SPECIFIC AIMS: The goal of this project is to determine the relationships among the clinical, cognitive, imaging, genetic and biochemical biomarker characteristics of the early (pre-dementia) stages of Alzheimer’s disease (AD). The project builds on the NIA-currently funded AD Neuroimaging Initiative (ADNI1), a collaboration between academia and industry to study biomarkers of AD and it also serves as a bridge to the renewal of ADNI (termed ADNI2). Herein we continue ADNI themes with new hypotheses informed by our results. Our model posits that AD begins with Amyloid β (Aβ) deposition in cortex, which leads to synaptic dysfunction, neurodegeneration, and cognitive/functional decline. This predicts that the earliest detectable changes (measured in the GO/ADNI projects) are those related to Aβ (Cerebrospinal fluid (CSF) and PET amyloid imaging). Subsequently neurodegeneration is detected by a rise of CSF tau species, synaptic dysfunction by FDG-PET and neuron loss indicated by atrophy most notably in medial temporal lobe (measured with MRI). These changes ultimately lead to memory loss, general cognitive decline and eventually dementia. Expression of each element of AD pathology (e.g. Aβ and tau deposits, atrophy) is influenced by modifying factors including age, APOE genotype, and cerebrovascular disease (white matter lesions detected by FLAIR MRI) and microbleeds (detected by T2* MRI). The results obtained from this GO grant, together with ADNI1 and ADNI2 will advance understanding of AD pathophysiology, improve diagnostic methods for early detection of AD and improve clinical trial design. We propose three global hypotheses based on cross sectional and longitudinal results (from ADNI1 data) described in Biostatistical Core (below). 1) Reductions of CSF Aβ and increased amyloid PET tracer retention are present in some cognitively normal asymptomatic individuals, indicating early stage AD neuropathology. 2) Subsequently, CSF tau increases accompanied by reduced brain glucose metabolism (assessed by FDG-PET) and an increased rate of medial temporal lobe atrophy (assessed by structural MRI), and 3) After changes in CSF Aβ, amyloid PET tracer retention, CSF tau, FDG PET and atrophy of the medial temporal lobe, signs and symptoms of cognitive decline appear, eventually progressing to dementia. Specific operational hypotheses are in the Biostatistics Core. This application will advance AD research by extending our infrastructure to perform amyloid imaging in all subjects and characterize an extended range of mild cognitive impairment (MCI) to broaden our understanding of the early stages of AD. Our specific aims are:

Aim 1: Define and characterize the stage of the AD spectrum that precedes MCI as currently enrolled in ADNI. 200 subjects in the mildest symptomatic phase of AD, defined here as early amnestic MCI (EMCI), will be enrolled to narrow the gap between normal controls (NC) and “late MCI (LMCI)” subjects currently enrolled in ADNI (see Table next page showing enrollment and assessments in GO and ADNI1). EMCI will be defined as individuals meeting clinical criteria for amnestic MCI, who score between 0.5 and 1.5 SD below the mean of NC on delayed paragraph recall performance. These 200 subjects will receive cognitive/clinical assessments at baseline, 6 months and 12 months. (Other measurements described below) All baseline visits are in year 1, the 6 months visits occur in Year 1 and 2. After GO, EMCI will be followed by the renewal of ADNI (ADNI2).

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

Aim 3: Continue longitudinal studies of 530 LMCI and NC from ADNI1 for an additional 2 years.

Aim 4: Analyze all existing and new clinical, biochemical, neuroimaging, genetic and other biomarker data. ADNI1 was funded largely as a data gathering grant. Funds were only provided to analyze baseline, 6 month, and 1 year data. ADNI1 subjects have been followed for 3-4 years and this application proposes an additional year of follow-up. Thus a large longitudinal data set will be comprehensively analyzed.

Summary: Taken together, the overall impact of this GO grant will be 1) increased knowledge concerning the sequence of events leading to AD dementia, 2) development of improved clinical and biomarker methods for early detection of AD 3) improved imaging and chemical biomarker methods for monitoring progression of AD, facilitating clinical trials of treatments to slow disease progression, ultimately contributing to the prevention of AD dementia. No other large multisite study in the world addresses these complex issues with the sample size and statistical power of this application. The innovation of this proposal lies in the longitudinal assessment of the spectrum from normal aging to AD using an integrated combination of clinical/cognitive, CSF/Plasma biomarker, MRI and amyloid/FDG PET measures, including a national network for F18 amyloid imaging. The Table (below) shows that evaluations and funds requested in the GO grant complement, and do not overlap with, current and proposed ADNI activities. This grant will generate 78 new jobs for year 1 and 2 and 48 additional new jobs for year 2 (see Budget Justification).
Research Area: Our broad goals are stated in the Specific Aims. This project will result in “research and development innovations” that will lead to early AD diagnosis, improved clinical trials, and ultimately treatments and preventions for AD.

Opportunity and Potential Impact: There are substantial gaps in scientific knowledge concerning the sequence of events in early AD, methods to diagnose AD, and how biomarkers should be used in AD treatment trials. The global community of elders at risk for AD is likely to benefit from this project. GO is exciting and innovative because GO/ADNI is the largest multisite project with the broad scope to address the use of biomarkers for early detection and for monitoring progression of AD. Furthermore, ADNI-like projects in Australia, Japan, Europe, and China demonstrate the far reaching impact of ADNI.

Approach: The approach of GO, which builds on ADNI, is described in the accompanying Table 1 and the sections which follow. In summary, the major goals of ADNI1 have been to: 1. Develop improved methods which will lead to uniform standards for acquiring longitudinal, multisite blood/CSF, MRI, and PET data on patients with AD, MCI, and matched NC. 2. Acquire a 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. Limited funds were provided for analysis of the baseline, 6 and 12 month data. 4. Test hypotheses based on the data. ADNI is a non-randomized natural history non-treatment study in which a total of 821 subjects including 221 NC, 400 individuals with MCI, and 200 subjects with mild AD were enrolled at 57 sites. All subjects have clinical/cognitive assessments and 1.5 T structural MRI. AD subjects (n=200) are studied at 0, 6, 12, and 24 months. MCI subjects (n= 400) are studied at 0, 6, 12, 18, 24, 36, and 48 months. NC (n=200) are studied at 0, 6, 12, 24, 36 and 48 months. 50% of subjects have FDG PET at the same time intervals, 25% of subjects have 3 T MRI (to compare 1.5 vs. 3T), and more than 50% of subjects have lumbar punctures at baseline, 12 months, and many continue to have annual LPs. 101 subjects are enrolled in a C-11 PIB sub study. All clinical data are collected at the Clinical Core at AD/UCSD. Plasma and CSF is collected/analyzed by the Biomarker Core at U Penn. MRI acquisitions are overseen by the MRI Core at the Mayo Clinic. PET acquisitions are overseen by the PET Core at UC Berkeley/U. Michigan. A genome wide association study (GWAS) was performed on all subjects. All clinical/cognitive/biomarker/genetic and raw and processed image data is archived by the Informatics Core at the UCLA Laboratory of Neuroimaging (LONI) and is available, without embargo, to all scientists on a public website. Enrollment completed Sept 2007. ADNI ends Sept 2010. Thus far there have been 37 peer reviewed publications, more than 40 additional publications submitted, and more than 100 abstracts presented at scientific meetings. ADNI was originally designed with a primary focus to validate biomarkers as measures of AD progression, but has evolved to also determine those biomarkers which detect AD at an early stage and indicate risk for cognitive decline/dementia. This project also provides considerable information concerning the pathophysiological series of events in the transition from normal aging, to mild symptoms/impairments, to dementia.

