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This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix 1. Alzheimer’s Disease Neuroimaging Initiative Coinvestigators

Paul Aisen, MD (UC San Diego, ADCS PI and Director of Coordinating Center Clinical Core, Executive Committee); Clifford R. Jack, Jr., MD (Mayo Clinic, Rochester, Core PI MRI, Executive Committee); Arthur W. Toga, PhD (UCLA, Core PI Informatics, Executive Committee); Laurel Beckett, PhD (UC Davis, Core PI, Biostatistics, Executive Committee) Anthony Gamst, PhD (UC San Diego, Executive Committee); Holly Soares, PhD (Pfizer, Chair, Industry Scientific Advisory Board [iSAB]); Robert C. Green, MD, MPH (Boston University, Chair, Data and Publication Committee [DPC]); Tom Montine, MD, PhD (University of Washington, Chair, Resource Allocation Review Committee); Ronald G. Thomas, PhD (UC San Diego, Clinical Informatics and Operations); Michael Donohue, PhD (UC San Diego, Clinical Informatics and Operations); Sarah Walter, MSc (UC San Diego, Clinical Informatics and Operations) ; Donald Dale, PhD (UC San Diego, MRI); Matthew Bernstein, PhD, (Mayo Clinic, Rochester, MRI); Joel Felmlee, PhD (Mayo Clinic, Rochester, MRI); Nick Fox, MD (University of London, MRI); Paul Thompson, PhD (UCLA School of Medicine, MRI); Norbert Schuff, PhD (UCSF MRI, MRI); Gene Alexander, PhD (Banner Alzheimer’s Institute, MRI); and Charles DeCarli, MD (UC Davis, MRI). Dan Bandy, MS, (CNMT Banner Alzheimer’s Institute, PET); Kewe Chen, PhD, (Banner Alzheimer’s Institute, PET); John Morris, MD (Washington University, St. Louis, Neuropathology Core); Virginia M.-Y. Lee, PhD, MBA (UPenn School of Medicine, Biomarkers); Magdalena Korecka, PhD (UPenn School of Medicine, Biomarkers); Karen Crawford (UCLA, Informatics); Scott Neu, PhD, (UCLA, Informatics); Danielle Harvey, PhD; (UC Davis, Biostatistics); John Kornak, PhD (UC Davis, Biostatistics); Andrew J. Saykin, PsyD (Indiana University Genetics); Tatiana M. Foroud, PhD (Indiana University, Genetics); Steven Potkin, MD (UC Irvine, Genetics); Li Shen, PhD (Indiana University, Genetics); Neil Buckholtz, PhD (National Institute on Aging/National Institutes of Health); Jeffrey Kaye, MD (Oregon Health and Science University, site investigator); Sara Dolen, BS (Oregon Health and Science University, site investigator); Joseph Quinn, MD (Oregon Health and Science University, site investigator); Lon Schneider, MD (University of Southern California, site investigator); Sonia Pawluczyk, MD (University of Southern California, site investigator); Bryan M. Spann, DO, PhD (University of Southern California, site investigator); James Brewer, MD, PhD (University of California-San Diego, site investigator); Helen Vanderguag, RN (University of California-San Diego, site investigator); Judith L. Heidebrink, MD, MS (University of Michigan, site investigator); Joanne L. Lord, LPN, BA, CCRC (University of Michigan, site investigator); Ronald Petersen, MD, PhD (Mayo Clinic, Rochester, site investigator); Kris Johnson, RN (Mayo Clinic, Rochester, site investigator); Rachelle S. Doody, MD, PhD (Baylor College of Medicine, site investigator); Javier Villanueva-Meyer, MD (Baylor College of Medicine, site investigator); Munir Chowdhury, MS (Baylor College of Medicine, site investigator); Yaakov Stern, PhD (Columbia University Medical Center, site investigator); Lawrence S. Honig, MD, PhD (Columbia University Medical Center, site investigator); Karen L. Bell, MD (Columbia University Medical Center, site investigator); John C. Morris, MD (Washington University, St. Louis, site investigator); Mark A. Mintun, MD (Washington University, St. Louis, site investigator); Stacy Schneider APRN, BC, GNP (Washington University, St. Louis, site investigator); Daniel Marson, JD, PhD (University of Alabama - Birmingham, site investigator); Randall Griffith, PhD, ABPP (University of Alabama - Birmingham, site investigator); David Clark, MD (University of Alabama - Birmingham, site investigator); Hillel Grossman MD (Mount Sinai School of Medicine, site investigator); Cheuk Tang PhD (Mount Sinai School of Medicine, site investigator); George Marzloff BS (Mount Sinai School of Medicine, site investigator); Leyla deToledo-Morrell, PhD (Rush University Medical Center, site investigator); Raj C. Shah, MD (Rush University Medical Center, site investigator); Ranjan Duara, MD (Wein Center, site investigator); Daniel Varon, MD (Wein Center, site investigator); Peggy Roberts CNA (Wein Center, site investigator); Marilyn S. Albert, PhD (Johns Hopkins University, site investigator); Julia Pedroso, MA (Johns Hopkins University, site investigator); Jaimie Toroney, BA (Johns Hopkins University, site investigator); Henry Rusinek, PhD (New York University, site investigator); Mony J de Leon, EdD (New York University, site investigator); Susan M De Santi, PhD (New York University, site investigator); P. Murali Doraiswamy, MD (Duke University Medical Center, site investigator); Jeffrey R. Petrella, MD (Duke University Medical Center, site investigator); Marilyn Aiello BS (Duke University Medical Center, site investigator); Christopher M. Clark, MD (University of Pennsylvania, site investigator); Cassie Pham, BS (University of Pennsylvania, site investigator); Jessica Nunez (University of Pennsylvania, site investigator); Charles D. Smith, MD (University of Kentucky, site investigator); Curtis A. Given II, MD (University of Kentucky, site investigator); Peter Hardy, PhD

