CONFIDENTIAL

ALZHEIMER'S DISEASE NEUROIMAGING PROTOCOL (ADNI)

PROTOCOL PRINCIPLE INVESTIGATOR	PROTOCOL CONSULTANTS
Ronald Petersen, M.D., Ph.D. **Mayo Clinic, Rochester, Minnesota** **Tel: (507) 538-0487** **Fax: (507) 538-6012** **peter8@mayo.edu**	**Marilyn Albert, Ph.D.** **Steven DeKosky, M.D.** **David Salmon, Ph.D.** **Pierre Tariot, M.D.**
ADNI PRINCIPAL INVESTIGATOR/ PROGRAM DIRECTOR	**COORDINATING CENTER (ADNI-CC)**
Michael W. Weiner, M.D. **University of California, San Francisco** **Tel: (415) 221-4810 x3642** **Fax: (415) 668-2864** **mweiner@itsa.ucsf.edu**	**Paul S. Aisen, M.D.** **University of California, San Diego** **Alzheimer's Disease Cooperative Study** **Tel: (858) 622-2028** **Fax: (858) 452-4291** **paisen@ucsd.edu**
LABORATORY OF NEUROIMAGING	**UNIVERSITY OF PENNSYLVANIA**
Arthur Toga, Ph.D. **LONI** **University of California, Los Angeles** **Tel: (310) 206-2101** **Fax: (310) 206-5518** **toga@loni.ucla.edu**	**Leslie M. Shaw, Ph.D.** **University of Pennsylvania Medical Center** **Department of Pathology and Laboratory Medicine** **Tel: (215) 662-6575** **Fax: (215) 662-7529** **shawlmj@mail.med.upenn.edu**
MAGNETIC RESONANCE IMAGING	**POSITRON EMISSION TOMOGRAPHY**
Clifford Jack, Ph.D. **Mayo Clinic, Rochester** **Tel: (507) 284-8548** **Fax: (507) 284 2405** **jack.clifford@mayo.edu**	**William Jagust, Ph.D.** **University of California, Berkley** **Tel: (510) 643-6537** **Fax: (510) 642-3192** **jagust@berkeley.edu**

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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**
ADL	**Activities of Daily Living**
ADNI	**Alzheimer's Disease Neuroimaging Initiative**
ADNI-CC	**Alzheimer's Disease Neuroimaging Initiative Coordinating Center**
ADC's	**Alzheimer's Disease Centers, under NIA**
APOE/APOE4	**Apolipoprotein (APOE) epsilon 4 (APOE4)**
Aβ	**Beta Amyloid**
CDR	**Clinical Dementia Rating**
CRF	**Case Report Form**
CSF	**Cerebral Spinal Fluid**
DNA	**Deoxyribonucleic Acid**
DSMB	**Data Safety Monitoring Board**
DSM-IV	**Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition**
ESI	**Electrospray Ionization**
FAQ	**Functional Activities Questionnaire (Activities of Daily Living)**
FDG	**Fluoro Deoxy Glucose**
GDS	**Geriatric Depression Scale**
HIPAA	**Health Insurance Portability and Accountability Act**
HPLC	**High-Performance Liquid Chromatography**
ICRP	**International Commission on Radiological Protection**
iPs	**Isoprostanes**
IRB	**Institutional Review Board**
LONI	**Laboratory of Neuroimaging**
LP	**Lumbar Puncture**
MCI	**Mild Cognitive Impairment**
MMSE	**Mini Mental State Examination**
MR/MRI	**Magnetic Resonance / Magnetic Resonance Imaging**
MS	**Mass Spectrometry**

NCRAD	**National Cell Repository for Alzheimer's Disease**
NIA	**National Institute on Aging, under the NIH**
NIH	**National Institutes of Health**
NINCDS/ADRDA	**National Institute of Neurological and Communicative Diseases and Stroke / Alzheimer's Disease and Related Disorders Association**
NL	**Normal**
NPI-Q	**Neuropsychiatric Inventory Questionnaire**
PET	**Positron-Emission Tomography**
PI	**Principal Investigator**
QA	**Quality Assurance**
QC	**Quality Control**
RPR	**Rapid Plasma Reagin Test**
SD	**Standard Deviation**
SSN	**Social Security Number**
T	**Tesla**
TFT's	**Thyroid Function Tests**

PROTOCOL SYNOPSIS

Title	**Alzheimer's Disease Neuroimaging Initiative (ADNI)**
Primary Objective	The major goals of the ADNI are to: 1. Develop improved methods which will lead to uniform standards for acquiring longitudinal, multi-site MRI and PET data on patients with Alzheimer's disease (AD), mild cognitive impairment (MCI), and elderly controls. 2. Acquire a generally accessible data repository which describes longitudinal changes in brain structure and metabolism. In parallel, acquire clinical cognitive and biomarker data for validation of imaging surrogates. 3. Develop methods which will provide maximum power to determine treatment effects in trials involving these patients. 4. Test a series of hypotheses based on the clinical and biomarker data as outlined in the statistical analysis section.
Study Design	This is a non-randomized natural history non-treatment study in which a total of 800 subjects including 200 normal controls, 400 individuals with MCI, and 200 subjects with mild AD will be recruited at approximately 50 sites in the United States and Canada for longitudinal follow up.
Sample Size	Eight hundred subjects from 50 sites from the United States and Canada.
Summary of Key Eligibility Criteria	Enrolled subjects will be between 55-90 (inclusive) years of age, have a study partner able to provide an independent evaluation of functioning, and will speak either English or Spanish. All subjects must be willing and able to undergo all test procedures including neuroimaging and agree to longitudinal follow up. Between twenty and fifty percent must be willing to undergo two lumbar punctures. Specific psychoactive medications will be excluded. General inclusion/exclusion criteria are as follows: 1. Normal subjects: MMSE scores between 24-30 (inclusive), a CDR of 0, non-depressed, non-MCI, and nondemented. 2. MCI subjects: MMSE scores between 24-30 (inclusive), a memory complaint, have objective memory loss measured by education adjusted scores on Wechsler Memory Scale

	Logical Memory II, 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. 3. Mild AD: MMSE scores between 20-26 (inclusive), CDR of 0.5 or 1.0, and meets NINCDS/ADRDA criteria for probable AD.
Procedures	All subjects will have clinical/cognitive assessments and 1.5 T structural MRI at specified intervals for 2-3 years. Approximately 50% of subjects will also have FDG PET scans at the same time intervals and 25% of subjects (who have not been scanned using PET) will have MRI at 3 Tesla. AD subjects (n=200) will be studied at 0, 6, 12, and 24 months. MCI subjects at high risk for conversion to AD (n= 400) will be studied at 0, 6, 12, 18, 24 and 36 months. Age matched controls (n=200) will be studied at 0, 6, 12, 24 and 36 months. 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 UCSD. U Penn will collect biomarker samples. All raw and processed image data will be archived at LONI.
Outcome Measures	1. Rate of conversion from MCI to AD. 2. Rate of volume change of whole brain, hippocampus, and other structural MRI measures. 3. Rates of change on each specified biomarker. 4. Rates of change of glucose metabolism for specified regions of interest on PET scanning. 5. Group differences for each imaging and biomarker measurement.
Statistical Considerations	As specified in the protocol. Additionally, many other statistical analyses not specified in the protocol will be carried out on this data set.
Sponsor	National Institutes of Health

ALZHEIMERS DISEASE NEURO-IMAGING INITIATIVE PROTOCOL

A. SPECIFIC AIMS

The overall goals of the Alzheimer's Disease Neuroimaging Initiative (ADNI) Clinical Protocol will be to recruit 800 subjects and carry out all clinical evaluations including: neuropsychological and clinical assessments, imaging studies and collection of biomarkers. To carry out this protocol we will:

A.1. SUPPORTIVE

1. Establish a network of clinical sites to recruit the subjects
2. Arrange communications among the sites and the various components of the ADNI
3. Develop a plan for the recruitment and retention of subjects
4. Develop and approve regulatory documents for the sites
5. Organize and conduct the training meeting to launch the protocol
6. Monitor and track enrollment to meet the recruitment goals of the ADNI
7. Collect clinical and neuropsychological data
8. Track collection of samples for biological markers including: plasma, serum, urine, DNA, cell lines and CSF
9. Assist in the scheduling and tracking of subjects undergoing imaging protocols
10. Arrange for adjudication for conversion from NL to MCI, NL to AD and MCI to AD
11. Store all clinical data
12. Perform quality control on clinical data
13. Monitor the sites
14. Reimburse sites for work performed
15. Share data on the web with the ADNI imaging units, PI, and serve as a back-up repository for the entire data set

A.2. HYPOTHESIS TESTING

The major goal of this initiative is the collection of data rather than its analysis. In addition to carrying out the clinical initiative, we will test several hypotheses based on the clinical and biomarker data. A few examples are listed below:

1. Rates of conversion from MCI to AD will average 10-15%/year.
2. Baseline scores on logical memory and apolipoprotein E (APOE) epsilon 4 (APOE4) status will predict conversion from MCI to AD.
3. Measures of global functioning such as activities of everyday living will be more sensitive than neuropsychological measures for predicting conversion from MCI to AD.
4. The rate of backcrossing from MCI to normal will be extremely low for this population.
5. Plasma isoprostanes will a) be related to disease severity and b) higher levels will predict a faster rate of decline.
6. Hippocampal volume and posterior cingulate glucose metabolic rate will predict rate of decline and conversion from MCI to AD.

B. BACKGROUND AND SIGNIFICANCE

B.1. WHY THE ADNI?

At present, the development of drugs for patients with AD is costly and requires a considerable length of time. Currently marketed drugs have been developed for symptomatic treatment of AD and trials can be completed in 6 months. Trials designed to slow the rate of decline necessary to demonstrate disease modification require at least one year of treatment or longer to see adequate clinical separation of groups. The development of drugs for subjects with mild cognitive impairment (MCI) takes longer since these subjects progress more slowly. Current MCI trials require 3-4 years to establish either a sufficient rate of clinical decline or a sufficient number of conversions from MCI to AD to complete a clinical trial (R.C. Petersen, 2003). Subjects with MCI are of particular interest since they represent a population at particularly high risk of converting to AD and a population in which secondary prevention trials can be carried out. In the case of normal subjects, conversion to AD is very slow, averaging only 1-2 % / year depending on the age of the cohort. Thus, primary prevention trials for AD require 3,000-6,000 subjects followed for 5 to 7 years to achieve sufficient clinical endpoints. These long timeframes are necessary because there is a great deal of variability in clinical endpoints based on slope analysis. For example, the standard deviation of the rate of change for the ADAS-Cog, the most widely used cognitive measure in AD trials, is 1 to 1.5 times the one year rate of change. In subjects with MCI or AD, volumetric images of the whole brain, hippocampus or entorhinal cortex have a smaller ratio of standard deviation of change to the rate of atrophy than clinical measures allowing for detection of a smaller effect size (Grundman et al., 2002). Thus, the development of suitable biomarkers that track the progression of the disease and reflect change in underlying pathology has two advantages over clinical/cognitive data: 1) They have greater statistical power to detect treatment effects, because of reduced measurement error and 2) They provide measurements which may directly (e.g., changes in brain size, measurements of CSF proteins) indicate rate of disease progression. Therefore, the use of such biomarkers could markedly speed drug development by providing an earlier signal of drug efficacy.

B.2. BIOMARKERS (BLOOD AND CSF)

The clinical diagnosis of AD is imprecise with an accuracy rate of ~90% using consensus criteria for probable AD, but definite AD requires autopsy confirmation, and diagnostic accuracy is far lower at early and pre-symptomatic stages of AD when confusion with other dementias is common (Frank et al., 2003; McKhann et al., 2001; Reckess, 2003; The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's, 1997). Since therapy is likely to be most effective at symptom onset, early diagnosis of AD is highly desirable before neurodegeneration becomes severe. Thus, there is a great need for blood and CSF biomarkers that substantially aid early diagnosis and track disease progression of AD and MCI. As reviewed elsewhere (Frank et al., 2003; Reckess, 2003; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association & NIAWG 1998), ideal AD biomarkers should detect a fundamental feature of AD neuropathology, be validated in autopsy-confirmed cases, have a diagnostic sensitivity >80% for detecting AD and a specificity of

>80% for distinguishing AD from other dementias. Moreover, assays using AD biomarkers should be reliable, reproducible, non-invasive, simple to perform and inexpensive. Further, validation of AD biomarkers requires confirmation by at least 2 independent studies from qualified investigators published in peer-reviewed journals. Finally, it would be useful if the biomarker also captured the beneficial effect of disease-modifying therapy. The lack of such validated, robust AD biomarkers impedes development of treatments for AD, and the studies proposed here are designed to resolve this problem. Due to funding constraints, the emphasis in the biomarker component is on biological sample collection and the establishment of a bank of biological fluids from the unique cohort of subjects followed here, but funds are available to perform APOE genotyping and to conduct studies of selected AD biomarkers, i.e. specific isoprostanes, tau, Aβ, sulfatides and homocysteine. However, it should be emphasized that the Steering Committee and a Resource Allocation Review Committee (RARC) will stimulate and oversee AD biomarker research funded from other public and private sources that will be conducted on the samples collected here. Thus, the most relevant biomarkers mentioned above will be studied; they were selected for high priority consideration based on a recent consensus of AD biomarker experts (Frank et al., 2003; Reckess, 2003), and advice from the biomarker advisors listed as consultants in this application. Other analytes to be included in subsequently funded studies are analytes such as Aβ precursor proteins, α1-antichymotrypsin, interleukin-6 (IL-6) and IL-6 receptor complex proteins, C-reactive protein, C1q protein, etc., since evidence indicates that assay of these analytes is likely to increase diagnostic accuracy of AD, enhance prediction of progressing from MCI to AD, or provide insights into pathways influenced by potential AD treatments (Frank et al., 2003; Reckess, 2003). For example, in clinical drug trials, compounds that reduce the level of tau might provide an early indication that the rate of neuronal damage has been slowed. However, since no single AD biomarker will serve all these purposes, it is important to develop a panel of AD biomarker assays that, in aggregate, provide the most informative diagnostic measures for the risk, onset and progression of AD. Preliminary studies of the AD biomarkers to be studied are summarized in section C. ADNI is in a powerful position to follow up on these preliminary studies to provide more definitive data on the utility of obtaining longitudinal measures of CSF tau and Aβ in the subset of the ADNI cohort who have agreed to LP for baseline and year one of the ADNI study

B.3. POPULATIONS FOR STUDY

B.3.a. MCI

In developing therapeutic strategies for cognitive impairment, most investigators believe that early intervention is desirable. Presumably by the time the degenerative process, e.g., AD, is fully developed, a great deal of neuronal damage has taken place. Consequently, in recent years the field of aging and dementia has moved toward identifying the earliest clinical signs of the degenerative process that is likely to evolve to AD. Mild cognitive impairment has come to represent this transitional zone between the cognitive changes of normal aging and very early AD (R. C. Petersen, 2003a).

