CONFIDENTIAL

ALZHEIMER'S DISEASE NEUROIMAGING PROTOCOL

GRAND OPPORTUNITY (ADNI-GO)

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PROTOCOL SYNOPSIS

Title	**Alzheimer's Disease Neuroimaging Initiative Grand Opportunity (ADNI-GO)**
Primary Objective	The major goals of ADNI-GO are to: 1. Define and characterize the stage of the AD spectrum that precedes MCI as currently enrolled in ADNI1 (late MCI or LMCI) by enrolling 200 subjects in the mildest symptomatic phase of AD, early amnestic MCI (EMCI, defined more specifically below). 2. Perform F18 amyloid imaging (^{18}F-AV-45) on the CN and LMCI subjects from ADNI1 (including those who had C-11 PIB) and the newly enrolled EMCI subjects. FDG PET will also be performed in association with F18 amyloid imaging. This establishes a national network for F18 amyloid imaging, and will test hypotheses concerning the prevalence and severity of brain amyloid accumulation and its relationship to current and previous changes of clinical state, MRI, FDG-PET, CSF and plasma biomarkers from ADNI1. 3. Collect 3T MRI on all newly enrolled subjects. MRIs will be collected at Baseline, Month 3, Month 6, and Month 12. 4. Continue longitudinal clinical/cognitive and 1.5T MRI studies of approximately 500 LMCI and Cognitively Normal subjects from ADNI1 for an additional 2 years. 5. Collect and analyze blood biomarkers from all newly enrolled EMCI and follow-up subjects. Collect and analyze CSF for biomarkers in all newly enrolled EMCI. Follow up subjects will be asked to agree to CSF collection even if they have not had CSF collected for the original ADNI. 6. Collect blood samples for DNA and RNA extraction. Newly enrolled subjects will also have samples collected for Cell Immortalization and APOE genotyping.
Study Design	This is a non-randomized natural history non-treatment study.

Sample Size	200 newly enrolled subjects from approximately 50 sites from the United States and Canada. Approximately 450-500 subjects will be followed from the original ADNI study.
Summary of Key Eligibility Criteria	Newly enrolled subjects will be between 55-90 (inclusive) years of age, have a study partner able to provide an independent evaluation of functioning, and will speak either English or Spanish. All subjects must be willing and able to undergo all test procedures including neuroimaging and agree to longitudinal follow up. 100% of the newly recruited EMCI subjects must be willing to undergo at least one lumbar puncture at baseline. Specific psychoactive medications will be excluded. *EMCI Subject Inclusion Criteria:* MMSE scores between 24-30 (inclusive), a memory complaint (reported by subject or informant), must have objective memory loss measured by education adjusted scores on delayed recall of one paragraph from Wechsler Memory Scale Logical Memory II (between approximately 0.5 and 1.5 SD below the mean of Cognitively Normal), a CDR of 0.5, absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living, and an absence of dementia. *Follow-up Subject Inclusion Criteria:* In order to meet inclusion for follow-up these subjects must have been originally diagnosed as either Mild Cognitive Impairment (MCI) or Cognitively Normal (CN), and be willing and able to continue to participate. Subjects will be asked to continue in the trial even if a diagnostic conversion occurs. *Exclusion for amyloid imaging with* ^{18}F-AV-45*:* Current or recent participation in any procedures involving radioactive agents such that the total radiation dose exposure to the subject in any given year would exceed the limits of annual and total dose commitment set forth in the US Code of Federal Regulations (CFR) Title 21 Section 361.1.
Procedures	All subjects will have clinical/cognitive assessments, biomarker collection, and imaging. A reduced battery of tests can be requested from the project directors if the subject is not able/willing to complete the full battery. All MRI and PET scans will be rapidly assessed for quality so that subjects may be rescanned if necessary. All clinical data will be collected, monitored, and stored by the Coordinating Center at University California San Diego. University of Pennsylvania will collect biomarker samples. All raw and processed image data will be archived at LONI.
Outcome	1. Rate of Decline as measured by: Cognitive tests, Activities of Daily

Measures	Living, and CDR Sum of Boxes. 2. Rate of conversion will be evaluated among all four groups 3. Rate of volume change of whole brain, hippocampus, and other structural MRI measures. 4. Rates of change on each specified biochemical biomarker. 5. Rates of change of glucose metabolism (FDG-PET) 6. Extent of amyloid deposition as measured by ^{18}F-AV-45. 7. Group differences for each imaging and biomarker measurement. 8. Correlations among biomarkers and biomarker change. 9. Subgroups analyses: APOE genotype, low CSF $A\beta_{42}$, positive amyloid imaging with ^{18}F-AV-45.
Sponsor	National Institute of Aging, National Institute of Health

SCHEDULE OF EVENTS (EMCI SUBJECTS)

Visit Name	Screen	Baseline	Month 3	Month 6	Month 12	Month 18
Visit Type	In-Clinic	In-Clinic	MRI	In-Clinic	In-Clinic	Telephone Check
Explain study	X					
Obtain consent	X					
Demographics, Family History, Inclusion and Exclusion Criteria	X					
Medical History, Physical Exam, Neurological Exam, Hachinski	X					
Vital Signs	X	X		X	X	
Height	X					
Screening Labs	X					
DNA Sample Collection for APOE Genotyping and GWAS		X				
Cell Immortalization Sample Collection		X				
American National Adult Reading Test		X				
Mini Mental State Examination	X			X	X	
Logical Memory I and II	X				X	
Everyday Cognition (ECog)		X		X	X	
Montreal Cognitive Assessment (MoCA)		X		X	X	
Category Fluency (Animals)		X		X	X	
Trails A & B		X		X	X	
Boston Naming Test (30-item)		X		X	X	
Auditory Verbal Learning Test		X		X	X	
Geriatric Depression Scale	X			X	X	
Clock drawing		X		X	X	
Neuropsychiatric Inventory Q		X		X	X	
ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)		X		X	X	
Clinical Dementia Rating Scale	X			X	X	
Activities of Daily Living (FAQ)		X		X	X	
Plasma and Serum Biomarker Collection		X		X	X	
RNA Sample Collection		X			X	
Concomitant Medications	X	X		X	X	X
Adverse Events	X	X		X	X	X
Diagnostic Summary		X		X	X	
3T MRI Imaging (100%)	X		X*	X	X	
^{18}F-AV-45 Amyloid Imaging (100%)		X				
FDG-PET Imaging (100%)		X				
CSF Collection by Lumbar Puncture (LP) (100%)		X				

*Month 3 MRI is timed from Screening MRI

SCHEDULE OF EVENTS (FOLLOW-UP CN AND LMCI SUBJECTS)

Visit name	Baseline	Month 6	Month 12	Month 18
Visit Type	In-Clinic	Telephone Check	In-Clinic	Telephone Check
Explain study	X			
Obtain consent	X			
Medical History, Physical Exam, Neurological Exam	X			
Vital Signs	X		X	
Mini Mental State Examination	X		X	
DNA Sample Collection for GWAS	X			
Logical Memory I and II	X		X	
Everyday Cognition (ECog)	X		X	
Montreal Cognitive Assessment (MoCA)	X		X	
Category Fluency (Animals)	X		X	
Trails A & B	X		X	
Boston Naming Test (30-item)	X		X	
Auditory Verbal Learning Test	X		X	
Geriatric Depression Scale	X		X	
Clock drawing	X		X	
Neuropsychiatric Inventory Q	X		X	
ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)	X		X	
Clinical Dementia Rating Scale	X		X	
Activities of Daily Living (FAQ)	X		X	
Plasma and Serum Biomarker Collection	X		X	
RNA Sample Collection	X		X	
Concomitant Medications	X	X	X	X
Adverse Events	X	X	X	X
Diagnostic Summary	X		X	
1.5T MRI Imaging (100%)	X		X	
^{18}F-AV-45 -Amyloid Imaging (100%)	X			
FDG PET Imaging (100%)	X			
CSF Collection by Lumbar Puncture (LP)	X			

Note: All subjects will be asked if they are willing to consent to at least one LP. Subjects who are not able or willing to have LP, MRI, FDG-PET, or ^{18}F-AV-45 Amyloid imaging will still be followed for cognitive and clinical assessments.

Study Glossary

AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale – Cognitive
ADC	Alzheimer's Disease Center
ADCS	Alzheimer's Disease Cooperative Study
ADEAR	Alzheimer's Disease Education & Referral Center, under the NIA
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADNI 1	Original NIH grant, funding began October 2004
ADNI 2	NIH study application submitted October 2009, if funded would begin October 2010.
ADNI-CC	Alzheimer's Disease Neuroimaging Initiative Coordinating Center
ADNI-GO	Alzheimer's Disease Neuroimaging Initiative – Grand Opportunity
ADC's	Alzheimer's Disease Centers (under NIA)
AE	Adverse Event
ANART	American National Adult Reading Test
ANOVA	Analysis of Variance
APOE/APOE4	Apolipoprotein (APOE) epsilon 4 (APOE4)
AVLT	Auditory Verbal Learning Test
Aβ	Beta Amyloid
ASL	Arterial Spin Labeling
BNT	Boston Naming Test
C-11 PIB	[N-methyl-11C]2-(4'-(methylamino)phenyl-6-hydroxy-benzothiazole
CDR	Clinical Dementia Rating
CRF	Case Report Form
CN	Cognitively Normal
CSF	Cerebral Spinal Fluid
CT	Computerized Tomography
DNA	Deoxyribonucleic Acid

DSMB	Data Safety Monitoring Board
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
DTI	Diffusion Tensor Imaging
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EMCI	Early Amnestic Mild Cognitive Impairment
ECog	Everyday Cognition
FAQ	Functional Activities Questionnaire (Activities of Daily Living)
^{18}F-AV-45	Florbetapir F 18
FDG	Fluoro Deoxy Glucose
fMRI	Functional Magnetic Resonance Imaging
GDS	Geriatric Depression Scale
FLAIR	Fluid Attenuation Inversion Recovery
GWAS	Genome Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
IR SPGR	Inversion Recovery Spoiled Gradient-Recalled
LMCI	Late Mild Cognitive Impairment
LONI	Laboratory of Neuroimaging
LP	Lumbar Puncture
MCI	Mild Cognitive Impairment
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MPRAGE	Magnetization Prepared Rapid Gradient Echo
MR/MRI	Magnetic Resonance / Magnetic Resonance Imaging
NCRAD	National Cell Repository for Alzheimer's Disease
NIA	National Institute on Aging, under the NIH