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Subjects and Methods

Participants

The Alzheimer’s Disease Neuroimaging Initiative (ADNI) is a multisite study using the modalities of amyloid-β (Aβ) imaging (with [18F] florbetapir), glucose metabolism (using [18F] fludeoxyglucose or FDG), MRI, cerebrospinal fluid (CSF), and cognitive measurements in aged controls, mild cognitive impairment (MCI) and Alzheimer disease (AD) patients. The study is supported by the National Institutes of Health, private pharmaceutical companies, and nonprofit organizations with approximately 50 medical center and university sites across the United States and Canada (http://adni.loni.usc.edu). Full inclusion/exclusion criteria are available at www.adni-info.org. To summarize, all participants were aged between 55 and 90 years, had completed at least 6 years of education, were fluent in English or Spanish, and had no further relevant neurological diseases.

All 401 ADNI MCI patients included in our study were enrolled in ADNI GO and ADNI2 and their baseline florbetapir and concurrent FDG PET scans were performed between September 2010 and May 2013. Assignment of MCI subjects the “early MCI” (EMCI) and “late MCI” (LMCI) groups was based on the patients’ education-adjusted scores on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) from the Wechsler Memory Scale – Revised (maximum score 25): EMCI diagnosis required a score of 3-6 (for 0-7 years of education), 5-9 (for 8-15 years of education), or 9-11 (for ≥ 16 years of education), while LMCI diagnosis required a score of ≤ 2, ≤ 4, or ≤ 8, respectively, for the same education categories.
Longitudinal cognitive function was assessed using the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) and total number of words correctly recalled on all five immediate recall trials of the Auditory Verbal Learning Test (AVLT).

**Florbetapir imaging and analysis**

Florbetapir ADNI data were acquired nationwide using various Positron emission tomography (PET) scanners at different sites. Briefly, images were obtained in four 5-minute frames 50 to 70 minutes after injection of approximately 10 mCi of \(^{18}\text{F}\) florbetapir; the four frames were co-registered to each other, averaged, interpolated to a uniform image and voxel size (160 x 106 x 96, 1.5 mm\(^3\)), and smoothed to a uniform resolution (8mm full width at half-maximum) to account for resolution differences between scanners \(^1\). Preprocessed florbetapir image data and co-registered structural MRI data were analyzed as follows: using Freesurfer version 4.5.0 \(^2\) MPRAGE scans of one structural 1.5T or 3T MRI scan within 2 months of florbetapir scans were segmented and parcellated into individual cortical regions, used to extract the mean florbetapir uptake from the gray matter of lateral and medial frontal, anterior, and posterior cingulate, lateral parietal, and lateral temporal regions.