Specifically, this application proposes 4 integrated approaches: 1) Enrolling 200 EMCI subjects, some of whom will have early biomarker signals of AD pathology. These subjects will have clinical/cognitive, blood/CSF/genetic, FDG and amyloid PET, and MRI measurements. This is a heretofore relatively unexplored population, and narrows the gap between NC and the LMCI subjects in ADNI1. 2) Performing F18 amyloid PET imaging on all NC, EMCI and LMCI, which will allow correlation and comparison of this modality with all of the other clinical/cognitive, neuroimaging, genetic, and biomarker data collected in ADNI1 and this GO project. 3) Extending the follow-up of LMCI and normal subject enrolled in ADNI1. 4) Analysis of all of the ADNI1 data that have not yet been analyzed (since ADNI1 was a data collection grant, and little funds provided for
analysis) as well as analysis of the data from this GO project, to test hypotheses and perform data explorations which are outlined in the Biostatistical Core below. The specific Methods are proposed below.

1) CLINICAL CORE: (CoPIs: Paul Aisen and Ron Petersen) Specific Aim 1: MCI has been recognized as the transitional state between the cognitive changes of aging and the full expression of the clinical dementia syndrome characterizing AD [1]. It is generally thought that disease-modifying interventions for AD will be most effective early in the disease process, but most current trials are conducted in the mild to moderate AD population. ADNI has demonstrated the feasibility of conducting studies on MCI subjects, and we have identified NC who appear to be at risk for progression (low CSF amyloid, high rates of brain atrophy). To be useful for drug development, it must be feasible to identify subjects with early-stage MCI. Our goal is to determine which CSF/plasma biochemical or imaging markers identify those EMCI subjects who subsequently decline, enabling future drug testing of this population. MCI subjects are classified as two subtypes, amnestic and non-amnestic MCI according to the degree to which memory is impaired the clinical syndrome. The amnestic MCI subtype is believed to be on the AD spectrum of pathology and represents the clinical stage prior to dementia [2]. Numerous studies have documented the annual progression rates from MCI to AD varying from 6-16% per year depending upon the manner in which the criteria are implemented and the source of the subjects [3]. A key factor influencing the progression rate pertains to the severity of the subjects within the MCI range. ADNI1 studies subjects with aMCI of moderate severity. The entry criteria episodic memory cutoff score approximated 1.5 standard deviations below the appropriate education mean for normal subjects in order to maximize the rate of progression to probable AD. The annual transition rate from aMCI to AD of 16%/ year is identical to the rate of the MCI, donepezil/vitamin E trial [4].

In order to recruit subjects at an earlier stage of cognitive impairment we will alter the memory cutoff scores. Thus, we include subjects at an earlier stage of the AD spectrum, at a cost of reduced specificity (i.e. subjects who do not have AD pathology or progress to develop another form of dementia). However, the imaging/ biochemical biomarkers obtained will enable us to identify risk factors for progression of cognitive decline/dementia. Progression will be tracked using the Clinical Dementia Rating scale (CDR) sum of boxes (CDR-SB), shown to be a robust measure of MCI decline in ADNI; longitudinal change in functional, behavioral and cognitive assessments, and time to dementia onset will also be followed.

The Clinical Core will be responsible for the recruitment and longitudinal assessment 200 milder aMCI subjects which will be labeled as early MCI or EMCI, while MCI from ADNI are designated as late MCI (LMCI). Subjects will have the same recruitment procedures, cognitive and behavioral instruments as performed in ADNI [5]. Enrolled subjects will be 55-90 years of age, have a reliable informant and will speak either English or Spanish. Certain psychoactive medications will be excluded. Identical training procedures for the administration of the CDR, AD Assessment Scale Cognitive Subscale, and the Neuropsychiatric Inventory-Q will be carried out as in ADNI1. Inclusion / exclusion criteria will be the same as in ADNI2. Enrollment will occur at most of the 50-60 clinical sites currently used in ADNI. To reach the goal of 200 subjects with EMCI, approximately four subjects will be enrolled/ site during the first year of the GO grant and will be re-evaluated in year 2 (see Table). Thus this enrollment is feasible. The specific inclusion criteria for EMCI will be as follows: MMSE score of ≥ 24, CDR-SB summary score 0.5 (memory box score ≥ 0.5), non-demented, in the judgment of the site clinician, delayed recall of one paragraph from the Logical Memory Test of the Wechsler Memory Scale-Revised with cutoff scores (derived from the National Alzheimer's Coordinating Center (NACC) database) by education as follows: ≥ 16 years: 9-11; 8-15 years: 5-9; 0-7 years: 3-6. The scores represent recall performance 0.5 - 1.5 SD below education adjusted means for one paragraph of the Logical Memory Test. EMCI subjects will have clinical and cognitive assessments and a 1.5 T-structural MRI at enrollment, 6 months and 12 months. In addition to the EMCI subjects, we will continue following ~ 530 subjects (180 normals and 350 MCI subjects) currently active in ADNI1 as part of year 6 of ADNI1 (at no cost to the GO grant in year In GO we will continue to see ~ 506 of these subjects in year 2, (assuming 5% dropout). The 200 EMCI subjects will undergo MRI scans in year 1 (at baseline and 6 months) and year 2 of GO, one F18 amyloid scan and FDG PET at baseline, and FDG PET at 1 year. The 530 ADNI carryforward subjects (including those who have had C-11 PIB) will undergo one F18 amyloid scan and the GO grant will also fund one FDG PET scan/ subject (50% of ADNI1 subjects receive FDG PET funded by ADNI1 in Year 6 of ADNI which is Year 1 of GO). Table 1 provides a description of the flow of subjects and the budgetary allocation of the procedures between ADNI and GO. The clinical evaluations for the EMCI and the normal and LMCI subjects followed in this GO project will include the ADNI1 assessments, as well as brief instruments (such as the MOCA and the AD8) that may be useful in the identification of very early stage AD in a general practice setting. The investigators at each site will be asked to adjudicate each subject according to the specified procedures in ADNI as to the clinical
diagnoses of normal, MCI (EMCI or LMCI) and dementia. All conversion decisions are reviewed by one of the 
Clinical Core directors and a central review committee.

In summary, treating subjects at the earliest stage of AD pathology is more likely to have a beneficial 
outcome. In EMCI, the problem is more difficult than in LMCI, because there is minimal functional 
impairment and thus the usual method for establishing clinical meaningfulness is unavailable emphasizing 
the value of biomarker endpoints. Characterization of the longitudinal cognitive and biomarker changes in 
the EMCI population will reveal feasible biomarkers for validation as surrogates. The ultimate validation will 
require long-term follow-up and correlation of longitudinal change to later clinical endpoints (e.g. conversion 
to dementia) or pathological verification of AD. Therefore, this GO program and ADNI2 will be critical for the 
process of validating biomarkers used for both for early detection of AD and monitoring of treatment effects 
in Phase II and III treatment trials.

