USING POSITRON EMISSION tomography (PET) to image fibrillar amyloid has begun to have transformational effects on the scientific study, early detection, and tracking of Alzheimer disease (AD) and on the evaluation of amyloid-modifying treatments. Amyloid imaging offers great promise to facilitate the evaluation of patients in a clinical setting. Because of their longer radioactive half-lives, 18F-labeled ligands are needed to make this technique widely available through commercial PET radiotracer distribution sites for use in research and clinical settings. Florbetapir F 18 [(E)-4-[(2-((2-(2-fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl]-N-methyl benzenamine; previously 18F-AV-45 and hereafter referred to as florbetapir F 18 when the tracer is named formally as a compound) is a PET ligand that has been shown in in vitro, ex vivo, and in vivo studies to measure cortical fibrillar β-amyloid (Aβ).

See also pages 1377 and 1398
Florbetapir F 18 has been studied in 6 human clinical studies registered with the US Food and Drug Administration. A total of 269 subjects have received florbetapir in 2 phase I studies and 3 phase II studies. A recent phase III study of 35 terminally ill participants compared florbetapir imaging and postmortem amyloid immunohistochemistry. Florbetapir-PET images were also obtained from 74 cognitively normal young adults at variable genetic risk of late-onset AD based on apolipoprotein E (APOE) genotype. In this study by Clark et al., 35 end-of-life patients with and without dementia demonstrated highly significant correlations between florbetapir-PET and subsequent immunohistochemistry measurements of fibrillar Aβ, using either blind visual ratings (r = 0.78) or automatically characterized cerebral–whole-cerebellar standard uptake value ratios (SUVRs) (r = 0.75). Florbetapir-PET visual ratings, using the median rating from 3 blinded readers, were found to have 96% accuracy for characterizing whether or not the research participants had fibrillar Aβ levels consistent with intermediate or high likelihood of pathologic AD (National Institute on Aging–Reagan Institute criteria).

In our study, we pooled data from the 4 registered phase I and II trials of florbetapir-PET imaging that used standard-dosing of florbetapir F 18 and nondynamic PET acquisition, permitting us to assess a large combined cohort of patients with probable AD, mild cognitive impairment (MCI), and age-matched older healthy controls (OHCs). We evaluated both continuous and binary measures of florbetapir-PET activity to assess global differences between clinical diagnostic groups, confirm expected patterns of regional distributions of fibrillar Aβ, and determine proportions of positive scans using cutoff thresholds for global cortical florbetapir F 18 activity. In doing so, we introduce empirically predetermined SUVR thresholds for defining florbetapir-PET positivity based on Avid Radiopharmaceutical’s previously reported study of expired end-of-life patients and a specificity cohort of young APOE ε4 (APOE4) noncarriers.

METHODS

PARTICIPANTS

A total of 210 participants, including 82 cognitively normal volunteers (ie, OHCs), 60 individuals with MCI, and 68 individuals with probable AD, were assessed at 31 US research sites. Florbetapir-PET scans were taken of all participants. We evaluated cognitively normal individuals who were 55 years of age or older. They were required to have no subjective cognitive complaints as corroborated by an informant report, to have a Mini-Mental State Examination (MMSE) score of 29 or greater, and to be cognitively normal based on psychometric testing. Participants with probable AD met National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for probable AD and had an MMSE score at screening in the range of 10 to 24. Participants with MCI had complaints of memory, cognitive decline corroborated by an informant, objective cognitive impairment or marginally normal performance with a documented history of high cognitive performance, generally preserved functional abilities and activities of daily living, a Clinical Dementia Rating scale global score of 0.5, and an MMSE score of greater than 24 and presented for initial diagnosis of cognitive impairment not more than 1 year from the day of the screening visit. APOE genotyping was performed for 155 participants as an optional procedure, in a double-blind fashion. Participants were excluded if they had other current clinically relevant neurologic or psychiatric illnesses, were receiving any investigational medications, or ever received an anti-amyloid experimental therapy. Trials were conducted in accordance with good clinical practice guidelines after approval from local institutional review boards. Study procedures were performed after written informed consent was obtained from study participants, authorized representatives, or both, according to local guidelines and degree of cognitive impairment.

