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Florbetapir (F18-AV-45) PET to assess amyloid burden in Alzheimer's disease dementia, mild cognitive impairment, and normal aging

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Abstract

Objective—To evaluate the performance characteristics of florbetapir F 18 PET in patients with Alzheimer's disease dementia (AD), mild cognitive impairment (MCI) and healthy control subjects (HC).

Methods—Florbetapir PET was acquired in 184 subjects (45 AD, 60 MCI, and 79 HC) within a multi-center phase 2 study. Amyloid burden was assessed visually and quantitatively and classified as positive or negative.

Results—Florbetapir PET was visually rated amyloid positive in 76% of AD, 38% of MCI and 14% of HC. 84% of AD, 42% of MCI, and 23% of HC were classified as amyloid positive using a quantitative threshold. Amyloid positivity and mean cortical amyloid burden were associated with age and APOE 4 carrier status.

Interpretation—The data are consistent with expected rates of amyloid positivity among individuals with clinical diagnoses of AD and MCI, and indicate potential value of florbetapir F 18 PET as an adjunct to clinical diagnosis.

Keywords

Alzheimer's disease; PET; amyloid

Introduction

Amyloid deposition is one of the defining neuropathologic findings of Alzheimer's disease (Braak 1997; Hyman 1997), and human amyloid imaging with PET now makes it possible to detect amyloid in vivo (Klunk 2004), relate it to clinical (Pike 2007; Rentz 2010), fluid

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biomarker (Fagan 2007), and autopsy (Bacskaï 2007; Ikonomic 2008) findings, and monitor attempts to alter the course the disease with anti-amyloid treatment (Rinne 2010). ¹¹C Pittsburgh Compound B (PiB) is the most widely adopted amyloid PET tracer and has been studied in specialized research centers for approximately 5 years. When applied at specialized AD research centers, PiB PET has been reported to indicate abundant deposition of amyloid in nearly all patients with clinical AD (Engler 2006), approximately half of patients with MCI (Forsberg 2007; Wolk 2009; Okello 2009), and in a variable fraction (25% - 50%) of apparently normal older individuals (Aizenstein 2008; Gomperts 2008; Sperling 2009; Becker 2011).

Since the clinical diagnosis of AD during life is frequently inaccurate outside of research centers (Pearl 1997), it is possible that amyloid PET could provide substantial clinical utility by accurately detecting the presence or absence of brain amyloid in individuals at-risk for AD. Indeed, investigators have recently proposed revised diagnostic criteria that are based in part on biomarker evidence of AD pathology, even in individuals with mild memory impairment who are not sufficiently impaired to meet criteria for dementia (Dubois 2007; Dubois 2010; Albert 2011; McKhann 2011). AD biomarkers are increasingly used for drug development in AD clinical trials, both for selecting participants and for evaluating target engagement. Effective disease-modifying therapies would probably be of greater benefit if diagnosis could be established earlier and treatment could be initiated prior to the end-stage of brain failure and dementia. If trends toward earlier, biomarker-based detection of AD pathology continue, the need becomes more acute for amyloid biomarkers that are widely available, readily standardized and easily acquired. In this connection, the major limitation of ¹¹C-PiB is the 20-minute half-life of the ¹¹C isotope, which restricts its use to research centers. In contrast, an ¹⁸F-based PET tracer, with a 110-minute half-life, can be prepared remotely and delivered far more widely.

Florbetapir F 18 ((E)-4-(2-(6-(2-(2-(2-[¹⁸F]fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylbenzenamine also known as ¹⁸F-AV-45) exhibits high affinity specific binding to amyloid plaques with a K_d of 3.1 nM. In-vitro autoradiography studies further confirm that when applied at tracer concentrations, ¹⁸F-AV-45 labels A β amyloid plaques in sections from patients with pathologically confirmed AD (Choi 2009). Early phase human PET studies indicate that florbetapir binds to amyloid vulnerable brain regions in AD patients, similar to reported binding with PiB in AD, with minimal cortical binding in normal control subjects (Wong 2010). More recently, the high correspondence of Florbetapir binding and A β amyloid plaque load has been reported in a postmortem series (Clark 2011).