2) PET CORE (PI: William Jagust): Specific Aim 2: FDG PET and F18 AV-45 Amyloid Imaging PET:
While PET amyloid imaging is a promising technology, many questions about its potential use remain 
unanswered. The goal of the PET core is to bring amyloid imaging to all ADNI sites and thereby provide a 
mechanism for large-scale studies that can both test the basic hypotheses as stated in the Biostatistical core, 
and provide a new approach to clinical trials. Imaging Aβ aggregates using PET has reached a level of maturity 
as evidenced by over 200 PubMed references on the topic. [11C]PIB (Pittsburgh compound B) is undoubtedly 
the most widely used PET amyloid imaging agent (with over 5,000 subjects studied at over 60 sites worldwide) 
and has been a component of ADNI as an “add-on” study that began in year 2 of the protocol and has enrolled 
approximately 100 subjects with longitudinal scans. Considerable data have been obtained and published 
using this compound (both in ADNI and other labs). However, the short half-life of 11C has limited its 
application to a small number of ADNI sites with cyclotrons and radiochemistry programs. The enthusiasm for 
amyloid imaging has prompted the development of a number of ligands labeled with the longer half-life 
radioisotope 18F (t1/2 = 109.8 min), which can be synthesized regionally and transported for use at clinical 
imaging centers within about a 6-8 hour radius of the manufacturing site. Of the several available agents, we 
have decided to collaborate with Avid Radiopharmaceuticals and employ the compound AV-45 (Florpiramine) 
for several reasons: (1) The compound has already undergone extensive human testing in 5 phase I and II 
FDA trials; (2) Avid is working with us (see attached letter from Dan Skovronsky) in assuring regulatory 
approval, protocol development, and public availability of all data (a standard ADNI practice); and (3) The Avid 
US manufacturing and distribution network will deliver the tracer to virtually every ADNI site in the US.

There is extensive preclinical 
and clinical experience with this 
tracer. It binds to Aβ with high 
affinity in vitro (Kd=3.1 nM) with 
low affinity for a host of other 
CNS receptors and an 
autoradiographic appearance 
that confirms plaque binding. 
Pharmacology and toxicology 
support human safety at the 
doses used in tracer studies. 
Peripheral metabolism does not 
complicate image interpretation 
or analysis. In humans, the 
tracer exhibits a multitude of 
characteristics associated with a 
fibrillar Aβ binding agent similar 
to PIB, including (1) extensive 
cortical binding in AD patients 
especially in frontal cortex and 
precuneus, and moderate non-
specific white matter binding in 
both normals and AD patients 
(Figure), (2) a high proportion of 
positive scans in AD, intermediate in MCI and lowest in NC in an age-related fashion, and (3) an association
between increased binding and the ApoE4 genotype. The kinetics of the tracer (Figure) and the relatively longer half-life of the radionuclide make imaging for brief periods shortly after injection a reasonable approach to generating standardized uptake value ratios (SUVRs).

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

Data acquisition: As noted, all currently enrolled normal and MCI ADNI participants, as well as the 200 new EMCI subjects will be studied in this protocol that includes both AV-45 and FDG imaging performed on 2 separate days. Scans may be performed in any order but both must be completed within a 2-week window to be included in the analysis. The AV-45 protocol will entail the injection of 10 mCi of [¹⁸F]AV-45 followed by an uptake phase of 50 min during which time the state of the subject is not important. At 50 minutes after injection subjects will be positioned in the scanner and 2 x 5 min frames of emission data will be collected. PET/CT scans will precede this acquisition with a CT scan for attenuation correction; PET-only scanners will perform a transmission scan following the emission scan. As we have done to date in ADNI, sites will be required to use a single iterative reconstruction for all scans that is optimized for the instrument and which cannot change during the protocol. The vast majority of sites are experienced with this; new sites will be instructed as part of the qualification procedure.

FDG scans will be acquired as they have been in the ADNI protocol to date: Subjects’ blood glucose is checked prior to scanning and must be < 180 mg/dL. After the injection of 5 mCi of tracer, subjects are in a quiet, dimly lit room with eyes and ears unoccluded for 30 min, after which they are placed in the scanner. Data are acquired as 6 X 5 min frames preceded by a CT scan or followed by a positron transmission scan.

Data flow and QC: All data will be uploaded to the UCLA Laboratory of Neuroimaging (LONI) as we have done to date with ADNI. Instruction in this protocol is provided as part of site qualification and all PET sites are currently familiar with this. Data are de-identified as part of the upload and placed into quarantine until they pass QC. Dr Koepppe’s laboratory at the University of Michigan is notified when new scans are uploaded, and QC is performed within 24 hours followed by pre-processing of the images. There will be several steps in the quality assurance and pre-processing of the AV-45 and FDG PET images that are obtained from the scanning sites. The aim of this work is not only to make sure that all PET scans are acquired and reconstructed using the appropriate protocols and that image quality is good, but to standardize the images from the different sites, and hence the different PET scanner vendors and models, as much as possible in order to reduce inter-site differences. The following are specific steps that will be taken: (1) Visual inspection of all images: both frames (temporal) and slices (spatial); (2) Extract and inspect header information; (3) Co-register all frames of the multi-frame studies to the first frame of the image set; (4) Assess motion by magnitude of translate/rotate parameters; (5) Recombine co-registered frames to create registered dynamic and registered average (averaged over all frames) image sets; (6) Reorient /resample images into a standard image matrix and image orientation (160x160x96 voxels 1.5 mm in all dimensions); (7) For all FDG scans, co-register all PET scans on each subject to baseline scan in standard image matrix; (8) Perform normalization on all image sets, based on global mean for FDG metabolic images or cerebellar gray matter for amyloid imaging; (9) Smooth images from all scanner models by amounts determined from Hoffman phantom scans to achieve uniform 8mm effective resolution; (10) Complete PET QA forms; (11) Upload post-processed PET images sets to LONI (image repository). As noted, these procedures have all been successfully employed as part of ADNI to date for both FDG and PIB tracers.

**PET Core Data Analysis:** While several methods have been used to analyze FDG and amyloid PET, there is no agreement concerning the relative value of these methods. Therefore a major goal of ADNI1 and GO has been to compare the results of different analysis methods. Thus, data will be analyzed by 4 groups that participated in ADNI. For the Jagust and Reiman labs, methods for FDG analysis developed during ADNI will...
be applied to all new FDG data as well as existing FDG data obtained in ADNI subjects after the 12 month time point (funding was only available for analysis up to 12 months), and all AV-45 data will be analyzed by these labs. For the Foster laboratory, FDG data will be analyzed only for the new EMCI subjects and AV-45 data will be analyzed on all subjects. For the Mathis laboratory, specific analyses will compare AV-45 to PIB.

**Jagust Laboratory (UC Berkeley):** The approach to analysis will use prespecified regions of interest (ROIs) to characterize retention of AV-45 and FDG scans in all participants. FDG data will be analyzed using methods defined as part of ADNI. This includes a set of ROIs that was specified by a literature meta-analysis generating MNI coordinates of brain voxels showing maximal effects between AD and NC patients for FDG scans. These regions were smoothed and thresholded, resulting in ROIs located in left and right lateral temporal cortex, parietal cortex and posterior cingulate/precuneus. An ROI template will be used to extract normalized (to pons/cerebellar vermis) counts from these ROIs for analysis of glucose metabolism. AV-45 data analysis will involve definition of ROIs using a template in standard space (AAL [6]) to extract counts from ROIs that widely sample cortex including prefrontal cortex, lateral parietal, medial parietal (precuneus/posterior cingulate) and temporal as well as a cerebellar ROI as a reference tissue. SUVRs will be calculated for each ROI and also averaged across all cortical ROIs to form a single index of tracer uptake. We will examine relationships between ROIs to define brain regions in which tracer retention appears earliest (i.e., in NC and most mildly affected EMCI), and we will compare retention in this region and the averaged region across subjects. We will also classify subjects as “+” or “-” for retention by using the iterative method to define a cutoff for positivity as published by Aizenstein [7].