Visual analysis was performed on axial, sagittal, and coronal slices, in an inverse gray scale, using software that permitted adjustment of image brightness and contrast to each reader’s specifications. Florbetapir positivity was defined as increased tracer uptake in cerebral cortex that was visually perceived as reduced or absent white matter/gray matter contrast in at least one cortical (frontal, parietal, temporal, occipital) region detectable on more than two adjacent scan slices. Reader 1 was first trained using an online electronic training tool produced by Avid Radiopharmaceuticals ([http://www.amyvidhcp.com/Pages/reader-training.aspx](http://www.amyvidhcp.com/Pages/reader-training.aspx)). Subsequently, both raters
independently read images of 37 amyloid-negative and amyloid-positive cases taken from a separate study of dementia; all these scans were obtained on a single PET scanner (a Siemens Biograph 6 PET/CT) from subjects recruited from a single site (the UCSF Memory and Aging center). These scans revealed very high interreader agreement ($\kappa = 0.83$); discrepant scans were subsequently reviewed together to achieve better conformity.

**Additional biomarkers**

Although nearly all subjects in this study underwent a single lumbar puncture (LP) at baseline, analysis of the CSF data for the entire sample was not complete at the time of analysis for this study (September 2014). Therefore we have included CSF Aβ1-42 data for 256 (64%) of all 401 MCI subjects that are currently available in this study. CSF Aβ1-42 was measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use–only reagents) immunoassay kit–based reagent 3-5. Analysis details and quality control procedures are available at [http://adni.loni.ucla.edu](http://adni.loni.ucla.edu). CSF aliquots were anchored to the baseline assay values derived from the original ADNI1 dataset to use the cutoff values for abnormal and normal Aβ1-42 established and validated for that assay 4,6.

Apolipoprotein E (APOE) genotyping was performed using DNA obtained from blood samples.

FDG image data were obtained at ADNI sites and were acquired 30 to 60 minutes postinjection, downloaded from the ADNI website after preprocessing (frame averaging, spatial alignment, interpolation to a standard voxel size, smoothing to a common resolution of 8 mm full width at half maximum), and then spatially normalized to standard $^{15}$O-H$_2$O PET template using SPM5 7. FDG mean uptake was determined within a set of
predefined meta regions of interest (metaROIs)\textsuperscript{8}. ROI-specific values were averaged and for each subject, a composite ROI measure (mean FDG) was generated from the mean of the metaROIs (right and left inferior temporal and lateral parietal regions, bilateral posterior cingulate-precuneus region) relative to the mean of a pons and cerebellar vermis reference region.

**Biomarker Cutoffs**

Florbetapir standardized uptake values ratio (SUVR) threshold is based on the upper limit of the 95%CI for the florbetapir distribution values in young healthy controls and is consistent with a separate autopsy-validated sample\textsuperscript{9,10}. CSF Aβ1-42 cutoff was derived from an autopsy validated baseline assay\textsuperscript{4}. The FDG threshold was deduced from a Receiver operating characteristic (ROC) analysis in a separate ADNI cohort including normal and AD subjects\textsuperscript{11}.

**Statistics**

Age, gender and education adjusted linear regression models were used to separately examine the main effect of a visual or quantitative florbetapir-positive baseline scan (dichotomous variable) on longitudinal cognitive function (ADAS-cog/AVLT modeled as continuous outcome at follow-up time points of 6, 12, 24, 36 months after florbetapir baseline scan). For those subjects remaining EMCI/LMCI regression models included all available cognitive scores determined during the whole observation period after the florbetapir scan, and for converters regression models included all follow-up time points when subjects were diagnosed with MCI and excluded all scores beginning at the visit at which dementia was diagnosed. Unstandardized regression coefficient B indicates cognitive score differences at baseline and at each follow-up time point for a florbetapir-positive baseline scan compared to a florbetapir-negative baseline scan.
Taking all cases, censored non-converters and censored converters into account, Kaplan-Meier plots were created to identify the distribution of the conversion rates over time for subjects with a florbetapir-positive and a florbetapir-negative baseline scan. Separate plots were generated for florbetapir data derived from visual analysis and SUVR measurements.

All analyses were performed using SPSS, version 22.0 and statistical significance was defined as $p \leq 0.05$. 
Results

Participants

Compared to non-converters, converters had poorer cognitive function at baseline \((p < 0.001)\), higher florbetapir SUVR values \((p < 0.001)\), lower CSF Aβ values \((p < 0.001)\), lower mean FDG values \((p < 0.001)\) and a higher frequency of APOEε4 carriers \((p < 0.001;\) Mann-Whitney test, \(\chi^2\) test).