In this communication we report the results of a multicenter phase 2 clinical trial in which we assessed brain amyloid burden by florbetapir F 18 PET utilizing both visual interpretation and quantitative measurement of florbetapir cortical uptake among HC, MCI and AD study participants. We also report the relationship of cortical amyloid burden as assessed by florbetapir F 18 PET to age and APOE ϵ 4 carrier status, as well as the clinical safety results from this study.

Methods

Subjects

A total of 184 subjects participated, including 45 AD, 60 MCI and 79 HC. AD diagnosis was made on the basis of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association Criteria (McKhann 1986). AD patients were included if the Mini-Mental State Examination (MMSE) score was between 10 and 24, inclusive (Folstein 1976). MCI diagnosis was made on the basis of 1) complaint of memory or cognitive decline corroborated by an informant;

2) CDR global score of 0.5; 3) objective evidence of cognitive impairment or marginally normal cognition with a documented history of high cognitive performance; 4) no obvious medical cause for the impairment; 5) subject not demented and criteria for AD not satisfied; 6) normal score on the Alzheimer's Disease Clinical Studies Consortium Activities of Daily Living (ADCS ADL); and 7) MMSE >24. HC were required to have MMSE $\geq$ 29 and have no evidence of cognitive impairment by history, verified by a knowledgeable study partner informant, and verified by neuropsychological testing. Apolipoprotein E (APOE) genotyping was performed at screening to determine the presence of the 2, 3, and 4 alleles.

Exclusions: Subjects were excluded if they had a history of neurodegenerative diseases other than AD (e.g., Parkinson's disease), normal pressure hydrocephalus, a history of drug or alcohol abuse. Subjects were excluded for cognitive impairment resulting from acute or chronic head trauma, subdural hematoma, hypoxic cerebral damage due to medical condition such as cardiac surgery, significant vitamin deficiency, a history of epilepsy, cerebral infection or cerebral neoplasia, significant endocrine or renal disease or mental retardation, and currently significant cardiovascular or psychiatric disease. MCI subjects were excluded if they had a documented diagnosis of MCI for greater than one year. Experimental amyloid targeting medications, current or past, and current drugs with narrow therapeutic windows (theophylline, warfarin, heparin and other anticoagulants) were excluded.

Subjects were excluded if they had a clinically significant infarct or possible multi-infarct dementia as defined by the National Institute of Neurological and Communicative Disorders and Stroke criteria. A volume-based high-resolution (at least $1.5 \times 1.5 \times 2.0$ mm) MRI including a T1-weighted sequence was performed at screening. MRI findings of two or more infarcts or lacunes, or severe white matter disease were exclusionary.

Each subject underwent physical and neurological examinations, and a battery of neuropsychological tests including the ADAS-COG (11-item cognitive subscale of the Alzheimer's Disease Assessment Scale) (Rosen et al. 1984), CDR (Morris 1993), Wechsler Logical Memory I and II Story A (immediate and delayed paragraph recall: LMI and LMII) (Wechsler 1987), Digit-Symbol Substitution (DSS) (Wechsler 1981), ADCS ADL Scale and Category Verbal Fluency (animals and vegetables; number generated in 60 seconds) (Morris et al. 1989). GDS was also assessed at screening (Yesavage et al. 1983).

The study was conducted at 24 centers. All participating sites' Institutional Review Boards approved the study and all participants or their appropriate representatives provided informed consent. Safety assessments included adverse event (AE) counts, pre- and post-dose clinical laboratory test results, vital sign measurements, and ECG results.

PET Methods

Florbetapir F 18 was prepared as described previously (Wong 2010). All participants received a single intravenous bolus of approximately 370 MBq (10 mCi) of Florbetapir F 18. After transmission data were acquired, a 10-minute PET acquisition for Florbetapir F 18 was acquired in 2×5 minutes frames beginning 50 minutes post-injection. The data was acquired using either ECAT HR+ PET scanner (Siemens), Discovery LS PET/CT scanner (General Electric), 16-slice Biograph PET/CT scanner (Siemens), or Advance PET scanner (GE). The reconstruction method was iterative (4 iteration 16 subsets) with a post-reconstruction Gaussian filter of 5mm. Images were reconstructed, corrected for scatter and attenuation using commercial software packages, and inspected at a central facility for adequacy of count statistics and absence of head motion.

