CONFIDENTIAL

ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE 2 (ADNI2) PROTOCOL (ADC-039)

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PROTOCOL SYNOPSIS

Title	**Alzheimer's Disease Neuroimaging Initiative 2 (ADNI2)**
Primary Objective	The major goals of ADNI2 are to: 1. Determine the relationships among clinical, imaging, genetic, and biochemical biomarker characteristics of the entire spectrum of Alzheimer's Disease (AD), as the pathology evolves from normal aging through very mild symptoms, to mild cognitive impairment (MCI), to dementia. 2. Inform the neuroscience of AD, identify diagnostic and prognostic markers, identify outcome measures that can be used in clinical trials, and help develop the most effective clinical trial scenarios. 3. Develop improved methods which will lead to uniform standards for acquiring longitudinal multi-site MRI and PET data on patients with AD, MCI, significant memory concern and elderly controls. 4. Perform longitudinal clinical, cognitive, MRI, PET (florbetapir F 18 and FDG), and blood and CSF biomarker studies on approximately 650 newly enrolled subjects in five diagnostic categories – cognitively normal (CN), significant memory concern (SMC), early MCI (EMCI), late MCI (LMCI), and mild AD. Continue these longitudinal studies for approximately 300 LMCI and Cognitively Normal subjects from ADNI1 and approximately 200 EMCI subjects from ADNI-GO for an additional 5 years. 5. Collect blood samples for DNA and RNA extraction. Newly enrolled subjects will also have samples collected for Cell Immortalization and APOE genotyping. 6. Validate the clinical diagnoses and imaging and biomarker surrogates through neuropathological examination of ADNI1, GO and ADNI2 participants who come to autopsy.
Study Design	This is a non-randomized natural history non-treatment study.
Sample Size	Approximately 650 newly enrolled subjects (150 CN, 100 SMC, 100 EMCI, 150 LMCI, and 150 mild AD, with a possibility of enrolling additional subjects should funding permit) from approximately 55 sites from the United States and Canada. Approximately 300 CN and LMCI subjects will be followed from the original ADNI1 study. Approximately 200 EMCI subjects will be followed from the ADNI-GO study.

Summary of Key Eligibility Criteria	Newly enrolled subjects will be between 55-90 (inclusive) years of age, have a reliable study partner able to provide an independent evaluation of functioning, and will speak either English or Spanish. They must be willing and able to undergo all test procedures including neuroimaging and lumbar puncture and agree to longitudinal follow up. Specific psychoactive medications will be excluded. Additional inclusion/exclusion criteria are as follows: *Cognitively Normal Subjects:* MMSE scores between 24-30 (inclusive), a CDR of 0, non-depressed, non-MCI, and non-demented, education adjusted scores on delayed recall of one paragraph from Wechsler Memory Scale Logical Memory II (≥9 for 16 or more years of education, ≥5 for 8-15 years of education, ≥3 for 0-7 years of education). *Significant Memory Concern Subjects:* MMSE scores between 24-30 (inclusive), a significant subjective memory concern reported by subject, informant, or clinician, CCI score ≥16 (based on first 12 questions), a CDR of 0, non-depressed, non-MCI, and non-demented, education adjusted scores on delayed recall of one paragraph from Wechsler Memory Scale Logical Memory II (≥9 for 16 or more years of education, ≥5 for 8-15 years of education, ≥3 for 0-7 years of education). *EMCI Subjects:* MMSE scores between 24-30 (inclusive), a subjective memory concern reported by subject, informant, or clinician, objective memory loss measured by education adjusted scores on delayed recall of one paragraph from Wechsler Memory Scale Logical Memory II (≥16 years: 9-11; 8-15 years: 5-9; 0-7 years: 3-6), 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. *LMCI Subjects:* MMSE scores between 24-30 (inclusive), a subjective memory concern reported by subject, informant, or clinician, objective memory loss measured by education adjusted scores on delayed recall of one paragraph from Wechsler Memory Scale Logical Memory II (≥16 years: ≤8; 8-15 years: ≤4; 0-7 years: ≤2), 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. *Mild AD Subjects:* MMSE scores between 20-26 (inclusive), a CDR of 0.5 or 1.0, and meets NINCDS/ADRDA criteria for probable AD. *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, early or late) 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 or they are no longer willing/able to continue with neuroimaging or LP procedures. *Exclusion for FDG PET scan and amyloid imaging with Florbetapir F 18 Injection:* 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 and genetic sample collection, and imaging. A reduced battery of tests is allowable if the subject is not able/willing to complete the full battery after the participant's original Baseline Visit. 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 of Southern California. University of Pennsylvania will receive and process biomarker samples and NCRAD will receive and process genetic samples. All raw and processed image data will be archived at LONI.
Outcome Measures	1. Rate of Decline as measured by: Cognitive tests, Activities of Daily Living, and CDR Sum of Boxes. 2. Rate of conversion will be evaluated among all five 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 florbetapir F 18 injection. 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 florbetapir F 18.
Sponsor	National Institute on Aging, National Institute of Health

For guidance on assessments and procedures conducted prior to the ADNI2 protocol changes implemented May 9, 2014, please refer to Appendix A (pages 48-58) of this protocol

Subjects followed under the ADNI2 protocol amendment 3 and thereafter, are those with the original diagnosis of CN, SMC, EMCI, LMCI (includes rollovers from ADNI1, ADNI-GO, and ADNI2). Subjects with the original diagnosis of AD are no longer being seen in-clinic. Periodic follow up by phone with AD subjects who have provisionally consented to the neuropath sub study or undecided is to occur to confirm if there is any change in their decision to participate or not in the neuropath sub study.

The list of schedule of events and procedures applies to all enrolled in ADNI2 including rollovers from ADNI1 and ADNI-GO.

Timing of visits is based on cohort and conversion status:

- In-clinic visits due every year: for subjects who enrolled with an original diagnosis of EMCI, or LMCI, as well as for subjects enrolled with an original diagnosis of CN or SMC who have since converted to EMCI, LMCI or AD at any point of the study (ADNI1, ADNI-GO, or ADNI2)
- In-clinic visits are due every two years: for subjects who enrolled with an original diagnosis of CN or SMC who have NOT converted to EMCI, LMCI, or AD
- Follow-up phone call every 6 months: for subjects who enrolled at any point of the study (ADNI1, ADNI-GO, ADNI2) with an original diagnosis of AD who have provisionally consented to neuropathology or are undecided
- Note: there are no longer the 6 month interim phone checks to be conducted across any cohort, which are different than the follow-up phone calls to AD subjects who have provisionally consented to neuropathology or are undecided.

Timing of procedures conducted during in-clinic visit:

- The MRI, LP and F18 amyloid PET scan should be conducted every 2 years across originally diagnosed CN, SMC, EMCI and LMCI subjects (regardless of conversion status) until the end of ADNI2.
- MRI, LP and F18 amyloid PET scan should coincide with the scheduled in-clinic visit.
    - If the next LP and F18 amyloid PET scans are due 1 year before the next scheduled in-clinic visit (for those participants on the biennial schedule), the participant should be brought in for their in-clinic visit 1 year earlier to coincide with the timing of the LP and F18 amyloid PET scan. The MRI scan should also be conducted at this in-clinic visit. Then their subsequent in-clinic visit (which will also include PET, LP and MRI) will be 2 years from that date.
    - If the LP, F18 amyloid PET scan, and MRI scan have been occurring on alternating years all three procedures should be conducted at the next in-clinic visit (which again the in-clinic visit may need to occur 1 year early – see above). This will require 1 or 2 of the procedures to potentially be 1 year ahead of schedule. Then their subsequent in-clinic visit will be 1 or 2 years from that date based on diagnosis and conversion status.

Note: In-clinic visits may occur over several days to accommodate all study procedures.

Please contact ATRI Clinical Operations if you have further questions.