MCI has received a great deal of attention in the literature recently and represents a major focus of research (Bennett et al., 2002; Larrieu et al., 2002; Luis, Loewenstein, Acevedo, Barker, & Duara, 2003; Petersen et al., 1999; Ritchie, Artero, & Touchon, 2001). Currently, there are at least 6 international drug trials involving 4,000-5,000 subjects under way for MCI, attesting to the importance of this group of subjects for study of interventional

strategies (R.C. Petersen, 2003). As such, the ADNI will focus on MCI subjects as the primary group of interest on whom to develop neuroimaging and biomarker measures. As important comparison groups, we will also recruit complements of normal and very mild AD subjects.

B.3.a.i. MCI ISSUES

While MCI is an important group of subjects to study, the topic is not without controversy (Larrieu et al., 2002; Luis et al., 2003; Petersen & Morris, 2003; Ritchie et al., 2001). One issue that is critical to the goals of the ADNI concerns the specific criteria to be used for defining MCI. In most of the clinical trials mentioned above, the criteria have focused on the amnestic form of MCI. The criteria for this type of MCI are as follows: 1) memory complaint, preferably corroborated by a study partner, 2) memory impairment relative to the appropriate reference group, 3) essentially normal general cognitive function, 4) largely preserved activities of daily living, and 5) not demented (R. C. Petersen, 2003b; Petersen et al., 1999).

The American Academy of Neurology has recently published an evidence-based medicine practice parameter paper on MCI using these criteria and has recommended that clinicians should identify these persons and follow them longitudinally since these subjects have a high likelihood of progressing to AD (Petersen, Stevens et al., 2001). The American Academy of Neurology recognizes that MCI subjects represent the clinical population of interest for most clinicians as new treatment strategies are being developed.

Recently, a group of international experts on MCI convened to assess the state of the field and concluded that while MCI was a useful construct for both research and clinical practice, it can be heterogeneous in its broader applications (Petersen, Doody et al., 2001). Recently multiple clinical subtypes have been recognized which help to more clearly define clinical outcome. For example, two major subtypes, amnestic and non-amnestic, have been recognized and each subtype can be further subdivided into single and multiple domain types. For the amnestic subtype, persons either have a primary memory deficit in relative isolation (single domain), or with other domain involvement (multiple domains). An individual in the latter group might have a memory impairment accompanied by subtle executive dysfunction but of insufficient severity to constitute significant functional decline and dementia. Both subtypes of amnestic MCI lead to AD at a high rate and will be enrolled in this protocol (Lopez et al., 2003; R. C. Petersen, 2003a). In a similar fashion, the non-amnestic subtype can be subdivided into single and multiple domains based on the number of non-memory cognitive domains involved. These subtypes likely do not progress to AD and will *not* be included in the present proposal.

B.3.a.ii. CLINICAL OUTCOME

Investigators from the Mayo Clinic have been following a large group of subjects with amnestic MCI for several years and have also documented an annual conversion rate to probable AD of approximately 12% per year (Petersen & Morris, 2003; Petersen et al., 1999). Investigators in the Religious Orders Study have followed subjects with amnestic features of the multiple domain type of MCI and have also demonstrated an increased risk of progressing to clinically probable AD (Bennett et al., 2002). The Alzheimer's Disease Patient Registry at the University of Washington in Seattle has followed subjects with a memory impairment over 48 months and has shown an increased rate of progression to AD as well (Bowen et al.,

1997). Investigators at New York University have been using the Global Deterioration Scale to categorize subjects and have found that those subjects with an intermediate level of impairment (Global Deterioration Scale 3) have an accelerated rate of progression to dementia of almost 25% per year (Flicker, Ferris, & Reisberg, 1991; Kluger, Ferris, Golomb, Mittelman, & Reisberg, 1999). Investigators at the Massachusetts General Hospital have used a community advertising procedure for recruiting subjects and have demonstrated a somewhat lower rate of progression but nevertheless an accelerated rate over the general population (Daly et al., 2000). A study of African Americans from Indiana also documented the importance of a memory impairment for progression to AD, but also noted some reversion to normal in some subjects (Unverzagt et al., 2001). Recent epidemiological studies from France have shown some variability in the stability of the classification of MCI, but in general, have also documented that the amnestic form of MCI has an increased rate of progression to clinically probable AD (Larrieu et al., 2002; Ritchie et al., 2001). Therefore, the ADNI will adopt criteria focusing on a memory impairment since numerous studies have indicated that these subjects have a high likelihood of progression to clinically probable AD.

B.3.a.iii. FACTORS AFFECTING CONVERSION

While most amnestic subjects will progress at a rate of 10-15% per year, certain factors have been shown to influence this rate of progression. For example, if subjects are carriers of the APOE4 allele, they have an increased rate of progressing to AD (Petersen et al., 1995). As it is well known in the literature, APOE4 is a risk factor for AD and consequently this has been shown to be a relevant predictive factor in MCI subjects as well. In addition, if amnestic MCI subjects have hippocampal formation atrophy on volumetric MRI studies, they have been shown to progress more rapidly (Jack, 2000). Consequently, both APOE genotyping and hippocampal formation volume will be parameters of interest in the current ADNI.

B.3.a.iv. PATHOLOGY OF MCI

There have been relatively few studies done on the neuropathology of MCI. A recent report from the Nun Study explored the relationship between AD neurofibrillary tau pathology and intermediate stages of cognitive impairment (Riley, Snowdon, & Markesbery, 2002). These investigators found a strong relationship between neurofibrillary tau pathology and cognitive state across the clinical spectrum, but they also noted that, by excluding other non-AD pathology, they may not be able to explain the total spectrum of findings. Another study done on subjects from the Religious Order Study found that using a classification of MCI which allowed for multiple cognitive domains to be impaired, 44% of their sample had an intermediate likelihood of AD pathology according to NIA-Reagan criteria (DeKosky et al., 2002). A study from Washington University describing the neuropathologic features of very mild AD subjects (CDR 0.5) demonstrated that 84% of these mild subjects had the neuropathologic features of AD (i.e. tau tangles and Aβ plaques) when they came to autopsy (Morris et al., 2001). Finally, a recent report from the Mayo Clinic indicated that of the 15 subjects on whom autopsy data were available who had died while their clinical classification was amnestic MCI, most of the subjects appeared to have "transitional" pathology (Petersen, 2002). That is, while a minority had fully developed AD at this time, most subjects had pathologic features of medial temporal lobe involvement and neocortical plaque and tangle pathology suggesting that had the subjects lived longer, they would have evolved to definite AD.

B.3.b. EARLY AD

Subjects with AD are of interest since these individuals are the group most frequently studied in rate of change trials for drugs designed to slow decline. On average, subjects with mild-moderate AD decline by about 4.25 (± 7.2) points on the ADAS-Cog (mean± SD) per year. In contrast, the annual rate of shrinkage of the hippocampus was -234 ±144 mm (mean ± SD) in this same cohort of 192 AD subjects (Jack et al 2003, Jack et al 2004). Thus, the standard deviation of the one year change for the ADAS-Cog, the most commonly used clinical measure in AD drug trials, was 1.7 times the annual rate of cognitive change. In contrast, for the hippocampus, the SD/one year rate of change was 0.6. Structural measures vary less than clinical measures, produce smaller coefficients of variation and hold the promise of having greater sensitivity for measurement of change.

B.3.c. NORMAL CONTROLS

Normal controls (NL) will be followed as a comparison group to establish:

a. Rates of change and learning in normals
b. Rate of brain atrophy in normals
c. Changes in metabolism on PET in normals
d. Normal levels and changes in biomarkers with aging.

By examining subjects who convert from normal to MCI, it may also be possible to identify the very earliest changes associated with the evolution of AD. While the numbers of subjects involved may be small, a detailed examination of their data may be highly informative.

C. PRELIMINARY STUDIES

C.1. ADCS MCI STUDY

In 1999, the ADCS launched a multi-center, randomized, double-blind, placebo-controlled, parallel trial to determine whether or not the use of vitamin E or donepezil would delay the conversion to AD in patients with MCI. Natural history data suggested that these individuals would convert to AD at a rate of 12-14% per year. The study was designed to follow subjects at six-month intervals for three years. Inclusion/exclusion criteria were developed to identify individuals with the amnestic form of MCI. Education-adjusted cut scores on logical memory were used to ensure that a true memory deficit existed and that the memory deficit was severe enough to ensure an adequate conversion rate to AD in the placebo-treated population. An adjudication committee was established to review data when a site believed that a subject had converted from MCI to AD. Biological samples for assessment purposes were collected at yearly intervals in the entire patient population, and a 25% subset underwent quantitative MRI studies at baseline, at the time of conversion, and at 36 months. A vigorous recruitment campaign was carried out and over 54,000 phone calls were handled by a centralized recruitment center. Both national and local advertising brought in 2,400 subjects for an in-clinic evaluation. Seven hundred and ninety completed successful screening, and 769 were baselined for this trial at 65 sites in the U.S. and Canada. Several extremely important pieces of information have emerged from the trial.

The baseline characteristics of the subjects were reported recently and demonstrated that the criteria for amnestic MCI could be implemented in a reliable fashion on a multi-center basis (Grundman et al., 2004). The clinical performance characteristics of these subjects were intermediate between those of normal control subjects and subjects with very mild AD. In addition, the baseline MR volumetric measurements of the hippocampal formation in the

subset of subjects participating in the MRI portion of the study were almost identical to the volumetric measurements on MCI subjects published in the literature (Grundman et al., 2003).

FIGURE 1. CONVERSION FROM MCI TO AD IN THE ADCS MCI TRIAL

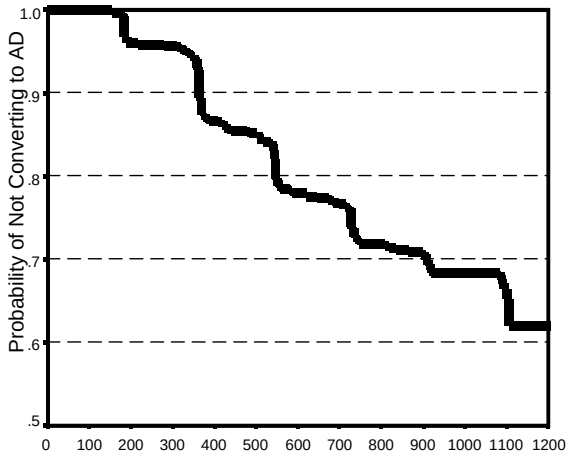
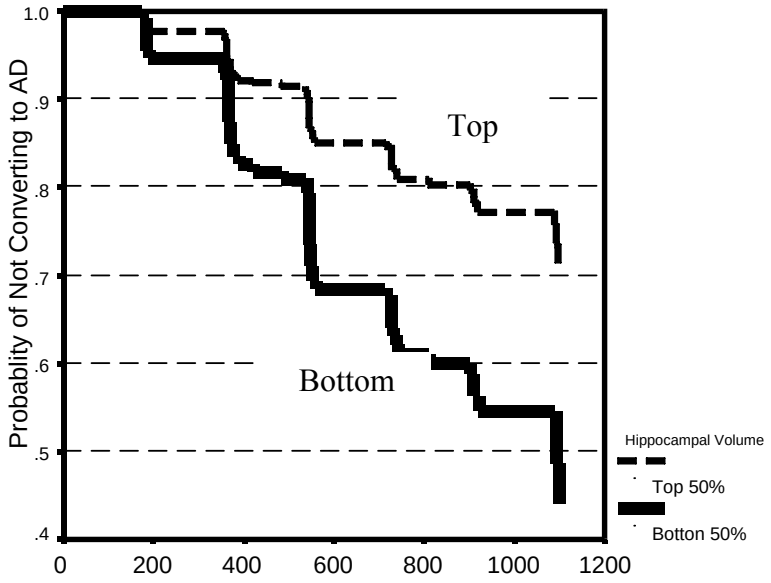
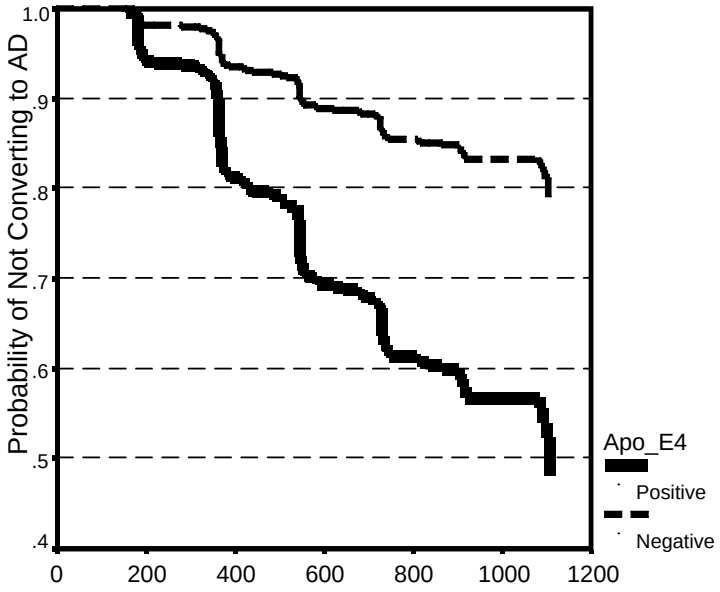