Visual Rating And Binary Classification Of PET Images

Semiquantitative Visual Rating—After a training session that included exemplar data sets, three nuclear medicine physicians with no access to clinical information, independently rated each PET image for amyloid burden based on successive levels of florbetapir retention from 0 to 4 as follows: (0) None: Predominantly white matter tracer retention with no appreciable cortical gray matter retention above cerebellar grey matter levels; (1) Low: evidence of increased tracer retention above cerebellar grey levels in 1 or 2 cortical grey regions; (2) Low-moderate: either (a) predominantly white matter pattern, but at least 2 cortical regions with increased retention relative to cerebellar grey, or (b) predominantly a cortical gray matter pattern, with most cortical areas mildly positive relative to cerebellum; (3) Moderate-high: specific cortical retention generally greater than or equal to white matter retention and at least one cortical area with greatly increased retention relative to cerebellar grey; (4) High: Specific cortical uptake greater than or equal to white matter background and multiple cortical areas with greatly increased retention relative to cerebellar grey.

Binary Classification—The visual reads were used to classify each data set as either visually positive for amyloid-beta (vAβ+) or visually negative for amyloid-beta (vAβ−). Visual rating scores of 2-4 were considered positive and 0-1 were considered negative. The majority classification from the 3 readers as either positive or negative was the primary endpoint for the analyses reported here.

To further evaluate inter-rater reliability, 5 private practice nuclear medicine physicians, with no previous experience in amyloid imaging, were trained to perform binary interpretation of Florbetapir PET scans and tested on a subset of 92 of the PET scans from the present study (20 AD, 20 HC, and 52 MCI; the full dataset from the present study was not available for testing because some cases were used in reader training).

Quantitative Image Analysis

For quantitative analysis, image data were aligned to a template previously constructed from an average of 10 HC and 10 AD subjects that had been spatially normalized to the MRI template in SPM2 (www.fil.ion.ucl.ac.uk/spm) MNI space. Standard ROIs were created using functional information from Florbetapir F18 early time point PET images (phase I study) and structural information from MRI, both normalized in MNI space, to identify predominantly grey matter regions. A voxelwise SPM contrast between 15 AD and 15 control subjects was then used to identify regions that had elevated tracer uptake in AD patients by comparison to HC, and ROIs were hand drawn on the normalized PET template to approximate an intersection of the gray matter and elevated tracer uptake regions in the target areas of interest. These ROI were saved as a template and applied to the normalized image for each patient in the present study. Florbetapir retention standardized uptake values (SUV) were measured from ROI for the following 6 target brain regions: frontal cortex, temporal cortex, precuneus, parietal cortex, anterior cingulate and posterior cingulate. Whole cerebellum was manually identified on the PET template using MRICron (www.cabiatl.com) and its mean SUV was computed and used to scale the 6 cortical ROIs, i.e., computing regional SUV ratio (SUVR), using MATLAB code. To evaluate the reference ROI between subjects, a gray matter cerebellum ROI was drawn on the same template and SUVs from cerebellar grey and whole cerebellum were correlated with each other and with subject age by diagnostic groups. The primary endpoint for quantitative evaluation of each subject was the mean of the SUVRs for the 6 cortical target regions (mcSUVR).

A threshold for amyloid positivity of 1.10 for the mcSUVR was determined based on the range of young healthy controls in a preceding study (i.e., 1.10 was the 95% CI upper limit

for no detectable amyloid in young healthy controls in that study (Joshi et al). PET data sets in the present study were therefore categorized as amyloid negative by quantitative cutoff (qA -) if the SUVR was less than 1.10 and as qA + if the SUVR was ≥ 1.10 .