SCHEDULE OF EVENTS (CN, SMC, EMCI, LMCI, and AD converter subjects)

Visit Name	**Month 6**[1]	**Ongoing Annual** [2 3]
Visit Type	Clinic	Clinic
Neuropath discussion/ Provisional consent		X [#]
Vital Signs*	X	X
Mini Mental State Examination*	X	X
Logical Memory I and II*		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		X
Neuropsychiatric Inventory Q	X	
ADAS-Cog 13 (w/ Delayed Recall and Number Cancellation)*	X	X
Clinical Dementia Rating Scale	X	X
Activities of Daily Living (FAQ)	X	X
Plasma and Serum Biomarker Collection*[4]	X	X
RNA Sample Collection*		X
Concomitant Medications	X	X
Adverse Events	X	X
Diagnostic Summary*	X	X
3T MRI Imaging (100%)*		X^
F18 amyloid PET scans (100%)*		X[z]
CSF Collection by Lumbar Puncture (LP) (100%)*		X[z]

[1] If not already completed, all 6 month in clinic visits will be conducted

[2] In-clinic visits will be conducted annually only for subjects who enrolled with an original diagnosis of EMCI, or LMCI, as well as for subjects who enrolled with an original diagnosis of CN or SMC who have since converted to EMCI, LMCI or AD. Subjects will be followed for a maximum of 60 months from baseline or from the time when they rolled into ADNI 2, after which they may be asked to consent to additional follow-up under a separate grant and protocol.

[3] In-clinic visits are due every two years for subjects who enrolled with an original diagnosis of CN or SMC who have NOT converted to EMCI, LMCI, or AD. Subjects will be followed for a maximum of 60 months from baseline or from the time when they rolled into ADNI 2, after which they may be asked to consent to additional follow-up under a separate grant and protocol.

[#] If participant has refused to participate in the neuropath program, no further inquiry should take place.

[*] Assessment must be done in-person

[4] Buffy Coat is removed from plasma sample and shipped to NCRAD for genetic analysis

^ MRI scan is to be conducted every other year, rather than annually, and occurs during the in-clinic visit when the subject is due for the LP and F18 amyloid PET scans (if applicable).

[z] F18 amyloid PET scans and LP are to be performed only every two years from baseline. If the next LP and F18 amyloid PET scans are due 1 year before the next scheduled in-clinic visit (for those participants on the biennial schedule), the participant should be brought in for their in-clinic visit 1 year earlier to coincide with the timing of the LP and F18 amyloid PET scan. If the LP, F18 amyloid PET scan, and MRI scan have been occurring on alternating years all three procedures should be conducted at the next in-clinic visit. This will require 1 or 2 of the procedures to potentially be 1 year ahead of schedule.

SCHEDULE OF EVENTS (Original diagnosis- AD subjects)

Visit Name	**Ongoing Month 6 Phone Follow-Up[1]**
Visit Type	Phone
Neuropath discussion	X

[1]Originally diagnosed AD subjects in ADNI1, ADNI-GO, and ADNI 2 that have consented to the Neuropath program or undecided will be contacted every 6 month by phone only to confirm if there is any change in their decision to participate or not in the neuropath sub study.

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
ADNI1	Alzheimer's Disease Neuroimaging Initiative-1
ADNI2	Alzheimer's Disease Neuroimaging Initiative-2
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 E (APOE) epsilon 4 (APOE4)
ATRI	Alzheimer's Therapeutic Research Institute
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
CCI	Cognitive Change Index
CDR	Clinical Dementia Rating
CN	Cognitively Normal
CSF	Cerebrospinal Fluid
CT	Computerized Tomography
dbGaP	database of Genotypes and Phenotypes
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 (under IND with Avid Radiopharmaceuticals, Inc.)
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
INN	International Non-proprietary Name
IRB	Institutional Review Board
IR SPGR	Inversion Recovery Spoiled Gradient-Recalled
LMCI	Late Mild Cognitive Impairment
LONI	Laboratory of Neuroimaging at USC
LP	Lumbar Puncture
SMC	Significant Memory Concern
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
NIAGADS	National Institute on Aging Genetics of Alzheimer's Disease Data Storage
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
USAN	United States Adopted Name
WMS-R	Wechsler Memory Scale – Revised
WML	White Matter Lesions
VIQ	Premorbid Verbal Intelligence

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1. **Introduction**

The overall goal of this project is to determine the relationships among the clinical, cognitive, imaging, genetic and biochemical biomarker characteristics of the entire spectrum of Alzheimer's disease (AD), as the pathology evolves from normal aging through very mild symptoms, to mild cognitive impairment (MCI), to dementia. ADNI2 continues the previously funded AD Neuroimaging Initiative (ADNI1), a public/private collaboration between academia and industry to study biomarkers of AD as well as a previously funded Grand Opportunities (GO) grant which supplements ADNI goals and activities. ADNI will inform the neuroscience of AD, identify diagnostic and prognostic markers, identify outcome measures that can be used in clinical trials, and help develop the most effective clinical trial scenarios.

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 ADNI1 and 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).

ADNI2 is focused on a establishing a broader understanding of biomarkers in a wider range of subjects in order to understand how these biomarkers can be used as both predictors and outcomes. Our model suggests that different imaging modalities and measurements and different biochemical markers will usefully serve as "predictors" (measurements which predict future change) and outcomes (measurements that detect change) at different stages in the transition from normal aging, to MCI, to dementia:

- Predictors – We hypothesize that the imaging/biomarker measurements most likely to predict decline in normal subjects, as well as subjects with significant memory concern will be measures of Aβ (CSF and PET), in combination with measures of CSF tau, FDG-PET, and MRI. While amyloid biomarkers may be useful predictors of decline in early MCI (EMCI), CSF tau, FDG-PET and MRI measures of regional atrophy, which likely change after amyloid markers change, may be more predictive. In late MCI (LMCI) and AD, we hypothesize that the most effective biomarkers for prediction of further decline will be FDG-PET, MRI, and cognition.
- Outcomes – We hypothesize that the biomarkers most likely to correlate with, and augment the utility of, cognitive and clinical measures as outcomes in clinical trials are FDG-PET and MRI measures of volume (especially of hippocampus and temporal cortex) at early stages and atrophy throughout the brain at later stages.

2. Study Aims

The study aims are focused on predictors, outcomes and clinical trial design, but fulfillment of these aims will add considerably to what is known about the basic neuroscience of AD in terms of progression and underlying mechanisms.

- **Aim 1:** Determine and define those biomarkers which best predict future cognitive decline and conversion to MCI/dementia at the various stages of the progression from normal cognition to dementia. These biomarkers may serve as predictive or early diagnostic markers, and could be used for selection of subjects or as covariates in future treatment or prevention trials.
- **Aim 2:** Determine and define those biomarkers that best serve as outcome measures to quantify the rate of progress at the various stages from controls to dementia. These biomarkers may serve as outcome measures in future treatment or prevention trials.
- **Aim 3:** Improve clinical trials by developing various clinical trial protocol scenarios, which use clinical, cognitive, and biomarker measures as selection criteria, as covariates, and as outcome measures, with maximum statistical power to detect treatment effects. Such scenarios would be developed for subjects with dementia, with MCI, with mild symptomatology, subjects with significant memory concern but whose neuropsychological test performance is within the normal range, and normal healthy controls.
- **Aim 4:** As clinical trials are moving toward less or asymptomatic individuals, a new cohort, subjects with significant memory concerns have been included to address this shift. Subjective memory concerns have been shown to be correlated with a higher likelihood of progression, thereby minimizing the stratification of risk among normal controls and addressing the gap between healthy elderly controls and MCI.
- **Aim 5:** Perform pathological examination on brains obtained by autopsy to validate the antemortum diagnoses.

3. Study Design

This is a non-randomized natural history non-treatment study. Approximately 650 Subjects will be enrolled at approximately 55 sites in the United States and Canada. Approximately an additional 300 subjects will be followed from the previous ADNI1 study and 200 subjects will be followed from ADNI-GO. Subjects will be followed up to 60 months under the ADNI2 protocol. At the end of this period subjects may be asked to consent to be followed long-term under a separate grant. For visits prior to May 9, 2014, clinical and cognitive assessments will be conducted annually along with 3T MRI and collection of plasma and serum for biomarkers. For visits after May 9, 2014, the frequency of clinical and cognitive assessments will vary based on original diagnosis and conversion status. As funding permits, F18 amyloid PET scans, MRI scans and CSF collection for biomarkers will occur biennially. Blood samples will be collected for genetic analysis. Neuropathological examination of brains obtained by autopsy will be performed if consent obtained.