NIH	National Institutes of Health
NINCDS/ADRDA	National Institute of Neurological and Communicative Diseases and Stroke / Alzheimer's Disease and Related Disorders Association
NPIQ	Neuropsychiatric Inventory Questionnaire
PET	Positron-Emission Tomography
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RARC	Resource Allocation Review Committee
REB	Research Ethics Board
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SD	Standard Deviation
T	Tesla
TFT's	Thyroid Function Tests
T2* GRE	T2 Star-Weighted Gradient-Echo
WMS-R	Wechsler Memory Scale – Revised
WML	White Matter Lesions
VIQ	Premorbid Verbal Intelligence

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1. Introduction

The goal of this project is to determine the relationships among the clinical, cognitive, imaging, genetic and biochemical biomarker characteristics of the early (pre-dementia) stages of Alzheimer's disease (AD). The project builds on the NIA-currently funded AD Neuroimaging Initiative (ADNI1), a collaboration between academia and industry to study biomarkers of AD. This project also serves as a bridge to the renewal of ADNI (termed ADNI2). Herein we continue ADNI themes with new hypotheses informed by our results. Our model posits that AD begins with amyloid β (Aβ) deposition in cortex, which leads to synaptic dysfunction, neurodegeneration, and cognitive/ functional decline. This predicts that the earliest detectable changes (measured in the ADNI-GO projects) are those related to Aβ (Cerebrospinal fluid (CSF) and PET amyloid imaging). Subsequently neurodegeneration is detected by a rise of CSF tau species, synaptic dysfunction by FDG-PET and neuron loss indicated by atrophy most notably in medial temporal lobe (measured with MRI). These changes ultimately lead to memory loss, general cognitive decline and eventually dementia. Expression of each element of AD pathology (e.g. Aβ and tau deposits, atrophy) is influenced by modifying factors including age, APOE genotype, and cerebrovascular disease (white matter lesions detected by FLAIR MRI) and microbleeds (detected by T2* MRI). The results obtained from this grant, together with ADNI1 and ADNI2 will advance understanding of AD pathophysiology, improve diagnostic methods for early detection of AD and improve clinical trial design.

We propose three global hypotheses based on cross sectional and longitudinal results (from ADNI1 data).

1) Reductions of CSF Aβ and increased amyloid PET tracer retention are present in some cognitively normal asymptomatic individuals, indicating early stage AD neuropathology.

2) Subsequently, CSF tau increases accompanied by reduced brain glucose metabolism (assessed by FDG-PET) and an increased rate of medial temporal lobe atrophy (assessed by structural MRI), and

3) After changes in CSF Aβ, amyloid PET tracer retention, CSF tau, FDG PET and atrophy of the medial temporal lobe, signs and symptoms of cognitive decline appear, eventually progressing to dementia.

2. Study Aims

This study will advance AD research by extending our infrastructure to perform amyloid imaging in all subjects and characterize an extended range of mild cognitive impairment (MCI) to broaden our understanding of the early stages of AD. Our specific aims are:

- **Aim 1:** Define and characterize the stage of the AD spectrum that precedes MCI as currently enrolled in ADNI. 200 subjects in the mildest symptomatic phase of AD, defined here as early amnestic MCI (EMCI), will be enrolled to narrow the gap between cognitively normal (CN) and "late MCI (LMCI)" subjects currently enrolled in ADNI. EMCI will be defined as individuals meeting clinical criteria for amnestic MCI, who score between 0.5 and 1.5 SD below the mean of CN on delayed paragraph recall performance. These 200 subjects will receive cognitive/clinical assessments at baseline, 6 months and 12 months. All baseline visits are in year 1, the 6 months visits occur in Year 1 and 2. After ADNI-GO, EMCI subjects will be followed by the renewal of ADNI (ADNI2).

- **Aim 2:** Perform ^{18}F-AV-45 amyloid imaging on the CN and LMCI subjects from ADNI1 (including those who had C-11 PIB) and the newly enrolled EMCI subjects. This establishes a national network for F18 amyloid imaging, and will test hypotheses concerning the prevalence and severity of brain amyloid accumulation and its relationship to current and previous changes of clinical state, MRI, FDG PET, CSF/ plasma biomarkers from ADNI1.

- **Aim 3:** Perform MRI imaging on all newly enrolled EMCI subjects (3T) and continue collecting MRI from all LMCI and CN (1.5T) at annual visits.
- **Aim 4:** Continue longitudinal clinical/cognitive and MRI studies of approximately 500 LMCI and Cognitively Normal (CN) subjects from ADNI1 for an additional 2 years.

- **Aim 5:** Collect and analyze Blood Biomarkers from all newly enrolled EMCI and follow-up subjects. Collect and analyze CSF for biomarkers in all newly enrolled EMCI. Longitudinal CSF collection is critical, but all follow up subjects will be asked to agree to a single LP.

- **Aim 6:** Collect blood samples for genome wide association studies (GWAS). Newly enrolled subjects will also have samples collected for Cell Immortalization and APOE genotyping.

3. Study Design

This is a non-randomized natural history non-treatment study. 200 Subjects will be enrolled and an additional 450-500 subjects will be followed from the previous ADNI study. Subjects will be followed for 18 months. At the end of this period subjects will be asked to consent to be followed long-term under a separate grant.

The overall impact of this grant will be 1) increased knowledge concerning the sequence of events leading to AD dementia, 2) development of improved clinical and biomarker methods for early detection of AD 3) improved imaging and chemical biomarker methods for monitoring progression of AD, facilitating clinical trials of treatments to slow disease progression, ultimately contributing to the prevention of AD dementia. No other large multisite study in the world addresses these complex issues with the sample size and statistical power of this application. The innovation of this proposal lies in the longitudinal assessment of the spectrum from normal aging to AD using an integrated combination of clinical/cognitive, CSF/Plasma biomarker, MRI and amyloid/FDG PET measures, including a national network for amyloid imaging with the ^{18}F-AV-45.

3.1 Site Personnel Requirements

Three staff functions (clinician, psychometrist, study coordinator) will be required to conduct the protocol at each site. At most sites, this will require three persons. At some sites, two persons may suffice. Details will be provided in the procedures manual.

- **Site Principal Investigator**. This person is responsible for ensuring that the local IRB approves the protocol and oversees all site activity for the study; this person may also serve as the study physician.
- **Study Physician.** This person is responsible for conducting or supervising the clinical evaluation of all participants, including physical and neurological examinations, reviewing adverse events, interpreting laboratory results; ensuring enrollment quotas and protocol adherence and for conversion determinations. The study physician will supervise project personnel and ensure that raters maintain a high level of skill and accuracy in conducting assessments.
- **Study Coordinator.** This person will be responsible for managing the day-to-day conduct of the trial, ensuring accurate administration of all instruments, maintaining online data and scheduling study procedures, processing laboratory samples, serving as liaison with the clinical monitor, and coordinating clinic visits. The study coordinator may perform several ratings, including the CDR. The ADAS-Cog rater must be a different person from the CDR rater.
- **Project interviewer/Psychometrician**. This person will have at least a

bachelor's degree in health care psychology, social work or a related field, and/or well-documented experience in administering interviews and neuropsychological tests.

3.2. Recruitment and Retention

ADNI has a multi-faceted recruitment plan in place; the overall goal being to raise awareness of ADNI trials among targeted populations. The ADNI will partner with NIA and coordinate with its ADEAR Center to take advantage of existing resources. The ADEAR center will also serve as the call center. In addition, a public relations/advertising firm will be consulted for broader coverage, and for seeking celebrity spokespersons and testimonies from other study participants or family members. The ADNI will determine the special requirements of each site and pattern their individual public relations support around those needs. In that context the ADNI will develop targeted messages in flyers, brochures, press releases, and presentations. Reference cards and online access to recruitment materials for the sites will also be available. Paid advertisements, direct mail and the Internet will be used as needed to supplement recruitment. A separate plan for minority recruitment is being developed. Enrollment will be monitored and tracked and additional support provided where appropriate. Additionally, the ADNI will provide background to sites on how to reach target audiences as well as assist in identifying them. Technical assistance will be offered to the sites on an ongoing basis.

Several steps will be taken to assure the high follow up rate that is essential to the validity of the study results. All staff members will be carefully instructed regarding the need for an expectation of full follow up participation and the process of removing barriers to participation. At entry, each participant, and a significant other informant will be queried regarding plans to change residence or leave the area. Frequent contact by telephone will be maintained by participants at a minimum of six-month intervals. Each participant will receive a thank you note following the clinical evaluation and a personalized greeting card on his or her birthday or on a major holiday. Progress of the study will be placed in a newsletter, distributed to the sites, for distribution to subject participants.

The goal of ADNI-GO is to obtain as close to 100% participation in lumbar puncture as is feasible. ADNI 1 aimed for 25% participation and achieved more than 50% participation. For ADNI-GO recruitment will be limited to those that consent to LP. Exceptions can be requested from the Protocol PI and may be considered in order to meet other goals such as minority recruitment.

Efforts to maximize LP participation will include training of site personnel at a meeting scheduled for January 2010. At this meeting, the ADCS Recruitment Core assisted by the PIs and staff at the most successful LP sites in ADNI 1 will share recruitment, consent and procedural methods. Training videos for both the recruitment/consent process and atraumatic LP techniques will be shared with all sites.

3.3. Data Collection and Monitoring

The Clinical Core will continue to be responsible for providing the operational infrastructure for this project, including the recruitment of 200 additional subjects with

EMCI, as well as the longitudinal follow-up of all remaining ADNI 1 normal and LMCI subjects. As in ADNI 1, this infrastructure will be provided by the ADCS Administrative, Clinical Operations, Medical and Data Cores at UCSD. The ADCS Cores occupy approximately 25,000 square feet of space across the street from the UCSD Medical School Campus.

Clinical data collection and monitoring are standardized with well-established and successful ADCS operating procedures. Data will be entered directly by sites using web-based data entry screens. Imaging data collection and monitoring are handled by LONI. Imaging data are handled using a combination of data entry via web-based forms and automated file transition modules embedded within the web-based Image Data Archive application. The file translation modules extract metadata directly from the imaging data, ensuring accuracy and reducing the amount of data entry required.

3.4. Data Sharing

In order to provide the clinical data from this project to Initiative investigators, the Pharmaceutical Industry and the public, the entire clinical data base (free of any identifying information such as name, address, or phone number) will be placed on a public web site, which will be appropriately linked to the imaging data base at LONI. The data base will be frequently updated, and all cleaned clinical data which is acquired by the ADNI-CC will be provided in real-time.