Statistical Methods

Descriptive statistics (mean and standard deviation for continuous variable and frequency and percentage for categorical variable) were provided on study subjects' demographic and baseline characteristics. For continuous variables, analysis of variance (ANOVA) was conducted to test the overall mean difference across the diagnosis groups. To further evaluate potential differences in mcSUVR across diagnostic groups, stepwise multiple linear regression models were conducted to adjust for potential covariates (APOE $\epsilon 4$, age, gender, education and clinical site); covariates that met had a $p < .05$ were retained in the final model. When a statistical significant overall difference was detected, pair-wise comparisons on means between diagnosis groups were conducted. For categorical variables, Pearson's Chi-square test was conducted to detect the general association across diagnosis groups. When a statistical significant overall difference detected, separate comparisons were conducted between different combinations of diagnosis groups in a pair-wise manner. Fisher exact test were conducted under the situation when a subcategory had frequency less than 5.

Results

The subject demographics and baseline characteristics for the three diagnostic groups recruited to the study can be found in Table 1. Cognitively normal subjects were younger, had higher mean MMSE scores and were more likely to be APOE $\epsilon 4$ non-carriers (71.8%) vs. clinically diagnosed AD patients (42.8%). MCI subjects generally had MMSE scores that were intermediate between the normal and clinically diagnosed AD group. Mean scores for all neuropsychological and functional performance measures differed by diagnostic group with better performance in the expected direction of HC>MCI>AD (Table 1).

The mean number of subjects recruited per site was 7.6 ± 7.9 , with each site averaging 1.9 ± 2.5 AD, 3.3 ± 3.5 MCI and 2.5 ± 3.0 HC subjects (a listing of the 24 participating sites can be found in the Appendix).

Florbetapir cortical retention was highest in subjects with AD, lowest in cognitively normal subjects, and intermediate in subjects with MCI, regardless of whether assessed with mcSUVR, visual ratings, or binary classification of the mcSUVR (Table 2). Group average mcSUVRs, using the whole cerebellum reference region, differed by diagnostic group and all pairwise comparisons were statistically significant ($p < 0.0003$; Table 2 and Figure 1). The diagnostic group differences in mcSUVR remained significant in a stepwise regression model that included APOE $\epsilon 4$, age, gender, education and clinical site as potential covariates (all $p < 0.0124$; AD vs. MCI: $p=0.0001$; AD vs. HC: $p<0.0001$; MCI vs. HC: $p=0.0124$). In this multivariate analysis, no covariates other than diagnostic group and APOE $\epsilon 4$ status ($P<0.0001$) significantly predicted mcSUVR. Similar to the mcSUVR analysis, regional SUVR analyses also showed significant differences for all individual regions that contributing to the mcSUVR (Table 3).

Visual ratings were also significantly related to diagnostic group, and were highly correlated across the entire sample with the mcSUVR ($r = 0.81$, $p < 0.0001$). Raters visually classified 76% of AD, 38% of MCI, and 14% of CN subjects as vA + (semiquantitative read 2, 3 or 4) and all comparisons between diagnostic groups were statistically significant (Fisher's exact test; $p = 0.0014$; Table 2). Similarly, 84% of AD, 42% of MCI, and 23% of CN were

classified as amyloid positive quantitatively (qA +) utilizing a binary classification with subjects considered qA + if they had a mcSUVR ≥ 1.10 (Table 2).

Overall agreement between the binary visual classification (vA + or vA -) and the mcSUVR based dichotomization (qA + or qA -) was 91% (i.e. there was agreement in 166/183 subjects including 67 vA +/qA + and 99 vA -/qA -) with a kappa of 0.81. Among the 9% of subjects (17/183) in whom the mcSUVR and visual classification differed, 16 were vA -/qA + and one was vA +/qA -. Among the 16 vA -/qA + subjects, there were 8 HC, 4 MCI and 4 AD subjects. vA -/qA + subjects had an average mcSUVR (1.16 $\pm$ 0.05) that was just slightly higher the mcSUVR threshold for a quantitatively positive classification (mcSUVR ≥ 1.10). Compared to vA -/qA - subjects, vA -/qA + subjects were significantly older (75.1 $\pm$ 6.8 vs. 68.5 $\pm$ 11.5; $p < 0.05$) and included a higher percentage of APOE $\epsilon 4$ subjects (33% vs. 17.2%; $p < ?$).