3.1 *Site Personnel Requirements*

At a minimum three staff functions (Site Principal Investigator, Study Coordinator, Psychometrician) will be required to conduct the protocol at each site. Additional functions are outlined below that may be covered by one staff member at certain sites, for other sites there will be multiple staff assigned. 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 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 should 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.
- **CDR Rater:** This person will render the CDR rating based on clinical assessment of participant and caregiver, but should remain blind to the ADAS-cog data.
- **ADAS- Cog Rater:** This person should be blinded to the CDR data**.**
- **Regulatory:** This person will be responsible for managing all regulatory related documents for the duration of the trial, including submitting all required regulatory documents to ATRI regulatory affairs.
- **Billing Remittance and Statement:** This person will be responsible for reviewing and verifying payments from the ATRI are in alignment with procedures completed, along with accepting and processing payments from the ATRI.
- **MRI Contact:** This person will be responsible for conducting phantom and human volunteer scans for site qualification purposes using the appropriate scanning sequence. As well as conducting participant and phantom MRI scans per protocol and ensuring the scans are uploaded to LONI in a timely manner.
- **PET Contact:** This person will be responsible for conducting phantom scans for site qualification purposes and as needed to assess for drift using the appropriate scanning sequence. As well as conducting participant PET scans per protocol and ensuring the scans are uploaded to LONI in a timely manner.

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. 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. ADNI will determine the special requirements of each site and pattern their individual public relations support around those needs. In that context 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, 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. It is recommended to maintain frequent contact with study participants as well as send thank you note following the clinical evaluation and a personalized greeting card on his or her birthday or on a major holiday to strengthen rapport and retention of study participants. Progress of the study may be placed in a newsletter, distributed to the sites, for distribution to study participants.

The goal of ADNI2 is to obtain as close to 100% participation in lumbar puncture as is feasible. ADNI1 aimed for 25% participation and achieved more than 50% participation. For ADNI2, 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.

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 approximately 650 additional subjects (150 cognitively normal, 100 subjects with significant memory concern, 100 with EMCI, 150 with LMCI, and 150 with AD, with a possibility of enrolling additional subjects should funding permit), as well as the longitudinal follow-up of all remaining ADNI-GO EMCI subjects and those subjects who originally enrolled in ADNI1 as cognitively normal and LMCI (regardless of whether a diagnostic conversion has occurred).

Clinical data collection and monitoring are standardized with well-established and successful ATRI 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 database (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 database at LONI. The database will be frequently updated, and all clinical data acquired by the ADNI-CC will be provided in real-time.

4. **Subject Selection**

4.1 Inclusion Criteria: NEW Subjects – CN, SMC, EMCI, LMCI, AD

	CN	SMC	EMCI	LMCI	AD
1.	Subject must be **free of memory complaints**, verified by a study partner, beyond what one would expect for age	Subject **must have a significant subjective memory concern** as reported by subject, study partner, or clinician.	Subject **must have a subjective memory concern** as reported by subject, study partner, or clinician.	Same as EMCI	Same as EMCI
1a.	n/a	Significant memory concern confirmed by Cognitive Change Index score ≥16 (from the first 12 questions)	n/a	n/a	n/a
2.	Normal memory function documented by scoring above education adjusted cutoffs on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) from the Wechsler Memory Scale – Revised (the maximum score is 25): **a. ≥ 9 for 16 or more years of education** **b. ≥ 5 for 8-15 years of education** c. **≥ 3 for 0-7 years of education**	Same as CN	Abnormal memory function documented by scoring within the education adjusted ranges on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) 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**	Abnormal memory function documented by scoring within the education adjusted ranges on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) from the Wechsler Memory Scale – Revised (the maximum score is 25): **a. ≤ 8 for 16 or more years of education** **b. ≤ 4 for 8-15 years of education** c. **≤ 2 for 0-7 years of education**	Same as LMCI
3.	Mini-Mental State Exam score between **24 and 30** (inclusive) (Exceptions may be made for subjects with less than 8 years	Same as CN	Same as CN	Same as CN	Mini-Mental State Exam score between **20 and 26** (inclusive) (Exceptions may be made for subjects with less than 8 years of education at the

	of education at the discretion of the project director)				discretion of the project director)
4.	Clinical Dementia Rating = **0**. Memory Box score must be **0**	Same as CN	Clinical Dementia Rating = **0.5**. Memory Box score must be at least **0.5**	Same as EMCI	Clinical Dementia Rating = **0.5 or 1.0**
5.	**Cognitively normal**, based on an absence of significant impairment in cognitive functions or activities of daily living	Same as CN	**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	Same as EMCI	NINCDS/ADRDA criteria for **probable AD**

	CN	SMC	EMCI	LMCI	AD
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 years) 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	Same as CN	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**	Same as EMCI	Same as EMCI

The following additional inclusion criteria apply to all diagnostic categories:

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. Completed six grades of 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 and RNA banking.
19. Agrees to collection of blood for biomarker testing.
20. Agrees to at least one lumbar puncture for the collection of CSF.

4.2. Exclusion Criteria: NEW Subjects – CN, SMC, EMCI, LMCI, AD

	CN	SMC	EMCI	LMCI	AD
1.	**Any significant neurologic 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	**Same as CN**	**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.	Same as EMCI	**Any significant neurologic disease other than 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.

The following additional exclusion criteria apply to all diagnostic categories:

2. Screening/baseline MRI scan 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. Currently treated with medication for obsessive-compulsive disorder or attention deficit disorder
6. History of schizophrenia (DSM IV criteria).
7. History of alcohol or substance abuse or dependence within the past 2 years (DSM IV criteria).
8. Any significant systemic illness or unstable medical condition which could lead to difficulty complying with the protocol.
9. Clinically significant abnormalities in B12, or TFTs that might interfere with the study. A low B12 is exclusionary, unless follow-up labs (homocysteine (HC) and methylmalonic acid (MMA)) indicate that it is not physiologically significant.
10. Residence in skilled nursing facility.
11. Current use of specific psychoactive medications (e.g., certain antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.). Current use of warfarin or dabigatran (exclusionary for lumbar puncture).
12. Current use of any other exclusionary medications
13. Investigational agents are prohibited one month prior to entry and for the duration of the trial.
14. Participation in clinical studies involving neuropsychological measures being collected more than one time per year.
15. Exclusion for FDG PET scan and amyloid imaging with florbetapir F 18: 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.

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

4.3 *Inclusion Criteria: Follow-up Subjects*

1. Must have been enrolled and followed in ADNI1 for at least one year or enrolled in ADNI-GO with original diagnosis of Cognitively Normal (CN), Mild Cognitive Impairment (MCI), or Early Mild Cognitive Impairment (EMCI) regardless of whether a diagnostic conversion has occurred since initial enrollment in ADNI.
2. Willing and able to continue to participate in an ongoing longitudinal study. A reduced battery of tests is allowable 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.

4.4. *Exclusion Criteria: Follow-up Subjects*

1. Subjects will not be able to participate in FDG PET scan and amyloid imaging with florbetapir F 18 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**

The frequency of visits is dependent on the subject's original diagnosis and conversion status. Please refer to the schedule of events section in the protocol for complete details.

Visits must be conducted within 2 weeks before or after the target date. Once the visit begins, all imaging studies, biofluid collection and cognitive and clinical assessments must take place within 2 weeks from the start of the visit (as applicable). Ideally all data will be collected in-clinic. With the exception of the Screen and Baseline visit, which must be completed in-person with both the subject and study partner, follow-up visits may be conducted by telephone if there are difficulties with scheduling in-person visits. See schedule of events section of the protocol and procedures manual for details on which assessments can be done over the phone.

If subjects are not willing or able to complete the full schedule of assessments at any visit, those assessments or procedures they are willing to complete should be conducted. If subjects are no longer willing or able to travel to the clinic for visits, as much information should be collected via telephone as possible. Where there is insufficient time to complete the full in-clinic visit, the order of priority assessments is:

- Clinical Dementia Rating Scale
- ADAS-Cog
- Mini Mental State Examination
- Logical Memory I and II
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- Vital Signs

5.1. *Procedures for CN, SMC, EMCI, LMCI, and AD converter Subjects*

5.1.1. Month 6 Visit

The Month 6 visit is timed 6 months from Baseline Visit day 1.

- 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

*****NPI-Q is **not** required for follow-up CN and LMCI subjects from ADNI1, and EMCI subjects from ADNI-GO

5.1.2. Annual Visit

After May 9, 2014, the in-clinic visits will be timed according to cohort, conversion status, and when subjects are due for LP and F18 amyloid PET scans, please refer to the schedule of events section in the protocol for complete details.

In-clinic visits will be conducted annually for subjects who enrolled with an original diagnosis of EMCI, or LMCI and for subjects who enrolled with an original diagnosis of CN or SMC who has since converted to EMCI, LMCI or AD. For CN and SMC subjects who have not converted to EMCI, LMCI or AD, the ongoing in-clinic visits will be conducted every 2 years, and when LP and F18 amyloid PET scans are due. If the next LP and F18 amyloid PET scans are due before the next biennial in clinic visit, the participant should be brought in for their in-clinic visit 1 year earlier to coincide with the timing of the LP and F18 amyloid PET scan. The MRI scan should also be conducted at this in clinic visit. However, if the LP, F18 amyloid PET scan, and MRI scan have been occurring on alternating years for CN or SMC who have NOT converted to EMCI, LMCI, or AD all three assessments should be conducted at the same in clinic visit. This will require 1 or 2 of the assessments to be 1 year ahead of schedule.