4. Subject Selection

4.1 Inclusion/Exclusion Criteria: EMCI

4.1.1. Inclusion Criteria: EMCI

1. Subject must have a memory complaint by subject or study partner that is verified by a study partner.

2. Abnormal memory function documented by scoring below the education adjusted cutoff on the Logical Memory II subscale (Delayed Paragraph Recall) from the Wechsler Memory Scale –Revised (the maximum score is 25):
   a. 9-11 for 16 or more years of education.
   b. 5-9 for 8-15 years of education.
   c. 3-6 for 0-7 years of education.

3. Mini-Mental State Exam score between 24 and 30 (inclusive) (Exceptions may be made for subjects with less than 8 years of education at the discretion of the project director.

4. Clinical Dementia Rating = 0.5. Memory Box score must be at least 0.5.

5. General cognition and functional performance sufficiently preserved such that a diagnosis of Alzheimer's disease cannot be made by the site physician at the time of the screening visit.

6. Stability of Permitted Medications for 4 weeks. In particular, subjects may:
   a. Take stable doses of antidepressants lacking significant anticholinergic side effects (if they are not currently depressed and do not have a history of major depression within the past 1 year).
   b. Estrogen replacement therapy is permissible.
   c. Gingko biloba is permissible, but discouraged.
   d. Washout from psychoactive medication (e.g., excluded antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.) for at least 4 weeks prior to screening.
   e. Cholinesterase inhibitors and memantine are allowable if stable for 12 weeks prior to screen.

7. Geriatric Depression Scale less than 6.

8. Age between 55-90 (inclusive).

9. Study partner is available who has frequent contact with the subject (e.g. an average of 10 hours per week or more), and can accompany the subject to all clinic visits for the duration of the protocol.

10. Visual and auditory acuity adequate for neuropsychological testing.

11. Good general health with no diseases expected to interfere with the study.

12. Subject is not pregnant, lactating, or of childbearing potential (i.e. women must be two years post-menopausal or surgically sterile).

13. Willing and able to participate in a longitudinal imaging study.

14. Hachinski less than or equal to 4.

15. Six grade education or has a good work history (sufficient to exclude mental retardation).

16. Must speak English or Spanish fluently.

17. Willing to undergo repeated MRIs (3Tesla) and at least two PET scans (one FDG and one Amyloid imaging) and no medical contraindications to MRI.

18. Agrees to collection of blood for GWAS, APOE testing and DNA banking.

19. Agrees to collection of blood for biomarker testing.

20. Agrees to at least one lumbar puncture for the collection of CSF.

4.1.2. Exclusion Criteria: EMCI

1. Any significant neurologic disease other than suspected incipient Alzheimer's disease, such as Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma followed by persistent neurologic defaults or known structural brain abnormalities.

2. Screening/baseline MRI scans with evidence of infection, infarction, or other focal lesions. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.

3. Presence of pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body.

4. Major depression, bipolar disorder as described in DSM-IV within the past 1 year. Psychotic features, agitation or behavioral problems within the last 3 months which could lead to difficulty complying with the protocol.

5. History of schizophrenia (DSM IV criteria).

6. History of alcohol or substance abuse or dependence within the past 2 years (DSM IV criteria).

7. Any significant systemic illness or unstable medical condition which could lead to difficulty complying with the protocol.

8. Clinically significant abnormalities in B12, or TFTs that might interfere with the study.

9. Residence in skilled nursing facility.

10. Current use of specific psychoactive medications (e.g.,certain antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.). Current use of warfarin (exclusionary for lumbar puncture).

11. Investigational agents are prohibited one month prior to entry and for the duration of the trial.

12. Participation in clinical studies involving neuropsychological measures being collected more than one time per year.

13. Exclusion for amyloid imaging with ^{18}F –AV-45: Current or recent participation in any procedures involving radioactive agents such that the total radiation dose exposure to the subject in any given year would exceed the limits of annual and total dose commitment set forth in the US Code of Federal Regulations (CFR) Title 21 Section 361.1.

14. Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the protocol director (Dr. Petersen).

4.2 Inclusion/Exclusion Criteria for Follow-up Subjects

4.2.1. Inclusion Criteria: Follow-up Subjects

1. Must have been enrolled and followed in ADNI for at least one year diagnosed as either Mild Cognitive Impairment (MCI) or Cognitively Normal (CN) regardless of whether a diagnostic conversion has occurred since enrolling in ADNI.

2. Willing and able to continue to participate in an ongoing longitudinal study. A reduced battery of tests can be requested from the project directors if the subject is not able/willing to complete the full battery.
3. Study partner is available who has frequent contact with the subject (e.g. an average of 10 hours per week or more), and can accompany the subject to all clinic visits for the duration of the protocol.

4.2.2. Exclusion Criteria: Follow-up Subjects

1. Subjects will not be able to participate in amyloid imaging with ^{18}F-AV-45 if the following is true: Current or recent participation in any procedures involving radioactive agents such that the total radiation dose exposure to the subject in any given year would exceed the limits of annual and total dose commitment set forth in the US Code of Federal Regulations (CFR) Title 21 Section 361.1.

5. Study Procedures

EMCI subjects who are enrolling in this study will go through a Pre-screening and Screening period as described below. Assessments completed at each visit are also listed. Subjects who are being followed from the first ADNI study will begin at the Baseline Visit for this study. The visit window is within 2 weeks of the target date, either before or after. All imaging studies, biofluid collection and cognitive and clinical assessments must take place within this 4-week timeframe.

5.1. Procedures for Newly Enrolled EMCI Subjects

5.1.1. Pre-screening

Sites will identify potential EMCI subjects through a variety of mechanisms: by reviewing subjects enrolled in ADCs, de novo recruitment, and referrals.

5.1.2. Screening Procedures

The purpose of the screening visit is to determine eligibility for the proposed study and to collect measures that will be used as a reference to assess change. Only newly enrolled EMCI subjects will have a screening visit. A standardized evaluation will be performed at each clinical site. Consent will be obtained before any portion of the screening visit is initiated. The MRI will be conducted only for subjects who meet inclusion criteria for all other screening assessments. A local clinical read of the MRI will be reviewed for eligibility. Eligibility will be determined according to the Inclusion/Exclusion criteria outlined above and confirmed by an ADCS clinical monitor before the subject can be brought back for Baseline.

- Explain study
- Obtain consent

- Demographics
- Family History
- Inclusion and Exclusion Criteria
- Medical History
- Physical Exam
- Neurological Exam
- Hachinski
- Vital Signs
- Height
- Screening Labs (hematology, chemistry panel, urinalysis, B12, TSH)
- Mini Mental State Examination
- Logical Memory I and II
- Geriatric Depression Scale
- Clinical Dementia Rating Scale
- Concomitant Medications
- Adverse Events
- MRI (3T)

5.1.3. Baseline Visit

The Baseline visit for the newly enrolled EMCI subjects must take place within 28 days of the Screening. All EMCI subjects will undergo lumbar puncture as part of the baseline visit upon consent for LP.

- Vital Signs
- DNA Sample Collection for APOE Genotyping and GWAS
- Cell Immortalization Sample Collection
- American National Adult Reading Test
- Everyday Cognition (ECog)
- Montreal Cognitive Assessment (MoCA)
- Category Fluency (Animals)
- Trails A & B
- Boston Naming Test (30-item)
- Auditory Verbal Learning Test
- Clock drawing
- Neuropsychiatric Inventory Q
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Activities of Daily Living (FAQ)
- Collection of Plasma and Serum Biomarkers
- RNA Sample Collection
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- ^{18}F-AV-45 Amyloid Imaging (100%)
- FDG-PET Imaging (100%)
- CSF Collection by Lumbar Puncture (LP)

5.1.4. Month 3 Visit

The Month 3 visit is timed 3 months from the date of the Screening MRI.

- MRI (3T)

5.1.5. Month 6 Visit

The Month 6 visit is timed 6 months from the Baseline Visit.

- Vital Signs
- Mini Mental State Examination
- Everyday Cognition (ECog)
- Montreal Cognitive Assessment (MoCA)
- Category Fluency (Animals)
- Trails A & B
- Boston Naming Test (30-item)
- Auditory Verbal Learning Test
- Geriatric Depression Scale
- Clock drawing
- Neuropsychiatric Inventory Q
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Clinical Dementia Rating Scale
- Activities of Daily Living (FAQ)
- Collection of Plasma and Serum Biomarkers
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- MRI (3T)

5.1.6. Month 12 Visit

The Month 12 visit will be timed 12 months from the Baseline Visit.

- Vital Signs
- Mini Mental State Examination
- Logical Memory I and II
- Everyday Cognition (ECog)
- Montreal Cognitive Assessment (MoCA)
- Category Fluency (Animals)
- Trails A & B
- Boston Naming Test (30-item)
- Auditory Verbal Learning Test
- Geriatric Depression Scale
- Clock drawing
- Neuropsychiatric Inventory Q
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Clinical Dementia Rating Scale

- Activities of Daily Living (FAQ)
- Collection of Plasma and Serum Biomarkers
- RNA Sample Collection
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- MRI (3T)

5.1.7. Month 18 Visit

The Month 18 visit will be a telephone check for all subjects.

- Concomitant Medications
- Adverse Events

There are no visits scheduled beyond 18 months because the ADNI-GO grant is only for 2 years funding which began on Oct 1 2009. EMCI subjects enrolled in ADNI-GO will be followed by the ADNI2 grant (assuming it is funded) after their 18 month visit or after Oct 1, 2011, whichever is earlier.

5.2. Procedures for Follow-up (LMCI and CN) Subjects

Subjects will be followed as long as they are willing regardless of whether a diagnostic conversion has occurred since first enrolled in ADNI. If subjects are not willing or able to complete the full schedule of assessments for this visit or any ADNI visit, a minimal battery can be requested from the Clinical Core PIs.

The minimum battery includes:

- Vital Signs
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Mini Mental State Examination
- Logical Memory I and II
- Clinical Dementia Rating Scale
- Concomitant Medications
- Adverse Events
- Diagnostic Summary

5.2.1 Baseline Visit

The baseline visit for ADNI-GO will be conducted at the subject's next projected annual follow-up visit, either 36, 48, or 60 months from their initial ADNI Baseline Visit. Longitudinal collection of CSF is critical, but all subjects will be asked to agree to a single LPs for CSF collection.