FLORBETAPIR-PET IMAGING ACQUISITION

Across the study sites, there were 14 different PET scanner models from 3 manufactures (Siemens, Washington, DC; General Electric, Little Chalfont, Buckinghamshire, England; and Philips, Andover, Massachusetts). Native-slice thickness ranged from 2 to 4.25 mm, with field of views from 550/153 to 700/153. All volunteers underwent a florbetapir-PET session that consisted of intravenous injection of approximately 10 mCi of florbetapir F 18 while resting quietly outside the PET scanner. After approximately 45 minutes, the participant was positioned in the scanner such that the entire brain (including the cerebellum) was in the field of view and a computed tomographic (for PET/computed tomographic scanners) or a PET attenuation scan (for PET-only scanners) was performed to allow estimation of attenuation factors. Starting at 50 minutes after injection, a 10-minute emission acquisition was performed (as a dynamic scan with two 5-minute frames). Images were reconstructed with an iterative reconstruction algorithm (4 iterations, 16 subsets), using a Gaussian filter of 5-mm full-width at half-maximum, and were saved as a series of $128 \times 128$ matrices with a voxel size of $2 \times 2 \times 2$ mm. Between-scanner variability in attenuation, scatter, and uniformity was corrected on the basis of Hoffman brain phantom scans acquired on each scanner.

IMAGE ANALYSIS

First, a region of interest (ROI) analysis was performed on individual PET images, spatially normalized into Montreal Neurological Institute (MNI) atlas space using statistical parametric mapping (SPM) software. For this analysis, no spatial smoothing was performed. Previously defined mean cortical and whole-cerebellar ROI templates were applied to all PET scans to calculate mean regional cerebral-to-cerebellar SUVRs. The ROIs were defined from 11 patients with AD and 15 age-matched healthy controls participating in an early phase I study, all of whom underwent 90-minute dynamic florbetapir-PET acquisitions (images excluded from our analysis) and structural magnetic resonance imaging. The whole-cerebellar reference ROI was hand drawn from mean group magnetic resonance imaging scans after they were spatially normalized to MNI atlas space. Flow maps from the first 10 minutes of PET data acquisition and a voxelwise comparison of between-group activity in patients with AD vs controls were used to identify key cortical regions of increased PET signal in MNI brain atlas space. After gray or white matter and cerebrospinal fluid space segmentation, by use of participants' MRI scans registered in MNI space, 6 cortical gray matter ROIs were defined from the Automated Anatomic Labeling Atlas or were manually delineated in gray matter regions that had prominent PET activity in patients with AD compared with controls: medial orbital frontal (Automated Anatomic

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Labeling), temporal, anterior, and posterior cingulate; parietal lobe; and precuneus (Figure 1). The average of these 6 regions was evaluated as a measure of global mean cortical florbetapir F 18 binding and was used as the primary outcome measure for ROI analyses. Between-group t tests and analyses of variance were used to assess mean cortical florbetapir differences. Linear regression was used to assess the interaction of mean cortical SUVRs and age. Differences in mean cortical SUVRs based on florbetapir F 18 levels typically as- signed to as any-amyloid levels), such that it exceeds that in young adults consistent with PATHAMY levels and any-amyloid levels of cortical amyloid were determined within each diagnostic group. Differences in proportions of positivity in a subgroup of OHCs (n = 54) who had APOE genotyping available was assessed between APOE carriers and noncarriers. To determine whether age was associated with binary florbetapir-PET positivity, the percentage of cognitively normal elderly participants with either any-amyloid or PATHAMY levels of Aβ was evaluated within age deciles using a x 2 linear-by-linear association (not corrected for cell sample size owing to meeting minimum cell size requirements). Differences in percentage of florbetapir positivity between diagnostic groups and between the 2 cutoff thresholds were compared using the nonparametric McNemar test, accounting for comparison of data from the same set of participants (ie, samples are not independent).

**RESULTS**

The OHC, MCI, and probable AD groups did not differ significantly in their age or sex distribution (Table). As expected, the patients with probable AD had significantly greater cognitive impairment on the MMSE, the AD Assessment Scale–cognitive subscale, and the Wechsler Memory Scale immediate recall test than did the patients with MCI or the OHCs (P < .001) (Table). There were significant differences among the probable AD, MCI, and OHC groups in their percentage of APOE carriers (51%, 39%, and 24%, respectively; P = .002). Fifty-four OHCs had APOE genotyping performed: 13 were found to be carriers, and 41 noncarriers; 28 participants opted out of genotyping.