FIGURE 2. CONVERSION FROM MCI TO AD AS A FUNCTION OF HIPPOCAMPAL VOLUME AT BASELINE



The combined group conversion rate to AD has been 16% per year, as predicted (Figure1). When a median split of baseline hippocampal formation volumes was done on those subjects participating in the MR portion of the study, hippocampal volumes strongly predicted the conversion rate consistent with the literature (Figure 2) (Grundman et al., 2003). In addition, the conversion to AD has been strongly influenced by APOE4 carrier status as has been demonstrated in the literature and which can be seen in Figure 3.

FIGURE 3. CONVERSION FROM MCI TO AD AS A FUNCTION OF APOE4 STATUS



This was a landmark trial that has defined a new patient population for study in clinical trials. The trial design has been widely copied by numerous pharmaceutical companies and other sponsors. The last subject completed the trial in January 2004. The database was locked in April 2004. The conduct of this study demonstrates the ability of the ADCS to: 1) enroll a large cohort of patients with MCI; 2) adjust inclusion/exclusion criteria so that an adequate number of subjects reached endpoint (conversion to AD); 3) collect and analyze suitable biomarkers; 4) standardize, obtain and analyze neuroimaging data in a multi-center trial. The same mechanism which has been successful at recruiting MCI subjects for the ADCS MCI treatment trial will be used to recruit subjects for the ADNI.

C.2. BIOMARKERS IN MCI AND AD

A variety of biomarkers have been identified that are associated with increased risk for AD. These are outlined below, but it should be emphasized that the final panel to be examined will be determined by the best available evidence at the time the analyses are conducted. This

decision will be made in conjunction with the Executive Committee and the Steering Committee of the ADNI.

Homocysteine is a sulfur-containing amino acid, derived from the metabolism of methionine (reviewed in Frank et al., 2003; Reckess, 2003). One of the first associations between homocysteine and AD came from a study comparing autopsied patients with AD versus controls. Homocysteine levels in the highest tertile were associated with a greater than fourfold increase in the relative risk of AD, while other studies showed that plasma homocysteine levels >14 μmol/L almost doubled the risk of AD. There are a variety of assays to measure homocysteine levels, including immuno-assays and HPLC-based methods which make it compelling to explore the utility of measuring homocysteine levels to aid in the early diagnosis of AD (Frank et al., 2003; Reckess, 2003).

Growing evidence implicates oxidative/nitrative damage in the pathogenesis of AD, and specific isoprostanes (iPs), i.e., 8,12-iso-iPF2α-VI are elevated in urine, blood and CSF of AD patients, the values for which correlate with memory impairments, CSF tau levels and the number of APOE4 alleles (Frank et al., 2003; Pratico et al., 2000; Pratico, Clark, Liun, Lee, & Trojanowski, 2002; Pratico, Uryu, Leight, Trojanoswki, & Lee, 2001; Pratico, V, Trojanowski, Rokach, & Fitzgerald, 1998; Reckess, 2003; Yao et al., 2003). This suggests that 8,12-iso-iPF2α-VI is a useful AD biomarker. Isoprostane levels can be measured in CSF, blood, urine and brain using HPLC/tandem mass spectrometry (MS) with electrospray ionization (ESI), and urine levels are expressed as ng per mg of creatinine while values in CSF, plasma and postmortem brain are normalized as described (Pratico et al., 2000; Pratico et al., 2002; Pratico et al., 2001; Pratico et al., 1998; Yao et al., 2003). Additional studies will confirm and extend these findings in larger cohorts of MCI and AD patients as well as determine if 8,12-iso-iPF2α-VI will be an informative analyte for monitoring the response of AD patients to new therapies in clinical trials.

New evidence suggests that levels of sulfatide may be indicative of AD pathogenesis (reviewed in Reckess, 2003). By screening with ESI/MS, sulfatide was identified as a potential AD biomarker of interest, and it decreases 93% in gray matter and 58% in white matter in MCI versus controls. Also, when normalized with phosphatidylinositol, CSF sulfatide distinguished non-demented individuals from those with very mild dementia with a sensitivity of 90% and a specificity of 100%. While preliminary, these exciting findings justify including sulfatide assays in studies supported by this grant.

Tau and Aβ are components of the two neuropathological diagnostic hallmarks of AD (tangles and plaques respectively), and they are the most frequently studied candidate diagnostic AD biomarkers where they are best studied in CSF using extensively characterized ELISAs (reviewed in Frank et al., 2003; Reckess, 2003). While thousands of living AD patients and as well as healthy controls have been studied (Arai et al., 1998; Arai, Morikawa et al., 1997; Arai et al., 1995; Arai, Terajima et al., 1997; Frank et al., 2003; Reckess, 2003), a recent examination of >100 subjects with autopsy-confirmed diagnoses showed that elevated CSF tau levels are associated with the presence of AD pathology and that CSF tau levels help discriminate AD from other dementing disorders. Furthermore CSF Aβ levels are decreased in AD and although less informative, CSF Aβ levels added diagnostic value to measures of CSF tau (Clark et al., 2003). Thus, it is time to validate the use of CSF tau and Aβ levels to monitor transitions form normal cognition to MCI and AD.

In summary, there is compelling evidence for the plausible diagnostic utility of the assays discussed above for the diagnosis of AD to justify further study of them in the ADNI as well as for future studies of the other analytes mentioned in recent consensus meetings (Frank et al., 2003; Reckess, 2003) that will be funded by other public/private grants. Finally, the selected citations listed here on AD and other biomarkers (Arai et al., 1998; Arai, Morikawa et al., 1997; Arai et al., 1995; Arai, Terajima et al., 1997; Clark et al., 2003; Frank et al., 2003; McKhann et al., 2001; Nanji, Khwaja, Tahan, & Sadrzadeh, 1994; Ness et al., 1999; Pratico et al., 2000; Pratico et al., 2002; Pratico et al., 2001; Pratico et al., 1998; Reckess, 2003; Reinke, Moore, & Nanji, 2000; The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's, 1997; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association & NIAWG 1998; Yao et al., 2003) document the experience and ability of the University of Pennsylvania investigators in the biomarker component of the ADNI to lead the studies proposed here.

C.3. ADCS COORDINATING CENTER

C.3.a. DATA MANAGEMENT AND QUALITY CONTROL

The ADCS Coordinating Center currently provides data management support for each ADCS protocol, including the generation of electronic case report forms, site training, data collection and quality control, study tracking, interim reporting to the Data and Safety Monitoring Board (DSMB), progress reports to the NIH and ongoing daily support to the PI. Data coordination using the secure intranet is administered using computer network address monitoring and layers of access control based on username/password and group/role designation. Online real-time reports are available for viewing at any time using a web browser to track the progress of study enrollment and ongoing study visits from site to site. Data collection and quality control are managed online. Reported data include enrollment statistics and demographics. Data are entered electronically into the online system using a web interface via online electronic forms with embedded real time data checking algorithms to provide immediate quality feedback. Entry for the ADNI trial will occur at the participating site. The ADNI system will mirror the current ADCS system.

In order to provide the clinical data from this project to Initiative investigators, the Pharmaceutical Industry and the public, the entire clinical data base (free of any identifying information such as name, address, phone number and SSN) will be placed on a public web site, which will be appropriately linked to the imaging data base at LONI. The data base will be frequently updated, and all cleaned clinical data which is acquired by the ADNI-CC will be on the website within 3 months.

Extensive backup systems for the ADNI-CC databases are in place. The databases are backed up onto the file server hard disks daily and to tape weekly. The backup tape is then stored off-site to protect the data against theft, or loss, such as fire, flood, or earthquake.

Quality assurance (QA): The QA program of the ADNI-CC is extensive. It includes development of clear and complete documentation of procedures and databases, cleaning of data and locking of completed databases prior to analysis, clinical monitoring of data both in-

house and at sites, as well as computerized data editing. All procedures for performing QC checks are fully documented and updated as needed.

Data are cleaned by Quality Control (QC) on an on-going basis, during the data collection phase of the protocol as well as after closure of the protocol. During each protocol, computerized data checks are used to confirm that subjects meet inclusion and exclusion criteria at the time of entry, identify missing or out-of-range items, identify missing forms from visit packets, identify any duplicate entries into the database, evaluate longitudinal consistency between visits, and track subject status in the protocol (active vs. discontinued). Requests for corrections are sent to sites and to the site's monitor. The sites are asked to provide missing information or to clarify contradictory responses, and to make the appropriate changes on the electronic record and to source documentation, creating an audit trail.

C.3.b. CLINICAL MONITORING

The monitors have an active role in screening review and approval and provide an educational facilitated interface between the sites and the coordinating center. This includes review of neuropsychological and behavioral ratings on a sampled basis with an emphasis on educating the study coordinator in proper procedures, review of source documents and meetings with study personnel. Thus, special monitoring emphasis will be placed on entry eligibility criteria and on assessment of conversions.

C.3.c. MEDICAL CORE

For this protocol, Dr. Ronald Petersen will assume the role of medical monitor backed up by Drs. Paul Aisen and Adam Fleisher. These individuals will have the following responsibilities: 1) answering queries regarding inclusion/exclusion criteria, 2) providing exceptions when needed, and 3) evaluating adverse events.

C.3.d. ADCS DATA AND SAFETY MONITORING COMMITTEE

The Data Safety and Monitoring Committee consist of three physicians and a biostatistician who review safety aspects of all of the ADCS protocol. The committee meets quarterly by telephone conference. Safety reports are prepared by the Medical Core for each committee meeting. The protocol PI and the Coordinating Center receive a report from the committee noting any concerns in any given protocol.

C.3.e. ADMINISTRATIVE CORE

The Administrative Core will develop and complete contracts with participating sites and will generate payments for work completed. The administrative core will also develop recruitment materials as well as recruitment and retention plans. All consent forms and regulatory documents are developed and tracked by the Administrative Core.

C.4. ADNI PREPARATORY STUDY

This study will be completed before the main ADNI study is initiated. During this preparatory phase, subjects with AD and normal controls (N=~60 AD and ~60 NL) will be recruited for testing on 6 platforms at 5 sites. These subjects will be recruited from existing ADC populations. They will be scanned using different MRI manufacturers (GE 1.5, 3.0T, Siemens 1.5 and 3.0T, and Phillips 1.5 and 3.0T) to standardize data collection across different vendors. Each AD subject will be scanned once. 40 NL subjects will be scanned

twice, in single scanning sessions separated by two weeks, while the remaining 20 NL subjects will be scanned four times back to back in one session, then again back to back in a second session 2 weeks later. In addition to using the preparatory phase of the study to determine the optimal scanning sequences to be used, considerable effort will be devoted to developing and testing QA and QC for the acquisition and transmission of MRI and PET images. Based on data derived from the preparatory phase, final sequences will be chosen for this study.

D. ADNI STUDY

D1. SYNOPSIS-ADNI STUDY

We propose a multi-center biomarker trial to identify biomarkers of disease progression that are most promising as surrogate endpoints in phase 2 and 3 clinical trials for the prevention and treatment of AD. We will enroll a total of 800 subjects with 400 subjects with MCI, 200 with early AD and 200 normal controls. Subjects with MCI and controls will be followed for 3 years, those with AD for 2 years. At 6 month intervals, all subjects will be seen in person or contacted by telephone. Subjects will undergo clinical and neuropsychological evaluations each time scanning is performed. All subjects will undergo repeated 1.5T MRI scanning (MCI-N=6 scans/subject, Controls-N=5 scans/subject, AD-N=4 scans/subject), 25% will undergo repeated 3T MRI scanning, and 50% will undergo repeated PET scanning with the same frequency of scanning used for the 1.5T MRI imaging. Blood and urine biomarkers will be collected at each interval when imaging data is collected from all participants. Immortalized cell lines will be established from all subjects at baseline. All subjects will be approached for LP so that a minimum of 20% and as many as 50% of each group will undergo lumbar puncture at baseline and at year 1 for analysis and storage of CSF.