THE FOLLOWING ARE MEASURED DURING THE ANNUAL 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
- 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
- Autopsy consent discussion

In addition to the above, the following procedures should be performed every two years:

- F18 amyloid PET scans (100%)
- CSF Collection by Lumbar Puncture (LP) (100%)
- MRI

5.2. *Procedures for original diagnosis AD Subjects in ADNI2 who have provisionally consented to neuropath or are undecided*

5.2.1. Ongoing Month 6 Phone Follow-Up

Telephone Follow-Up calls will be performed at six-month intervals to confirm if there is any change in the subject's decision to participate or not in the neuropath sub study.

5.3. *Early Termination Visit*

If a subject wishes to exit the study, a termination visit will be scheduled. This may include all evaluations normally performed at the Annual Visit, including CSF collection by lumbar puncture, and F18 amyloid PET scans as funding permits. Early termination visits will not be conducted for original diagnosis AD participants that will no longer be followed. Please contact your Clinical Monitor or ATRI Clinical Operations group for guidance on what specific procedures should be conducted at an early termination visit for all other subjects that elect to exit the study.

5.4. *Retrieved drop-outs*

Unless a subject withdraws consent, subjects who miss visits will be encouraged to come in for subsequent visits. Refer to the Procedures Manual for further discussion of options for reduced testing batteries.

5.5. *Nursing Home Placement*

If a subject is placed in a skilled nursing home, the Participant Demographics eCRF will reflect this. Subject should continue to be followed as scheduled and all assessments as outlined in the schedule of events should be completed, to the extent possible. If the subject withdraws consent, a termination visit should be conducted, consisting of evaluations normally performed at the Annual Visit, that may include CSF collection by lumbar puncture, and F18 amyloid PET scan as funding permits. Early termination visits will not be conducted for original diagnosis AD participants that will no longer be followed. Please contact your Clinical Monitor or ATRI Clinical Operations group for guidance on what specific procedures should be conducted at an early termination visit for all other subjects that elect to exit the study.

6. **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 significant memory concern (SMC), 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 (ADC's) 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, 2003): 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. The performance of the MoCA will be followed to determine its ability to differentiate among the three cognitive groups.

6.1.2. Everyday Cognition (ECog) (Farias et al, 2008): 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 an informant-rated questionnaire comprised of multiple subscales and takes approximately ten minutes to administer. Participants themselves will be asked to complete a self-reported version of the same questionnaire. 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. The performance of the ECog will be followed to determine its ability to differentiate among the four 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, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two overlapping 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. 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 30-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.

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. Where a full CDR interview is not possible, the abbreviated CDR (Davis, 991) can be utilized.

6.1.13. Activities of Daily Living | Functional Assessment 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 on a scale from 0 to 3. Scores are summed across items to provide a total disability score (higher scores = greater impairment; maximum score = 30).

6.1.14. Neuropsychiatric Inventory (Cummings et al, 1994) The NPI is a well-validated, reliable, multi-item instrument to assess psychopathology in AD based on an interview with a caregiver or qualified study partner (defined in this study as having direct contact > 2 days/week). The interview is also relatively brief (15 minutes). These properties make it well suited for a multicenter trial. It evaluates both the frequency and severity of 10 neuropsychiatric features including delusions, hallucinations, dysphoria, anxiety, agitation/aggression, euphoria, disinhibition irritability, lability, apathy, and aberrant motor behavior. Frequency assessments range from 1 (occasionally, less than once per week) to 4 (very frequently, once or more per day or continuously) as well as severity (1= mild, 3 = severe). The overall score and the score for each subscale are the product of severity and frequency.

6.1.15. 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 NPIQ is a shorter version that does only the screening questions and the severity rating for each domain. The maximum score is 36.

6.1.16. 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 questions that the subject is asked to answer yes or no on the basis of how they felt over the past week. The more benign items asked 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.

6.1.17. Cognitive Change Index (Adapted from Saykin et al, 2006): The Cognitive Change Index is a subset of items that are focused on memory concerns adapted from a longer panel of self- and informant-report measures originally used to calculate a cognitive complaint index. The Cognitive

Change Index is a self-report scale, consisting of 20 items that the subject rates on a scale from 1 to 5 as to whether there has been no change or some degree of change in each of these functions compared to 5 years ago. The total score for the entire self -report can range from 20 (no change) to 100 (severe change). Only the first 12 questions that specifically address memory concerns are used to calculate the CCI-Memory Total Score in ADNI2, which has a range from 12 (no change) to 60 (severe change).

6.2. *Biofluid Collection*

The ADNI2 procedures manual will provide more detailed instructions for collection, processing and shipment of all biofluid samples. Samples will be collected so as to accommodate the assay of the broadest range of the best antecedent biomarkers/analytes. Since some key analytes such as Aβ are known to stick to polystyrene and glass, polypropylene tubes will be utilized for the collection and storage of plasma, serum, and CSF. Fasting overnight (minimum 6 hours) is required for plasma, serum and CSF sample collection. Only water is permitted until blood draws and the LP procedure are completed. The methods used to assay homocysteine, tau, p-tau and Aβ are identical to those used in ADNI1.

6.2.1. Cell Immortalization Samples

Blood samples will be collected for Cell Immortalization at Baseline for all newly enrolled 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 overnight under ambient conditions to the National Cell Repository for Alzheimer's Disease (NCRAD).

If a sample does not successfully establish, subjects will be asked to agree to re-sampling.

6.2.2. DNA Sample collection for GWAS, APOE Genotyping and Epigenetic Analysis

Whole blood is collected for GWAS at Baseline for both newly enrolled subjects and follow-up subjects who did not have a sample collected for GWAS under ADNI-GO. APOE genotyping will be done only for the newly enrolled subjects using this sample. A single 10 mL EDTA tube of whole blood will be sent ambient, overnight, to NCRAD for genotyping.

The genetics core will request a re-sampling if the sample condition is compromised or if there is poor sample yield.

In order to measure longitudinal epigenetic changes such as DNA methylation profile and chromatin remodeling, the buffy coat from the plasma collected for biomarker analysis will be aliquoted and sent to NCRAD for processing and banking.

6.2.3. RNA Sample Collection

In order to measure gene expression across time, a RNA sample will be collected at multiple time points during the course of the study (refer to schedule of events). Three (3) 2.5 mL tubes will be collected at each visit using kits supplied by the ADNI-CC. Samples will be shipped overnight under ambient conditions to NCRAD.

6.2.4. Plasma and Serum for Biomarkers

All samples will be collected in the morning before breakfast and after an overnight fast (minimum 6 hour fast).

Plasma is collected in a uniform fashion using EDTA as anti-coagulant. Once blood is collected into two 10 mL EDTA plastic tubes, it is mixed thoroughly, then centrifuged. 10 mL of the plasma sample is transferred to a labeled polypropylene vial, frozen on dry ice for 20 minutes. The buffy coat will also be extracted from each plasma tube and shipped to the National Cell Repository (NCRAD) overnight under ambient conditions.

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 (if applicable) collected for the same visit and shipped 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 (minimum 6 hour 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. Approximately the first 2 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 will be collected into polypropylene collection tubes and transferred to polypropylene shipping tubes as outlined in the Procedures manual.

CSF samples for Penn AD Biomarker Laboratory are to be frozen upright on dry ice for at least 20 minutes before being packaged along with the frozen plasma and serum. CSF samples are shipped overnight frozen on dry ice to the Penn AD Biomarker Fluid Bank Laboratory.

CSF samples for local laboratory analysis are shipped ambient, day of collection.

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 to 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 are thawed and aliquots transferred to plastic vials, bar code labeled, and placed in designated locations in the -80°C freezers. All samples are inventoried and tracked. A database has been 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 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 is used on the sample tracking form that is used by the technologist when processing and storing samples. This is being 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. Removal of samples also is tracked on the database, including the date removed and the recipient center.