- Explain study
- Obtain consent

- Medical History
- Physical Exam
- Neurological Exam
- Vital Signs
- DNA Sample Collection for GWAS
- Mini Mental State Examination
- Logical Memory I and II
- Everyday Cognition (ECog)
- Montreal Cognitive Assessment (MoCA)
- Category Fluency (Animals)
- Trails A & B
- Boston Naming Test (30-item)
- Auditory Verbal Learning Test
- Geriatric Depression Scale
- Clock drawing
- Neuropsychiatric Inventory Q
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Clinical Dementia Rating Scale
- Activities of Daily Living (FAQ)
- Plasma and Serum Biomarker Collection
- RNA Sample Collection
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- MRI (1.5T) (100%)
- ^{18}F-AV-45 Amyloid Imaging (100%)
- FDG-PET Imaging (100%)
- CSF Collection by Lumbar Puncture (LP)

5.2.2. Month 6 Visit

The Month 6 visit is a telephone check for Follow-up Subjects, reviewing the following with the subject and study partner:

- Concomitant Medications
- Adverse Events

5.2.3. Month 12 Visit

The Month 12 visit will be timed 12 months from the Baseline Visit.

- Vital Signs
- Mini Mental State Examination
- Logical Memory I and II
- Everyday Cognition (ECog)
- Montreal Cognitive Assessment (MoCA)
- Category Fluency (Animals)
- Trails A & B
- Boston Naming Test (30-item)

- Auditory Verbal Learning Test
- Geriatric Depression Scale
- Clock drawing
- Neuropsychiatric Inventory Q
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Clinical Dementia Rating Scale
- Activities of Daily Living (FAQ)
- Plasma and Serum Biomarker Collection
- RNA Sample Collection
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- MRI (1.5T) (100%)

5.2.4. Month 18 Visit

The Month 18 visit will be a telephone check reviewing the following with the subject and study partner:

- Concomitant Medications
- Adverse Events

There are no visits scheduled beyond 18 months because the ADNI-GO grant is only for 2 years funding which began on Oct 1 2009. Subjects who participate in ADNI-GO will be followed by the ADNI2 grant (assuming it is funded) after their 18 month visit or after Oct 1, 2011, whichever is earlier.

5.3. Early Termination Visit

If a subject wishes to exit the study, a termination visit will be scheduled. This will include all evaluations normally performed at the Month 12 visit.

5.4. Retrieved drop-outs

Subjects missing visits will be encouraged to return for subsequent visits. Unless a subject withdraws consent, subjects who miss visits will be encouraged to come in for subsequent visits.

5.5. Nursing Home Placement

If a subject is placed in a skilled nursing home, the subject status report will reflect this. All assessments will be completed, to the extent possible. If the subject withdraws consent to continue in the study, a termination visit will be conducted.

6. Assessments

The following assessments are only performed in the EMCI group, as there is already data on the follow-up subjects as part of their initial visits in ADNI 1.

- Screening Labs
- Height
- ANART
- Cell Immortalization
- APOE

Refer to the prior section for timing and frequency of administration.

6.1. Clinical and Cognitive Assessments

The tests and scales chosen for use in this protocol were selected because: (1) they represent the domains of interest in this patient population; (2) they will adequately sample cognitive domains of interest in subjects who are cognitively normal (CN), have MCI or AD; (3) they can measure change over two to three years in these patient populations; (4) subjects enrolled will not demonstrate floor or ceiling effects; (5) they are reasonably efficient and can meet the practical demands of the proposed study; and (6) they were utilized previously in the ADCS MCI trial and worked well. All of these instruments are widely used in multi-center trials studying CN, MCI, and early AD subjects. Additionally, they are being used by Alzheimer Disease Centers as part of their collection of a Uniform Data Set thereby reducing the amount of testing that subjects will need to undergo who are enrolled in both ADC's and ADNI.

6.1.1. Montreal Cognitive Assessment (MoCA) (Nasreddine et al, 2005): The Montreal Cognitive Assessment test (MoCA) is a brief cognitive assessment designed to detect subjects at the MCI stage of cognitive dysfunction. This instrument has been shown to have adequate sensitivity and specificity in clinical settings to detect suspected MCI. The MoCA is believed to be more sensitive than general screening instruments such as the MMSE or the Short Test of Mental Status. The MoCA can be administered in approximately ten minutes.

6.1.2. Everyday Cognition (ECog) (Farias et al, 2009): For a functional assessment, we have selected the Measurement of Everyday Cognition (ECog). This instrument has been developed to assess functional impairment of a very mild nature as can be seen in MCI. The ECog is a informant-rated questionnaire comprised of multiple subscales and takes approximately ten minutes to administer. Previous research on this instrument indicates that ECog correlates well with established measures of functional status and global cognition but only weakly with age and education. ECog was able to differentiate among cognitively normal (CN), MCI and AD subjects. Results of ECog suggest that it is a useful tool for the measurement of general and domain-specific everyday functions in the elderly. However, the performances of the MoCA and ECog will be followed to determine their ability to differentiate among the three cognitive groups.

6.1.3. Mini-Mental State Exam (MMSE) (Folstein, Folstein, & McHugh, 1975): The MMSE is a fully structured screening instrument frequently used for Alzheimer's disease drug studies. The scale evaluates orientation to place, orientation to time, registration (immediate repetition of three words), attention and concentration (serially subtracting seven beginning with 100), recall (recalling the previously repeated three words), language (naming, repetition, reading, writing, comprehension), and visual construction (copy two intersecting pentagons). The MMSE is scored as the number of correctly completed items with lower scores indicative of poorer performance and greater cognitive impairment. The total score ranges from 0 to 30 (perfect performance).

6.1.4. Alzheimer's Disease Assessment Scale-Cognitive (ADAS-COG) 13 (Rosen, Mohs, & Davis, 1984): The ADAS-COG is a structured scale that evaluates memory (word recall, word recognition), reasoning (following commands), language (naming, comprehension), orientation, ideational praxis (placing letter in envelope) and constructional praxis (copying geometric designs). Ratings of spoken language, language comprehension, word finding difficulty, and ability to remember test instructions are also obtained. The test is scored in terms of errors, with higher scores reflecting poorer performance. Scores can range from 0 (best) to 70 (worse). Delayed Word Recall and Number Cancellation will be conducted in addition to the eleven standard ADAS-Cog Items.

6.1.5. Logical Memory Test I and II (Delayed Paragraph Recall) (D Wechsler, 1987): The Logical Memory test that will be used is a modification of the episodic memory measure from the Wechsler Memory Scale-Revised (WMS-R) (D Wechsler, 1987). In this modified version, free recall of one short story (Story A) that consists of 25 bits of information will be elicited immediately after it is read aloud to the subject and again after a thirty-minute delay. The total bits of information from the story that are recalled immediately (maximum score = 25) and after the delay interval (maximum score = 25) are recorded. A retention or "savings" score can be computed by dividing the score achieved during delayed recall by the score achieved during immediate recall.

6.1.6. Boston Naming Test (Kaplan, Goodglass, & Weintraub, 1983): This measure of visual confrontation naming requires the subject to name objects depicted in outline drawings. In our modification of the full BNT, only 30 items are presented (the odd-numbered items from the full 60-item test). The drawings are graded in difficulty, with the easiest drawings presented first. If a subject encounters difficulty in naming an object, a stimulus cue and/or a phonemic cue is provided. The number of spontaneous correct responses (maximum score = 30) and spontaneous plus semantically-cued correct responses (maximum score = 30) are recorded. The number of perceptual errors, circumlocutions, paraphasic errors, and perseverations can also be used to evaluate the subjects' language performance.

6.1.7. Category Fluency Test (Butters, Granholm, Salmon, Grant, & Wolfe, 1987): This is a measure of verbal fluency in which the subject is asked to generate examples from the semantic categories (animals) in successive one-minute trials. The primary performance

measure is the number of correct, unique examples generated for the two categories. Perseveration (repetitions of a correct item) and intrusion (non-category items) errors are also noted.

6.1.8. Clock Drawing Test (Goodglass & Kaplan, 1983): In the "command" condition of this visuoperceptual constructional task, the subject is given a blank sheet of 8 1/2" X 11" paper and instructed to "Draw a clock, put in all of the numbers, and set the hands for 10 after 11." After that task is completed, the "copy" condition ensues in which the subject attempts to copy a drawing of a clock with the hands set at ten past eleven. A quantitative score (maximum total score = 10) is derived for each drawing by adding the scores of three separate features: a maximum of 2 points is given for the integrity of the clock face; a maximum of 4 points for the presence and sequencing of the numbers; a maximum of 4 points for the presence and placement of the hands. A qualitative analysis can also be performed to note the presence of conceptual, perseverative, stimulus bound, and spatial arrangement errors. The Clock Drawing Test is effective for discriminating between subjects with AD and normal elderly individuals (Cahn et al., 1996).

6.1.9. American National Adult Reading Test (ANART) (Nelson & O'Connell, 1978): The ANART is a method for estimating premorbid verbal intelligence (VIQ) in demented patients based upon their ability to read words aloud, a skill that is thought to remain relatively preserved until the later stages of Alzheimer's disease (Nelson & O'Connell, 1978). The test requires patients to read and correctly pronounce 50 "irregular" words that do not follow common rules of phonography and orthography. The correct pronunciation of such words depends solely on previous familiarity and cannot be accomplished by applying common grammatical rules (e.g., the word 'naive' might be pronounced 'nave' if common English grammatical rules were employed). Thus, the ability to correctly pronounce progressively less common irregular words suggests a large premorbid vocabulary that is correlated with a high premorbid VIQ. The 50 irregular words of the ANART are printed on a single sheet of paper which is presented to the subject who is instructed to read each word aloud. The number of mispronounced words is recorded by the examiner (maximum errors = 50). Premorbid VIQ can be estimated by applying a formula derived by Grober and Sliwinski: [118.2 - .89 (ANART errors) + .64 (years of education)](Grober & Sliwinski, 1991).

6.1.10. Rey Auditory Verbal Learning Test (Rey, 1964): The AVLT is a list learning task which assesses multiple cognitive parameters associated with learning and memory. On each of 5 learning trials, 15 unrelated words (all nouns) are presented orally at the rate of one word per second and immediate free recall of the words is elicited. The number of correctly recalled words on each trial is recorded. Following a 20-minute delay filled with unrelated testing, free recall of the original 15 word list is elicited. Finally, a yes/no recognition test is administered which consists of the original 15 words and 15 randomly interspersed distracter words. The number of target "hits" and false positive responses are recorded. Two equivalent alternate forms of the test will be used across test sessions so that subjects will be exposed to the same word list as infrequently as possible.