In the full sample, age significantly correlated with mcSUVR ($r = 0.25$; $p < 0.0006$), however, this was due largely to correlation between age and mcSUVR in the HC group ($r = 0.32$; $p < 0.005$; Figure 2); age was not significantly correlated with mcSUVR in MCI ($r = 0.16$; $p = 0.21$) or in AD ($r = 0.01$; $p < 0.97$). In cognitively normal subjects, rates of A positivity by visual read increased with age: 5.3%, 10.5%, 15.0%, and 25.0% of cognitively normal subjects aged 50 to 59 ($N = 19$), 60 to 69 ($N = 19$), 70 to 79 ($N = 20$), and 80 years or more ($N = 20$), respectively, were vA +. Similarly, 5.3%, 15.8%, 25.0%, and 40.0% of cognitively normal subjects aged 50 to 59 ($N = 19$), 60 to 69 ($N = 19$), 70 to 79 ($N = 20$), and 80 years or more ($N = 20$), respectively, were qA +. Florbetapir binding did not differ according to gender using either mcSUVR measures or visual reads.

Subjects with the $\epsilon 2/\epsilon 3$ APOE genotype ($N=18$) were all vA - and had low mean cortical SUVRs regardless of age or clinical diagnostic group (Figure 3). Subjects with either $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$ genotype had a higher proportion of vA + and tended to have higher mcSUVR, particularly among those who were older or had cognitive impairment (MCI or AD). The overall difference in mcSUVR between APOE groups (carrier vs. non-carrier) reached statistical significance for subjects with AD ($P=0.0017$) and MCI ($P<0.0001$) and approached, but did not reach, statistical significance in cognitively normal subjects ($P=0.0776$) (Figure 1). Looking only within qA + subjects with known APOE genotypes (a subset of the subjects in Table 1), there was no difference in mcSUVR between carriers and non-carriers in the AD group (64% carriers, $p>0.34$) or in the HC group (38% carriers, $p>0.97$). However, among the qA + MCI subjects (67% carriers), mcSUVR was significantly higher in carriers (mcSUVR mean difference = 0.23; $p<0.003$)

We compared cognitive test scores between the vA + and vA - groups, as well as the qA + and qA - groups. Across all subjects, irrespective of visual or quantitative classification methods, there were statistically significant differences between A + and A - groups on all cognitive tests ($P<0.0001$), with subjects rated as A - performing better than subjects rated as A + (Table 4). Within diagnostic groups (Table 5), the most consistent differences in cognitive performance between vA + and vA - subjects were seen in the HC group, discussed in more detail in a separate communication (Sperling et al, submitted). Within the AD and MCI groups, differences in cognitive performance were more variable. AD subjects rated as vA + had significantly poorer scores on LMI, DSS and ADAS-COG than subjects rated as vA -. Within the MCI group, subjects rated as vA + had statistically significantly poorer scores on DSS and ADCS-ADL than subjects rated as vA -. We also compared those in the MCI group with an amnesic profile, performing at least 1 SD below normal (score ≤ -1) on the LMI test to those performing above this threshold. There were no significant differences between the amnesic and nonamnesic subjects in the proportion of subjects rated vA + or qA +.

The inter-rater correlation for raters trained to rate amyloid burden level on the semi-quantitative scale (rating from 0 to 4) ranged from 0.759 to 0.826 between any 2 raters ($P < 0.0001$ for each pair of raters). The overall (Fleiss) kappa for the derived binary rating was 0.58 (0.49-0.66). For the derived binary rating, two of the 3 readers (reader 1 and reader 3) had excellent agreement with the majority score and with each other, yielding a kappa coefficient of 0.86. The third rater consistently assigned higher amyloid burden scores, which resulted in more cases receiving a positive rating on the binary read, yielding kappa values of 0.46 and 0.48 in comparison to the other raters.

