADS protocol. 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 both participant cohorts (EOAD 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 and tau PET summary measures, blood biomarker and selected genomic data. The actual imaging files will be stored at LONI. The WGS 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 (florbetaben) or cerebellum gray matter (flortaucipir) regions to quantify amyloid and tau PET data. 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. 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.

15.1.2 Unique LEADS data

Completed LEADS database eCRFs will be submitted according to LEADS ATRI 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 Imaging process to ensure that no participant identification 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.

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 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, LONI's server will be connected to the GAAIN server. 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 on the specialized LEADS website linked to LONI's

homepage (www.loni.usc.edu) 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

17.1 Data Request Procedures

In order to receive access to the LEADS data, investigators will be asked to provide a written request via the electronic data request form available on the GAAIN and LEADS IDA websites, 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 Administrative Core and any relevant Core Leaders as outlined below. The number, type and disposition of the requests will be tracked by the Administrative Core and a data sharing report will be generated for reports to the NIA. Each data request will specify the data elements required for the planned analyses and the specific aims and hypotheses of the project. A brief analysis plan will also be required as well as publication and other (i.e., submission of grant proposals, etc.) will be required.

17.2 Clinical, Imaging and Genetic Data Request Review process

The LEADS Resource Sharing Committee will conduct a two-step review of all data requests. Formal requests should be submitted via GAAIN website or the LEADS Image and Data Archive site at LONI. To avoid overlapping effort, investigators will be encouraged to use the search option on the LEADS request website to see what other requests might be similar to theirs. These requests will be sent to a designated

Administrative Core mailbox and subjected to initial review. After this initial review the request will be forwarded to the relevant ad-hoc committee members for their input.

17.3 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 a Specimen Access 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.

17.4 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 will be completed by 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 and GCP 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 prior to starting study procedures.

20.3 Confidentiality and HIPAA 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. No research information other than MRI scans and reports will be entered into the participants' medical records files, unless required by local site-specific practice.

The study data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsoring institution. 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, the FDA, the NIA, and the OHRP.

Information about 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 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.

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).

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 EOAD and ADNI LOAD groups. 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.

Aim 4

Research-based whole genome sequencing (WGS) will be performed in a research laboratory. Established pipelines will be used for processing and annotating sequencing variants. A case control design will be used. Sequence data from the EOAD cases in this study will be combined with publicly available WGS 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 EOAD as compared with CNs.

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 400 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

EOAD Participants

Procedure \ Visit	Screening	Amyloid Status Disclosure	Baseline	Genetics Status Disclosure	Month 12	Month 24	Early Termination[m]
Window Period (days)	≤ 45			See footnote **a**	+/- 30[l]	+/- 30[l]	
Informed Consent	X						
Vital Signs	X		X		X	X	X
Neurological and Physical Exams	X				X	X	X
Demographics & Medical History	X				X	X	X
Clinical Diagnosis and Clinical Symptom Assessment	X				X	X	X
Family History	X						
Early Developmental History Questionnaire			X				
Autoimmune History Questionnaire			X				
Concomitant Medications	X				X	X	X
NACC UDS and FTLD Modules, and Neuropsychological Battery[b]	X				X	X	X
MMSE	X						
Digit Symbol	X						
ADAS-Cog13	X				X	X	X
Rey Auditory Verbal Learning Test (RAVLT)	X				X	X	X
TabCat: Flanker, Line Length, Line Orientation, and Match			X		X	X	X
CDR, NPI-Q, GDS, FAS, Social Norm Questionnaire, and Behavior Observation Checklist	X				X	X	X
ECG[c]	X				X	X	
Pregnancy Test[d]	X		X		X		
3T MRI/fMRI[e,f]			X[e,f]		X	X	X
PET Scan: Amyloid (florbetaben)	X				X		
PET Scan: Tau (flortaucipir)			X		X		
Adverse Events		X	X		X	X	X
Genetic Counseling	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	
Amyloid Status Disclosure		X					
Wellness Check[h]			X		X	X	X
Study Participation Report[i]			X		X	X	X
Blood for DNA, and RNA			X		X	X	X
Blood for PBMC			X		X	X	X
Blood for AD Biomarkers[j]			X		X	X	X
Blood for Genetic testing			X				