6.1.11. Trail Making Test: Parts A and B (Reitan, 1958): Part A consists of 25 circles numbered 1 through 25 distributed over a white sheet of 8 1/2" X 11" paper. The subject is

instructed to connect the circles with a drawn line as quickly as possible in ascending numerical order. Part B also consists of 25 circles, but these circles are either numbered (1 through 13) or contain letters (A through L). Now the subject must connect the circles while alternating between numbers and letters in an ascending order (e.g., A to 1; 1 to B; B to 2; 2 to C). The subject's performance is judged in terms of the time (in seconds) required to complete each trail and by the number of errors of commission and omission. The time to complete Part A (150 second maximum) and B (300 second maximum) will be the primary measures of interest (testing is stopped if the maximum time is reached). Although both Parts A and B depend on visuomotor and perceptual-scanning skills, Part B also requires considerable cognitive flexibility in shifting from number to letter sets under time pressure. Both parts of the Trail-Making Test are available in multiple forms of equal difficulty for purposes of repeated evaluation.

6.1.12. Clinical Dementia Rating (CDR) (Berg, 1988): The CDR describes five degrees of impairment in performance on each of 6 categories of cognitive functioning including memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. The ratings of degree of impairment obtained on each of the 6 categories of function are synthesized into one global rating of dementia (ranging from 0 to 3), with more refined measure of change available by use of the Sum of Boxes. Reliability and validity has been established, as has high inter-rater reliability. This will be used as a global measure of severity of dementia.

6.1.13. Activities of Daily Living | Functional Activities Questionnaire (FAQ) (Pfeffer, Kurosaki, Harrah, Chance, & Filos, 1982): Based on an interview with a caregiver or qualified partner, a subject is rated on their ability to carry out ten complex activities of daily living: 1) manage finances, 2) complete forms, 3) shop, 4) perform games of skill or hobbies, 5) prepare hot beverages, 6) prepare a balanced meal, 7) follow current events, 8) attend to television programs, books or magazines, 9) remember appointments, and 10) travel out of the neighborhood. Each activity is rated as 0 (does without difficulty), 1 (needs frequent advice or assistance), or 2 (someone has taken over the activity). Scores are summed across items to provide a total disability score (higher scores = greater impairment; maximum score = 20). If an activity was never or very rarely performed premorbidly, it is not rated and a pro-rated proportional score can be derived [achieved score / (20 – 2 times the number of items rated as never performed)].

6.1.14. Neuropsychiatric Inventory Q (NPIQ) (Kaufer et al., 2000): The Neuropsychiatric Inventory (NPI) is a well-validated, reliable, multi-item instrument to assess psychopathology in AD based on an interview with a caregiver or qualified partner. The interview is also relatively brief (15 minutes). These properties make it well suited for a multicenter trial. The NPIQ is a shorter version that does only the screening questions and the severity rating for each domain. The maximum score is 36.

6.1.15. Geriatric Depression Scale (Sheikh & Yesavage, 1986): The Geriatric Depression Scale (Short Form) is a self report scale designed to identify symptoms of depression in the elderly. The scale consists of 15 printed questions that the subject is asked to answer by circling yes or no on the basis of how they felt over the past week. The items are presented on a single page with more benign items presented first. Answers to 5 of the items are negatively oriented

for depression (e.g., Do you feel full of energy?) and 10 positively oriented (e.g., Do you often feel helpless). One point is given for each appropriate positive or negative answer indicative of a symptom of depression, for a possible total of 15 points. Total scores of 0-5 are considered normal and scores of 6-15 are considered depressed.

6.1.16. Concomitant Medications

All approved anti-dementia therapies will be permitted provided doses are stable for 4 weeks prior to screening. Vitamin E is permitted. Only antipsychotics with anti-cholinergic properties, chronic use of sedatives or anxiolytics specified in the procedures manual are prohibited at entry. Use of experimental drugs is prohibited one month prior to screen.

6.1.17. Vital Signs

Weight, Seated Blood Pressure, Seated Pulse, Respirations, Temperature will be collected at each clinic visit. Height will be measured at Screening. Prior to and immediately after injection of ^{18}F-AV-45 the following vitals will be measured: Seated Blood Pressure, Seated Pulse, Respirations and Temperature.

6.1.18. Diagnostic Summary

At each in clinic visit, the study physician will determine the current diagnosis of the ADNI subject. Following procedures established in ADNI, uniform application of the diagnostic criteria across sites will be insured by having each subject's record monitored and reviewed by a Central Review Committee. The Central Review Committee will verify each subject's eligibility and conversion to MCI or AD or an alternate diagnosis. Subjects who have converted from one stage of disease to another, or from normal to a disease state, will continue to be followed in this protocol.

6.2. Biofluid Collection

The study procedure manual will provide more detailed instructions for collection, processing and shipment of all biofluid samples for ADNI-GO. Polypropylene tubes will be utilized for the collection and storage of plasma, serum, and CSF, since some key analytes such as Aβ are known to stick to polystyrene and glass. Only water is permitted until blood draws and the LP procedure are completed. Blood, (separated into plasma and serum), and CSF will be collected so as to accommodate the assay of the broadest range of the best antecedent biomarkers/analytes. The methods used to assay homocysteine, tau, p-tau and Aβ are identical to those used in ADNI1.

Fasting overnight is only required for plasma, serum and CSF sample collection.

6.2.1. Cell Immortalization Samples

Blood samples will be collected for Cell Immortalization at Baseline for all newly enrolled EMCI subjects after passing screening criteria for this study. Whole blood will be collected in two ACD-A 8.5 mL tubes. Samples will be shipped ambient, day of collection, by overnight delivery to the National Cell Repository for Alzheimer's Disease (NCRAD).

Upon arrival at NCRAD the blood sample is placed in a centrifuge and spun to separate the sample into three main layers: the red blood-cell layer, the plasma layer, and the buffy coat, which contains the white-blood cells. The white-blood cells are needed to establish cell lines

and obtain DNA. To establish cell lines, the white-blood cells are placed in a flask along with a solution that allows permanent cell growth. The cells are incubated at 37°C (body temperature) ranging from four weeks to three months. The cell-containing solution is then divided and transferred into two larger flasks for further cell growth. It takes approximately one week for the cells to divide to the desired number. The cells are then placed in a vial along with a preservative. Each vial holds approximately 1 milliliter of solution containing $1x10^7$ (10,000,000) cells. The cells are gradually cooled to freezing temperatures. The slow freeze prevents damage to the cell line. The frozen cells are bar code labeled and stored in a tank filled with liquid nitrogen at -316°F. Cells can be preserved this way indefinitely and thawed at any time for additional propagation.

If a sample does not successfully establish, subjects will be asked to agree to resampling.

6.2.2. DNA Sample collection for GWAS and APOE Genotyping

Whole blood is collected for GWAS at Baseline for both newly enrolled EMCI subjects and follow-up subjects. APOE genotyping will only be done for the newly enrolled EMCI subjects, using this sample.

A single 10 mL EDTA tube of whole blood will be sent ambient, overnight, and on the day of collection to the National Cell Repository for AD (NCRAD) for genotyping.

The genetics core will request a resampling if the sample condition is compromised or if there is poor sample yield. The blood collected may be used for generating a cell line for follow-up subjects, if the original cell lines created for ADNI are compromised.

6.2.3. RNA Sample Collection

In order to measure gene expression across time, a RNA sample will be collected at Baseline and Month 12 from all ADNI-GO subjects.
Three (3) 2.5 mL tubes will be collected at each visit using kits supplied by the ADNI CC and following these steps:

a. If the PAXgene Blood RNA Tube is the only tube to be drawn, a small amount of blood should be drawn into a "Discard Tube" (A) prior to drawing blood into the PAXgene Blood RNA Tube. Otherwise, the PAXgene Blood RNA Tube should be the last tube drawn in the phlebotomy procedure.
b. Using a BD (Becton, Dickinson and Company) Vacutainer® Safety-Lok™ Blood Collection Set, collect blood into the PAXgene Blood RNA Tube using your institution's recommended standard procedure for venipuncture.
c. Hold the PAXgene Blood RNA Tube vertically, below the blood donor's arm, during blood collection.
d. Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.

e. Gently invert the PAXgene Blood RNA Tube 8 to 10 times.

Samples will be shipped overnight day of collection under ambient conditions to the National Cell Repository for AD (NCRAD).

6.2.4. Plasma and Serum for Biomarkers

All samples will be collected in the morning before breakfast and after an overnight fast.

Plasma is collected in a uniform fashion using EDTA as anti-coagulant. Once blood is collected into two 10 mL EDTA plastic tubes, as described in the procedures manual, it is mixed thoroughly, then centrifuged for 15 min. at ~3000 rpm. 10 mL of the plasma sample is transferred to a labeled polypropylene vial, frozen on dry ice for 20 minutes.

Serum is obtained after allowing the samples collected in two 10 mL plain red top plastic tubes to clot at room temperature, and it is spun as above for plasma preparation, aliquoted into polypropylene shipping containers, frozen on dry ice for 20 minutes.

After freezing on dry ice, plasma and serum are packaged with the CSF collected for the same visit and shipped day of collection overnight on dry ice to Penn AD Biomarker Fluid Bank Laboratory.

6.2.5. CSF for Biomarkers

All samples will be collected in the morning before breakfast and after an overnight fast.

The ADNI–preferred method for obtaining CSF is lumbar puncture with a small caliber atraumatic needle (22 gauge Sprotte needle) and collection by gravity into a polypropylene container. To clear any blood from minor trauma associated with needle insertion, the first 1-2 mL of CSF are discarded (or more if needed) to eliminate blood, and then 20 mL of CSF are collected from each patient for use and treatment in the following manner:

1. The first 3 mL will be used for standard tests such as cell counts, glucose, and total protein with determinations done at local laboratories.
2. The remaining CSF (17 mL) will be collected into polypropylene collection tubes and transferred to polypropylene shipping tubes as outlined in the Procedures manual.

CSF is frozen upright on dry ice for at least 20 minutes before being packaged, along with the frozen plasma and serum. CSF samples are shipped frozen on dry ice, day of collection, via Federal Express overnight delivery to the Penn AD Biomarker Fluid Bank Laboratory.

The day after the Lumbar Puncture each study participant or a person designated to speak for them will be contacted by phone 24 hours after the Lumbar Puncture to confirm the participant's well being and query about any new adverse events.

6.2.6. Laboratory Procedures at University of Pennsylvania

When plasma, serum and CSF samples are received in the Penn AD Biomarker Fluid Bank Laboratory, they will be thawed and aliquots transferred to plastic vials, bar code labeled, and placed in designated locations in the -80°C freezers. All samples will be inventoried and

tracked using commercially available software. A database will be created and used for the inventory of stored samples, in conjunction with a bar code reading system. Bar code labels affixed to each sample vial will contain the following information: sample ID# (to preserve confidentiality), date of collection and processing, total initial volume collected, sample type (plasma, serum, CSF), volume, aliquot number, freezer, shelf, rack, box, location in the box. A bar code label will be used on the sample tracking form that is used by the technologist when processing and storing samples. This will be done to avoid manual entry errors of sample numbers. When the data are entered into the database the bar code label is scanned in and the sample aliquots entered. Removals of samples will also be tracked on the database, including the date removed and the recipient center.