In efforts to provide more definitive data on the utility of obtaining longitudinal measures of CSF tau and Aβ in the LP subset of ADNI, consent will be obtained from any ADNI participants that have consented to LPs at baseline and year-1 to do a third LP at the year-2 visit. Additionally, any AD subject that has had a lumbar puncture will be asked to consent to continued annual clinic visits with an LP. Those Normal Controls and MCI subjects who are willing will be asked to consent to annual follow-up clinic visits which will include 1.5T MRI imaging (See schedule of events). Telephone checks will be conducted 6 months after these visits. If these subjects participated in the LP substudy they will be asked to consent to another LP at each annual visit. Normal Controls who have been randomized to the PET study will be asked to consent to an annual PET scan.

The overall objectives are to collect the required data for current and future analyses and to carry out a limited set of analyses as outlined in the Specific Aims (A2) and statistical analysis section (D11c).

D.2. MR SITE QUALIFICATION

Each site will undergo qualification testing for MR during the Preparation Phase. The procedures for site qualification will be identical for 1.5T and 3T scanners. Each scanner

ultimately used in the execution phase needs to undergo the same procedure. So, a site scanning at both 1.5T and 3T will need to qualify both scanners. The MRI site qualification process consists of two parts – phantom and human scanning. In terms of human scanning, each site will image a volunteer subject with the protocol and send the images to LONI. Each parameter in each of the pulse sequences in the protocol will be checked at Mayo. In the event that the protocol has not been performed according to protocol, the site will be asked to perform another human volunteer scan. This will be repeated as many times as necessary until the site has demonstrated exact execution of the MR protocol in a volunteer subject, at which point they will have passed the human scanning portion of MR site qualification. The volunteers do not need to be elderly controls; in fact scanning for site qualification may be more easily performed with normal younger volunteers. In the event that repeat attempts are needed, repeat scans need not be on the same volunteer subject. Once a site has demonstrated perfect execution of the protocol, the protocol will be stored permanently on the scanner at that site that will be used in the study.

D.3. SUBJECT RECRUITMENT AND RETENTION

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

D.4. INCLUSION/EXCLUSION CRITERIA

D.4.a. GENERAL

All enrolled subjects will be between 55 and 90 (inclusive) years of age, have a study partner able to provide an independent evaluation of functioning and will speak either English or Spanish. All subjects must be willing and able to undergo all testing procedures including neuroimaging and agree to longitudinal follow-up. All subjects will be approached for LP's and a minimum of 20% must be willing to undergo 2 lumbar punctures. Certain psychoactive medications will be excluded. General inclusion criteria are:

1. Normal subjects: MMSE scores between 24-30 (inclusive), a CDR of 0, non-depressed, non-MCI and non-demented.
2. MCI subjects: MMSE scores between 24-30 (inclusive), a memory complaint, have objective memory loss measured by education-adjusted scores on Wechsler Memory

Scale-Revised (WMS-R) Logical Memory II, a CDR of 0.5, absence of significant enough levels of impairments in other cognitive domains so that criteria for dementia are not met, largely preserved activities of daily living, and an absence of dementia.

a. Categorization as to amnestic MCI at screen using MMSE, logical memory and CDR.
b. Subcategorization of amnestic MCI, single domain or multiple domain at the baseline using neuropsycological test scores by reference to the MCI flow diagram in figure 4.
c. Mild AD: MMSE scores between 20 – 26 (inclusive), CDR of 0.5 or 1.0`, and meet NINCDS/ADRDA criteria for probable AD.

D.4.b.MCI

The MCI group will be the cohort of most interest. The ADCS screened 2,400 subjects for its MCI protocol: 790 MCI subjects met screening criteria and 769 were entered in our ADCS MCI trial. Using rigorous inclusion/exclusion criteria, the conversion rate to AD in this cohort was 16%/year. The most important inclusion criterion was the use of education-adjusted cutoff scores on logical memory to make certain that subjects have a true memory deficit and represent amnestic MCI, the cohort most likely to develop AD. This protocol will use almost identical inclusion criteria but will also allow the enrollment of amnestic MCI individuals with impairment in non-memory domains who do not meet clinical criteria for dementia. Exclusion criteria are less restrictive since many drugs excluded in the MCI Study may be allowed in this biomarker protocol (Grundman et al., 2004). Inclusion/exclusion criteria for AD subjects and normal controls closely match those we have previously used in other instrument or drug protocols (Aisen et al., 2003) with appropriate adjustments so that the three groups do not overlap.

For our present purposes we will focus on the amnestic subtype of MCI. Once the clinical subtype has been determined (Figure 4), then the clinician needs to address the issue of putative etiology of the syndrome. By means of the history from the participant and the study partner, the clinician then determines if the suspected etiology is of a degenerative, vascular, metabolic or other cause (Figure 5). If the suspicion is of a degenerative etiology and the clinical subtype is amnestic, then the likely outcome will be AD. The ADNI will focus on this combination of clinical presentations.

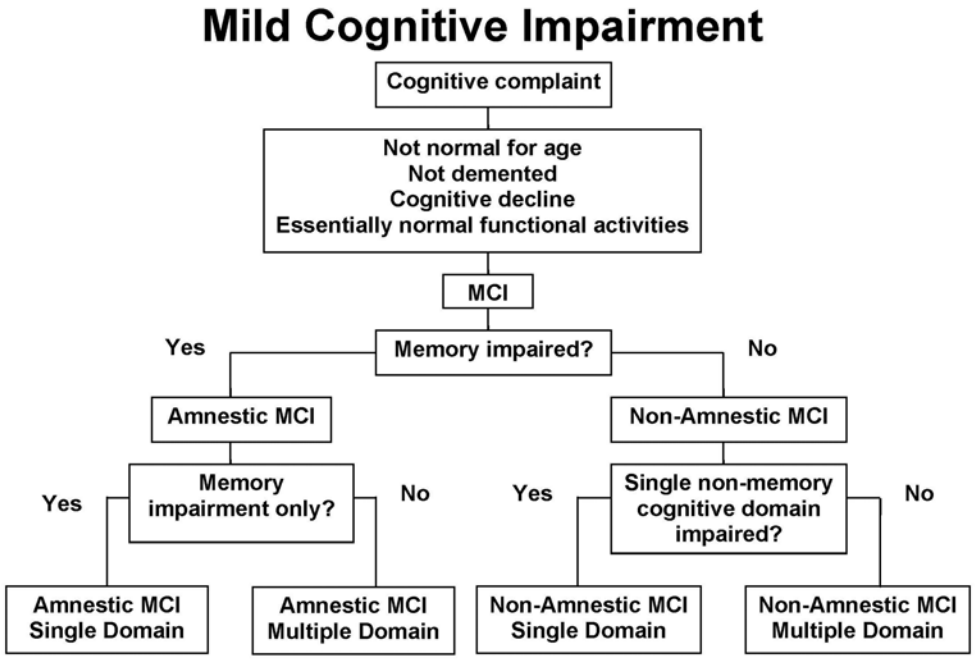
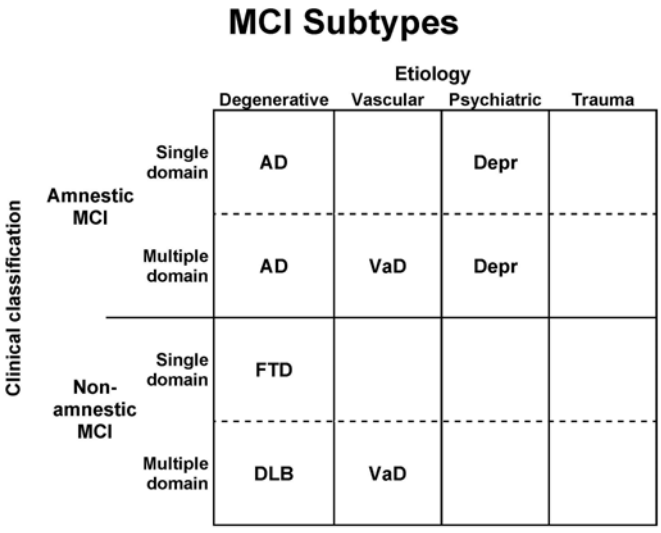


FIGURE 4. CLASSIFICATION OF MCI

FIGURE 5. SUBTYPES OF MCI



For this protocol, MCI subjects with isolated amnestic MCI and amnestic MCI multidomain can be enrolled. Operationally, these subjects should meet the following criteria:

1. Cognitive complaint: By history
2. Not normal
   Not demented: Both by CDR 0.5 and MMSE $\geq$ 24
   Cognitive decline: By history and CDR
   Essentially normal functional activities: FAQ (no cutoff) and CDR

3. If 1 and 2 above fulfilled, subject has MCI
4. Memory impaired?: Delayed recall of one paragraph of Logical Memory (cutoff scores by education)
5. If #4 "yes" then diagnosis is Amnestic MCI
6. To determine if other domains are involved, consider the participant's performance on the following without cutoff scores:

Language:	Boston Naming Test Category Fluency
Attention/Exec Function:	Trails A and B Digit Symbol Substitution
Visuospatial:	Clock copy and drawing Supplement these assessments with performance on ADAS-Cog

7. If any additional domains impaired (by clinical judgment) then diagnosis is Amnestic MCI multiple domain. If other domains normal, then diagnosis is Amnestic MCI single domain. Either is acceptable.
8. Not depressed: Geriatric Depression Scale: < 6
9. Not vascular: Hachinski ≤ 4

Eligibility for enrollment is based on a combination of scores from the MMSE, LM delayed recall, CDR, Geriatric Depression Scale and Hachinski as well as clinical judgment.

D.4.c SUMMARY OF INCLUSION/EXCLUSION CRITERIA

INCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
Memory Complaints		Memory complaint by subject or study partner that is verified by a study partner.	Memory complaint by subject or study partner that is verified by a study partner.
Memory Function	Normal memory function documented by scoring at specific cutoffs on the Logical Memory II subscale (delayed Paragraph Recall) from the Wechsler Memory Scaled - Revised (the maximum score is 25):	Abnormal memory function documented by scoring below the education adjusted cutoff on the Logical Memory II subscale (Delayed Paragraph Recall) from the	Same as MCI

INCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
	a) more than or equal to 9 for 16 or more years of education b) more than or equal to 5 for 8-15 years of education c) more than or equal to 3 for 0-7 years of education.	Wechsler Memory Scale – Revised (the maximum score is 25): a) less than or equal to 8 for 16 or more years of education b) less than or equal to 4 for 8-15 years of education c) less than or equal to 2 for 0-7 years of education.	
MMSE	Mini-Mental State Exam score between 24 and 30 (inclusive) (Exceptions may be made for subjects with less than 8 years of education at the discretion of the project director).	Mini-Mental State Exam score between 24 and 30 (inclusive) (Exceptions may be made for subjects with less than 8 years of education at the discretion of the project director).	MMSE between 20 and 26 (inclusive) (Exceptions may be made for subjects with less than 8 years of education at the discretion of the project director).
CDR	Clinical Dementia Rating = 0. Memory Box score must be 0.	Clinical Dementia Rating = 0.5. Memory Box score must be at least 0.5.	Clinical Dementia Rating = 0.5, 1.0
General Cognition	Cognitively normal, based on an absence of significant impairment in cognitive functions or activities of daily living.	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.	NINCDS/ADRDA criteria for probable AD.
Hachinski	Modified Hachinski score of less than or equal to 4.	Modified Hachinski score of less than or equal to 4.	Modified Hachinski score of less than or equal to 4.
Age	Age between 55 and 90 (inclusive).	Age between 55 and 90 (inclusive).	Age between 55 and 90 (inclusive).
Stability of Permitted medications	Permitted medications stable for at least 4 weeks prior to screening. In particular:	Permitted medications stable for at least 4 weeks prior to screening. In particular:	Permitted medications stable for at least 4 weeks prior to screening. In particular:

INCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
	a) Subjects may 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 2 years) b) Estrogen replacement therapy is permissible c) Gingko biloba is permissible, but discouraged d) Washout from psychoactive medication (e.g., excluded anti-depressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.) for at least 4 weeks prior to screening.	a) Subjects may 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 anti-depressants, 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 4 weeks prior to screen	a) Subjects may 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 anti-depressants, 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 4 weeks prior to screen.
Geriatric Depression Scale	Geriatric Depression Scale score of <6	Geriatric Depression Scale score of <6	Geriatric Depression Scale score of < 6
Study partner	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.	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.	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.

INCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
Visual and auditory acuity	Adequate visual and auditory acuity to allow neuropsychological testing.	Adequate visual and auditory acuity to allow neuropsychological testing.	Adequate visual and auditory acuity to allow neuropsychological testing.
General Health	Good general health with no additional diseases expected to interfere with the study.	Good general health with no additional diseases expected to interfere with the study.	Good general health with no additional diseases expected to interfere with the study.
Pregnancy/Childbearing Potential	Subject is not pregnant, lactating, or of childbearing potential (i.e. women must be two years post-menopausal or surgically sterile).	Subject is not pregnant, lactating, or of childbearing potential (i.e. women must be two years post-menopausal or surgically sterile).	Subject is not pregnant, lactating, or of childbearing potential (i.e. women must be two years post-menopausal or surgically sterile).
Testability	Willing and able to complete all baseline assessments. Willing and able to participate in a 3-year protocol.	Willing and able to complete all baseline assessments. Willing and able to participate in a 3-year protocol.	Willing and able to complete all baseline assessments. Willing and able to participate in a 2-year protocol.
Commitment to neuroimaging and providing study samples	Willing to undergo MRI 1.5 Tesla neuroimaging (PET and MRI 3Tesla are optional) and provide DNA for ApoE assessments and banking as well as plasma samples at protocol specified time points.	Willing to undergo MRI 1.5 Tesla neuroimaging (PET and MRI 3Tesla are optional) and provide DNA for ApoE assessments and banking as well as plasma samples at protocol specified time points.	Willing to undergo MRI 1.5 Tesla neuroimaging (PET and MRI 3Tesla are optional) and provide DNA for ApoE assessments and banking as well as plasma samples at protocol specified time points.
Commitment to provide CSF samples	Willing to provide CSF for biomarker studies at protocol specified time points (optional).	Willing to provide CSF for biomarker studies at protocol specified time points (optional).	Willing to provide CSF for biomarker studies at protocol specified time points (optional).
Education	Completed 6 grades of education (or had a good work history sufficient to exclude mental retardation).	Completed 6 grades of education (or had a good work history sufficient to exclude mental retardation).	Completed 6 grades of education (or had a good work history sufficient to exclude mental retardation).

INCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
Language	Fluent in English or Spanish.	Fluent in English or Spanish.	Fluent in English or Spanish.

EXCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**

EXCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
Significant neurologic disease	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.	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.	Any significant neurologic disease other than Alzheimer's disease including 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.
Neuroimaging	Screening/baseline MRI scans with evidence of infection, infarction, or other focal lesions. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.	Screening/baseline MRI scans with evidence of infection, infarction, or other focal lesions. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.	Screening/baseline MRI scans with evidence of infection, infarction, or other focal lesions. Subjects with multiple lacunes or lacunes in a critical memory structure are excluded.
MRI exclusions	Presence of pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body.	Presence of pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body.	Presence of pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin or body.

EXCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
Psychiatric disorders/psychotic features	Major depression, bipolar disorder as described in DSM-IV within the past 1 year. History of schizophrenia (DSM IV criteria).	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. History of schizophrenia (DSM IV criteria).	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. History of schizophrenia (DSM IV criteria).
Alcohol abuse	History of alcohol or substance abuse or dependence within the past 2 years (DSM IV criteria).	History of alcohol or substance abuse or dependence within the past 2 years (DSM IV criteria).	History of alcohol or substance abuse or dependence within the past 2 years (DSM IV criteria).
Significant medical illness	Any significant systemic illness or unstable medical condition which could lead to difficulty complying with the protocol.	Any significant systemic illness or unstable medical condition which could lead to difficulty complying with the protocol.	Any significant systemic illness or unstable medical condition which could lead to difficulty complying with the protocol.
Clinically significant laboratory abnormalities	Clinically significant abnormalities in B12, RPR, or TFTs that might interfere with the study.	Clinically significant abnormalities in B12, RPR, or TFTs that might interfere with the study.	Clinically significant abnormalities in B12, RPR, or TFTs that might interfere with the study.
Residence	Residence in skilled nursing facility.	Residence in skilled nursing facility.	Residence in skilled nursing facility.

EXCLUSION CRITERIA			
Item	**NL**	**MCI**	**AD**
Concomitant medications	Current use of specific psychoactive medications (e.g., certain antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.). Current use of warfarin.	Current use of specific psychoactive medications (e.g., certain antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.). Current use of warfarin.	Current use of specific psychoactive medications (e.g., certain antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.). Current use of warfarin.
Investigational agents	Prohibited one month prior to entry and for the duration of the trial.	Prohibited one month prior to entry and for the duration of the trial.	Prohibited one month prior to entry and for the duration of the trial.
Multiple trial participation	Participation in clinical studies involving neuropsychological measures being collected more than one time per year.	Participation in clinical studies involving neuropsychological measures being collected more than one time per year.	Participation in clinical studies involving neuropsychological measures being collected more than one time per year.
Exceptions by project director	Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the project director.	Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the project director.	Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the project director.

D.5. CONDUCT OF THE LONGITUDINAL STUDY

D.5.a.CONDUCT OF STUDY: HUMAN SUBJECTS, ETHICAL AND REGULATORY CONSIDERATIONS

This study will be conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki, US 21CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards, and pursuant to state and federal HIPAA regulations. Written informed consent for the study must be obtained from all subjects and/or authorized representatives and study partners before protocol-specific procedures are carried out.

D.5.b. INSTITUTIONAL REVIEW BOARD

Institutional Review Boards must be constituted 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 hospital/University/independent IRBs for protocol review and approval. Verification of IRB 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 the study partners and will be transmitted and validated by the ADNI-CC in order to obtain approval for shipment of study supplies to study sites. This approval must refer to the study by exact protocol title and number, identify documents reviewed, and state the date of review. IRBs must be informed by investigators of all subsequent protocol amendments and of serious or unexpected adverse events 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.

D.5.c. INFORMED CONSENT AND HIPAA COMPLIANCE

The principles of informed consent in the current edition of the Declaration of Helsinki and applicable HIPAA privacy notifications will be implemented before protocol procedures are carried out. Informed consent will be obtained in accordance with US 21 CFR 50.25. Information should be given in both oral and written form as deemed appropriate by the sites' IRB. Subjects, their relatives, guardians or authorized representatives and study partners must be given ample opportunity to inquire about details of the study. The consent form generated by the investigator with the assistance of the ADNI-CC must be approved, along with the protocol, and HIPAA privacy notifications by the IRB and be acceptable to the ADNI-CC. Consent forms must be in a language fully comprehensible to the prospective subjects and/or their authorized representatives and the study partner. Informed consent will be documented by the use of a written consent form approved by the IRB and signed by the participant and/or an authorized representative and the study partner. The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations. The form may be read to the subject and/or authorized representative and study partner, but, in any event, the investigator will give the subject and/or authorized representative and study partner adequate opportunity to read it before it is signed. In either case the signature confirms that the consent is based on information that has been understood. Each subjects' signed informed consent and/or HIPAA research authorization must be kept on file by the investigator for possible review by regulatory authorities and/or ADNI-CC 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.

D.5.d. INFORMED CONSENT FOR BIOMARKERS, GENETIC MATERIAL AND IMAGING DATA

The informed consent will not only cover consent for the trial itself, but for the genetic research, biomarker studies, biological sample storage and imaging scans as well. The consent for storage will include consent to access stored data, biological samples, and imaging data for secondary analyses. Consent forms will specify that DNA and biomarker samples are for research purposes only; the tests on the DNA and biomarker samples are not diagnostic in nature and participants will never receive results. MRI scan findings of clinical significance, determined by the site radiologist, will be shared with participants. The informed consent and/or HIPAA notification will specify that:

- University of California, San Diego (UCSD) will receive and store all research data;

- The University of California, Los Angeles Laboratory of Neuroimaging (UCLA/LONI) will receive MRI and PET images;
- The University of Pennsylvania (UPENN) AD Biomarker Fluid Bank Laboratory will receive and store biomarker samples;
- The National Cell Repository for Alzheimer's Disease (NCRAD) will receive blood samples and prepare and store immortalized cell lines from them;
- The University of California, Los Angeles Laboratory of Neuroimaging (LONI) will house a full set of all the data as will UCSD.
- All data will be made available to: the pharmaceutical industry, academic investigators and other interested parties in the public domain. A policy for distribution of data has been developed.

To ensure the ability to broadly share data, the consent documents should include the following wording:

"By signing this consent you are authorizing the use of your data for large scale, multi-center studies that will combine data from similar populations. These multi-center studies are being conducted by the Alzheimer's Disease Neuroimaging Initiative (ADNI), a neuroscience consortium of universities and research institutes. Your data will be stored with a coded research identifier to protect your identity. Only de-identified data, which does not include anything that might directly identify you, will be shared with ADNI members and the general scientific community for research purposes. This data will be entered into linked databases at UCLA and UCSD to be used from this date and going forward."

D.5.e. PROCEDURES TO MAINTAIN CONFIDENTIALITY OF GENETIC MATERIAL, BIOMARKER SAMPLES AND IMAGING DATA.

Genetic research and storage of genetic material. The de-linking of the sample from the subject occurs at the time the blood is sent to the UPENN investigators and to the National Cell Repository for Alzheimer's Disease (NCRAD). All samples will be inventoried and tracked using commercially available software. A database will be created and used for the inventory of stored samples in conjunction with a bar code reading system. Bar code labels affixed to each sample vial will contain the following information: sample ID# (to preserve confidentiality), date of collection and processing, total initial volume collected, sample type (urine, plasma, serum, CSF), volume, aliquot number, freezer, shelf, rack, box, location in the box. A bar code label will be used on the sample tracking form. Immortalized cell lines will be prepared at NCRAD and APOE genotyping using DNA obtained from subjects blood cells will be performed at UPENN. However, neither the ADNI-CC nor UPENN or NCRAD will have information regarding the participant's name and thus are unable to link the DNA analysis results to the person. Also, since the results are not ever transmitted to the site that enrolled and followed the subject, the site will be equally unable to link the results to the subject. 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 subject in relation to biological specimens from that participant. However, there will be no link to research done on these specimens with subjects' names. It is important to note that the linkage is between DNA research data and study research data, and the linkage will take place at only one of our data centers. The data centers (UCSD, UCLA) do not have any

record of the names of the study subjects, or of specific medical identifiers such as clinical medical record numbers. The participating sites do not receive APOE results or any DNA results, and do not have access to the database in which these results are stored. Therefore, even though DNA results can be linked to clinical research data for purposes of analyses, there is no way to achieve linkage of DNA test results to names of subjects.

The procedures for patient confidentiality will be approved by the IRB of UPENN. The protection of patient confidentiality and the use of stored DNA specimens for APOE genotyping and immortalized cell lines will be in accordance with the rules and procedures established by the UPENN IRB and at NCRAD. The DNA for APOE genotyping is banked in a locked freezer at UPENN dedicated to the ADNI and immortalized cell lines are stored in a tank of liquid nitrogen. The samples are without a link to the identity of the donor subject. All samples are identified by a bar code.

Specific procedures for requesting and accessing immortalized cells will be created by RARC of the ADNI in accordance with recommendations proposed in the NBAC Human Biological Materials Report. These DNA guidelines were 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.

MRI and PET imaging and data storage.

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

Biomarker Samples and Research

Blood samples will be labeled by bar coding samples. Subject's names will not be provided to the UPENN investigators. Samples will be stored by bar code number and no other identifying information will be provided.

D.5.f. 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. Even though no drugs are involved, there is a potential for adverse events related to participation in this study. Thus, our DSMB will review safety data collected on a quarterly basis including adverse events and laboratory surveillance. After reviewing emerging safety data, the DSMB can make recommendations regarding the conduct/continuation of this trial.

D.5.g. PRE-SCREENING

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

D.5.h. 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. A standardized evaluation will be performed at each clinical site. Consent will be obtained before any portion of the screening visit is initiated. Demographics, family history, physical exam, neurologic exam, and Hachinski ischemic score will be obtained. Vital signs and blood for screening labs (hematology, chemistry panel, urinalysis, B12, RPR, TSH) will be collected. Blood will also be collected to determine APOE genotype. Subjects will undergo a series of evaluations including the Geriatric Depression Scale, a Mini Mental State Examination and WMS-R Logical Memory, immediate and delayed conditions. A Clinical Dementia Rating Scale will be obtained as well as concurrent medications. The first imaging study, a 1.5T MRI will be obtained. Subjects meeting eligibility will be scheduled to return for a baseline visit. Eligibility will be determined according to the Inclusion/Exclusion criteria outlined above. Essentially, from a cognitive perspective, if the subjects have a Mini-Mental State Exam score of $\geq$ 24 and meet WMS-R Logical Memory II criteria and are judged to not be demented by the site clinicians after reviewing the other cognitive data, the subjects will be considered eligible for entrance as a MCI subject.

D.5.i. BASELINE VISIT

At the baseline visit, blood for immortalized cell lines and the first set of biomarkers will be obtained. An ANART will be obtained along with a neuropsychological battery. A minimum of 20% of each cohort will undergo lumbar puncture as part of the baseline visit. However, the ADNI will strive to obtain LP's on 50% of the cohort. Shortly after the baseline visit, 25% of the subjects in each cohort will undergo a 3T MRI scan while 50% will undergo an FDG PET scan.

D.5.j. FOLLOW UP VISITS

Follow up visits will be carried out at six month intervals either in person or by telephone contact as proscribed in the flow sheets. A complete battery of clinical and neuropsychological measures and biomarkers will be collected at each time point that imaging studies are collected in order to correlate change on imaging studies with change in clinical measures. A lumbar puncture will be repeated at annually (in a subset of subjects). A synopsis of the study visits appear on the following pages.As part of the optional extension of study subjects, MCI and NL subjects will be asked to consent to an additional telephone check 6 months and clinic visit with collection of biomarkers and 1.5T MRI annually. Normal Controls in the PET substudy will be asked to have an annual PET scan. Any subject who previously agreed to Lumbar Puncture (LP) and who had at least a single LP will be asked to agree to additional annual Lumbar Punctures. 3T imaging will not be offered or performed after the M36 visit.

D.5.k. EARLY TERMINATION VISIT

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

D.5.l. UNSCHEDULED VISIT

Unscheduled visits will be discouraged. However, if needed, unscheduled visits will be tailored to the specific issue after discussion with the protocol PI.