The inter-rater correlation for amyloid burden level among the 5 readers newly trained to perform a binary interpretation showed an overall mean accuracy on the previously reported autopsy cases in excess of 90%, consistent with the high accuracy/AUC obtained for the three readers in the original study (Clark, 2011), and also showed high inter-rater consistency on both the autopsy cases from that study (kappa=0.82, 95% CI 0.71-0.92), and the cases from the present study (kappa = 0.88, 95% CI 0.81 to 0.94). Most importantly, this improved agreement did not come at the expense of a change in overall interpretation. The majority read score from the five new raters disagreed with the majority read score from the original three raters in only 3 of 92 cases, resulting in a kappa of 0.93 (95% CI 0.85 to 1.0). The intra-rater agreement among these 5 readers was also excellent with a median kappa= 0.94 (range from 0.8 to 1).

Florbetapir was safe and well tolerated. Twenty treatment-emergent adverse events (TEAE) were experienced by 17 subjects (9.2%), but no TEAE was considered severe or considered to have a probable relationship to study drug (Table 6).

DISCUSSION

This phase 2 study of the amyloid PET agent Florbetapir F 18, used both qualitatively (visual read) and quantitatively derived definitions of amyloid positivity. Consistent with other studies that have used PET amyloid tracers or CSF markers for amyloid, the proportion of subjects classified as amyloid positive by both the visual read and the quantitative evaluation increased with diagnostic category (HC<MCI<AD), age and APOE 4 carrier status.

Using the quantitative cut point (mcSUVR=1.10) that has been histopathologically validated (Clark et al 2011), 84% of clinically diagnosed AD patients, 42% of MCI subjects and 23% of clinically normal subjects were amyloid positive. Using the qualitatively derived binary classification, somewhat lower values of 76%, 38% and 14% amyloid positive were obtained, respectively. These results are consistent with the range of amyloid positive AD, MCI and clinically normal subjects observed in previous autopsy case studies (Gearing 1995; Lim 1999; Pearl, 1997; Rasmussen 1996; Bennett, 2005; Petersen, 2006; Hulette, 1998; Knopman, 2003; Price and Morris, 1999; Schmitt, 2000).

The percentage of subjects rated positive, particularly for the AD and MCI groups was lower than in some previous studies using other PET amyloid tracers (e.g., Rowe 2007; Rowe 2011; Gomperts 2008; Vandenberg Annals 2010; Barthels Lancet Neurology 2011). There may have been several factors contributing to this difference. First, the percentage of subjects who were APOE 4 carriers in the present study (39% of MCI and 53% of AD) is lower than in APOE 4-enriched multi-center research studies such as ADNI (e.g., MCI: 54% carriers and 74% A +; AD: 69% carriers and 91% A +; Shaw 2009) and AIBL (e.g., MCI: 55% carriers and 68 % A +e; AD: 69% carriers and 98% A +; Rowe 2011). Given that APOE 4 is strongly associated with increased probability of amyloid positivity in present and previous trials, the lower percentage of 4 carriers in the present study is

consistent with the lower rate of A + classification in this study. Second, site-specific practices and protocol-directed selection criteria may have contributed to the lower observed rate of amyloid positive cases. The rate of A + classification in the present AD group, in particular, appeared to be influenced in part by one center that accounted for half the A – AD subjects. Notably, the A – AD in the present study had significantly less impairment than the A + AD on the LMI, DSS and particularly the ADAS, suggesting that at least some of these subjects may have had fairly mild cognitive impairments, which are more difficult to diagnose accurately. Along the same lines, it is notable that the MCI subjects in the present study, in contrast to previous studies including ADNI and AIBL, were required by the inclusion criteria to have been diagnosed within one year of screening (approximately 50% received the MCI diagnosis at screening) and not required to perform below a certain threshold level of impairment on objective cognitive testing. It is likely therefore that the AD and MCI subjects in the present study represented more heterogeneous groups of individuals who on average had milder levels of impairment. Such individuals might be more typical of those who would present in a clinical setting for initial diagnosis or might be considered for clinical trials outside major academic centers.