6.2.7. Sharing of Banked DNA, RNA, Plasma, Serum and CSF

Specific procedures for requesting and accessing DNA, RNA, or plasma serum or CSF have been created by the Resource Allocation Review Committee (RARC) of the ADNI in accordance with recommendations proposed in the National Bioethics Advisory Commission (NBAC) Human Biological Materials Report.

6.3 Magnetic Resonance Imaging (MRI)

6.3.1. Site Qualification

Each site will undergo qualification testing for MRI. The procedures for site qualification will be identical for 1.5T and 3T scanners. Site qualification will not be required for 1.5T MRI if the machine the site is using has already be certified by the ADNI MRI Core, and has not experienced any software upgrades.

The MRI site qualification process consists of two parts – phantom and human scanning. In terms of human scanning, each site will image a volunteer subject with the protocol and send the images to LONI. Each parameter in each of the pulse sequences in the protocol will be checked at Mayo. In the event that the protocol 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 subject, 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 subject. 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.

6.3.2. Data Acquisition

CN and MCI subjects carried forward from ADNI into the GO grant are scanned with the original ADNI protocol on the existing ADNI 1.5T scanner at that site in order to maintain optimum longitudinal consistency in this longitudinal cohort. Imaging for this group will occur annually, within 2 weeks before or 2 weeks after the in-clinic assessments.

All EMCI subjects newly enrolled into the GO grant will be scanned using a more modern and also expanded protocol using a 3T scanner. These subjects will be scanned at Screening, 3 months from the Screening MRI, and then within 2 weeks before or after the Month 6 and Month 12 visits.

As in ADNI 1, the MRI protocols will be customized for each MRI vendor and each vendor platform that must be supported in ADNI. The protocols will be delivered to each site by Mayo for electronic installation. The ADNI/GO 3T MR acquisition is divided into a core protocol and a set of experimental sequences.

The Core MRI protocol consists of 3 types of sequences that are acquired in every subject and on every MRI vendor system these are: (1) structural MRI, (2) FLAIR, and (3) T2* GRE. MRI measurements of brain structure have been shown to demonstrate brain atrophy (which correlates with neuron loss) in MCI and AD and increasing rates of brain atrophy as subjects become more impaired. Therefore, structural MRI is used as a measure of the rate of disease progression, and possibly as a measure of treatment effect, in AD treatment trials. Major accomplishments of ADNI1 were to establish uniform methods for data collection in large multisite trials, using scanners of different vendors, comparison of 1.5 and 3 Tesla, and comparison of several different image analysis methods. Structural MRI (MPRAGE/IRSPGR) will continue in the GO grant and the data will be used both as a measure of the rate of change as well as a predictor of future change, in EMCI and in the LMCI and CN from ADNI1. Cerebro-vascular disease (especially white matter lesions (WMLs) will be assessed with FLAIR. Recently, iron imaging especially micro bleeds (T2* GRE); has been used in anti-amyloid clinical trials, because of the association of microbleeds with anti-amyloid therapy; this will be measured with T2* GRE.

The experimental sub studies consist of three different types of sequences: (1) DTI, (2) ASL, (3) resting state functional connectivity. Each of these will be acquired on only one MRI vendor system – ie the experimental sequences will be vendor specific.

The data will be used to test the hypotheses in the Biostatistics core as well as to examine relationships between: 1) baseline and rates of change of structural MRI to clinical, PET, and plasma/CSF biomarker measures 2) baseline MRI WMLs and microbleeds and cognitive measures. 3) MRI assessments of microbleeds/ WMLs and PET and CSF measures of brain amyloidosis. 4) baseline/rate of change of ASL, DTI and resting state connectivity MRI and FDG PET.

The specific objectives of the MRI core include: 1) Obtaining high quality multi-site data that is consistent over time, and across different MRI systems. 2) Perform appropriate image quality control throughout the study. 3) Qualify (and re-qualify after upgrades) each scanner on the GO MRI protocol. 4) Correct specific classes of image artifacts in each image acquired; imaging intensity nonuniformity, image warping due to gradient nonlinearity, and scaling changes over time. 5) Monitor each scanner longitudinally in the study using the ADNI phantom. Unlike ADNI, measurements from the phantom will not be used to modify accompanying human images. 6) Perform quantitative measurements of all images.

6.3.3. Clinical Read of MRIs

The research site is responsible to obtain a read from a local radiologist for each MRI completed for the ADNI Protocol. This read must be retained in source documents and a de-identified report included in the forms saved in the clinical database.

6.3.4. Data Management and Quality Control

Images are uploaded by site users to the Laboratory of Neuroimaging (LONI) at the University of California Los Angeles (UCLA). Each MRI will be assessed in terms of quality control by the MRI Core. Quality control for MRI will result in failure of some scans. However, some scans will need to be repeated. Repeat scans must be scheduled within four (4) weeks of the visit date.

6.5. ^{18}F-AV-45 Amyloid and FDG-PET Imaging Protocol

A separate but linked protocol for ^{18}F-AV-45 will be submitted by each site. Procedures are also described in this section.

6.5.1. Site Qualification

We will employ the methods utilized for site qualification in ADNI to date to select sites. This protocol was implemented at the start of the PET Core activities with FDG and requires all sites to image an 18F-filled (generally with FDG) Hoffman brain phantom on two sequential days using the protocol identical to that required for human imaging. This enabled us to ascertain the characteristics of the scanner (particularly resolution and uniformity) and assured that sites were capable of performing the protocol for acquisition and image reconstruction. Almost all sites will participate in the ^{18}F-AV-45 protocol and are already qualified for PET imaging. We did not require re-qualification when we instituted the C-11 PIB protocol and we will not do so for the ^{18}F-AV-45 protocol. About 10 ADNI sites which were not in the original PET protocol now wish to participate and these (as well as any others that change scanners during the protocol) will be required to qualify. Any such site will be provided with a Hoffman phantom (we have purchased 4 at the start of ADNI) and will be provided with a technical manual (developed as part of ADNI) for the data acquisition. All phantom images will be forwarded to Dr Koeppe at U. Michigan for review and qualification.

6.5.2. Data Acquisition

As noted, all currently enrolled normal and MCI ADNI participants, as well as the 200 new EMCI subjects will be studied in this protocol that includes both ^{18}F-AV-45 and FDG imaging performed on 2 separate days. Scans may be performed in any order but both must be completed within a 2-week window to be included in the analysis and reimbursed. The ^{18}F-AV-45 protocol will entail the injection of 10 mCi of [18F]AV-45 followed by an uptake phase of 50 min during which time the subject may wait in a quiet room. At 50 minutes after injection subjects will be positioned in the scanner and 4 x 5 min frames of emission data will be collected for a total of 20 minutes of imaging time. PET/CT scans will precede this acquisition with a CT scan for attenuation correction;

PET-only scanners will perform a transmission scan following the emission scan. As we have done to date in ADNI, sites will be required to use a single iterative reconstruction for all scans that is optimized for the instrument and which cannot change during the protocol. The vast majority of sites are experienced with this; new sites will be instructed as part of the qualification procedure.
FDG scans will be acquired as they have been in the ADNI protocol to date: Subjects will be asked to fast for at least four (4) hours prior to the scanning session. Subjects' blood glucose is checked prior to scanning and must be < 180 mg/dL. After the injection of 5 mCi of tracer, subjects are in a quiet, dimly lit room with eyes and ears unoccluded for 30 min, after which they are placed in the scanner. Data are acquired as 6 X 5 min frames preceded by a CT scan or followed by a positron transmission scan.

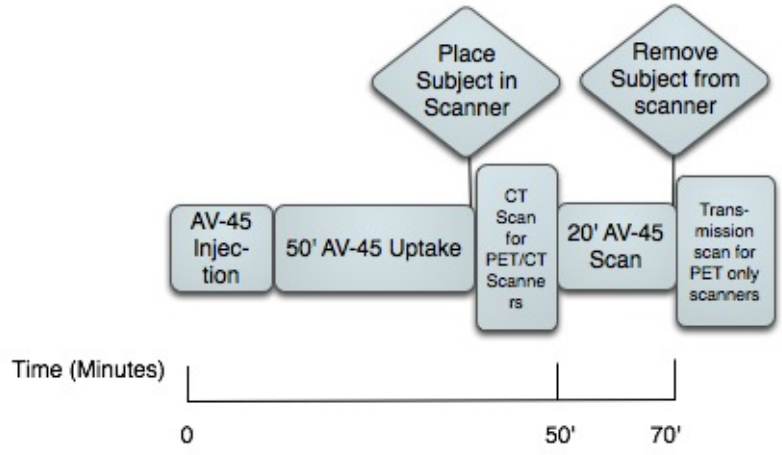
6.5.3. Data Management and Quality Control

All data will be uploaded to the UCLA Laboratory of Neuroimaging (LONI) as we have done to date with ADNI. Instruction in this protocol is provided as part of site qualification and all PET sites are currently familiar with this. Data are de-identified as part of the upload and placed into quarantine until they pass QC. Dr Koeppe's laboratory at the University of Michigan is notified when new scans are uploaded, and QC is performed within 24 hours followed by pre-processing of the images. There will be several steps in the quality assurance and pre-processing of the ^{18}F-AV-45 and FDG PET images that are obtained from the scanning sites. 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.

The following are specific steps will be taken:
(1) Visual inspection of all images: both frames (temporal) and slices (spatial);
(2) Extract and inspect header information;
(3) Co-register all frames of the multi-frame studies to the first frame of the image set;
(4) Assess motion by magnitude of translate/rotate parameters;
(5) Recombine co-registered frames to create registered dynamic and registered average (averaged over all frames) image sets;
(6) Reorient /resample images into a standard image matrix and image orientation (160x160x96 voxels 1.5 mm in all dimensions);
(7) For all FDG scans, co-register all PET scans on each subject to baseline scan in standard image matrix;
(8) Perform normalization on all image sets, based on global mean for FDG metabolic images or cerebellar gray matter for amyloid imaging;
(9) Smooth images from all scanner models by amounts determined from Hoffman phantom scans to achieve uniform 8mm effective resolution;
(10) Complete PET QA forms;
(11) Upload post-processed PET images sets to LONI (image repository). As noted, these procedures have all been successfully employed as part of ADNI to date for both FDG and C-11 PIB tracers.