**Reiman Laboratory (Banner Alzheimer's Institute):** AV-45 scan data and >12 month FDG uptake declines will be analyzed using a voxel-based approach developed for ADNI FDG-PET data using an empirically predefined statistical ROI (stat-ROI). This strategy has been widely presented and discussed at several research meetings, has recently been extended to voxel-based analyses of MRI data by the Thompson and Alexander labs, and was demonstrated to be associated with significantly improved statistical power and freedom from multiple comparisons. We will apply bootstrap with replacement to ADNI’s “training set” subjects to empirically predefine a set of voxels reliably associated with regional-to-whole brain CMRgl increases (the spared/reference ROI) and a set of voxels reliably associated with declines (the decline stat-ROI) for each patient group and each follow-up duration. For AV-45, we will characterize a single cerebral-to-cerebellar AV-45 SUVR z-score, an integrated index over all cerebral voxels, in each subject using the stat-ROI strategy and data from previously studied young-adult NCs, compare subjects from each of the four groups in terms of this continuous measurement, characterize the percent of subjects in each group with an abnormal SUVR and compare our between-group effect size to that generated using the standard anatomical ROI and ratio methods employed by the Jagust laboratory.

**Foster Laboratory (University of Utah):** The Foster laboratory will continue its role in ADNI focusing on individual image analysis and processing data using 3-dimensional stereotactic surface projection (3D-SSP) using Neurostat, a free, non-commercial analysis program [8]. In brief, this method projects peak values along a vector perpendicular to the brain surface directly onto a surface map and compares values from a reference set of identically derived images from cognitively normal individuals to make 3D-SSP images in standard space that define each voxel in a given subject based on its z transformation. For FDG analyses in the newly recruited EMCI subjects, we will characterize the number of abnormal pixels, which has previously been shown to be highly predictive of conversion of ADNI MCI subjects to AD. In addition, all AV-45 scans will be analyzed using this approach along with construction of a comparative database of normal subjects based upon an iterative outlier approach used in [11C]PIB studies. Both the extent of abnormality (by evaluating the number of voxels that fall above a predefined Z value) and the dichotomization of subjects as “+” or “-” for AV-45 uptake will be used as variables in analyses.

**Mathis Laboratory (University of Pittsburgh):** The key analyses in this laboratory will quantitatively compare outcome measures of the currently widely used [11C]PIB amyloid tracer to those of the proposed [18F]AV-45 tracer in the same subjects. There are currently 89 ADNI subjects (18 NC, 55 MCI and 16 AD) enrolled in a longitudinal [11C]PIB protocol at 12 ADNI sites who will return for follow up PIB scans during the GO grant. We will obtain [18F]AV-45 scans on all of these individuals within 6 months of the PIB study, which is an acceptable time interval given the minimal change in signal over durations of even 1-2 years of follow up [9]. These ADNI subjects will have had extensive PIB data (2-3 scans prior to the current scan) analyzed by this laboratory. Data will be analyzed using regions defined on an MCI template as we have done with ADNI PIB data, and then on a voxel level by using SPM to compare PIB and AV-45 data using a paired-test analysis. We will also compare results from the MCI template to results obtained in the Jagust lab using the AAL template. Analyses
will also be compared for atrophy-corrected data using coregistered MPRAGE MRIs smoothed to the final resolution of the PET images and correcting SUVR images on a voxel level.

3) **MRI CORE (PI: Clifford Jack):** MRI measurements of brain structure have been shown to demonstrate brain atrophy (which correlates with neuron loss) in MCI and AD and increasing rates of brain atrophy as subjects become more impaired. Therefore, structural MRI is used as a measure of the rate of disease progression, and possibly as a measure of treatment effect, in AD treatment trials. Major accomplishments of ADNI I were to establish uniform methods for data collection in large multisite trials, using scanners of different vendors, comparison of 1.5 and 3 Tesla, and comparison of several different image analysis methods. Structural MRI (MPRAGE/IRSPGR) will continue in the GO grant and the data will be used both as a measure of the rate of change as well as a predictor of future change, in EMCI and in the LMCI and NC from ADNI I. Cerebro-vascular disease (especially white matter lesions (WMLs)) will be assessed with FLAIR. Recently, iron imaging especially micro bleeds (T2* GRE); has been used in anti-amyloid clinical trials, because of the association of microbleeds with anti-amyloid therapy; this will be measured with T2* GRE. Finally, there is increasing interest in ASL MRI perfusion imaging since it is thought to provide similar functional information to FDG PET. A subset of subjects will be studies with ASL.

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

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

Important differences between the GO grant and ADNI include a “two track” approach whereby NC and MCI subjects carried forward from ADNI into the GO grant are scanned with the ADNI II protocol on the existing ADNI 1.5T scanner at that site in order to maintain optimum longitudinal consistency in this longitudinal cohort. Subjects newly enrolled into the GO grant will be scanned using a more modern and also expanded protocol which includes the following features. Where possible, scanning will be done at 3T. The 3D T1 weighted sequence will be performed with acceleration on 3T multi channel systems, and on 1.5T 32 channel systems. Whenever possible, we will use the ADNI I1 MP-RAGE for the structural T1-weighted scan. Due to MP-RAGE IP issues, we will employ a closely-related product pulse sequence (e.g. 3D IR-FSPGR sequence on GE systems) if necessary, while retaining the MP-RAGE sequence on Siemens/Philips systems. The dual-echo fast spin echo sequence that was used for vascular pathology detection in ADNI will be replaced by a T2-weighed FLAIR, which will be accelerated using parallel imaging by a factor of 2 at 3T. A T2*-weighted gradient echo (GRE) sequence for iron/micro hemorrhage detection. It’s not currently possible to perform a multisite arterial spin labeling (ASL) study with a uniform acquisition sequence across platforms. Therefore an ASL sequence will be added, only for subjects imaged on Siemens 3 T Trio and Verio scanners using uniform product sequences, producing comparable data across sites. Current results from ADNI indicate no consistent or significant differences at 3T vs. 1.5T for structural MRI. Therefore, newly enrolled subjects will be scanned at 3T and will enable acceleration of 3D T1 weighted and FLAIR acquisitions. Future clinical trials will likely include both 1.5T and 3T MRI, emphasizing the importance of our approach and comparisons between field strengths. After careful consideration we are not including resting BOLD fMRI or DTI because of lack of standardization, minimal longitudinal data demonstrating diagnostic value, and questionable relevance to clinical trials.