6.3 *Magnetic Resonance Imaging (MRI)*

6.3.1. Site Qualification

Each site must be qualified for MRI. If the machine being used has already been certified by the ADNI MRI Core and has not experienced any software upgrades, re-qualification will not be required. The procedures for site qualification consist 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

All subjects newly enrolled into ADNI2 and EMCI subjects carried forward from ADNI-GO will be scanned using the ADNI-GO 3T scanning protocol. All subjects carried forward from ADNI1 will continue with 1.5T MRI scanning protocol unless and until the MRI Core makes a decision that the site should perform 3T MRIs on all participants. Please refer to the schedule of events section in the protocol for details on the timing of each MR scan.

As in ADNI1, 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 MRI protocol will consist of FDA approved imaging sequences.

The data will be used to test the hypotheses in the ADNI Biostatistics Core as well as to examine relationships between baseline and rates of change of MRI to clinical, PET, and plasma/CSF biomarker measures.

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 ADNI2 MRI protocol. 4) Monitor each scanner longitudinally in the study using the ADNI phantom, as needed. 5) 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 scanned and submitted to the ADNI-CC.

6.3.4. Data Management and Quality Control

Images are uploaded by site users to the Laboratory of Neuroimaging (LONI) at the University of Southern California). 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 which may need to be repeated. Repeat scans must be scheduled within four (4) weeks of the visit date.

6.4. *F18 amyloid PET scans and FDG-PET Imaging*

Please note that the previous convention of referring to the amyloid imaging tracer as ^{18}F-AV-45, has been updated to the United States Adopted Name (USAN), florbetapir F 18. Outside the United States the International Non-proprietary Name (INN) is used, florbetapir ($^{\mathbf{18}}$F).

6.4.1. Site Qualification

Each site must be qualified for PET. If the machine being used has already been certified by the ADNI PET Core and has not experienced any major software upgrades, re-qualification will not be required. Qualification of the PET scanner applies to both the FDG-PET and F18 amyloid PET scans imaging protocols. Qualification will employ the same methods utilized for site qualification in ADNI. Sites will be provided with a Hoffman brain phantom (if one is not available to the site) and a technical manual for the data acquisition using ^{18}F, generally FDG. The phantom must be scanned on two sequential days using the protocol identical to that required for human imaging. This enables the PET Core to ascertain the characteristics of the scanner (particularly resolution and uniformity) and assure that sites are capable of performing the protocol for acquisition and image reconstruction. All phantom images will be forwarded to PET Core QC group for review and qualification.

6.4.2.Data Acquisition for the florbetapir F 18 study

CN and MCI subjects carried forward from ADNI1, EMCI subjects carried forward from ADNI-GO and all newly enrolled subjects will be studied in this protocol that includes F18 amyloid PET scans. Scans must be completed within 2 weeks before or 2 weeks after the in-clinic assessments at Baseline and at two-year intervals.

F18 amyloid PET scan Standard Protocol

The *F18 amyloid PET scan* protocol will entail the injection of 10 mCi (370 MBq) (+/- 10%) of [18F]AV-45 administered by intravenous bolus injection (over 5 to 10 seconds). In some cases, due to scheduling, transit, manufacturing issues or site specific guidelines, florbetapir F 18 doses less than 10 mCi – 10% may be injected into the subject. However, if the dose is below 5 mCi, no injection should occur and the subject should be rescheduled. Dose administration is followed by an uptake phase of 50 minutes during which time the subject may wait in a quiet room.

Depending on the type of scanner, the subject should be placed on scanning table to permit enough time to obtain a CT scan and then begin the emission acquisition on time. PET-only systems will acquire a transmission scan following the emission acquisition. The PET scan must begin at approximately 50 minutes post-florbetapir F 18 injection. Brain images will be acquired continuously for a period of 20 minutes as four 5-minute frames. 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 four 5-minute frames. If there is a repeat scan, a second transmission scan can be done after the emission acquisition. PET/CT scans will precede this acquisition with a CT scan for attenuation correction. 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. This information is detailed in the PET technical manual.

F18 amyloid PET scan "Early Frames Add On" Study

Enrollment for the ADNI2 early frames add-on study has been met and this sub study is closed. Please do not conduct any further early frame add-on sequences, even if participants have been consented.

6.4.3 FDG Protocol

As of May 9, 2014, no further FDG PET scans will be conducted under the ADNI 2 protocol.

6.4.4 F18 amyloid PET Scans and FDG-PET Data Management and Quality Control

Images are uploaded by site users to the Laboratory of Neuroimaging (LONI) at the University of Southern California. Data are de-identified as part of the upload and placed into quarantine until they pass quality assurance evaluation conducted by the PET Core. There will be several steps in the quality assurance and pre-processing of the F18 amyloid PET scans 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 site (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) Perform normalization on all image sets, based on global mean for FDG-PET metabolic images or cerebellar gray matter for amyloid imaging;
(8) Smooth images from all scanner models by amounts determined from Hoffman phantom scans to achieve uniform 8mm effective resolution;
(9) Complete PET QA forms;
(10) Upload post-processed PET images sets to LONI (image repository).

Quality control for florbetapir F 18 will result in failure of some scans. Many of these scans are expected to be useful if the data are reprocessed. Sites must retain all raw, unprocessed images at least until image quality control has been performed and the determination has been made as to whether the image requires reprocessing. 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 the FDG and F18 amyloid PET scans were repeated. Repeat scans must be conducted within 4 weeks of the visit date.

6.5. *Neuropathology*

6.5.1 Discussing Autopsy and Obtaining Provisional Consent:

An ADNI clinician will lead a discussion about autopsy with all participants (CN, SMC, EMCI, LMCI, and AD) during the consent process, at baseline and at every in-clinic visit thereafter, as well as during the ongoing 6 month follow-up phone check visits for the AD cohort who have provisionally consented to neuropathology or are undecided (study partners and families are welcomed in the discussion and required for AD participants). There are 3 objectives of the discussion: 1) to convey information about the value of brain autopsy in confirming the clinical diagnosis and advancing knowledge regarding MCI and AD; 2) to initiate consideration of the individual's wishes concerning an autopsy; and 3) to answer questions, misconceptions, or concerns about autopsy. The involvement of the physician in these discussions emphasizes the importance of autopsy. The discussions are repeated at each ADNI visit (unless the participant has clearly refused autopsy), both to ensure the participant's wishes regarding brain donation are carried out and that family members and/or participant's Durable Power of Attorney (DPOA) are aware of the participant's wishes. There is no pressure on an individual to decide; they are encouraged to involve family members, clergy, physicians, or other appropriate persons in their decision-making. Participants are assured that a decision not to have autopsy in no way jeopardizes their research participation or any other patient rights. It is important to note that autopsy will not interfere with funerary arrangements nor will it be a financial burden to the participant's family. As a supplement to this discussion, the ADNI-NPC has developed an Autopsy brochure which dispels some of the common myths and concerns regarding autopsy and a Brain Donation letter which explains the importance of autopsy and brain donation in lay language. We encourage clinicians to use these tools when discussing autopsy with ADNI participants.

6.5.2 After Obtaining Provisional Consent:

When voluntary consent is granted, more detailed information should be provided to the participant about procedures to follow at time of death, including telephone numbers to call and other guidelines. The ADNI-NPC has developed autopsy notification materials including wallet cards and letters to primary care physicians and nursing homes to communicate the participant's wishes regarding autopsy. Participants are strongly encouraged to share this information with next-of-kin, legally authorized representatives (e.g. Durable Power of Attorney or DPOA), and private physicians. In many states, final legal authorization by the DPOA or next-of-kin must be obtained at the time of death. As ADNI is a multi-center study involving sites in the US and Canada, please be sure to follow state and local laws regarding autopsy consent procedures.

It is important to emphasize to ADNI participants and caregivers the procedure for notifying the ADNI site at the time of death so that the autopsy protocol may be initiated. Wallet cards should be given to all participants that list contact information for the person they should notify at the time of death.

6.5.3 At the Time of Expiration:

Once your site has been notified of the death of an ADNI participant, please follow the autopsy procedures as outlined in the ADNI-NPC manual or the specific autopsy procedures developed for your site.

7. **Adverse Events Reporting**

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

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 eCRF. 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 in turn will trigger an alert to the appropriate Medical Safety personnel and Protocol Project Director, which will lead to the initiation of the creation of the report. A notification will be sent to all participating sites and the DSMB once the report is available.

Sites will report based on local IRB requirements.

Avid Radiopharmaceuticals and Eli Lilly will be notified of any SAE that occurs within 48 hours of the florbetapir F 18 PET imaging procedure, and any SAE that is deemed attributable to the florbetapir F 18 scan by the investigator.If an SAE is felt to be unexpected (not previously reported or described by Avid/Eli Lilly) and related, Avid/Eli Lilly will notify the investigator in writing. The investigator should forward this notification to the IRB within 24 hours of receipt.