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Procedure \ Visit	Screening	Amyloid Status Disclosure	Baseline	Genetics Status Disclosure	Month 12	Month 24	Early Termination[m]
Lumbar Puncture (optional)[j]			X		X	X	X
LP Safety Labs (CBC, coagulation profile)[k]	X				X	X	X

a) Timing for result disclosure is not set and dependent on the availability of the WGS results.
b) The NACC UDS and FTLD batteries includes demographics, clinical, cognitive, functional, behavioral assessments.
   - Version C2 the NACC UDS Neuropsychological Battery will be used in this study
   - Only the FTLD "Required" forms are mandatory in this study
c) Safety requirement for flortaucipir PET scan.
d) If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed. Only participants with a negative result will be eligible for scanning.
e) The first MRI scan can be done either at screening or baseline.
f) If a MRI was not completed within previous 12 months, the study MRI must be used to rule out space occupying lesion, another reason for increased intracranial pressure, and Arnold Chiari malformation prior to the lumbar puncture procedure. See section 7.6.3 for more detail.
g) At the Screening Visit, participants will view a video that will discuss the implications of mutations in known ADAD loci (e.g. in *APP*, *PSEN1*, *PSEN2*). The participant will also be asked if they would like to speak with a genetic counselor 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. Written informed consent will be obtained on all participants prior to genetic testing. If a known mutation is found and confirmed in CLIA laboratory, the participant will be contacted and referred to the site's genetic counselor for result disclosure, and appropriate counseling and discontinued from the LEADS study.
h) To be completed within 48 hours post flortaucipir administration and lumbar puncture. See section 7.1.4 for more detail
i) To be provided to participants within 60 days of receiving results. See section 7.1.8 for more detail.
j) Should be collected after a minimum 6-hour fast (preferably in the morning after an overnight fast).
k) Mandatory for participants who consented to the lumbar procedure. See section 7.6.3 for more detail.
l) Month 12 and 24 visit dates are timed from Day 1 of the baseline visit procedures collection date and should commence within 365 days **+/- 30 days** from the previous visit. However, a PET scan of the same modality (tau to tau or amyloid to amyloid) should occur 365 days **+ 60 days** prior to the previous PET scan of the same modality. Consult the ATRI Coordinating Center for guidance on scheduling participant visits.
m) Contact ATRI Coordinating Center for guidance on what specific procedures should be conducted at this visit.

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Cognitively Normal Participants

Procedure \ Visit	Screening	Baseline	Month 12	Early Termination[j]
Window Period (days)	≤ 45	+/- 30[i]	+/- 30[i]	
Informed Consent	X			
Vital Signs	X	X	X	X
Neurological and Physical Exams	X		X	X
Demographics and Medical History	X		X	X
Clinical Diagnosis and Clinical Symptom Assessment	X		X	X
Family History	X			
Early Developmental History Questionnaire		X		
Autoimmune History Questionnaire		X		
Concomitant Medications	X		X	X
NACC UDS and FTLD Modules and Neuropsychological Battery[a]	X		X	X
MMSE	X			
Digit Symbol	X			
ADAS-Cog13	X		X	X
Rey Auditory Verbal Learning Test (RAVLT)	X		X	X
TabCat: Flanker, Line Length, Line Orientation, and Match		X	X	X
CDR, NPI-Q, GDS, FAS, Social Norm Questionnaire, and Behavior Observation Checklist	X		X	X
ECG[b]	X			
Pregnancy Test[c]	X	X		
3T MRI/fMRI[d,e]		X[d,e]		
PET Scan: Amyloid (florbetaben)	X			
PET Scan: Tau (floratаucipir)		X		
Adverse Events		X	X	X
Wellness Check[f]		X	X	X
Blood for DNA and RNA		X	X	X
Blood for PBMC		X	X	X
Blood for AD Biomarkers[g]		X	X	X
Lumbar Puncture (optional)[h]		X		
LP Safety Labs (CBC, coagulation profile)[i]	X			

a) 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
- Only the FTLD "Required" forms are mandatory in this study

b) Safety requirement for flortaucipir PET scan.

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c) If a female is not surgically sterile or post-menopausal by two years, a pregnancy test will be performed. Only participants with a negative result will be eligible for scanning.
d) The MRI scan can be done either at screening or baseline.
e) If a MRI was not completed within previous 12 months, the study MRI must be used to rule out space occupying lesion, another reason for increased intracranial pressure, and Arnold Chiari malformation prior to the lumbar puncture procedure. See section 7.6.3 for more detail.
f) To be completed within 48 hours post flortaucipir administration and lumbar puncture. See section 7.1.4 for more detail
g) Should be collected after a minimum 6-hour fast (preferably in the morning after an overnight fast.
h) Mandatory for participants who consented to the lumbar procedure. See section 7.6.3 for more detail.
i) Month 12 visit date is timed from Day 1 of the baseline visit procedures collection date and should commence within 365 days **+/- 30 days** from the previous visit.
j) Contact ATRI Coordinating Center for guidance on what specific procedures should be conducted at this visit.

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