**Image Corrections:** All 3D T1 weighted images acquired for the GO grant will undergo correction for image nonuniformity, warping due to gradient nonlinearity, and scaling change over time, using an automated pipeline tested and validated on over 1,000 MRI studies obtained at the Mayo Clinic. Our results document tangible benefits of these image correction steps. Correcting image scaling changes results in a reduction of the needed sample size to detect a 25% rate reduction in AD subjects of 10-12% when compared to scans in which gradient scaling changes have not been corrected. Similarly, correction of gradient nonlinearity results in a reduction in the sample size needed to detect a 25% rate reduction in NC, MCI, and AD subjects of 9-10%,
when compared to the same scans in which gradient and warping has not been performed. Finally, offline filtering to reduce intensity non-uniformity results in a significant improvement in the precision of longitudinal measurements, particularly at 3T and on images acquired on multi array coils.

**Image Analysis:** While several methods have been used to analyze structural MRI to measure rates of change of brain structure, there is no agreement concerning the relative value of these methods. Therefore a major goal of ADNI1 and GO has been to compare the results of different analysis methods. Conclusions from initial analysis of ADNI1 data are: The best performing measures in terms of longitudinal precision (smallest sample size needed to detect a 25% rate reduction in AD and MCI subjects) were rates of hippocampal atrophy using Freesurfer software and temporal lobe atrophy using tensor-based morphometry. The longitudinal measures with the greatest correlations with longitudinal change in general cognition (ADAS cog) were the whole brain and ventricular BSI measures and also Freesurfer ventricular volume expansion. Therefore, all images existing in ADNI and new MRIs acquired by the GO grant will be analyzed by the following different processing groups (several groups in ADNI have been dropped because of low statistical power and the need to focus efforts).

A) **Tensor-based morphometry** (Paul Thompson) 3D maps of rates of atrophy (as a percent per year) will be derived by fluid registration of each follow-up scan to the baseline scan using an extensively validated nonlinear image registration algorithm [10, 11]. After fluidly aligning maps of annualized atrophic rates to a geometrically-centered mean anatomical template, we will fit general linear models and longitudinal mixed models to map voxel-by-voxel correlations with diagnosis, cognitive decline, and pathological biomarkers, and false discovery rates (FDR) will be used for multiple comparisons correction. To boost power, numeric summaries of atrophic rates will be computed in a statistically pre-defined region-of-interest within the temporal lobes showing greatest effect sizes in independent training data.

B) **Cortical thickness and subcortical ROIs** (Michael Weiner/Norbert Schuff) will be performed with the probabilistic-based FreeSurfer (FS) software. The results obtained from ADNI thus far show that hippocampal volume loss over time, measured by FS, has extremely high statistical power to detect potential treatment effects. These efforts using FS will be assisted by Dr. Bruce Fischl of MGH, one of the primary developers of FS, who will be available for consultations related to FS process optimization. In short, the FreeSurfer pipeline consists of five stages: an affine registration with Talairach space, an initial volumetric labeling, bias field correction, non-linear alignment to the Talairach space, and a final labeling of the volume. The fully automated labeling of volumes is achieved by warping a population based brain atlas to the target brain and by maximizing an a-posteriori probability of the labels given specific constraints. A full description of the FreeSurfer processing steps can be found in Ref [12]. The procedures have been extensively validated. Volume measurements of about 96 anatomical brain regions will be computed, including the hippocampus, which showed the most prominent volume change in previous studies [13]. In addition to volumes, thickness, curvature and other geometric measures will be computed for cortical regions. For longitudinal measurements of change, a Markov chain based protocol will be applied [13], in which past measurements are used as priors for current measurements. We have demonstrated that a Markov chain approach can drastically reduce within subject variability in longitudinal data, increasing the sensitivity to detect volume change.

C) **Ventricular and whole brain boundary shift integral (BSI)** (Nick Fox) measures on 3D T1 weighted (T1-w) images from the EMCI cohort. Each individual’s 3D T1-w image will have the brain and ventricular regions delineated using a validated semi-automated method to give an approximate volume for each time point. Follow-up scans will be affine registered to baseline incorporating scaling and differential bias corrections. Brain and ventricular volume change will be measured using the BSI. The BSI calculates the 3D integral of change in the boundary of the region of interest directly from the registered, normalized and subtracted images and has been shown to be sensitive to early neurodegenerative loss [14] and applicable to intervals of only 6 months. In addition, we will investigate hippocampal BSI and non-linear registration (fluid) [15] to track change within the medial temporal lobe. These exploratory analyses will assess change based upon baseline hippocampal regions derived from other processing labs and also explore the use of other automated template-based region definition [16].

D) **Structural Abnormality iNDex (STAND)** (Clifford Jack) STAND is an algorithm that extracts atrophy information from individual patient’s 3D T1 MRI scans and assigns a continuous STAND-score to the scan based on the degree of atrophy in comparison to patterns extracted from a large library of clinically well characterized subjects’ MRI scans [17]. Algorithm training and voxel section is based on a linear support vector machine classifier. In Mayo subjects who underwent ante mortem MRI and went on to autopsy, the rank correlation between Braak NFT stage and STAND-scores was 0.62 (p<0.0001) [18]. Analyzing ADNI data...
using this method, the correlation with clinical group membership, general cognitive performance and functional performance was better with STAND than CSF biomarkers (Aβ 42, t-tau, p-tau). The ability to predict future progression from MCI to AD in ADNI was better with STAND than CSF biomarkers.

E) Cerebrovascular disease and white matter lesions (Charles DeCarli) Measures of white matter lesions (WMls) will be quantified using the same methods that were applied to ADNI. All base protocol sequences will be used for detection of white matter hyperintensities (WMH) and for manual infarct ratings; i.e., the 3DT1 and DSE sequences for subjects carried over from ADNI 1, and the 3DT1 and FLAIR sequences for new subjects scanned with the 3T ADNI GO protocol. The validated, fully-automated WMH detection method aligns the imaging data to an elderly template image, where WMHs are identified on a per-voxel basis based on image intensities and prior knowledge of the probability of WMH occurrence at each location in the brain [19]. A trained and validated expert will determine the gross locations, sizes, and etiologies of MRI-evident infarcts using the same reliable, repeatable protocol that has been used for ADNI and a variety of other studies, including the Framingham Heart Study.

F) Abnormal tissue iron deposits (Microbleeds) (Clifford Jack) Pulse sequence options are T2* GRE or susceptibility weighted images (SWI or SWAN). The number and location of microbleeds and other abnormal iron deposits will be quantified by visual review and entered into a data form. Preliminary data indicate that up to 20% of subjects entered into typical AD therapeutic trials may have such abnormal features.

G) Quantitative analysis of perfusion ASL MRI (Clifford Jack/Norbert Schuff/Michael Weiner) ASL MRI detects regional pattern of reduced brain perfusion in AD and MCI [20]. Furthermore, alterations in brain atrophy and hypoperfusion in AD can be discordant [21], suggesting that ASL-MRI provides information complementary to structural MRI. The goal of this sub study is to demonstrate feasibility of ASL-MRI in a multisite setting and to compare the results to FDG PET. To reduce variability in ASL-MRI data, the processing for this study will include: 1) co-registration of ASL-MRI to the corresponding structural MRI data by fluid registration algorithm to correct for brain atrophy and gray/white matter partial volume effects; 2) intensity normalization to reduce distortions from the RF bias field, 3) intensity calibration to the global mean value of perfusion to account for global variations in cerebral blood flow from scan to scan and 4) spatial normalization to MNI brain atlas space using nonlinear transformations to perform voxelwise group analyses. We will tests voxelwise change of ASL perfusion over time, and compare the results with FDG PET, using an in-house developed software package written in R (http://www.r-project.org/) and initially designed for that includes mixed effects statistical libraries to account for within subject variations over time [22]. In addition, we will perform longitudinal ROI analysis of perfusion change, and compare with FDG PET.