Quality control for both FDG and ^{18}F-AV-45 will result in failure of some scans. Many of these scans are expected to be useful if the data are reprocessed. However, based on our experience in ADNI, approximately 1-2% of scans will need to be repeated. For this reason, our dosimetry projections include the radiation exposure that a subject would encounter if both the FDG and ^{18}F-AV-45 scans were repeated.

6.5.4. ^{18}F-AV-45 Imaging Protocol



Please refer to the separate linked A-14 Protocol for a more detailed description of ^{18}F-AV-45 procedures.

^{18}F-AV-45 will be administered by intravenous bolus injection (over 10 to 20 seconds). Approximately 50 minutes following the injection, the subject will be placed on the scanning table with their head in a comfortable head holder and moved into the scanner. Brain images will be acquired continuously for a period of 20 minutes. The images will be immediately assessed for technical validity. If considered inadequate, the subject will have an additional 20 minutes of continuous imaging, collected as two 5-minute frames. Transmission scans should be done prior to the scans to allow for reconstruction of the images after the scan. If there is a repeat scan, transmission can be done after the scan.

Vital signs will be taken in a supine position immediately prior to administration of florbetapir F 18 (within 5 minutes prior to injection) and again at the end of the study visit, prior to discharge (approximately 70 minutes after florbetapir F 18 administration);

Follow-up
Each study participant or a person designated to speak for them will be contacted by phone 24 to 48 hours after the imaging session to confirm their well being and query them about any new adverse events.

7. Adverse Events Reporting

All subjects will be evaluated for adverse events at each clinical visit and telephone check.

7.1. Definition of an Adverse Event

An adverse event is any adverse change from the subject's baseline condition including clinical or laboratory tests, or abnormalities that occur during the course of the study after consent.

7.2. Following up on Adverse Events

The investigator is obliged to follow subjects with AE's until the events have subsided, the conditions are considered medically stable, or the participants are no longer available for follow up. Subjects who discontinue due to adverse events will be treated and followed according to established medical practice. All pertinent information will be entered into the electronic CRF. All serious adverse events (SAEs) will be reported to the independent Data Safety Monitoring Board.

Serious adverse events include any event that is fatal, life threatening, significantly or persistently disabling or incapacitating, results in hospitalization, prolongs a hospital stay, or is associated with a congenital abnormality or birth defect. In addition, any experience which the investigator regards as serious, or which would suggest significant hazard, contraindication, side effect, or precaution associated with participation in the study should be reported as a serious adverse event.

7.3. Reporting Serious Adverse Events

Any such experience due to any cause, which occurs during the course of the investigation or within 30 days of the last study visit, must be reported to the Project Director within 24 hours after learning of the event. This is in turn will trigger a report to be distributed to all participating sites, IRBs, and the NIA.

7.4. Data and Safety Monitoring Board

The ADNI-CC currently has an active Data and Safety Monitoring Board (DSMB) that reviews the safety of all subjects enrolled in trials on an ongoing basis. The DSMB will review serious adverse event reports on a quarterly basis.

8. Statistical Considerations

The first broad class of analyses will test hypotheses about cross-group differences at baseline or in one-number change summaries like boundary shift integrals. Example: EMCI is intermediate between NC and MCI clinically (Clinical Core) and in imaging measures (MRI, PET Cores). We will address these comparisons using standard linear models if the

outcome is normal and homoscedastic (e.g. ANOVA to compare hippocampal volume) and generalized linear models otherwise. Models for cross-group comparisons may also be adjusted for important covariates such as APOE4+, age, education, or other markers.

The second broad group of analyses will test hypotheses about trajectories of change and their predictors and relationships, including trajectories both for cognitive measures and for biomarkers. For example, the PET core hypothesizes that FDG measures of glucose metabolism will predict cognitive decline, and the Biomarker Core hypothesizes that baseline CSF markers will predict metabolic decline and cortical atrophy. The first analytic step will be to develop longitudinal models that provide accurate descriptions of the overall patterns of change in outcome measure and heterogeneity of trajectories, while still allowing for possible missing data, unequal spacing of observations, and within-person correlation. For most outcomes, we will use random effects repeated measures models for longitudinal data. If the assumptions of these models are systematically violated for some outcomes we will consider a generalized linear models approach instead (this was necessary in ADNI-1 for CDR and MMSE.) We will also test for non-linear trajectories, for example accelerating rates of change over the longer-term follow-up in ADNI-GO. The longitudinal models will then form the basis for addition of predictors, adjustment for covariates, and examination of predictor effects both on starting level and rates of change. The scientific hypotheses and available data will determine our approach to handling predictors that may themselves change over time. Time-varying covariates may be used to see whether current level of the predictor correlates directly with current level of the outcome, beyond the value of knowing the baseline predictor level. More elaborate models can take variation into account while estimating the relationship between two trajectories for two different processes. In some cases, we will only have two time points on an outcome of interest; in this case, we can calculate change scores (difference, percent change) and fit ordinary regression models with the change summary as the outcome.

A third set of analyses will address hypotheses about conversion (MCI to AD, CN to MCI) and other time-to-event data. For these analyses, the primary approach will be survival analysis, as used in ADNI-1. We anticipate using Cox proportional hazards models but will consider alternatives such as accelerated failure time models if the Cox assumptions are violated.

High-dimensional data are a general problem in imaging, and genetics data also run the risk of large numbers of predictors. We plan to continue the training-set, test-set approach first implemented in ADNI-1 to avoid bias, and to include genetics analyses in the same paradigm. All models will be carefully validated both analytically and graphically, and alternatives considered as needed. If generalized linear models are insufficiently flexible, we will explore other model families both for the fixed and the stochastic parts of the model. C.3.

9. Ethics

9.1 Human Subjects, Ethical and Regulatory Considerations

This study will be conducted according to Good Clinical Practice guidelines, US 21CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards (IRBs) / Research Ethics Boards (REBs), and pursuant to state and federal HIPAA regulations. Written informed consent for the study must be obtained from all participants

and/or authorized representatives and the study partners before protocol-specific procedures are carried out.

9.2. Institutional Review Board / Research Ethics Boards

Institutional Review Boards and Research Ethics Boards must be constituted and their authority delegated through the institution's normal process of governance according to applicable State and Federal requirements for each participating location. The protocol will be submitted to appropriate Boards and their written unconditional approval obtained and submitted to Regulatory Affairs at the ADNI-CC prior to commencement of the study. The ADNI-CC will supply relevant data for investigators to submit to their IRBs/REBs for protocol review and approval. Verification of IRB/REB unconditional approval of the protocol and the written informed consent statement with written information to be given to the participants and/or their authorized representatives and study partners and will be transmitted and validated by the ADNI-CC in order to obtain approval for shipment of study supplies and worksheets to study sites. Sites' approval must refer to the study by exact protocol title and number, identify documents reviewed, and state the date of review. IRBs/REBs must be informed by investigators of all subsequent protocol amendments and of serious or unexpected adverse experiences occurring during the study that are likely to affect the safety of the participants or the conduct of the study. IRB approval for such changes must be transmitted in writing to the ADCS.

9.3. Informed Consent and HIPAA Compliance

Informed consent will be obtained in accordance with US 21 CFR 50.25, the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada, ICH Good Clinical Practice and applicable HIPAA privacy notifications will be implemented before protocol procedures are carried out. Information should be given in both oral and written form as deemed appropriate by the Site's IRB.

Participants, their relatives, guardians or authorized representatives and study partners must be given ample opportunity to inquire about details of the study. The consent form generated by the investigator with the assistance of the CC must be approved, along with the protocol, and HIPAA privacy notifications by the IRB/REB and be acceptable to the CC. Consent forms must be in a language fully comprehensible to the prospective participants and/or their authorized representatives and study partners. Informed consent will be documented by the use of a written consent form approved by the IRB/REB and signed by the participant and/or an authorized representative and study partner. Consent must be documented by the dated signature of the participant and/or authorized representative pursuant to local regulations. Each participant's signed informed consent and/or HIPAA research authorization must be kept on file by the investigator for possible review by regulatory authorities and/or ADCS monitors. HIPAA privacy requirements will be met by either inclusion of required HIPAA text within the IRB-approved consent document or by separate HIPAA research authorization, pursuant to local regulations.

9.4. Informed consent for biomarkers, genetic material, and imaging data.

The informed consent will not only cover consent for the trial itself, but for the genetic research, biomarker studies, biological sample storage and imaging scans as well. The consent for storage will include consent to access stored data, biological samples, and imaging data for secondary analyses. Consent forms will specify that DNA and biomarker samples are for research purposes only; the tests on the DNA and biomarker samples are not diagnostic in nature and participants will never receive results. MRI scan findings of clinical significance, determined by the site radiologist, will be shared with participants The informed consent and/or HIPAA notification will specify that the ADCS will receive and store all research data; that Mayo Clinic Rochester will receive MRI images, the University of Michigan will receive and store PET images, the University of Pennsylvania Alzheimer's Disease Biomarker Fluid Bank Laboratory will receive and store biomarker samples, the National Cell Repository for AD (NCRAD) located at Indiana University will receive and store blood, DNA and RNA samples and develop and store immortalized cell lines, while the University of Los Angeles Laboratory of Neuroimaging will house a full set of all the data. All data will be made available to: the pharmaceutical industry, academic investigators and other interested parties in the public domain. A policy for distribution of data will be developed.

9.5. Procedures to maintain confidentiality

9.5.1. Genetic research and storage of genetic material

The ADNI laboratories at the University of Pennsylvania and at the National Cell Repository for AD (NCRAD) will follow procedures to ensure that no reasonable chance of the subject's genetic data can be linked to her/his identity. Confidentiality will be will be sincerely attempted, but cannot be guaranteed.

The de-linking of the sample from the participant occurs at the time the blood is sent to the University of Pennsylvania and the National Cell Repository for AD. All samples at both institutions will be inventoried and tracked using commercially available software. At the University of Pennsylvania, a database will be created and used for the inventory of stored samples in conjunction with a bar code reading system. Bar code labels affixed to each sample vial will contain the following information: sample ID# (to preserve confidentiality), date of collection and processing, total initial volume collected, sample type (plasma, serum, CSF), volume, aliquot number, freezer, shelf, rack, box, location in the box. A bar code label will be used on the sample tracking form.