While it is clear that recruitment and screening factors can influence the observed rate of amyloid positivity, other possibilities should be considered. Case reports in which amyloid negative PET has been correlated with amyloid positive histopathology highlight the unusual but important circumstance in which low levels of abeta pathology were detected but were insufficient to support a diagnosis of AD pathology under current guidelines (Leinonen 2008; Cairns 2009). Moreover, Rosen et al. reported the rare circumstance in which PiB failed to bind *in vitro* to an unusual strain or form of abeta (Rosen 2009). While isolated, these reports indicate potentially important limitations in sensitivity and specificity that may apply to the detection of minimal amyloid changes or to atypical amyloid forms. Additional research, including multiple biomarker studies with long term cognitive follow-up and autopsy verification continue to be needed to determine whether low levels of amyloid reflect the earliest stages of Alzheimer's type pathology or normal variants on aging.

The 3 readers utilized in the present study appeared more conservative and potentially less sensitive to the presence of tracer accumulation/amyloid pathology by comparison to the quantitative analysis, although one reader did show a higher overall rate of positivity than the others. This study was conducted as part of a Phase II trial, prior to the evaluation of florbetapir imaging in patients followed to autopsy (Clark et al., 2011). The availability of the autopsy verified cases from the Clark study has led to further improvements in visual read criteria and training methods, reducing the difference between the read and the quantitative evaluation.

In addition, further developments in quantitative evaluation, including algorithmic and software developments could provide a basis for the incorporation of the SUVR into the interpretation process, in order potentially to improve reliability. The most valuable information regarding the threshold for positivity on these subjects will come from follow-up studies of cognitive status.

Nevertheless, even in this Phase II study, consistent trends were seen for both the visual read and quantitative analysis (mcSUVR) results with respect to diagnostic group, age and APOE genotype (see below). Moreover, as will be described in a separate report, measurement of amyloid burden by visual read and amyloid burden measured by mcSUVR yielded similar predictive relationships with cognitive deterioration over an 18-month interval (Murali, Neurology in press). Thus, both the visual read and the SUVR analysis appear to provide consistent and meaningful associations with risk factors and cognitive performance.

In the present study age was related to amyloid deposition in the HC group. Previous reports have indicated that the prevalence of abnormal abeta deposition rises in normal aging (Pontecorvo and Mintun, 2011; Ikonomic, 2008; Price and Morris, 1999; Jack, 2009; Pike, 2007; Rowe NBA 2010; Becker 2011). However, it is clear from the present data that there are substantial numbers of older individuals in whom measurable abeta deposition is not detected, suggesting that increasing abeta deposition is not a necessary consequence of aging. In contrast to the HC cohort, in the AD group, the A + patients were more evenly distributed across the age range and were more likely to carry the APOE4 allele. APOE4 was present in the HC sample at close to the expected level for the general population, but was overrepresented in both the MCI and AD groups, as is commonly observed. Across all diagnostic groups and within diagnostic groups, mean amyloid burden (SUVR) was higher according to carrier status, as previously reported (Rowe 2007; Reiman 2009; Morris 2010).

In summary, data from the phase 2 trial of florbetapir are consistent with rates of amyloid positivity seen in autopsy case studies of cognitively normal individuals and individuals with clinical diagnoses of AD and MCI. The probability of an amyloid positive florbetapir PET scan varied as expected with risk factors for amyloid accumulation, including clinical diagnosis, age and APOE genotype. These results support the recent findings indicating florbetapir PET signal as measured by visual read or cortical to cerebellum SUVR is correlated with brain amyloid burden as assessed by immunohistochemistry or silver stain (neuritic plaque density) at autopsy (Clark 2011). Together these results suggest florbetapir PET may have value as an adjunct to clinical diagnosis.

APPENDIX: Site Clinical Investigators

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Figure 1.
Mean Cortical SUVR by diagnostic group and ApoE 4 status

Figure 2.
Mean Cortical SUVr in Healthy Controls by age

Figure 3. Average Mean Cortical SUVR by APOE 4 genotype and diagnostic group. Error bars show the upper 95% CI using the standard error of the mean.