4) BIOMARKER CORE (Pls: John Trojanowski and Leslie Shaw): Because of substantial interest concerning biochemical analysis of plasma and CSF to identify early AD pathology and assess risk for future progression, this Core will continue measurements of amyloid and tau species begun in ADNI-1 [23] Data will be used to test biostatistical hypotheses. This Core will coordinate all aspects of biofluid collections, sample labeling, documentation of all samples to assure identification accuracy/sample integrity, report discrepancies and assure compliance with stated volumes. Plasma will be collected at each visit and CSF will be collected annually on ~60% of subjects. Functions performed for each sample will be: log into the new LDMS location created specifically for the ADNI GO EMCI subject’s plasma and CSF samples and aliquots prepared from these samples. Once aliquote into polypropylene aliquot tubes and labeled, all samples will be transferred into appropriate racks, locations, in -80 °C freezers with tracking of samples via LDMS software. Upon completion of the ongoing validation study for the xMAP-Luminex/Innogenetics immunoassay for plasma Aβ1-42/1-40 the assay will be implemented for the 800 ADNI subjects’ plasma samples collected at the BASELINE visit and their YEAR 1 visit (total of 1600 plasma samples, not funded by the ADNI1). The BASELINE and YEAR 1 plasma samples collected from the EMCI study group will be assayed for plasma Aβ1-42/1-40 using the Luminex/Innogenetics immunoassay (400 samples). All CSF samples collected from the ~ 60% of consented EMCI subjects at baseline and 1 year will be assayed for Aβ1-42, t-tau and p-Tau181p using the xMAP-Luminex/Innogenetics assay system extensively validated in the ADNI study (240 samples). Every analytical result for plasma Aβ1-42/1-40 and CSF Aβ1-42, t-tau and p-Tau181p will be reviewed and quality controlled by a second individual assigned to this task in the Biomarker Core to insure the quality of each individual result and the accuracy of data transfers from the Luminex analyzer to the working database. Concerning estimations of diagnostic utilities for each biomarker test, methods and procedures to be used here are now well defined, up and running in the Biomarker Core as evidenced by several recent publications from ADNI-1 [23] and publications on optimal methods to measure and interpret plasma Aβ1-42/1-40 using the Luminex/Innogenetics immunoassay [24]. The importance of these measurements is that our proposed model, and recent reports in
the literature, suggests that changes related to Aβ (both CSF and plasma measures as well as amyloid imaging with PET) may be followed by tau tangle formation associate with a rise in CSF tau species. Biomarker data will be integrated with clinical and imaging data to elucidate pathophysiological steps in the onset of EMCI and MCI and their progression to AD and to improve methods for early detection of AD and inform clinical trial design with high statistical power.

5) GENETICS CORE (PI: Andrew Saykin): Genetic variation plays a strong role in risk for developing AD [25]. However, despite numerous genetic studies of AD, there has been no previous study of any size which examines genetic risk factors for rates of progression. Therefore, a high density genome-wide association study (GWAS) with over 600,000 markers was performed on the DNA of the ADNI cohort (818 samples) and publicly released on the UCLA/LONI/ADNI/website. Preliminary studies show promising results of identifying genetic effects on brain integrity using imaging as phenotypes. The Genetics Core provides a central point of communication linking the many parties within and outside of ADNI interested in the interface of genetics, brain imaging and dementia. The Core has the following plan for sample processing, genotyping and dissemination: (1) receive and bank blood samples, extract DNA, and perform ApoE genotyping for the proposed 200 new early MCI (EMCI) participants; (2) develop and store immortalized cell lines on the new sample; (3) perform genotyping on the new sample using the same Illumina Human 610 Quad array as ADNI; (4) perform quality control, sample verification and organization and work with the Informatics Core to make these genotypes rapidly available to the scientific community; (5) disseminate cell line derived DNA. Furthermore a control, sample verification and organization and work with the Informatics Core to make these genotypes publicly released on the UCLA/LONI/ADNI/website. Preliminary studies show promising results of identifying genetic effects on brain integrity using imaging as phenotypes. The Genetics Core provides a central point of communication linking the many parties within and outside of ADNI interested in the interface of genetics, brain imaging and dementia. The Core has the following plan for sample processing, genotyping and dissemination: (1) receive and bank blood samples, extract DNA, and perform ApoE genotyping for the proposed 200 new early MCI (EMCI) participants; (2) develop and store immortalized cell lines on the new sample; (3) perform genotyping on the new sample using the same Illumina Human 610 Quad array as ADNI; (4) perform quality control, sample verification and organization and work with the Informatics Core to make these genotypes rapidly available to the scientific community; (5) disseminate cell line derived DNA. Furthermore a comprehensive, hypothesis-guided and data-driven mapping analysis will be performed using both genetic data and the multidimensional phenotypic data collected on the ADNI1/GO cohort. The Core will perform comprehensive analysis of genetic influences on baseline data in order to identify novel risk markers and validate established markers that predict conversion and progression. Examples include diagnostic class and continuous neuropsychological measures, clinical rating scales, structural, functional, and molecular neuroimaging variables and phenotypes, as well as other biomarkers from blood, urine and CSF assays. Further the Core will perform comprehensive analysis of genetic influences on longitudinal data, which heretofore has not been possible on such a large scale. This has the potential to identify genetic features associated with rate and characteristics of progression and ultimately of treatment response. The core will also evaluate copy number variation (CNV) features related to diagnosis and other phenotypes, and complete preliminary analyses of the new EMCI cohort during year two of the GO project. The third plan of the core is to serve as a central resource, point of contact and planning group for genetics in ADNI, providing organizational and informational resources for investigators, industry partners and other parties interested in analyses of the ADNI genetics data, including conference calls for working groups and interested parties, and liaison to the recently funded NIA AD Genetics Consortium, World Wide ADNI investigators collecting and analyzing GWAS data, and other cohort studies that could serve as replication samples or collaborate in other synergistic research activities. The core will formulate potential post genomic screening analyses, emphasizing functional annotation of SNPs/genes, CNV regions, and functional pathway analyses to place emerging results within a systems biology perspective to guide further assays and analyses. The core will also identify future directions such as targeted deep sequencing based on the emerging GWA studies within ADNI and beyond as well as gene expression, gene-gene interaction, and epigenetic analyses. The data from the Genetics Core will not be directly used for hypothesis testing, but is exploratory in nature. Nevertheless, the high value of the genetics data is the opportunity for correlation with longitudinal clinical/cognitive/biochemical and imaging data.

6) NEUROPATHOLOGY CORE (PIs: John Morris and Nigel Cairns): The ultimate validation of AD diagnosis is pathology. The ADNI-Neuropathology Core (ADNI-NPC) objectives are : 1) providing and implementing training materials/protocols to assist clinicians in obtaining voluntary consent for brain autopsy. Protocols are established for obtaining autopsy consent and neuropathology services at all sites. 2) maintaining a central laboratory to provide uniform neuropathological assessments in all autopsied participants in accordance with standard criteria and to promote clinical-neuroimaging-neuropathological correlations; 3) determining the relationship between the molecular neuropathology, structural, and functional changes, especially the [11C]Pittsburgh Compound-B (PIB) amyloid imaging (PIB-PET) and Avid Radiopharmaceuticals compound [18F]AV-45; 4) maintaining a state-of-the-art resource for fixed and frozen brain tissue obtained from autopsied ADNI participants to support the Biomarker Core, and to provide investigators with access to the tissue and data for research purposes; and 5) interacting with the Biostatistics Core to ensure appropriate entry of the Core's data into ADNI's database, promote data sharing and collaborative research. The NPC does not interfere with or supersede neuropathological activities at any ADNI site. It uses brain tissue obtained at the
sites to provide a uniform neuropathological assessment to support the clinical classifications and ADNI/GO aims. Thus far there have been 13 deaths of ADNI participants, 8 of whom have come to autopsy (61.5%).