**MEAN CORTICAL SUVR MEASUREMENTS**

Mean (SD) cortical-to-whole-cerebellar SUVRs were significantly different among the 3 groups and in the expected direction: 1.39 (0.24) for the probable AD group, 1.17 (0.27) for the MCI group, and 1.05 (0.16) for the OHC group (P = 2.9 × 10^-13) (Figure 2). There were no...
significant linear relationships between mean cortical SUVR and age in the AD group \((P = .17)\) or in the MCI group \((P = .73)\), but there was an age-related increase in mean cortical SUVR in the OHC group \((P = .002\), with a regression slope of 0.005\). Among OHCS, APOE4 carriers had a higher mean (SD) cortical SUVR than did noncarriers \((1.14 [0.2] \text{ vs } 1.03 [0.16]; P = .048)\).

**STATISTICAL PARAMETRIC MAPPING**

Statistical brain maps revealed significantly greater regional–to–whole-cerebellar florbetapir SUVRs among the 3 groups (with the probable AD group having the highest SUVRs and the OHC group having the lowest, with voxelwise \(P\) values ranging from \(.05\) to \(4 \times 10^{-15}\), uncorrected for multiple comparisons) (Figure 3). The pattern of SUVR increases in the AD and MCI groups was consistent with that previously reported in amyloid PET studies, with preferential uptake in the precuneus, the posterior cingulate, the parietal lobe, and the temporal and frontal cortex (Figure 3). \(^3\,^{15}\,^{17}\) Group differences remained significant when controlling for the effects of APOE4, but with attenuated statistical power (voxelwise \(P\) values ranging from \(.05\) to \(4 \times 10^{-9}\) with similar distribution patterns.

**PERCENTAGE OF FLORBETAPIR POSITIVITY ASSOCIATED WITH PATHOLOGIC LEVELS OF AMYLOID IN AD AND ANY-AMYLOID LEVELS ABOVE NORMAL**

Evaluation of mean cortical florbetapir levels in the PATHAMY group and in the group of young adult APOE4 noncarriers established predetermined florbetapir-PET criteria associated with a neuropathological diagnosis of (intermediate-to-high likelihood) AD \((SUVR \geq 1.17)\) for the PATHAMY group or having an any-amyloid level above that typically seen in young adult APOE4 noncarriers \((SUVR > 1.08)\) (Figure 4). The percentage of pa-
tients in each clinical diagnostic group who were at or above criteria for PATHAMY SUVR levels was significantly different for each group (80.9% of patients with probable AD, 40.0% of patients with MCI, and 20.7% of OHCs; \( P < 1.0 \times 10^{-7} \)). Similarly, those individuals meeting florbetapir positivity criteria above any-amyloid levels were different for each group (85.3% of patients with probable AD, 46.6% of patients with MCI, and 28.1% of OHCs; \( P < 1.0 \times 10^{-7} \)) (Figure 4). The mean percentage of individuals with cortical florbetapir levels falling between PATHAMY and any-amyloid levels did not differ substantially among diagnostic groups (4.4% of patients with probable AD, 6.7% of patients with MCI, and 2.4% of OHCs; \( P = .76 \)) (Figure 2). APOE4 carriers among the OHC group had more than twice the percentage of florbetapir-PET positivity compared with noncarriers: of 13 APOE4 carriers, 4 (30.8%) had PATHAMY levels, and 6 (46.1%) had any-amyloid levels; of 41 APOE4 noncarriers, 6 (14.6%) had PATHAMY levels, and 9 (21.9%) had any-amyloid levels (Figure 4). However, these differences did not reach statistical significance \( (P = .19 \) for PATHAMY level and \( P = .09 \) for any-amyloid level). Lastly, florbetapir positivity increased by age decile among OHCs for both positivity thresholds \( (P = .05 \) for PATHAMY level and \( P = .01 \) for any-amyloid level) (Figure 5). The percentage of positivity by age decile among OHCs for PATHAMY levels of florbetapir was 5.9% for those 55 to 60 years of age, 15.8% for those 61 to 70 years of age, 27.3% for those 71 to 80 years of age, and 29.2% for those 81 years of age or older (Figure 5). Within the MCI and AD groups, there were no statistical differences in the proportion of florbetapir-PET positivity using the PATHAMY threshold vs the any-amyloid threshold \( (P = .25 \) for AD group and \( P = .13 \) for MCI group). For the OHC group, however, more positivity was detected when using the more liberal any-amyloid threshold than when using the PATHAMY threshold \( (P = .03 \) (Figure 4). However, OHC APOE subgroups did not show this difference in percentage of positivity between thresholds.