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 and NIA representative will meet in person or by conference call on a quarterly basis.

Additionally, the DSMB will be informed of the occurrence of any serious adverse events within 7 days of being reported to the Coordinating Center. The DSMB may at any time request additional information from the Coordinating Center.

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 CN 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 ADNI1 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 ADNI2. 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 ADNI1. 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 ADNI1 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.

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 regulations. Written informed consent and HIPAA authorizations 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 ADNI-CC.

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 and ICH Good Clinical Practice. Applicable HIPAA privacy notifications will be implemented and HIPAA authorizations signed 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 must be approved, 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 authorization must be kept on file by the investigator for possible review by regulatory authorities and/or Clinical 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, Genetic Data and Imaging Data.*

The informed consent will not only cover consent for the trial itself, but for the genetic samples/data/storage, biomarker samples/data/storage, and imaging scans/data/storage as well. Consent for storage will include consent to access stored data, biological samples, and imaging data for secondary analyses. Consent forms will specify that genetic and biomarker samples are for research purposes only; the tests on the genetic and biomarker samples are not diagnostic in

nature. Data and results of these studies will not be communicated to study participants, relatives of participants, personal physicians or insurance companies. (Reference: MOU for the Alzheimer's Disease Sequencing Project). MRI scan findings of clinical significance, determined by the site radiologist, will be shared with participants. The informed consent and/or HIPAA authorizations will specify that:

- The ATRI at the University of Southern California (USC) will receive and store all research data;
- 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 Core Laboratory will receive and store biomarker samples;
- NCRAD will receive and store blood, DNA, RNA and Buffy Coat samples and develop and store immortalized cell lines;
- LONI at the University of Southern California (USC) will house a full set of all the data;
- All data will be made available to the pharmaceutical industry, academic investigators and other qualified parties in the public domain.

To maintain consistency and ensure the ability to broadly share data, the consent documents should include the following language:

> "By signing this consent you are authorizing the use of your data and biological materials 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 and biological samples will be stored with a coded research identifier to protect your identity. Only de-identified data, which does not include anything that might d i r e c t l y identify you, will be shared with ADNI members and the general scientific community for research purposes. This data will be entered into study databases to be used from this date and going forward. Genetic data may be made available on NIH-approved, s e c u r e databases."

9.5. *Procedures to Maintain Confidentiality*

Each participant's identity and participation in this study will be kept confidential in the study databases. All records will be stored in a computer file and access will be restricted to research staff. All questionnaires and other forms generated from the data collection will be kept in locked file cabinets, and only the investigators will have access to this information. Participant names will not be used. Rather, we will assign a code number that will be associated with all information collected. Information presented or published for scientific purposes will not include participant names. Research records are maintained in locked paper files and secured computer files, available only to research staff and institutional personnel as part of routine audits.
Information is coded and password protected. Access is restricted to the authorized scientific investigators.

9.5.1. Genetic and Phenotypic Data and Genetic Material

All ADNI sites will independently obtain a Certificate of Confidentiality from NIA as part of the site approval process. The protections provided by the Certificate will be outlined in the Informed Consent documents.

De-identified samples and data will be sent to NCRAD. DNA, RNA , Buffy Coat and cell lines will be stored indefinitely at NCRAD. In addition to the blood sample, year of birth, family history of dementia, and diagnosis will be sent to NCRAD. Identity of participants will not be shared with NCRAD or with any investigators. Data that has been stripped of all identifying information from genetic analysis will be kept separate from the clinical and demographic data stored at NCRAD and can only be accessed by authorized individuals.

Certain datasets may be stored in the database of Genotypes and Phenotypes (dbGaP http://www.ncbi.nlm.nih.gov/gap) that was developed to archive and distribute the results of studies that have investigated the interaction of genotype and phenotype or the NIA Genetics of Alzheimer's Disease Data Storage Site NIAGADS (http://www.niagads.org/). Data are stored under strict security provisions, including multiple firewalls, separate servers, and data encryption protocols. Investigators and their sponsoring institutions seeking access to data in the NIH supported genetic data repositories must submit a data access request. For dbGaP, investigators are approved by a Data Access Committee (DAC) for access to specific datasets for a specific use(s); a similar process if followed for NIAGADS. Data can only be accessed through an NIH- approved login process. In addition, for approved use of the data investigators and their institutions agree to store the requested data securely and to not share the requested data with third parties.

The NIA "Policies And Guidance For Sharing Of Resources And Data From Studies On The Genetics Of Alzheimer's Disease" (http://www.nia.nih.gov/research/dn/alzheimers-disease-genetics-sharing-plan) states that NIAGADS, along with other NIA-approved sites, will make these genetic data and associated phenotypic data available to qualified investigators in the scientific community for secondary analysis in accordance with standards established by NIA.

"Genetic Data" is defined as de-identified data derived from genotyping, mutation analysis, single nucleotide polymorphisms (SNPs) and other genetic analyses of biomaterials and Associated Phenotypic Data, stripped of all personal identifiers and thus with no reasonable chance of being linked to the individuals from whom they were obtained".

Associated "Phenotypic Data" is defined as de-identified data on family structure, age, sex, vital status, psychopathology, diagnosis, and other clinically relevant associated phenotypic information, stripped of all personal identifiers and thus with no reasonable chance of being linked to the individuals from whom they were obtained.

Datasets are stored in the NIH GWAS data repository under strict security provisions, including multiple firewalls, separate servers, and data encryption protocols. Investigators and their sponsoring institutions seeking access to data in the NIH GWAS data repository must submit a data access request that specifies both the data to which access is sought and the planned research use, and agree to the terms of access set forth in the Data Use Certification (DUC). Investigators are approved by a Data Access Committee (DAC) for access to specific datasets for a specific use(s). Data can only be accessed through the NIH login process. In addition, the DUCs include a provision that approved users and their institutions agree to store the requested data securely and to not share the requested data with third parties.

The de-linking of the sample from the participant occurs at the time the blood is sent to the National Cell Repository for AD. All samples will be inventoried and tracked.

Immortalized cell lines, DNA, Buffy Coat and RNA will be prepared at The National Cell Repository for AD. A unique bar-code number is affixed to all purple top, buffy coat and RNA

specimen tubes as well as affixed to the Sample Form/Draw Sheet. The yellow top tubes are labeled with a kit number and 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/Buffy Coat 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.

The ADNI-CC and NCRAD will not have information regarding the participant's name and thus are unable to link the genetic 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 genetic research data and study research data, none of which includes identifying information. The data centers 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 genetic results can be linked to clinical research data for purposes of analyses, the only linkage of genetic test results to names of participants that can happen is at the clinical site.

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 genetic specimens will be in accordance with the rules and procedures established by the Indiana University IRBs. 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.

9.5.2 *Biomarker Data and Storage of Biomarker Material*

At the University of Pennsylvania, a database has been created and is 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.

The ADNI-CC and University of Pennsylvania will not have information regarding the participant's name and thus are unable to link the biomarker analysis results to the person.

Samples handled by the Biomarker Core are banked in a locked freezer dedicated to the ADNI study at the University of Pennsylvania. The samples are without a link to identity of the participant from whom the sample came.

9.5.3. Genetic and Biomarker Samples and Research

Specific procedures for requesting and accessing genetic and biomarker specimens have been created by the Resource Allocation Review Committee (RARC) of the ADNI in accordance with recommendations proposed in the NBAC Human Biological Materials Report. These 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.

9.5.4. 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 Technical Manual and the PET Technical 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. *Florbetapir F 18 Injection and FDG-PET*

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 most up-to-date and complete information regarding the use of florbetapir F 18 can be found in the investigator's brochure or Amyvid™ package insert.

Florbetapir F 18 Injection is now approved by the United States Food and Drug Administration (FDA) to estimate beta-amyloid neuritic plaque density in adult patients with cognitive impairment who are being evaluated for AD and other causes of cognitive decline. The FDA approved Florbetapir F 18 Injection based on the results of completed registration studies in 496 subjects per the product package insert.