7) INFORMATICS CORE (PI: Arthur Toga): The overall goal of the Informatics Core is to provide all the raw, preprocessed and post processed ADNI1/GO data to the worldwide scientific community without embargo, in an organized and user friendly fashion. Medical image data from all modalities collected for this study, both from the 200 new EMCI subjects and the approximately 530 ADNI subjects undergoing [F]AV-45 and FDG scanning, will be sent to the LONI archive where they will be centrally stored and made available to qualified investigators. The archive, which already contains the raw and processed image data from ADNI1, will be customized for the GO project as necessary to ensure validity of entered subject information and proper de-identification of the image data. Mechanisms for integrating quality control results will be implemented, and the existing database will be expanded to support the specific needs of the GO project. This expansion will involve the integration of genetic and other non-imaging data as well as results of the [F]AV-45 analysis. The archive application will be expanded to improve data discovery providing users with more flexibility and control in finding and interacting with the stored information. Additionally, the informatics team will develop mechanisms for bridging between the ADNI2 and GO projects such that qualified investigators will have access to data spanning both projects. The existing viewing tool will be modified to better represent four dimensional datasets such as F18 Amyloid PET. The release of all data through the LONI website has been a major factor in the widespread use of ADNI data leading to the success of the GO/ADNI projects.

8) BIOSTATISTICS CORE (PI: Laurel Beckett): The Biostatistics Core, in collaboration with other GO investigators will address the specific aims of GO through analysis of data on the new EMCI cohort (Aim 1) combined with newly collected data from [F]AV-45 amyloid imaging on ADNI1 and the EMCI group (Aim 2), from extended longitudinal follow-up of ADNI 1 (Aim 3) and from newly processed image and biochemical biomarker data collected in ADNI1 (Aim 4). Data analysis will combine existing longitudinal ADNI1 data (including data available on AD subjects) with the newly collected data to provide extended longitudinal follow-up on NC and MCI subjects and greater power. Cognitive, clinical, and structural MRI data is already available through ADNI1 from at least three time points for NC, LMCI, and AD subjects and is planned as part of the ADNI-GO data collection for EMCI at three time points. In addition, FDG-PET data are available on approximately 50% of the NC, MCI, and AD subjects in ADNI1 from at least three time points. CSF from at least two time points will be is now available 50-60% of the subjects on ADNI1 and will be forthcoming from the EMCI in GO. AV-45 amyloid imaging will only be available at a single time point for the subjects included in ADNI-GO. Below we operationalize the three global hypotheses (GH) from Specific Aims into specific testable hypotheses about the imaging and biochemical biomarkers, clinical grouping and conversion, and cognitive change, as follows:

**GH1: Reductions of CSF Aβ and increased amyloid PET tracer retention (C-11 PIB and AV-45) are present in some cognitively normal asymptomatic individuals, indicating early stage AD neuropathology.**

1.1. In cross sectional analysis, CSF Aβ levels and amyloid tracer retention (PIB, AV-45) will differ across groups, with NC having the highest CSF Aβ levels and lowest F18 amyloid tracer retention with the AD subjects having the lowest CSF Aβ and highest F18 amyloid tracer retention, and the two MCI groups in between (CSF Aβ: NC > EMCI > LMCI > AD; amyloid: NC < EMCI < LMCI <AD) moderated by ApoE genotype. ApoE4 prevalence in EMCI subjects will be between NC and LMCI. CSF Aβ levels and amyloid tracer retention may be moderated by ApoE4 genotype by cerebrovascular disease (CVD measured as white matter lesions (WML) from FLAIR MRI and by microbleeds (from T2* MRI).

1.2. CSF Aβ levels will decline on average longitudinally, with rates consistent with an S-shaped curve across the course of disease, beginning in some asymptomatic people, changing most rapidly well before the onset of dementia and moderated in part by CVD, microbleeds, and ApoE4 genotype.

1.3. Measures of positivity for AV-45 (across PET analysis labs), C-11 PIB measures (Mathis lab), and CSF Aβ will be highly correlated. Change in PIB will correlate with change in CSF Aβ.

**GH2: Subsequently, CSF tau increases accompanied by reduced brain glucose metabolism (FDG-PET) and an increased rate of medial temporal lobe atrophy (MRI)**

2.1. Cross sectionally, CSF tau, FDG PET, and medial temporal lobe volume will differ across diagnostic groups showing worse levels on average in the AD subjects relative to the NC with the EMCI and LMCI in between (CSF tau: NC < EMCI < LMCI < AD; FDG-PET and medial temporal lobe volume: NC > EMCI > LMCI > AD). Differences in these measures will be less between NC and EMCI and greater between LMCI and AD, compared to the CSF Aβ and amyloid PET measures reflecting a later development in the disease process;
differences in levels measured by FDG PET between AV-45+ and AV-45- people will differ across diagnoses, with NC showing the smallest differences between AV-45+ and AV-45+ individuals (NC < EMCI < LMCI).

2.2. Longitudinally, CSF tau, FDG PET, and medial temporal lobe volumes will show declines that begin after changes of CSF Aβ and amyloid PET tracer retention; presence of ApoE4 alleles, WMLS reflecting CVD, and microbleeds will increase the rates of decline.

2.3. CSF tau, FDG PET and medial temporal lobe volume will correlate both cross-sectionally and longitudinally, consistent with a pattern of concurrent change.

GH3: After changes in CSF Aβ, amyloid PET tracer retention, CSF tau, FDG PET and atrophy of the medial temporal lobe, signs and symptoms of cognitive decline appear (moderated by Apoe4 and CVD), eventually progressing to dementia.

3.1. Rates of cognitive decline will increase across the diagnostic categories (NC < EMCI < LMCI < AD), with EMCI intermediate between the NCs who will show little decline and the LMCI, and moderated by Apoe4 genotype, microbleeds, and CVD.

3.2. Medial temporal lobe atrophy measures (cross-sectional and longitudinal) are correlated with an increased rate of decline in cognitive and functional measures in EMCI, LMCI and AD, and with a greater risk of conversion from LMCI to dementia.

3.3. Medial temporal lobe atrophy measures will be more proximal to, and thus more strongly correlated with, cognitive decline and conversion from LMCI to AD than CSF Aβ or amyloid PET measures, but not more proximal than CSF tau or FDG PET measures. In the EMCI and LMCI cohorts, but not in the mild AD cohort, measures of brain amyloid (CSF and amyloid PET tracer retention) will be associated with subsequent cognitive changes.

Finally, although not hypothesis testing, the data from GO/ADNI will be used to construct various clinical trial designs (for both proof of concept and registration trials) which use GO/ADNI clinical/cognitive data and imaging and blood/csf biomarkers for subject selection, as covariates in the linear model, and as outcome variables.