**To our knowledge, our study presents the largest analysis of multicenter \(^{18}\)F amyloid PET data currently reported. These results robustly support the ability of florbetapir-PET SUVRs to characterize amyloid levels in clinically probable AD, MCI, and OHC groups using both continuous and binary quantitative measures of amyloid burden. Between-group comparisons revealed increased florbetapir F 18 positivity associated with clinical severity in distribution patterns consistent with known fibrillar amyloid deposition in AD.\(^{11,13,16}\) In addition, clinical severity was distinguishable by florbetapir PET even when controlling for the effects of APOE4 gene status. However, OHCs had higher levels of mean cortical florbetapir SUVRs among APOE4 carriers compared with noncarriers, and normal aging was associated with an increase in florbetapir F 18 binding. By using florbetapir positivity cutoff thresholds that were based on patients with neuropathologically confirmed AD and low-risk young individuals, we introduce criteria to determine whether an image is consistent with amyloid levels associated with an intermediate-to-high likelihood of pathologic AD or with having any identifiable presence of cortical amyloid.

Although the etiology of AD has not been definitively established, converging evidence suggests that the A\(\beta\) peptide plays an important role in its pathogenesis.\(^{11,16-21}\) Given the recently reported correlation of florbetapir F 18 with underlying cortical amyloid burden,\(^2\) we applied criteria established from the florbetapir au-
topy and the young specificity cohort study to this larger data set. Our findings are consistent with previous clinical-pathological comparisons in AD and with previous amyloid imaging studies. Clinical-pathological comparison studies demonstrate that between 10% and 30% of patients with clinically diagnosed AD lack AD pathology at autopsy.\textsuperscript{22-24} We found 19.1% of patients with clinical AD to have florbetapir levels below that associated with pathologic AD and 14.7% of patients with clinical AD to have no evidence of amyloid at all. Similarly, in cognitively normal older adults, although large autopsy studies are not available, previous carbon 11--labeled Pittsburgh Compound B [\textsuperscript{11}C]PiB studies report that 20% to 51% of healthy elderly adults have elevated levels of amyloid binding on PET imaging,\textsuperscript{13,25-28} compared with our findings of 21% to 28%.

Advanced age and the \textit{APOE} \textepsilon \textcorner gene are the most potent known risk factors for AD and the subsequent presence of neurofibrillary tangles and amyloid plaques in the brain.\textsuperscript{29-33} Our findings with florbetapir are consistent with previous [\textsuperscript{11}C]PiB imaging studies that demonstrate an association between the \textit{APOE} \textepsilon \textcorner gene and amyloid PET load in cognitively normal adults.\textsuperscript{34,35} Although mean cortical SUVs were higher in APOE4 carriers compared with noncarriers, the proportion of florbetapir-PET positivity between carriers and noncarriers did not reach statistical significance. This may have been due to the small sample size of APOE4 carriers. Similar to our findings, increasing age among healthy elderly controls has been shown to be associated with increasing amyloid binding in recent large studies of [\textsuperscript{11}C]PiB PET.\textsuperscript{27,35}

The choice of thresholds for determining florbetapir positivity should be hypothesis driven and applied within the context of the clinical or scientific question at hand. For instance, a more conservative threshold (such as that associated with pathologic AD) might be used in the clinical setting to determine whether or not a person meets florbetapir-PET criteria for neuropathologically significant cerebral amyloidosis. However, a more liberal criteria (eg, florbetapir levels above those seen in low-risk young individuals) might identify those individuals in the earliest stages of amyloid accumulation, providing a group that might be especially responsive to presymptomatic amyloid-modifying treatments for AD. Establishing standards for image acquisition, cerebral and reference ROIs, and cutoff thresholds is needed to facilitate the comparison of data among different subjects and the comparison of findings from different investigators.