D.5.m. RETRIEVED DROP-OUTS

Subjects missing visits will be encouraged to return for subsequent visits. Unless a subject withdraws consent, subjects who miss visits will be encouraged to come in for subsequent visits, with priority placed on the final visit.

D.5.n. PHONE CHECKS

Phone checks will occur as indicated in the schedule of events in order to maintain contact with the subject. Update demographic information and update AE information.

D.5.o. NURSING HOME PLACEMENT

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

SCHEDULE OF EVENTS (NORMAL SUBJECTS)

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Visit name	**Screen**	**Baseline**								
Time (months)	**0**	**1**	**6**	**12**	**18**	**24**	**30**	**36**	**42[3]**	**48[3]**
Explain Study	x								x	
Obtain Consent	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		x		x
Screening Labs	x									
APOE	x									
American National Adult Reading Test		x								
Mini Mental State Examination	x		x	x		x		x		x
Logical Memory I and II	x			x		x		x		x
Digit Span		x	x	x		x		x		x
Category Fluency		x	x	x		x		x		x
Trails A & B		x	x	x		x		x		x
Digit Symbol		x	x	x		x		x		x
Boston Naming Test		x	x	x		x		x		x
Auditory Verbal Learning Test		x	x	x		x		x		x
Geriatric Depression Scale	x			x		x		x		x
Clock drawing		x	x	x		x		x		x
Neuropsychiatric Inventory Q		x	x	x		x		x		x
ADAS-Cog		x	x	x		x		x		x
Clinical Dementia Rating Scale	x		x	x		x		x		x
Activities of Daily Living (FAQ)		x	x	x		x		x		x
Collect and process biomarkers		x[1]	x	x		x		x		x
Concomitant Medications	x	x	x	x		x		x		x
Subject Payments	x	x	x	x		x		x		x
Phone Contact					x		x		x	
Adverse Events	x	x	x	x	x	x	x	x	x	x
Diagnostic Summary	x	x	x	x		x		x		x
MRI (1.5 T) (100%)	x		x	x		x		x		x
MRI (3 T) (25%)		x	x	x		x		x		
PET (50%)		x	x	x		x		x		x
LP (minimum of 20%)		x		x		x[2]		x[2]		x[3]

[1]Includes blood draw for Immortalized cell lines
[2]Optional LP for subjects consenting to the CSF extension study
[3]Additional years for follow-up are planned, depending on funding, IRB approval and consent of participants. This includes optional LP for subjects consenting to the CSF extension study.

SCHEDULE OF EVENTS (MCI SUBJECTS)

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Visit name	**Screen**	**Baseline**								
Time (months)	**0**	**1**	**6**	**12**	**18**	**24**	**30**	**36**	**42[3]**	**48[3]**
Explain study	x								x[3]	
Obtain consent	x								x[3]	
Demographics, Family History, Inclusion and Exclusion Criteria	x									
Medical History, Physical Exam, Neurological Exam, Hachinski	x									
Vital Signs	x	x	x	x	x	x		x		x
Screening labs	x									
APOE	x									
American National Adult Reading Test		x								
Mini Mental State Examination	x		x	x	x	x		x		x
Logical Memory I and II	x			x		x		x		x
Digit Span		x	x	x	x	x		x		x
Category Fluency		x	x	x	x	x		x		x
Trails A & B		x	x	x	x	x		x		x
Digit symbol		x	x	x	x	x		x		x
Boston Naming Test		x	x	x	x	x		x		x
Auditory Verbal Learning Test		x	x	x	x	x		x		x
Geriatric Depression Scale	x			x		x		x		x
Clock drawing		x	x	x	x	x		x		x
Neuropsychiatric Inventory Q		x	x	x	x	x		x		x
ADAS-Cog		x	x	x	x	x		x		x
Clinical Dementia Rating Scale	x		x	x	x	x		x		x
Activities of Daily Living(FAQ)		x	x	x	x	x		x		x
Collect and process biomarkers		x[1]	x	x	x	x		x		x
Concomitant Medications	x	x	x	x	x	x		x		x
Subject payments	x	x	x	x	x	x		x		x
Phone contact							x		x	
Adverse events	x	x	x	x	x	x	x	x	x	x
Diagnostic Summary	x	x	x	x	x	x		x		x
MRI (1.5 T) (100%)	x		x	x	x	x		x		x
MRI (3 T) (25%)		x	x	x	x	x		x		
PET (50%)		x	x	x	x	x		x		
LP (minimum of 20%)		x		x		x[2]				x[3]

[1]Includes blood draw for Immortalized cell lines

[2]Optional LP for subjects consenting to the CSF extension study
[3]Additional years for follow-up are planned, depending on funding, IRB approval and consent of participants. This includes optional LP for subjects consenting to the CSF extension study.

SCHEDULE OF EVENTS (AD SUBJECTS)

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Visit name	**Screen**	**Baseline**					
Time (months)	**0**	**1**	**6**	**12**	**18**	**24**	**36[3]**
Explain study	x						x
Obtain consent	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	x
Screening labs	x						
APOE	x						
American National Adult Reading Test		x					
Mini Mental State Examination	x		x	x		x	x
Logical Memory I and II	x			x		x	x
Digit Span		x	x	x		x	x
Category Fluency		x	x	x		x	x
Trails A & B		x	x	x		x	x
Digit symbol		x	x	x		x	x
Boston Naming Test		x	x	x		x	x
Auditory Verbal Learning Test		x	x	x		x	x
Geriatric Depression Scale	x			x		x	x
Clock drawing		x	x	x		x	x
Neuropsychiatric Inventory Q		x	x	x		x	x
ADAS-Cog		x	x	x		x	x
Clinical Dementia Rating Scale	x		x	x		x	x
Activities of Daily Living(FAQ)		x	x	x		x	x
Collect and process biomarkers		x[1]	x	x		x	x
Concomitant Medications	x	x	x	x		x	x
Subject payments	x	x	x	x		x	x
Phone contact					x		
Adverse events	x	x	x	x	x	x	x
Diagnostic Summary	x	x	x	x		x	x
MRI (1.5 T) (100%)	x		x	x		x	
MRI (3 T) (25%)		x	x	x		x	
PET (50%)		x	x	x		x	
LP (minimum of 20%)		x		x		x[2]	x[3]

[1]Includes blood draw for Immortalized cell lines
[2]Optional LP for subjects consenting to the CSF extension study
[3]Additional annual clinic visits are planned, depending on funding, IRB approval and consent and only for those AD subjects agreeing to the optional LP

D.5.p. CONCOMITANT MEDICATIONS

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

D.5.q. SAFETY ASSESSMENTS

All subjects will be evaluated for adverse events at each visit.

D.5.r. BIOMARKER COLLECTION

The methods described below will be utilized for the collection, aliquoting, storage, archiving, tracking of all samples collected from subjects in this grant. In addition to the screening labs described earlier in this application, and the AD biomarkers summarized above, lymphoblastoid cell lines will be established and the APOE genotype will be determined. To accomplish this, we will: (A) Generate EBV immortalized cells of all selected subjects using blood cells sent in 2 ACD-A 8.5 mL tubes by overnight delivery at room temperature to arrive at the National Cell Repository for Alzheimer's Disease (NCRAD) within 24 hours of sample harvesting. Upon arrival at NCRAD the blood sample is placed in a centrifuge and spin to separate the sample into three main layers: the red blood-cell layer, the plasma layer, and the buffy coat, which contains the white-blood cells. The white-blood cells are needed to establish cell lines and obtain DNA. To establish cell lines, the white-blood cells are placed in a flask along with a solution that allows permanent cell growth. The cells are incubated at 37°C (body temperature) ranging from three weeks to three months. The cell-containing solution is then divided and transferred into two larger flasks for further cell growth. It takes approximately one week for the cells to divide to the desired number. The cells are then placed in a vial along with a preservative. Each vial holds approximately 1 milliliter of solution containing $1x10^7$ (10,000,000) cells. The cells are gradually cooled to freezing temperatures. The slow freeze prevents damage to the cell line. The frozen cells are bar code labeled and stored in a tank filled with liquid nitrogen at -316°F. Cells can be preserved this way indefinitely and thawed at any time for additional propagation. (B) Genotype all selected subjects for APOE allele status using DNA extracted from peripheral blood cells from all selected subjects that are collected in 1 EDTA plastic tube (10 mL) sent by express mail to the UPENN AD Biofluid Bank Laboratory by overnight delivery at room temperature as described (5). (C) Perform analysis of selected analytes: isoprostanes (blood, CSF, urine), homocysteine (blood and CSF), sulfatides (CSF), Aβ (CSF), and tau (CSF).

D.5.r.i. BIOLOGICAL FLUIDS TO BE COLLECTED

Polypropylene tubes will be utilized for collection and storage, since some key analytes such as Aβ are known to stick to glass and others may do so as well, although this may not yet be known. Also, all samples will be collected in the morning before breakfast and after an overnight fast. Only water is permitted until blood draws and the LP procedure are completed. Blood, (separated into plasma and serum) urine, and CSF will be collected so as

to accommodate the assay of the broadest range of the best antecedent biomarkers/analytes. The methods used to assay homocysteine, isoprostanes, sulfatide, tau, and Aβ are the same as those previously discussed in preliminary studies.

D.5.r.ii. SAMPLE COLLECTION, ALIQUOTING AND STORAGE

Urine is obtained and spun for 10 min at 1000 rpm to eliminate cells (see procedures manual). Urine is kept at 4°C once collected and during centrifugation. A 10 mL aliquot is pipetted into a plastic vial, labeled, frozen within 1 h, placed in the shipping container on dry ice and shipped by express mail (e.g. Federal Express)

Plasma is collected in a uniform fashion using EDTA as anti-coagulant. Once blood is collected into two 10 mL EDTA plastic tubes, as described in the procedures manual, it is mixed thoroughly, then centrifuged for 15 min. at ~3000 rpm. 10 mL of the plasma sample is transferred to a labeled plastic vial, frozen, and placed in the shipping container with dry ice. The blood sample is kept at 4°C at all times during the preparation of plasma prior to freezing. Serum is obtained after allowing the samples collected in two 10 mL plain red top plastic tubes, as described in the procedures manual, to clot at room temperature, and it is spun as above for plasma preparation, frozen and placed in the shipping container with dry ice.

CSF is obtained with the recommended use of a small caliber atraumatic needle (e.g. 24 or 25 gauge Sprotte needle). Syringes (generally using multiple 5 cc syringes) should only be used with a side port needle and are used to withdraw CSF from subjects in a lateral decubitus or sitting position, according to the preference of the subject. To clear any blood from minor trauma associated with needle insertion, the first 1-2 ml of CSF are discarded (or more if needed) to eliminate blood, and then 20 ml of CSF are collected from each patient for use and treatment in the following manner:

1. The first 3 ml will be used for standard tests such as cell counts, glucose, and total protein with determinations done at local laboratories
2. The remaining CSF will be collected and processed as outlined in the Procedures manual.

All collected samples, after placement into the shipping container on dry ice (except for samples for immortalized cell lines and for ApoE genotyping both of which are shipped at room temperature) are sent the same day as collected via express mail with overnight delivery to the Penn AD Biomarker Fluid Bank Laboratory. When samples are received in the Laboratory, they will be thawed and aliquots transferred to plastic vials, bar code labeled, and placed in designated locations in the -80°C freezers. All samples will be inventoried and tracked using commercially available software. A database will be created and used for the inventory of stored samples, in conjunction with a bar code reading system. Bar code labels affixed to each sample vial will contain the following information: sample ID# (to preserve confidentiality), date of collection and processing, total initial volume collected, sample type (urine, plasma, serum, CSF), volume, aliquot number, freezer, shelf, rack, box, location in the box. A bar code label will be used on the sample tracking form that is used by the

technologist when processing and storing samples. This will be done to avoid manual entry of sample numbers in order to avoid manual entry errors. When the data are entered into the database the bar code label is scanned in and the sample aliquots entered. Removals of samples will also be tracked on the database, including the date removed and the recipient center.

D.5.s. IMAGING STUDIES

D.5.s.i. MRI SCANS

1.5 and 3T MRI scans will be collected according to a standardized protocol and transmitted to LONI at UCLA for storage. Scan time will be about 45 minutes per subject per session. See procedures manual for details of the scan collection.

D.5.s.ii. PET IMAGING PROTOCOL

- Subjects will be studied after a 4 hour fast (water only).
- Subjects will have blood glucose measured. Plasma glucose must be ≤ 180 mg/dL for FDG to be injected.
- An intravenous catheter will be placed in one arm for injection of [^{18}F]FDG. If the quantitative protocol is used, a second i.v. catheter will be placed in the opposite arm.
- Subjects will be injected with 5.0 ± 0.5 mCi of [^{18}F]FDG.
- One of two PET imaging protocols will be used.
    - Qualititave: imaging begins at 30 min post injection, and the scan is acquired as six 5-min frames
    - Quantitative: imaging begins at injection, and the scan is acquired as 27 frames over 60 min. Five venous blood samples will be drawn and counted for both plasma glucose and [^{18}F]FDG throughout the 60 min study.