To date, florbetapir F 18 has been tested in approximately 4,691 subjects in completed and ongoing research studies. The most common side effect in completed studies involving 555 subjects was headache. Additional uncommon side effects reported were nausea, dysgeusia (bad taste in the mouth), flushing, pruritus (itching), urticaria (hives), and infusion site rash. Musculoskeletal (muscle and bone) pain in the neck, shoulder, and back, fatigue, anxiety, claustrophobia (fear of being in closed or narrow spaces), insomnia (inability to sleep),

dizziness, chills/feeling cold, and hypertension (high blood pressure) were also reported. Although the side effects from florbetapir F 18 noted so far have been relatively limited, participants may experience side effects that are not listed above. Because these events could be related in part to the PET scan apparatus and procedures, careful attention should be taken to make the subject aware of the planned procedures and to maximize subject comfort in the scanner. Previous human clinical trials have revealed no consistent and clinically significant pattern of changes in vital signs, ECG or laboratory changes.

The possibility exists for a rare reaction to any of the drugs or procedures to which the participant will be exposed. The full potential for drug-drug interactions is not presently known. There is no data on the effects of florbetapir F 18 in human perinatal development. In the event of a study related adverse event, subjects should not be discharged from the imaging facility until the event has resolved or stabilized.

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 florbetapir F 18 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**	**Florbetapir F 18 rad/mCi**	**Florbetapir F 18 rad/10 mCi**	**Total Annual Dose from Study**	**Total Annual Dose if Both Scans Repeated**
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 wall	0.32	1.6	0.1	1	2.6	5.2
Uterus	0.062	0.31	0.058	0.58	0.89	1.78
Effective Dose	0.07	0.35	0.069	0.69	1.04	2.08
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. Use of anxiolytic agents for completion of MRI scans is at the discretion of site clinicians, in consultation with project director.

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, and bleeding into the CSF space. 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 6 months of start up time, 12 months of enrollment and up to 60 months of follow up (After May 9, 2014, participants with the original diagnosis of AD under ADNI 2 will

no longer be followed by in-clinic visits. AD participants that have consented to the Neuropathology Program or are undecided will be followed by phone every 6 months as outlined in the schedule of events section in the protocol). The ADNI2 study will therefore be active until July 31, 2016.

At the end of the funding for this 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 ADNI2 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**

For genetics, genomics, and related data, data will be shared with other researchers pursuant to the NIA Alzheimer's Disease Genetics sharing Policy: http://www.nia.nih.gov/research/dn/alzheimers-disease-genetics-sharing-plan

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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Appendix A

Assessments and procedures conducted prior to the ADNI2 protocol changes implemented May 9, 2014

SCHEDULE OF EVENTS (New CN, SMC, EMCI, LMCI Subjects)

Visits prior to May 9, 2014

Visit Name	Screen	Baseline	Month 3	Month 6	Ongoing Annual[1]	Ongoing 6 Month Interim[1]
Visit Type	Clinic	Clinic	MRI	Clinic	Clinic	Phone
Explain study*	X					
Obtain consent*	X					
Neuropath discussion/ Provisional consent	X	X			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				
Cognitive Change Index[5]	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		X			X	
Neuropsychiatric Inventory Q				X		X
ADAS-Cog 13 (w/ Delayed 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*[4]		X		X	X	
RNA Sample Collection*		X			X	
Concomitant Medications	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X
Diagnostic Summary*	X	X		X	X	
Telephone Check – Participant Status						X
3T MRI Imaging (100%)*	X		X[2]	X	X	
Florbetapir F 18 Amyloid Imaging (100%)*		X			X[3]	
FDG-PET Imaging (100%)*		X			X[3]	
CSF Collection by Lumbar Puncture (LP) (100%)*		X			X[3]	

*Assessment must be done in-person

[1] Subjects will be followed for 54 months from baseline, after which they will be asked to consent to additional follow-up under a separate grant and protocol.
[2] Month 3 MRI is timed from Screening MRI
[3] FDG-PET, Florbetapir F 18 Amyloid Imaging, and LP are to be performed only every two years from baseline, as funding permits.
[4] Buffy Coat is removed from plasma sample and shipped to NCRAD for genetic analysis**.**
[5] CCI may be conducted over the phone. Verbal consent must be obtained prior to conducting in order to be acquired under ADNI2.

SCHEDULE OF EVENTS (Newly Enrolled AD Subjects in ADNI 2)
Visits prior to May 9, 2014

Visit Name	Screen	Baseline	Month 3	Month 6	Month 12	Month 18	Month 24	Ongoing 6 Month Follow-up[1]
Visit Type	Clinic	Clinic	MRI	Clinic	Clinic	Phone	Clinic	Phone
Explain study*	X							
Obtain consent*	X							
Neuropath discussion/ Provisional consent	X	X			X		X	X
Demographics, Family History, Inclusion and Exclusion Criteria*	X							
Medical History, Physical Exam, Neurological Exam, Hachinski*	X							
Vital Signs*	X	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						
Cognitive Change Index[4]	X							
Mini Mental State Examination*	X			X	X		X	
Logical Memory I and II*	X				X		X	
Everyday Cognition (ECog)		X		X	X		X	
Montreal Cognitive Assessment (MoCA)*		X		X	X		X	
Category Fluency (Animals)*		X		X	X		X	
Trails A & B*		X		X	X		X	
Boston Naming Test (30-item)*		X		X	X		X	
Auditory Verbal Learning Test*		X		X	X		X	
Geriatric Depression Scale	X			X	X		X	
Clock drawing*		X		X	X		X	
Neuropsychiatric Inventory		X			X		X	
Neuropsychiatric Inventory Q				X		X		X
ADAS-Cog 13 (w/ Delayed Recall and Number Cancellation)*		X		X	X		X	
Clinical Dementia Rating Scale	X			X	X		X	
Activities of Daily Living (FAQ)		X		X	X		X	
Plasma and Serum Biomarker Collection*[3]		X		X	X		X	
RNA Sample Collection*		X			X		X	
Concomitant Medications	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X
Diagnostic Summary*	X	X		X	X		X	
Telephone Check - Participant Status						X		X
3T MRI Imaging (100%)*	X		X[2]	X	X		X	
F18 amyloid PET scans (100%)*		X					X	
FDG-PET Imaging (100%)*		X					X	
CSF Collection by Lumbar Puncture (LP)(100%)*		X					X	

*Assessment must be done in-person

[1] Subjects will be followed for 54 months from baseline, after which they will be asked to consent to additional follow-up under a separate grant and protocol.

[2] Month 3 MRI is timed from Screening MRI

[3] Buffy Coat is removed from plasma sample and shipped to NCRAD for genetic analysis.

[4] CCI may be conducted over the phone. Verbal consent must be obtained prior to conducting in order to be acquired under ADNI2.

SCHEDULE OF EVENTS (Follow-up CN and LMCI from ADNI 1, EMCI subjects from ADNI-GO) Visits prior to May 9, 2014

Visit Name	Initial	Ongoing 6 Month Interim[1]	Ongoing Annual Follow-up[1]
Visit Type	Clinic	Phone	Clinic
Explain study*	X		
Obtain consent*	X		
Neuropath discussion/Provisional consent*	X		X
Demographics*	X		
Medical History*	X		
Vital Signs*	X		X
Mini Mental State Examination*	X		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	X		X
Neuropsychiatric Inventory Q		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*[4]	X		X
DNA Sample Collection for GWAS (if not previously obtained)*	X		X
RNA Sample Collection*	X		X
Concomitant Medications	X	X	X
Adverse Events	X	X	X
Diagnostic Summary*	X		X
Telephone Check – Participant Status		X	
MRI Imaging (100%)[2*]	X		X
F18 amyloid PET scans (100%)*	X[3]		X[3]
FDG-PET Imaging (100%)*	X[3]		X[3]
CSF Collection by Lumbar Puncture (LP) (100%)*	X[3]		X[3]

*Assessment must be done in-person

[1] Subjects will be followed for 54 months from initial ADNI2 visit, after which they will be asked to consent to additional follow-up under a separate grant and protocol.

[2]All EMCI participants enrolled in ADNI-GO will continue with 3T MRI imaging. All CN and LMCI participants enrolled in ADNI1 will continue with 1.5T MRI imaging unless and until decision made by MRI Core that the site should perform 3T MRIs on all participants.

[3]FDG-PET, F18 amyloid PET scans, and LP are to be performed every two years, as funding permits.

[4] Buffy Coat is removed from plasma sample and shipped to NCRAD for genetic analysis..

1. **Study Procedures**

New subjects who are enrolling in this study will go through a Pre-screening and Screening period as described below. Subjects who are being followed from the first ADNI study and from ADNI-GO will continue to be seen annually in this study and contacted semi-annually via telephone. Assessments completed at each visit are listed below. Visits must be conducted within 2 weeks before or after the target date. Once the visit begins, all imaging studies, biofluid collection and cognitive and clinical assessments must take place within 2 weeks from the start of the visit.. Ideally all data will be collected in-clinic. With the exception of the Screen and Baseline visit, which must be completed in-person with both the subject and study partner, if there are difficulties with scheduling follow-up visits some assessments may be done on the telephone.