Agreement between dichotomous analyses of Aβ biomarkers

Discordance between visual florbetapir analysis and SUVR measurements occurred in 53 participants and a visual inspection of all 53 discordant cases revealed 9 subjects with high focal and asymmetric cortical florbetapir retention, 8 with lower but preserved contrast between white and gray matter, 7 with global atrophy and 5 cases with poor image quality. An additional 13 exhibited florbetapir uptake in tissues that appeared to be non-cortical such as the venous sinus (see Figure 1).

There were no group differences between participants with discordant \((n=53)\) and concordant \((n=348)\) visual florbetapir ratings and florbetapir SUVR measurements with respect to gender, age, APOEε4 status, baseline cognitive function, mean FDG and florbetapir SUVR and CSF Aβ values \((p > 0.05;\) Mann-Whitney test, \(\chi^2\) test). Only 3 of the 53 subjects \((6\%)\) with discordant qualitative and SUVR amyloid image assessments converted to AD (see Figure 1C for an example).

ROC analysis revealed that the current SUVR cutoff \((1.11)\) yielded a sensitivity of 94\% and a specificity of 81\% \((\text{area under the curve (AUC) 0.96 [95\%CI 0.94-0.98]})\) relative to a Reference Standard using visually defined normal (florbetapir-negative) subjects from visually defined abnormal (florbetapir-positive) subjects. A slightly more conservative
SUVR threshold of 1.17 would define scans read as florbetapir-negative or florbetapir-positive with sensitivity and specificity of 90% relative to visual ratings.

To compare the sensitivity and specificity of the amyloid biomarkers under consideration (visual and quantitative amyloid PET analysis), an external reference to determine $A\beta$ positivity was needed. Taking here CSF $A\beta$ as the Reference Standard resulted in a higher sensitivity but lower specificity for SUVR measurements when compared to visual florbetapir PET analysis (supplemental Table 1).

Intermethod agreement between amyloid biomarkers was substantial even if more conservative CSF $A\beta$ cutoffs (174 pg/ml & 157 pg/ml $^{12}$) and a higher amyloid PET SUVR threshold (1.24 $^{12}$) were applied.

**Prediction of longitudinal cognitive function and MCI to AD conversion**

As indicated by standardized regression coefficients a positive florbetapir status, assessed by visual reads and SUVR measurements, was significantly associated with baseline and longitudinal cognitive function determined by ADAS-cog (supplemental Table 2). Unstandardized regression coefficients of age, gender and education adjusted linear regression models predicted significant differences on the ADAS-cog at baseline and all follow-up times between visual florbetapir-positive and visual florbetapir-negative MCI status (supplemental Table 2). Evidence was comparable for florbetapir SUVR measurements (supplemental Table 2), and for baseline AVLT and its longitudinal changes (data not shown).

Within the mean follow-up period of 1.6 years (all participants) 30 visual florbetapir-positive (all florbetapir-positive by SUVR) MCI subjects and 5 visual florbetapir-negative (all florbetapir-negative by SUVR) MCI cases converted to AD (supplemental Figure 1).
Kaplan-Meier analysis indicated that nearly 50% of the visual/quantitative baseline florbetapir-positive cases will convert to AD in around 2 years (supplemental Figure 1).

Of all 205 visual florbetapir-negative MCI subjects 7 (3 EMCI, 4 LMCI) progressed to AD (3%), representing 11% of all 61 converters. Of all 180 SUVR florbetapir-negative MCI subjects 8 (4 EMCI, 4 LMCI) progressed to AD (4%), representing 13% of all 61 converters. Of all 90 CSF Aβ-negative MCI subjects 4 (1 EMCI, 3 LMCI) progressed to AD (4%), representing 11% of all 37 converters with available CSF data. All 4 CSF Aβ-negative converters were also visual florbetapir-negative and SUVR florbetapir-negative. Of the 7 (8) visual florbetapir-negative (SUVR florbetapir-negative) converters, 6 (86%) were negative on both – visual florbetapir reads and quantitative florbetapir analysis.
eTable 1. Sensitivity, specificity and predictive values for A\(\beta\) negativity and A\(\beta\) positivity assessments by visual florbetapir analysis and florbetapir SUVR measurements considering CSF A\(\beta\) negativity and A\(\beta\) positivity estimates as Reference Standard.