Our analytic strategy will have four phases.

Phase I is description of longitudinal trajectories within and across the full clinical spectrum. In this phase, we will use a mixed model “growth-curve” approach to characterize the range of longitudinal paths followed by imaging and biochemical markers among those who start as normal, LMCI and AD. We will use these models to estimate the location of EMCI participants within the spectrum on different measurements hypothesized to correlate with disease progression, and test whether they are indeed intermediate (Hypotheses 1.1, 2.1). We will determine the extent to which heterogeneity in trajectories can be accounted for by age, the presence of ApoE4 alleles, evidence of cerebrovascular disease (measured from FLAIR), and microbleeds (measured from T2* MRI) and other demographic and genetic variables (Hypothesis 1.2). We will characterize the shape of the underlying trajectories of marker change and assess whether it is consistent with that hypothesized in hypotheses 1.2 and 2.2. We will assess the correlations of CSF Aβ, PIB, AV-45 (G1.3) and other marker relationships using standard statistical measures of agreement (hypotheses 1.3).

Phase II is testing of hypotheses about the pathophysiological sequence of human AD. We will build on the longitudinal models developed for separate markers in Phase I, which will be used to help quantify the relative level of progression represented by specific imaging and fluid biomarker values. We will combine our previous models to fit multivariate longitudinal models for correlated data [26]. We will assess the degree to which the level of progression in one marker precedes or lags that in another, or is concurrent, in the relevant diagnostic categories (hypotheses 2.2, 2.3). We will assess the role of ApoE genotype, microbleeds, and cerebrovascular disease by adding covariates.

Phase III will test hypotheses about the correlation of imaging and fluid biomarkers to cognitive decline or conversion to EMCI, LMCI or dementia. One testing strategy will use mixed models for longitudinal cognitive function data (AD, LMCI, EMCI, and possibly NC if longer follow-up reveals more change than in the first 24 months, Hypothesis 3.1) and will test the cognitive change hypotheses of 3.2. A second strategy will fit interval-censored survival models for time to conversion from LMCI to AD (and from normal to EMCI or LMCI if sufficient conversions take place). In each of these models, we will test hypotheses about the pathophysiological steps by assessing the role of biomarker baseline levels and rates of change as predictors and as mediators in the models. We will test which markers are more proximal to the clinical outcomes (mediating variables) by including multiple markers as predictors; a marker that mediates in the pathophysiology would be more strongly correlated with outcome, assuming comparable levels of measurement error (3.3). The analyses of Phase III will potentially offer supporting evidence for the sequence
of changes by showing that one biomarker not only reaches more advanced stages earlier, but also that its effects on cognition and conversion are mediated by a hypothesized later stage of progression.

Phase IV will assess the feasibility of conducting proof of concept and pivotal trials of disease-modifying agents using continuous measures of cognition (e.g., ADAS-cog12) and clinical status (e.g. CDR-SB) in an enriched, high-risk MCI subgroup defined by amyloid biomarkers. We expect that the initial one-year longitudinal EMCI data will confirm the feasibility of disease-modification trials in the subjects defined by CSF Aβ, amyloid PET tracer retention or other biomarkers. To verify these hypotheses, a number of trial designs will be evaluated, including various selection criteria and covariates; various continuous and dichotomous outcome measures; and linear, non-linear and survival regression models.

Sample size and power: Although space does not permit a detailed power analysis for each of the clinical/cognitive/CSF/blood/PET/MRI measurements, the ADNI-GO analyses will have excellent power for all the comparative and correlational hypotheses described above through the combination of the ADNI1 and the ADNI-GO data. For overall comparisons across the 4 diagnostic groups, we will have >80% power to detect a difference of 0.33 standard deviation (SD) units in level (cross-sectional hypotheses 1.1, 2.1) and a difference of 0.35 SD units in rates of change (longitudinal hypotheses 1.2, 3.1) between LMCI and NL with EMCI in between biomarkers measured on all participants, and >80% power to detect a difference of 0.48 SD in level and 0.5 SD units in change for CSF biomarkers ascertained on an approximate 50-60% subsample (0.35 SD in level and 0.38 SD units in 3-group rates of change using all subjects and 0.5 SD units in level and 0.53 SD units in change if AD is included for a four group comparison; differences are between NC and mild AD with EMCI and LMCI in between). Correlational studies will have >80% power to detect an association accounting for 1.1% of the variation for analyses across the entire group and 2.2% of the variation in a 50-60% subsample. Subgroup (within a diagnostic category) correlational analyses will still have > 80% power to detect an association accounting for 5% of the variation in the outcome, with the exception of the analyses using the CSF biomarker data, which will have >80% power to detect an association accounting for 9% of the variation.

Timeline, Milestones, Expected Measurable Outcomes and Deliverables: Our timeline for the proposed research is as follows: Upon notification of a favorable priority score (we will not wait for NOA), our Clinical Core will immediately prepare new Protocols and Consent Forms which will be modifications of existing Protocols and Consent forms at the sites (2-3 weeks), which will be sent to the 50-60 performance sites and subcontracts will be initiated. Simultaneously, regulatory procedures for FDA approval of incorporation of AV-45 into the ADNI protocol will begin. Based on our experience, the average time for approval at the sites is 3 months. Thus we expect that enrollment of new EMCI subjects would begin in Oct-Nov 2009 and would be completed by Oct 2010, allowing for 1 yr follow-up. At the same time, Avid Radiopharmaceuticals will be prepared for delivery of [18F]AV-45 to the PET centers as soon as the consent forms are approved and signed by subjects. Thus [18F]AV-45 PET scanning of all existing ADNI subjects and newly enrolled EMCI subjects can be completed by the end of 2011. GO funds will provide for MRI, PET, CSF/plasma analysis to be completed prior to Sept 1, 2011 so that the Biostat Core can finish all of the analyses by Oct 1, 2011. The specific deliverables will be: 1) all data will be available on the LONI website without embargo for use by the worldwide scientific community 2) the stated hypotheses will be tested and reported 3) clinical trial designs will be developed and validated. The results should significantly advance use of biomarkers for early detection, diagnosis, and as outcome measures in clinical trials, leading to more effective treatment/prevention of AD.

Long Term Sustainability Plan: The new infrastructure support in this application is the delivery of [18F]AV-45 to the PET centers, which will provide a long-term, sustainable infrastructure for amyloid imaging technology in academic medical centers. The long term follow-up using clinical/cognitive, plasma/CSF, MRI and PET of the EMCI (newly enrolled) and LMCI and NC (being carried forward from ADNI) rests in a successful renewal of the ADNI grant. The application will be submitted Nov 1 2009. We believe that the perceived success of ADNI1 evidenced by numerous publications, abstracts, development of ADNI-like projects in other countries, use of ADNI data by many investigators outside of ADNI, use of ADNI data for design/power of clinical, trials by academia and industry all suggest that the ADNI renewal will be favorably reviewed. The Foundation for NIH is currently in active fund raising discussions with the ADNI industry and non profit partners regarding ADNI2 and feel that it is highly likely that $20 million will be provided by the private sector. This will be in addition to the $40 million that will be requested from NIA for ADNI2, bringing the total requested funds (direct plus indirect) for ADNI2 to $60 million. The current organizational structure of ADNI will be continued for the GO grant and in ADNI2.
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