Immortalized cell lines, DNA and RNA will be prepared at The National Cell Repository for AD. A unique bar-code number is affixed to all purple top and RNA specimen tubes as well as affixed to the Sample From/Draw Sheet. The yellow top tubes are spun down immediately. All transfer tubes, vessels and storage vials are prelabeled prior to sample processing. NCRAD maintains a secure database for tracking all incoming ADNI samples. The only information that will be maintained in this database is an Individual number (to preserve confidentiality), Kit number (assigned to all tubes that come in a single shipment for an individual), DNA/RNA 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.

Neither the ADNI-CC, NCRAD nor the University of Pennsylvania will have information regarding the participant's name and thus all three are unable to link the DNA analysis results to the person.. To gain the maximum utility for research on genetic material and biological markers, the ADNI-CC will be able to analyze clinical research data collected on each participant in relation to biological specimens from that participant. However, there will be no link to research done on these specimens with participants' names. It is important to note that the linkage is between DNA research data and study research data, none of which includes identifying information. The data centers (UCSD, UCLA) do not have any record of the names of the study participants, or of specific medical identifiers such as clinical medical record numbers.. Therefore, even though DNA results can be linked to clinical research data for purposes of analyses, the only linkage of DNA test results to names of participants that can happen is at the clinical site.

The procedures for patient confidentiality will be approved by the IRB of the University of Pennsylvania. Since NCRAD is an NIH specimen repository whose focus is on 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 patient confidentiality and the use of stored DNA specimens will be in accordance with the rules and procedures established by the University of Pennsylvania and Indiana University IRBs. Samples handled by the Biomarker Core are banked in a locked freezer dedicated to the ADNI study at the University of Pennsylvania. Similarly, NCRAD stores all samples in a secure freezer within a secure facility at Indiana University. The samples are without a link to identity of the participant from whom the sample came. All samples are bar coded and identified by a bar code.

Specific procedures for requesting and accessing DNA will be created by the Resource Allocation Review Committee (RARC) of the ADNI in accordance with recommendations proposed in the NBAC Human Biological Materials Report. These DNA guidelines have also been developed in accordance with the American Society for Human Genetics' position paper on the NBAC report and the Ad Hoc Committee on Stored Tissue of the College of American Pathologists.

> "By signing this consent you are authorizing the use of your data for large scale, multi-center studies that will combine data from similar populations. These multi-center studies are being conducted by the Alzheimer's Disease Neuroimaging Initiative (ADNI), a neuroscience consortium of universities and research institutes. Your data will be stored with a coded research identifier to protect your identity. Only de-identified data, which does not include anything that might directly identify you, will be shared with ADNI members and the general scientific community for research purposes. This data will be entered into linked databases at UCLA and UCSD to be used from this date and going forward. Genetic study data may also be made available through the Database of Genotype and Phenotype following NIH policy"
>
> Please refer here to established NIH guidelines: http://www.ncbi.nlm.nih.gov/gap

9.5.2. Biomarker Samples and Research

Biomarker fluid samples will be labeled by bar coding samples. Subject's names will not be provided to the University of Pennsylvania investigators. Samples will be stored by bar code number and no other identifying information will be provided.

9.5.3. MRI and PET Imaging and Data Storage

MRI and PET scans will be labeled according to each site's imaging machine capabilities using ADNI subject identifiers and scanner specific series descriptions as detailed in the MRI Procedures Manual and the PET procedures manual. All efforts will be made to have scans sent with this information. All scans will undergo a de-identification process, which is embedded within the LONI Imaging process to ensure that no subject identification information is present in the image files. MRI scan findings of clinical significance, determined by the site radiologist, will be shared with the subject and the subject's local physician.

10. **Potential Risks**

10.1. FDG-PET and ^{18}F-AV-45

The primary risk related to PET is that of radiation exposure associated with the CT scan or transmission scan and the injected radiotracers. There is also minor risk associated with the venipuncture and radioisotope injection (pain and bruising or painful infiltration of a failed injection). The estimated absorbed radiation dose for [^{18}F]-FDG (rad/mCi) for a 70kg adult is presented in the table below. These estimates were calculated from human data (Jones et al., 1982) and used the data published by the International Commission on Radiological Protection for [^{18}F] FDG for a 70 kg adult with assumptions on biodistribution from Jones, et al, 1982 and using MIRDDOSE 2 software ("International Commission on Radiological Protection for 18[F] FDG," 1987). The critical organ is the urinary bladder wall, followed by heart, spleen and pancreas.

This radiation dose is not 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 every day risks. No PET studies will be performed on pregnant or potentially pregnant women, as the protocol requires female subjects to be postmenopausal as a condition of participation.

The clinical safety experience with ^{18}F-AV-45 is described in the Investigator's Brochure. In general, ^{18}F-AV-45 has been well tolerated. One serious adverse event has been reported; a patient tripped and fell four days after ^{18}F-AV-45 administration and was hospitalized for surgical repair of a broken arm. The most common adverse events were shoulder pain, nausea and anxiety/claustrophobia, all deemed not related to the investigational agent. The expected scattered changes in vital signs, laboratory, and

electrocardiogram (ECG) values were seen, but no consistent and clinically significant changes were observed.

However, the possibility exists for a rare reaction to any of the drugs or procedures to which the participant will be exposed. A physician will therefore be available at all times during the study and an emergency cart will be in close proximity. If adverse effects would occur, medical intervention will be provided

As with any investigational study, there may be adverse events or side effects that are currently unknown and it is possible that certain unknown risks could be permanent, serious, or life-threatening. However, if any new risks become known in the future participants will be informed of them. Participation in this study may involve some added risks or discomforts, which are outlined below.

The dosimetry schedule below includes a projection for exposure should both the FDG and ^{18}F-AV-45 scans require repeating. This would be done in the eventuality of scans that do not pass quality control and cannot be salvaged through reprocessing.

10.1.1. Dosimetry Table

Organ	FDG rad/mCi	FDG rad/5 mCi	^{18}F-AV-45 rad/mCi	AV45 rad/10 mCi	Total Dose from Study	Total Dose from Repeating Both Scans
Adrenals	0.048	0.24	0.05	0.5	0.74	1.48
Brain	0.07	0.35	0.037	0.37	0.72	1.44
Breasts	0.034	0.17	0.023	0.23	0.4	0.8
Galbladder wall	0.049	0.245	0.529	5.29	5.535	11.07
Lower Large Intestine Wall	0.051	0.255	0.103	1.03	1.285	2.57
Small Intestine	0.047	0.235	0.242	2.42	2.655	5.31
Upper Large Intestine wall	0.046	0.23	0.276	2.76	2.99	5.98
Heart wall	0.22	1.1	0.048	0.48	1.58	3.16
Kidneys	0.074	0.37	0.048	0.48	0.85	1.7
Liver	0.058	0.29	0.238	2.38	2.67	5.34
Lungs	0.064	0.32	0.032	0.32	0.64	1.28
Muscle	0.039	0.195	0.032	0.32	0.515	1.03
Ovaries	0.053	0.265	0.065	0.65	0.915	1.83
Pancreas	0.096	0.48	0.053	0.53	1.01	2.02
Red marrow	0.047	0.235	0.053	0.53	0.765	1.53
Skin	0.03	0.15	0.022	0.22	0.37	0.74
Spleen	0.14	0.7	0.033	0.33	1.03	2.06
Testes	0.041	0.205	0.025	0.25	0.455	0.91
Thymus	0.044	0.22	0.027	0.27	0.49	0.98
Thyroid	0.039	0.195	0.025	0.25	0.445	0.89
Urinary bladder	0.32	1.6	0.1	1	2.6	5.2

wall						
Uterus	0.062	0.31	0.058	0.58	0.89	1.78
Effective Dose			0.069	0.69		
Total Body	0.043	0.215	0.043	0.43	0.645	1.29

10.2. MRI

There are no proven biologic risks associated with MRI scanning. All subjects will be rigorously screened by MR personnel to be certain that they do not have any medical contraindications for MRI which include metallic foreign bodies in the brain or eye or cardiac pacemaker. This safety screening is part of routine clinical practice at MRI centers and is performed before any subject is permitted to enter the scanning room. However there is a slight risk that someone will accidently bring metal into the MRI scanner room, which might be pulled into the MRI magnet and injure the subject. There is a slight risk of anxiety due to claustrophobia and noise. Any subject who experiences anxiety when placed into the MR scanner will be removed from the scanner, offered reassurance by the MR tech doing the scan, and offered the option of continuing or terminating the study. If the subject decides that the anxiety associated with MRI is uncomfortable for them and they wish to terminate the scan, then the examination will be ended at that time. There will be no attempt to coerce subjects to complete exams that they are uncomfortable with. No anxiolytic agents will be given, as this is a voluntary research protocol.

10.3. Lumbar Puncture

Lumbar puncture may be associated with pain during the performance of the procedure. This is usually temporary and confined to the lower back. Headache may occur in about 5% of elderly people who undergo lumbar puncture. Less commonly, in about 1-4% of subjects, a persistent low-pressure headache may develop, probably due to leakage of CSF. Lower rates of post-LP headache have been noted in elderly patients, and when atraumatic (Sprotte) needles are used. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly a blood patch (injection of some of the subject's blood to patch the CSF leak) may be needed. Potential but rare risks of lumbar puncture include infection, damage to nerves in the back, bleeding into the CSF space, and death. The risk of these is much less than 1%.

10.4. Blood Draw

The risks of blood draw include pain from the needle, bruising or infection at the site of venipuncture, or fainting as a response to blood draw.

11. Overall Study Timetable

This study involves 4 months of start up time, 8 months of enrollment and 12 months of follow up. It will therefore be active for 2 years. The first 4 months will be consumed by submission of protocol to local IRBs, verification of approval, certification of site personnel and imaging facilities in performance of the trial, establishing laboratory contracts, distribution of material to sites, preparation and implementation of training meetings and manuals, eCRFs and initial recruitment efforts. The recruitment will last 8 months. At the

end of the funding for this 2 year project, subjects will be asked to consent to long-term follow up under a separate grant.

12. Inclusion of Women and Minorities

Women and members of minority groups will be actively recruited during this protocol. Based on the participating sites data regarding enrollment of minorities, we expect 12% of subjects enrolled will be minorities. This is close to the aged minority population in the U.S. which is 14%.

13. NIH Data Sharing Policy

Data from this research will be shared with other researchers pursuant to the 02/26/2003 "NIH Final Statement on Sharing Research Data." The ADCS grant contains a data sharing policy consistent with the goals of the NIH, but which also respects the rights of commercial partners. The NIH policy on data sharing can be found online at: **http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html**

NIH believes that data sharing is important 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 subjects' rights and confidentiality, identifiers will be removed from the data before they are shared.

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