<table>
<thead>
<tr>
<th></th>
<th>CSF A(\beta)1-42+, n</th>
<th>CSF A(\beta)1-42+, n</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tr>
<td></td>
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<td>[95% CI]</td>
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<td>[95%CI]</td>
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<td><strong>Visual analysis</strong></td>
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<tr>
<td>Florbetapir-negative</td>
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<td>35</td>
<td>79</td>
<td>96</td>
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<td>[72-85]</td>
<td>[88-99]</td>
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<td>[62-79]</td>
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<td>Florbetapir-positive</td>
<td>4</td>
<td>131</td>
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<td><strong>SUVR measurements</strong></td>
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<tr>
<td>Florbetapir-negative</td>
<td>81</td>
<td>25</td>
<td>85</td>
<td>90</td>
<td>94</td>
<td>76</td>
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<td>[78-90]</td>
<td>[81-95]</td>
<td>[89-97]</td>
<td>[67-84]</td>
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<tr>
<td>Florbetapir-positive</td>
<td>9</td>
<td>141</td>
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</table>

CI, confidence interval, CSF, cerebrospinal fluid, n, number, NPV, negative predictive value, PPV, positive predictive value, SUVR, florbetapir standardized uptake values ratio.
**eTable 2.** Linear regression models to predict longitudinal cognitive function by a [\(^{18}\)F] florbetapir-positive scan in MCI.

<table>
<thead>
<tr>
<th>Florbetapir+ scan (dichotomous predictor variable)</th>
<th>ADAS-cog (continuous outcome variable)</th>
<th>Adj R(^2)</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>0.13</td>
<td>2.82**</td>
<td>0.42</td>
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<tr>
<td></td>
<td>6-months follow-up</td>
<td>0.20</td>
<td>3.46**</td>
<td>0.45</td>
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<td></td>
<td>12-months follow-up</td>
<td>0.14</td>
<td>3.63**</td>
<td>0.55</td>
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<td></td>
<td>24-months follow-up</td>
<td>0.19</td>
<td>2.61**</td>
<td>0.63</td>
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<td></td>
<td>36-months follow-up</td>
<td>0.38</td>
<td>2.71*</td>
<td>1.10</td>
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<tr>
<td>SUVR measurements</td>
<td>Baseline</td>
<td>0.12</td>
<td>2.56**</td>
<td>0.42</td>
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<td>6-months follow-up</td>
<td>0.14</td>
<td>2.43**</td>
<td>0.47</td>
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<td></td>
<td>12-months follow-up</td>
<td>0.08</td>
<td>2.40**</td>
<td>0.57</td>
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<td>24-months follow-up</td>
<td>0.17</td>
<td>2.00**</td>
<td>0.61</td>
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<tr>
<td></td>
<td>36-months follow-up</td>
<td>0.41</td>
<td>2.77*</td>
<td>0.94</td>
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</table>

Baseline ADAS-cog refers to the test session closest to florbetapir scan and the respective follow-up periods correspond to the time intervals between florbetapir scan and each test session. All models include age, gender and education as covariates. Adjusted R\(^2\) represents the amount of variance in the outcome explained by the respective model relative to the total variance in the outcome.
available data: ADAS-cog baseline, n=255/145 (EMCI/LMCI), 6-months follow-up, n=244/130 (EMCI/LMCI), 12-months follow-up, n=240/118 (EMCI/LMCI), 24-months follow-up, n=150/38 (EMCI/LMCI), 36-months follow-up, n=46/0 (EMCI/LMCI)

ADAS-cog, Alzheimer’s Disease Assessment Scale-Cognitive Subscale, Adj, adjusted, SE, standard error, SUVR, florbetapir standard uptake values ratio, **p ≤ 0.001, *p < 0.05.
**eFigure.** Kaplan-Meier Survival Plots for MCI to AD Conversion.

Graphs indicate the cumulative MCI probability (y-axis) over time (x-axis) in case of a visual (A) or quantitative (B) florbetapir-negative scan (blue curve) and in case of a visual (A) or quantitative (B) florbetapir-positive scan (red curve) at baseline. Estimated median conversion time, defined as time interval during which around 50% of the MCI subjects will have converted, accounts for around 2 years in case of a florbetapir-positive baseline scan (dotted black lines in A & B). As the cumulative MCI probability of those subjects with a florbetapir-negative baseline scan did not drop below 0.5 (y-axis), no median conversion time was assessed for those cases.
Reference List