- Subjects will also received a transmission scan. This can either be a short CT scan for PET/CT systems, or a rotating positron source for PET only scanners.
- All PET data for the subject will be de-identified and sent to the data repository maintained at LONI at UCLA.
- Subjects will return for repeat PET scans at the intervals outlined in the "Schedule of Events" specific to the subject's classification.
- See procedures manual for further details

D.5.t. ENDPOINT DETERMINATION

The conversion from normal to MCI or AD, and conversion from MCI to AD is of considerable interest. We estimate that the most frequent conversion will be from MCI to AD which will occur at approximately 10-15% per annum. Thus, allowing for a 10% drop-out rate, using an estimated conversion rate of 12%, we estimate that there will be 119 conversions from MCI to AD during the course of the trial. All conversions from one state to another will initially be made by clinicians at each site then reviewed by a central review committee.

Diagnostic classification at each site will be a multiple step procedure. First, a study clinician (nurse or physician) will assess the subject by performing a clinical interview. The clinical interview should be performed at each visit without referring to other neuropsychological testing, adverse effects, and laboratory data. After interviewing the subject and study partner, the clinician will complete the Clinical Dementia Rating.

Second, a psychometrist will perform the Alzheimer's Disease Assessment Scale–Cognitive (ADAS-COG), the neuropsychological battery, and the Mini-Mental State Examination (MMSE). A nurse or psychometrist will administer the FAQ, GDS and NPI-Q.

A nurse or physician will collect the intervening adverse events, concurrent medications, and perform vital signs. Biomarker samples will be acquired, when relevant.

After the visit is complete, a physician who is an experienced neurologist or psychiatrist will determine the best diagnosis (NL, MCI, AD, or other). The physician who determines the diagnosis may also serve as the study clinician performing the CDR. The diagnosing physician will review the medical history, examine the CDR ratings (global and box scores), GDS, FA, and other laboratory tests. He or she will also review the neuropsychological test information, including scores on the MMSE and ADAS-COG as well as each cognitive domain measured by the neuropsychological tests to determine if there is significant impairment or deterioration. Based on the results of the clinical, neuropsychological and laboratory information, the physician may then make a diagnosis and, if appropriate, further classify the diagnosis of AD into Probable AD or Possible AD. For the purposes of this study Possible AD refers to situations where a second disorder is present that may cause dementia but is *not* considered to be *the* primary cause of the dementia (i.e. AD is considered to be the primary cause). If the subject has developed another diagnosis other than AD, which is believed to be the primary etiology for cognitive impairment or dementia, this will also be specified.

Operationalized NINCDS-ADRDA Criteria for Probable or Possible AD

1. Memory impairment established by neuropsychological testing (using the standardized test battery).
2. Clear evidence of impairment or deterioration in memory and another cognitive domain as evidenced by worsening performance on the MMSE, ADAS-COG or neuropsychological test battery.
3. Evidence of continued decline from a previous level of functioning through a collateral source and structured clinical examination (CDR, CDR Sum of Boxes, GDS), or assessment of activities of daily living
4. Absence of clinical or laboratory evidence of another disorder that could account for memory and cognitive decline or if a second disorder is present, it is not considered to be the primary cause of the dementia.

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

D.6. STUDY-SPECIFIC PROCEDURES

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 normal, 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 normals, MCI, and early AD subjects. Additionally, they are being used by Alzheimer Disease Centers as part of their collection of a Uniform Data Set thereby reducing the amount of testing that subjects will need to undergo who are enrolled in both ADC's and ADNI.

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

Alzheimer's Disease Assessment Scale-Cognitive (ADAS-COG) (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).

Logical Memory Test (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.

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 (either the odd- or even-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.

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 each of two semantic categories (animals and vegetables) in successive one-minute trials. The primary performance measure is the number of correct, unique examples generated for the two categories. Perseveration (repetitions of a correct item) and intrusion (non-category items) errors are also noted.

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).

Digit Span Test (D. Wechsler, 1981): The Digit Span subtest from the WAIS-R requires the subject to repeat sequences of single-digit numbers which are read aloud by the examiner. In the Forward condition, the subject must repeat the digits in the same order; in the Backward condition, the digits must be repeated in the reverse order. The lengths of the sequences increase progressively from three to nine digits in the Forward condition, and from two to eight digits in the Backward condition, with two trials presented for each sequence length. Testing is terminated when the subject misses both trials at a given sequence length. A point is awarded for each sequence correctly produced, so the maximum score for each condition is 14 points.

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 (AMNART errors) + .64 (years of education)](Grober & Sliwinski, 1991).

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

Trail Making Test: Parts A and B (Reitan, 1958): Part A consists of 25 circles numbered 1 through 25 distributed over a white sheet of 8 1/2" X 11" paper. The subject is instructed to connect the circles with a drawn line as quickly as possible in ascending numerical order. Part B also consists of 25 circles, but these circles are either numbered (1 through 13) or contain letters (A through L). Now the subject must connect the circles while alternating between numbers and letters in an ascending order (e.g., A to 1; 1 to B; B to 2; 2 to C). The subject's performance is judged in terms of the time (in seconds) required to complete each trail and by the number of errors of commission and omission. The time to complete Part A (150 second maximum) and B (300 second maximum) will be the primary measures of interest (testing is stopped if the maximum time is reached). Although both Parts A and B depend on visuomotor and perceptual-scanning skills, Part B also requires considerable cognitive flexibility in shifting from number to letter sets under time pressure. Both parts of the Trail-Making Test are available in multiple forms of equal difficulty for purposes of repeated evaluation.

Digit Symbol Substitution Test (D. Wechsler, 1981): This subtest from the WAIS-R consists of 110 small blank squares (presented in seven rows) each randomly paired with one of nine numbers (1 to 9) printed directly above it. Above the row of blank squares is a printed "key" that pairs each of the numbers 1 through 9 with an unfamiliar symbol. Following a short series of practice trials, the subject must use the key to fill in the blank squares in order (working left to right across the rows) with the symbol that is paired with the number above it, working as quickly as possible for 90 seconds. The number of blank squares filled in correctly within the time limit is the measure of interest (Maximum raw score = 110). This test engages multiple cognitive abilities including attention, psychomotor speed, complex scanning, visual tracking, and immediate memory.

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.

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

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

Geriatric Depression Scale (Sheikh & Yesavage, 1986): The Geriatric Depression Scale (Short Form) is a self report scale designed to identify symptoms of depression in the elderly. The scale consists of 15 printed questions that the subject is asked to answer by circling yes or no on the basis of how they felt over the past week. The items are presented on a single page with more benign items presented first. Answers to 5 of the items are negatively oriented for depression (e.g., Do you feel full of energy?) and 10 positively oriented (e.g., Do you often feel helpless). One point is given for each appropriate positive or negative answer indicative of a symptom of depression, for a possible total of 15 points. Total scores of 0-5 are considered normal and scores of 6-15 are considered depressed.

D.7. STEPS TO MAINTAIN A HIGH RATE OF FOLLOW-UP PARTICIPATION

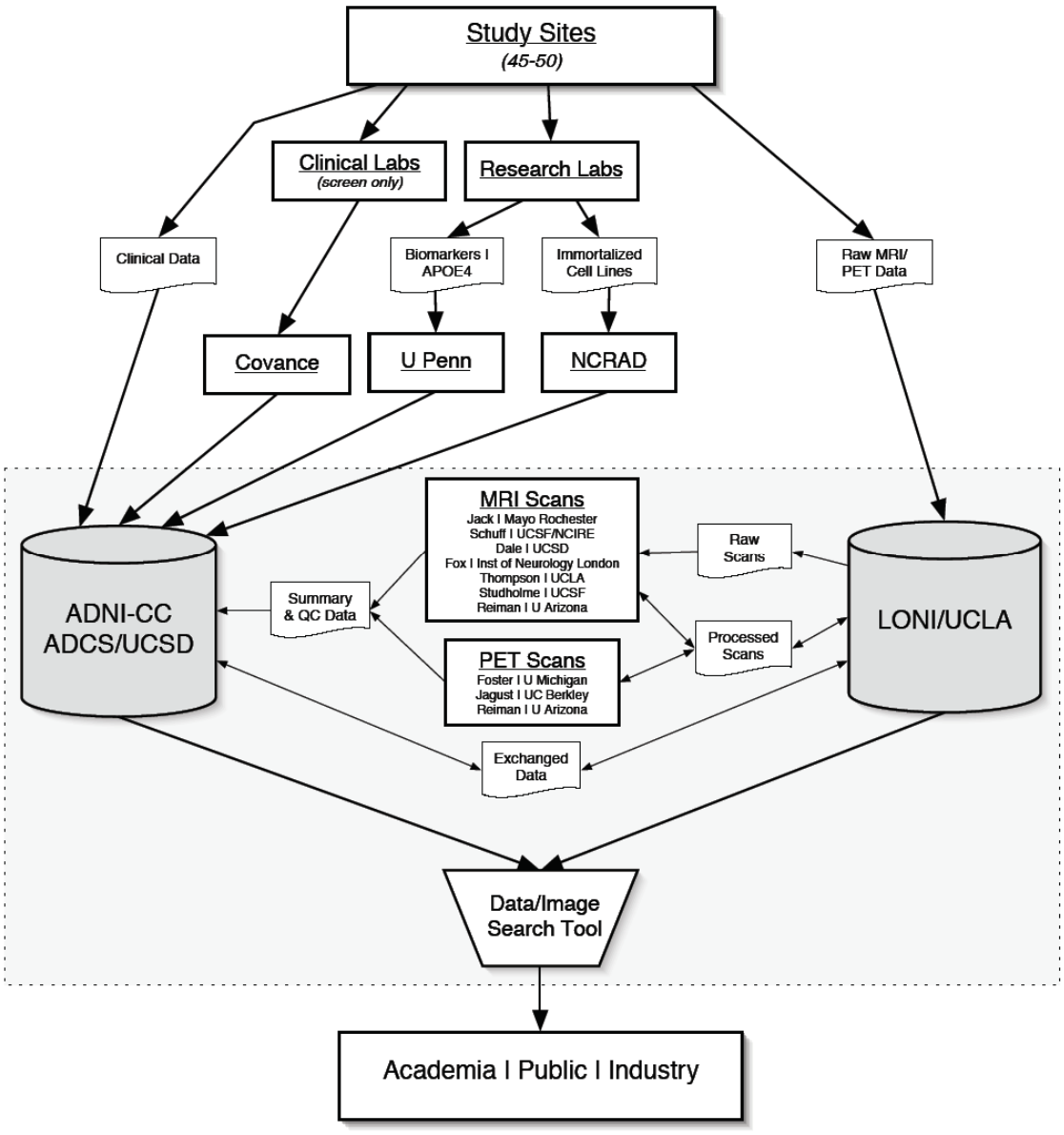
Several steps will be taken to assure the high follow up rate that is essential to the validity of the study results. All staff members will be carefully instructed regarding the need for an expectation of full follow up participation and the process of removing barriers to participation. At entry, each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

D.8. DATA COLLECTION AND MONITORING

Clinical data collection and monitoring are standardized with well-established and successful ADCS operating procedures. The ADCS currently plans to handle data by direct data entry from 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. The following figure illustrates the flow of imaging and clinical data for this study.

FIGURE 6. FLOW OF DATA



D.9. POTENTIAL RISKS

D.9.a. PET

The primary risk related to PET is that of radiation exposure. There is also minor risk associated with the venipuncture and radioisotope injection (pain and bruising or painful infiltration of a failed injection).

A subset of subjects will undergo a quantitative PET study that will require blood sampling. This sampling could result in pain or bruising.

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.

Organ	Dose (rad/mCi)	Dose/5mCi	Dose to Normal subjects	Dose to MCI Subjects	Dose to AD subjects
Bladder Wall	0.32	1.6	8	9.6	6.4
Heart Wall	0.22	1.1	5.5	6.6	4.4
Pancreas	0.096	0.48	2.4	2.88	1.92
Spleen	0.14	0.7	3.5	4.2	2.8
Lungs	0.064	0.32	1.6	1.92	1.28
Kidneys	0.074	0.37	1.85	2.22	1.48
Ovaries	0.053	0.265	1.325	1.59	1.06
Uterus	0.062	0.31	1.55	1.86	1.24
LLI Wall	0.051	0.255	1.275	1.53	1.02
Liver	0.058	0.29	1.45	1.74	1.16
Gallbladder	0.049	0.245	1.225	1.47	0.98
Small Intestine	0.047	0.235	1.175	1.41	0.94
ULI Wall	0.046	0.23	1.15	1.38	0.92
Adrenals	0.048	0.24	1.2	1.44	0.96
Testes	0.041	0.205	1.025	1.23	0.82
Red Marrow	0.047	0.235	1.175	1.41	0.94
Thymus	0.044	0.22	1.1	1.32	0.88
Thyroid	0.039	0.195	0.975	1.17	0.78
Muscle	0.039	0.195	0.975	1.17	0.78
Bone Surfaces	0.041	0.205	1.025	1.23	0.82
Breast	0.034	0.17	0.85	1.02	0.68
Skin	0.03	0.15	0.75	0.9	0.6
Brain	0.07	0.35	1.75	2.1	1.4
Other tissues	0.042	0.21	1.05	1.26	0.84

For a single 5mCi study that will be used in this project, the bladder wall will receive a dose of 1.6 rad which is still well below the dose guidelines of 5 rad published in the Code of Federal Regulations (21CFR §361.1). The protocol involves the following number of 5 mCi injections for each subject type:

AD – 4 injections (baseline, 6, 12, 24 months)
Controls – 5 injections (baseline, 6, 12, 24, 36 months)
MCI – 6 injections (baseline, 6, 12, 18, 24, 36 months)

Thus, the entire study protocol will result in a cumulative bladder dose of 9.6 rad for MCI subjects, also well beneath the cumulative dose recommendations of 15 rad (21CFR §361.1). Doses are lower for other organs and other subject groups.