In our report, we proposed 2 thresholds for determining florbetapir positivity based on 2 separate convenience samples: one threshold intended to be consistent with the neuropathological diagnosis of intermediate or high likelihood AD and another consistent with any substantial identifiable cortical amyloid. Statistical methods such as interquartile range\textsuperscript{15,36} or hierarchical cluster analysis\textsuperscript{27} can be used to establish positivity thresholds based on clinically defined cohorts of healthy elderly adults or patients with probable AD. Herein, however, we had the opportunity to base thresholds on a pathological gold standard and on a cohort extremely unlikely to have any cortical amyloid pathology.\textsuperscript{3,9} Notably, we found no statistical outliers in the mean cortical SUVR distribution for either of these 2 cohorts. Despite this, these convenience samples may not represent the full range of florbetapir F 18 levels seen in a larger community sample. Clinicopathologic correlate studies in larger community-based samples with a broader distribution of SUVRs may be needed to more definitively establish standard thresholds.

Use of the whole cerebellum as a reference region for SUV calculations has not been proven to be superior to other noncortical brain regions, such as the pons or cerebellar gray matter. A whole-cerebellar reference region was chosen here because it rarely contains fibrillar amyloid plaques, and cortical regions on PET scans typically contain a mixture of white and gray matter tissue, which is matched by the whole-cerebellar region. Therefore, theoretically, the whole cerebellum should be a suitable measure of neutral nonspecific florbetapir binding.\textsuperscript{37,38} The use of pons or cerebellar gray matter as reference regions may potentially further minimize the effects of nonneocortical amyloid and nonspecific white matter binding, respectively.\textsuperscript{13,35,36,38} In addition, it is certainly possible that healthy elderly controls have different nonspecific binding compared with the young cohort used here as an exemplar of normal.

Another limitation to our study may include a potential cohort selection bias. This cohort is a combined sample from several clinical studies with specific selection criteria intended to identify relatively homogenous diagnostic samples. Although data collection was done in a consistent prospective manner, this cohort was not intended to represent a broad community-based clinical practice setting. Guidance for appropriate clinical use of florbetapir requires further community-based data.

In conclusion, our analysis provides additional support for the emerging role of florbetapir-PET imaging in the assessment of fibrillar A\textsubscript{beta} burden and underscores the need for standardization of definitions for positive amyloid scans. Herein, we introduced the use of positivity thresholds associated with an intermediate-to-high likelihood of fibrillar A\textsubscript{beta} pathology vs one related to at least minimal elevations in fibrillar A\textsubscript{beta} above that seen in young low-risk individuals. Our study analyzes pooled data in an effort to better understand and optimize the use of florbetapir PET for clinical research and diagnostic assessments in AD.

Accepted for Publication: May 10, 2011.

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**Published Online:** July 11, 2011. doi:10.1001/archneurol.2011.150

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**Financial Disclosure:** Dr Fleisher has received research support for nonrelated projects from Avid Radiopharmaceuticals and Eli Lilly and is a consultant for Eli Lilly. Mr Joshi and Drs Clark, Mintun, Pontecorvo, and Skovronsky are employees of Avid Radiopharmaceuticals, a wholly owned subsidiary of Eli Lilly. Dr Doraiswamy has received research support or advisory fees from Avid, Lundbeck, Medivation, TauRx, Bayer, GE Healthcare, BMS, Astra, Shering, Neoptix, Neuronetrix, Alzheimer’s Foundation, and Sonexa and owns stock in Sonexa. Dr Johnson has received research support or advisory fees from GE Healthcare, Bayer, Janssen Alzheimer Immunotherapy, and Avid Radiopharmaceuticals. Dr Reiman has received research support for nonrelated projects from Avid Radiopharmaceuticals and Eli Lilly.

**Funding/Support:** This study was supported by funding from Avid Radiopharmaceuticals, a wholly owned subsidiary of Eli Lilly.

**Additional Information:** All data analyses were performed independently by the Banner Alzheimer’s Institute (a 503c not-for-profit organization) with consultation provided by Avid Radiopharmaceuticals. Drs Fleisher, Chen, Ayutyanont, and Reiman, Ms Liu, and Messrs Roontiva and Thiyyagura had full access to the data set reported herein and unrestricted publication rights. All data collection was done as part of multisite-registered trials funded by Avid Radiopharmaceuticals, many of which Banner Alzheimer’s Institute participated in. No financial compensation was obtained from Avid Radiopharmaceuticals for the analyses reported herein.

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