Where there is insufficient time to complete the full visit, the order of priority assessments is:

- Clinical Dementia Rating Scale
- ADAS-Cog
- Mini Mental State Examination
- Logical Memory I and II
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- Vital Signs

1.1. *Procedures for Newly Enrolled Subjects*

1.1.1. Pre-screening

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

1.1.2. 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 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 eligibility criteria for all other screening assessments as determined by both a site investigator and clinical monitor. A local clinical read of the MRI will be reviewed for eligibility and scan must pass MRI QC evaluation. Eligibility will be determined according to the Inclusion/Exclusion criteria outlined above and confirmed by a 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
- Cognitive Change Index
- Logical Memory I and II
- Geriatric Depression Scale
- Clinical Dementia Rating Scale
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- Autopsy consent discussion
- MRI (3T) – Screening MRI only to be conducted after confirmation from clinician and monitor that the subject has met all other inclusion/exclusion criteria.

1.1.3. Baseline Visit

The Baseline visit for the newly enrolled subjects must take place within 28 days of the Screening.

- 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
- 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
- F18 amyloid PET scans (100%)
- FDG-PET Imaging (100%)
- CSF Collection by Lumbar Puncture (LP) (100%)
- Autopsy consent discussion

1.1.4. Month 3 Visit

The Month 3 visit is timed 3 months from the date of the Screening MRI. After September 17, 2013 all M3 MRIs were canceled and are no longer to be conducted.

- MRI (3T)
- Adverse Events
- Concomitant Medications

1.1.5. Month 6 Visit

The Month 6 visit is timed 6 months from Baseline Visit day 1.

- 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) * After September 17, 2013 all M3 MRIs were canceled and are no longer to be conducted.

1.1.6. Annual Visit

The Annual visits will be timed every 12 months from Baseline Visit day 1. See section 5.1.8 regarding ongoing follow-up for AD participants.

- 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
- 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)
- Autopsy consent discussion

In addition to the above, the following procedures should be performed every two years as funding permits:

- F18 amyloid PET scans (100%)
- FDG-PET Imaging (100%)
- CSF Collection by Lumbar Puncture (LP) (100%)

1.1.7. 6 Month Interim Telephone Check

The 6 Month Interim Telephone visits will be timed at 18, 30, 42, and 54 months from Baseline visit day 1. See section 5.1.8 regarding ongoing follow-up for AD participants.

- Concomitant Medications
- Adverse Events
- Participant Status

1.1.8. AD Participants- Ongoing Follow-up

AD participants will be seen as described in sections 5.1.1 through 5.1.7 until the Month 24 Clinic visit. After that, telephone checks only will be performed at six-month intervals to obtain key demographic and participant status information as well as to discuss provisional autopsy consent and logistical support for autopsy.

1.2. *Procedures for Follow-up Subjects: CN, LMCI, EMCI*

Subjects will be followed as long as they are willing regardless of whether a diagnostic conversion has occurred since first enrolled in ADNI1 or ADNI-GO. Every attempt should be made to retain participants for longitudinal follow-up as long as possible. If subjects are not willing or able to complete the full schedule of assessments at any visit, those assessments or procedures they are willing to complete should be conducted. If subjects are no longer willing or able to travel to the clinic for annual visits, as much information should be collected via telephone as possible.

1.2.1 Visit Scheduling

The ADNI2 visits will occur every six months. The timing of all visits will be projected from the subject's original Baseline visit - under ADNI1 for CN and LMCI continuing participants and under ADNI-GO for continuing EMCI participants. ADNI2 Initial and Annual visits should

be timed at 12-month intervals from the original Baseline visit day 1. ADNI2 6 Month Interim Telephone visits should be timed at 12-month intervals starting 6 months from the original Baseline visit day 1.

1.2.2 Initial Visit

The initial visit for ADNI2 will be conducted at the subject's next projected annual follow-up visit from their initial ADNI1 or ADNI-GO Baseline Visit. Longitudinal collection of CSF is critical and all subjects will be asked to agree to at least one LP for CSF collection, but is not required in order to continue to ADNI2.

- Explain study
- Obtain consent
- Demographics
- Medical History
- 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
- ADAS-Cog 13 (with Delayed Word Recall and Number Cancellation)
- Clinical Dementia Rating Scale
- Activities of Daily Living (FAQ)
- Plasma and Serum Biomarker Collection
- DNA Sample Collection for GWAS (only if not previously obtained)
- RNA Sample Collection
- Concomitant Medications
- Adverse Events
- Diagnostic Summary
- MRI (3T or 1.5T) (100%) - All EMCI participants enrolled in ADNI-GO will continue with 3T MRI imaging. All CN and LMCI participants enrolled in ADNI1 will continue with 1.5T MRI imaging unless and until decision made by MRI Core that the site should perform 3T MRIs on all participants.
- Autopsy consent discussion

<u>In addition to the above, the following procedures should be performed every two years as funding permits:</u>

- F18 amyloid PET scans (100%)
- FDG-PET Imaging (100%)
- CSF Collection by Lumbar Puncture (LP) (100%)

1.2.3. 6 Month Interim Telephone Check

The 6 Month Interim Telephone visits will be timed at the 6-month point between annual visits.

- Concomitant Medications
- Adverse Events
- Participant Status

1.2.4. Annual Visit

Ongoing annual visits will be conducted after completion of the ADNI2 Initial Visit. Visits will be scheduled based on original Baseline date for either ADNI1, ADNI-GO or ADNI2.

- 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
- 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 (3T or 1.5T) (100%) - All EMCI participants enrolled in ADNI-GO will continue with 3T MRI imaging. All CN and LMCI participants enrolled in ADNI1 will continue with 1.5T MRI imaging unless and until decision made by MRI Core that the site should perform 3T MRIs on all participants.
- Autopsy consent discussion

<u>In addition to the above, the following procedures should be performed every two years as funding permits:</u>

- F18 amyloid PET scans (100%)
- FDG-PET Imaging (100%)
- CSF Collection by Lumbar Puncture (LP) (100%)

2. F18 amyloid PET scans "Early Frames Add On" Study

2.1. Site Qualification for the F18 amyloid PET scans "Early Frames Add On" Study

Eligible sites must be collecting florbetapir F 18 images on a scanner capable of rapid dynamic images (peak 4 per min) for a total of 16 frames. Some scanners acquire such data as list mode. Also, sites must have the capacity for injection with simultaneous scan start (typically requires two technologists).

2.2. F18 amyloid PET scans "Early Frames Add On" Study

Rationale:
PET data obtained immediately following injection of rapidly diffusing tracers such as florbetapir F 18 contain information about both blood flow and binding. This "early frames add-on" study will collect additional F18 amyloid PET scans data for 20 min immediately after injection of a participant's regular dose for comparison to later image times in that same participant and to their FDG scan.

It is anticipated that approximately 100 participants distributed across the categories of normal, SMC, EMCI, LMCI and AD that are currently enrolled as an ADNI2 participant will participate in the "early frames add on" study. In order to be eligible, the study participant must be seen at a site participating in the "early frames add on study", have or be expected to have, an FDG scan using the same scanner at the same study time point as the F18 amyloid PET scan and must be able to easily tolerate the additional 20 minutes of PET imaging and provide informed consent for this additional scanning.

Study data will be collected with the participant's standard F18 amyloid PET scan session. Instead of waiting the 50 minutes for the uptake phase, the participant will be positioned in the scanner prior to injection. For PET/CT systems a CT will be performed prior to injection. For PET-only systems a transmission scan will be performed prior to injection. At the time of injection, a dynamic scan will be initiated. Thus, there is no additional radioactive tracer being administered for the "early frames add on" study. The scan will be 20 min long comprised of the following sequence 4 x 15 sec, 4 x 30 sec, 3 x 60 sec, 3 x 120 sec, 2 x 240 sec. Following this acquisition, subjects will be removed from the scanner, and then repositioned shortly before the 50 min time point for the standard acquisition, whereby a second CT scan would be obtained and then begin the emission acquisition on time for the florbetapir F 18 standard protocol. PET-only systems will acquire a transmission scan following the emission acquisition. Reconstruction of dynamic images will be identical to that done on data obtained at 50 min.

3. FDG Protocol

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 (+/- 10%) 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 (for PET/CT systems) or followed by a positron transmission scan (for PET-only systems).