In the initial ADNI protocol we decided to limit the radiation exposure for each subject to a maximum of 35 mCi (or 7 PET studies) over the 3 year scanning period, which will keep the dosage for target organs well below acceptable limits (~ 11 rads). This permitted 1 additional PET study to be performed for quality control reasons in an MCI subject, 2 in a control subject, and 3 in an AD subject, in the event that studies need to be repeated.

D.9.a.1
Extension of PET Imaging in Normal Controls

Subjects who enrolled in the original cohort of ADNI subjects as Normal Controls will be asked to consent to annual PET scans as part of the ADNI Extension. For a single 5mCi study that will be used in this project, the bladder wall will receive a dose of 1.6 rad which is still well below the dose guidelines of 5 rad published in the Code of Federal Regulations (21CFR §361.1).
Under no circumstances may the radiation dose to an adult research subject from a single study or cumulatively from a number of studies conducted within one year be generally recognized as safe if such dose exceeds:

WHOLE BODY / ACTIVE BLOOD FORMING ORGANS / GONADs / EYE
* Single Dose = 3 rem
* Annual & Total Dose Commitment = 5 rem

OTHER ORGANS
* Single Dose = 5 rem
* Annual & Total Dose Commitment = 15 rem

The research site is responsible to report any overdosing of subjects as a protocol violation. Using real time distributed data entry and reporting, the ADCS tracks and reports on total radiation exposure for all participants in the ADNI study. The ADCS will alert the participating research sites if any subject's dosing exceeds 90% of 5/15 rad within a calendar year.

D.9.b. 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. There is a slight risk of anxiety due to claustrophobia and noise. Any subject who experiences anxiety when placed into the MR scanner will be removed from the scanner, offered reassurance by the MR tech doing the scan, and offered the option of continuing or terminating the study. If the subject decides that the anxiety associated with MRI is uncomfortable for them and they wish to terminate the scan, then the examination will be ended at that time. There will be no attempt to coerce subjects to complete exams that they are uncomfortable with. No anxiolytic agents will be given, as this is a voluntary research protocol.

D.9.c. LUMBAR PUNCTURE

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

D.9.d. 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.

D.10. PERSONNEL REQUIREMENTS

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

- **Site Principal Investigator**. This person is responsible for ensuring that the local IRB approves the protocol; this may be the study physician.
- **Study Physician.** This person is responsible for conducting or supervising the clinical evaluation of all participants, including physical and neurological examinations, reviewing adverse events, interpreting laboratory results; ensuring enrollment quotas and protocol adherence and for conversion determinations. The study physician will supervise project personnel and ensure that raters maintain a high level of skill and accuracy in conducting assessments.
- **Study Coordinator.** This person will be responsible for managing the day-to-day conduct of the trial, ensuring accurate administration of all instruments, maintaining online forms 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.
- **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.

D.11. ADVERSE EVENTS

D.11.a. DEFINITION.

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.

D.11.b. FOLLOWING UP ON ADVERSE EVENTS

The investigator is obliged to follow subjects with AE's until the events have subsided, the conditions are considered medically stable, or the participants are no longer available for follow up. Subjects who discontinue due to adverse events will be treated and followed according to established medical practice. All pertinent information will be entered into the electronic CRF. All adverse events will be reported to an independent Data Safety Monitoring Board. Adverse events will be rated as mild, moderate or severe. This will also pertain to abnormal laboratory values.

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.

D.11.c. REPORTING SERIOUS ADVERSE EVENTS

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

D.12. STATISTICAL CONSIDERATIONS

D.12.a STUDY ASSIGNMENT

Depending on the subject's consent the subject will be enrolled in the arm with the lowest percentage of target enrollment. To ensure the minimum number of CSF samples are collected, sites will not be allowed to enroll their third participant in each arm (NL, MCI, AD) unless two of the first 3 have agreed to lumbar puncture. Extensive reporting and monitoring of enrollment progress will feed back to the ADNI-CC to ensure recruitment goals are met.

D.12.b STATISTICAL POWER

The sample sizes chosen (NL=200, MCI=400, AD=200) are designed to meet the needs of the hypotheses being tested for the proposed imaging and biochemical biomarkers.

Between-diagnostic group analyses: These analyses will mainly be confirmatory analyses to verify that the imaging measures and biomarkers behave as expected, differing across diagnostic categories in level and rate of change. An effect size in these analyses describes how the variance of the group means compares to the common variance of the observations within each group. With the chosen sample sizes, we will have 80% power to detect an effect size of 0.01, at level alpha=0.05 (0.02, at level alpha=0.01) between rates of change in the 3 clinical groups for MRI and biomarkers, 0.03 (0.04) for PET, and 0.05 (0.08) for MRI at 3 Tesla.

Within diagnostic group analyses: The primary focus of this proposal is to provide imaging and biomarker information that will help in planning future clinical trials, which are likely to be conducted within a particular diagnostic category. Therefore, we present power separately for MCI and the smaller groups of AD and normals. These analyses will have two primary goals: 1) to assess the strength of association between imaging summaries and clinical measures, and 2) to better understand the between- and within-subject variation to correlate longitudinal change in imaging with longitudinal change in clinical measures. We will have 80% power to detect an association attributed to the imaging or biomarker measures accounting for 2.1% at level alpha=0.05, (3.2%, at level alpha=0.01) of the variability in the clinical measures or imaging measures, 4.2% (6.2%) for PET, and 8.2% (11.9%) for MRI at 3 Tesla within the MCI patients and 4.2% (6.2%) for MRI, 8.2% (11.9%) for PET, and 15.4% (21.9%) for MRI at 3 Tesla within the normal or AD subjects.

We will have 80% power to detect a correlation of 0.15 at level, alpha=0.05 (0.18, at level, alpha=0.01) between MRI or biomarker and the clinical outcome, 0.21 (0.25) for PET, and 0.29 (0.35) for 3-Tesla MRI in the MCI subjects and 0.21 (0.25) for MRI, 0.29 (0.35) for PET, and 0.39 (0.47) for 3-Tesla MRI in the AD or normal subjects. Under a multivariate normal assumption, we would, for example, be able to construct 95% confidence intervals around an estimated correlation of 0.5 of the following: (0.42, 0.57) for MRI, (0.38, 0.60) for PET, and (0.33,0.64) for 3-Tesla MRI within the MCI subjects and (0.38,0.60) for MRI, (0.33,0.64) for PET, and (0.24,0.69) for 3-Tesla MRI within the normal or AD subjects.

D.12.c. STATISTICAL ANALYSES FOR HYPOTHESES

Numerous hypotheses based on the clinical and biomarker data will be evaluated. Analyses by gender and minority status will also be conducted as required by NIH policy. Five examples will be discussed here:

1. Rates of conversion from MCI to AD will average 10-15%/year. Annual conversion rates will be assessed using Kaplan-Meier product limit estimators at the conclusion of the investigation. To estimate the anticipated precision of the incidence estimates, a sensitivity analysis of the 95% confidence interval for conversion rate from MCI to AD was conducted using life table probabilities.

The 95% confidence intervals for annual conversion rate and overall conversion rate through 3 years was calculated with 2 different true conversion rates (8, 12%) and two different sample sizes (300, 400). An annual drop-out rate of 10% was assumed.

N	Annual Rate (%)	3 Year Rate (%)	3 Year Rate Confidence-Interval (%)	Half-Width (%)
300	8	22.1	16.6 - 27.6	5.5
300	12	31.9	25.7 - 38.0	6.2
400	8	22.1	17.4 - 26.9	4.7
400	12	31.9	26.5 - 37.2	5.3

It can be seen from this analysis that the MCI to AD conversion rates at three years can be anticipated to have associated 95% confidence intervals with approximately half width of 5.5%. In other words the confidence interval lower and upper bounds for either annual or 3-year rates will be within 5 percentage points of the estimated annual rate in the above 4 examples.

2. Baseline scores on logical memory and APOE4 status will predict conversion from MCI to AD. These two predictors will be evaluated as baseline predictors of conversion using the Cox proportional hazards model. The underlying assumptions of the model will be assessed. It is anticipated that the sample size in the MCI group will be adequate to make this assessment. A power analysis based on Pearson's correlation coefficient (transformed using Fisher's Z) to assess the power to detect a univariate predictor indicates that with 400 subjects a correlation as low as 0.2 will be detected with 94% power. A correlation of 0.2 is selected as an effect size of clinical relevance.

3. Measures of global functioning, such as activities of everyday living, will be more sensitive than neuropsychological measure for predicting conversion from MCI to AD. Cox modeling will be used to assess the relative predictive ability of standard global and cognitive measures.

4. The rate of backcrossing from MCI to normal will be extremely low for this population. The backcrossing rate will be assessed using survival analysis techniques i.e. Kaplan-Meier curves. With 400 subjects, it is anticipated that it will be possible for the rates to be estimated with confidence intervals of plus or minus five percentage points (two standard deviations) based on a sensitivity analysis.

5. Plasma isoprostanes will be analyzed as a potential predictor of disease state. Analysis of Covariance will be used to compare the baseline isoprostane levels between the three ADNI disease severity groups. Based on results from Pratico et al (2000, 2002), the estimated mean baseline plasma isoprostane levels for AD, MCI and Normal are .66, .44 and .19 ng/ml, respectively. The common standard deviation is estimated as 0.16. The estimated power for the omnibus comparison of isoprostane levels in the three disease severity groups is greater than .99. Other Biomarkers as

well as isoprostane will be assessed as predictors of cognitive decline. Contemporary longitudinal regression methods will be employed to assess the predictive ability of baseline isoprotane levels. Main effect assessment will be made using Generalized Estimating Equation models. Additionally potential interactions between isoprostane levels and ADNI group (AD, MCI, Normal) will be evaluated.

D.13. OVERALL STUDY TIMETABLE

This study involves 6 months of start up time, 12-18 months of enrollment and 36 months of follow up. It will therefore be active for 5 years. The first 6 months will be consumed by submission of protocol to local IRBs, verification of approval, certification of site personnel and imaging facilities in performance of the trial, establishing laboratory contracts, distribution of material to sites, preparation and implementation of training meetings and manuals, CRF and initial recruitment efforts. The recruitment will last 12-18 months with follow up of up to 36 months. After 5 years, additional data cleaning and analysis will still be ongoing.

With the extension of this protocol and the continued follow-up of subjects the ADNI study will now be active for 6 years (until September 2010). At this point the subjects who agree will be enrolled in ADNI2, if it is funded, for long-term followup.

E. 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%.

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Appendix 1. CLINICAL PROTOCOL AMENDMENT 1

Summary of Amendment to Protocol

1. In efforts to provide more definitive data on the utility of obtaining longitudinal measures of CSF tau and Aβ in the LP subset of ADNI, consent will be obtained from the 150 ADNI participants (i.e. 50 controls, 50 MCI and 50 AD subjects) that have consented to LPs at baseline and year-1 to do a third LP at the year-2 visit.

Appendix 2. CLINICAL PROTOCOL AMENDMENT 2

Summary of Amendments to Protocol

1. In order to optimize the impact of the ADNI study and the CSF already collected, any participant who has had at least one Lumbar Puncture will be asked to consent to additional annual LPs. The Protocol has been updated throughout to reflect this.
2. Extended Follow-up of Alzheimer's Disease Subjects: Only those subjects enrolled in the primary ADNI as Alzheimer's Disease Subjects who agree to a 4th Lumbar Puncture at Month 36 will be followed for additional visits. If these subjects agree to annual LPs, they will continue to be followed with Telephone checks every 6 months and annual clinic visits with LPs. No imaging will be conducted for these subjects. Protocol has been updated throughout.
3. MCI Subjects: Any MCI subject willing to continue will have annual clinic visits with 1.5 T MRI imaging. If these MCI subjects had at least one previous Lumbar Puncture, they will be asked to consent to additional annual LPs. Protocol has been updated throughout.
4. Normal Controls Subjects: Any Normal Control (NL) subject willing to continue will have annual clinic visits with 1.5 T MRI imaging. If these NL subjects had at least one previous Lumbar Puncture, they will be asked to consent to additional annual LPs. If these NL subjects were randomized to the PET arm of the study, they will be asked to consent to annual PET imaging as well. Protocol has been updated throughout.
5. Telephone checks will be conducted 6 months after these visits, in order to maintain contact with the subject and study partner, document any adverse events, and ensure continued interest in the ADNI study. Protocol has been updated throughout.
6. 3T Imaging will not be continued past the Month 36 visit.
7. Risks Section: Normal Control Subjects agreeing to annual PET scans will have a dose of 5mCi per scan, which will result in well below the annual limits of exposure according to the Code of Federal Regulations (21 CFR 361.1). The ADCS will continue to track radiation exposure and notify sites if dose reaches 90% of this limit.
8. Overall Study Timetable: The overall timetable of the study is now extended until September 2010. At this time the ADNI 2 Protocol will be implemented and any subject willing will be enrolled for long-term follow-up.
9. Appendix 1 and 2 Added to ensure changes made to the ADNI protocol are clearly defined for Research Sites and their IRBs**.**