Table 1

Baseline Subject Characteristics

	Clinically Diagnosed AD	Mild Cognitive Impairment	Cognitively Normal	p-value	p<0.05
	(N=45)	(N=60)	(N=78)		
Age in years	75.38 (9.21)	71.68 (10.23)	69.40 (11.11)	P=0.0099	<i>a</i>
Gender: Female N (%)	19 (42.2%)	33 (55.0%)	44 (56.4%)		
Male	26 (57.8%)	27 (45.0%)	34 (43.6%)	P=0.2816	
Education (years)	14.53 (3.03)	15.00 (2.42)	15.23 (2.32)	P=0.3438	
Race					
Asian	0	0	1 (1.3%)		
Black or African American	1 (2.2%)	2 (3.3%)	4 (5.1%)		
Caucasian	41 (91.1%)	58 (96.7%)	71 (91.0%)		
Native American/Alaskan Native	0	0	0		
Other	3 (6.7%)	0	2 (2.5%)	P=0.3834	
ApoE genotype				P = 0.0055	<i>a</i>
2/2	0	0	0		
2/3	2 (5.0%)	4 (7.3%)	12 (16.7%)		
2/4	0	0	0		
3/3	17 (42.5%)	29 (52.7%)	44 (61.1%)		
3/4	13 (32.5%)	20 (36.4%)	12 (16.7%)		
4/4	8 (20%)	2 (3.6%)	4 (5.6%)		
Missing	5	5	6		
Mini Mental State Exam	20.60 (3.85)	27.37 (1.78)	29.55 (0.50)	p<0.0001	<i>a, b, c</i>
CDR-SB	5.98 (2.78)	1.57 (0.98)	0.03 (0.11)	p<0.0001	<i>a, b, c</i>
Activities of Daily Living	61.96 (10.80)	74.20 (5.36)	77.19 (1.66)	p<0.0001	<i>a, b, c</i>
Digit Symbol Substitution	18.84 (12.48)	38.52 (11.16)	48.85 (9.86)	p<0.0001	<i>a, b, c</i>

	Clinically Diagnosed AD	Mild Cognitive Impairment	Cognitively Normal	p-value	p<0.05
	(N=45)	(N=60)	(N=78)		
WMS Delayed Recall	1.51 (2.77)	8.63 (4.59)	12.50 (3.74)	p<0.0001	a,b,c
WMS Immediate Recall	3.89 (3.74)	10.48 (3.87)	13.72 (3.17)	p<0.0001	a,b,c
Verbal Fluency Animals	10.02 (4.72)	15.83 (4.42)	19.92 (4.65)	p<0.0001	a,b,c
Verbal Fluency Vegetables	5.93 (3.28)	11.42 (3.57)	13.97 (3.65)	p<0.0001	a,b,c
ADAS-COG	22.04 (9.80)	9.52 (5.02)	4.71 (2.39)	p<0.0001	a,b,c

Values provided are means (SD) unless otherwise noted

p-value: test of overall association

^aAD vs CN

^bMCI vs. CN

^cAD vs. MCI

Mean cortical SUVR (mcSUVR), and amyloid positive classification by visual rating (vA +) and SUVR threshold (qA +) in AD Dementia, MCI and NCs

Table 2

	AD	MCI	NC	
	(N=45)	(N=60)	(N=78)	P value
				Pairwise Comparisons
Mean cortical SUVR (mcSUVR)	1.40 (0.27)	1.20 (0.28)	1.05 (0.16)	$P_a < 0.0001$ $P_b = 0.0003$ $P_c < 0.0001$
A + by visual classification				0.0001^b
No. (%)	34 (76)	23 (38)	11 (14)	$P_a < 0.0001$ $P_b = 0.001$
[95%CI % A +] ^c	[61,86]	[27,51]	[8,24]	$P_c = 0.0001$
A + by SUVR cutoff (1.10) classification				$< 0.0001^b$
No. (%)	38 (84)	27 (45)	18 (23)	$P_a < 0.0001$ $P_b = 0.01$
[95% CI % A +] ^c	[71,92]	[33,58]	[15,34]	$P_c < 0.0001$

p-value: test of overall association; P_a: AD vs CN; P_b: MCI vs. CN; P_c: AD vs. MCI

^a P-value from ANOVA

^b P-values are from a Fisher exact test comparing the proportion of A + subjects.

^c Wilson 95% CI on the proportion of subjects classified as A +.

Table 3

Mean regional cortical SUVR by Diagnostic Group

	Clinically Diagnosed AD (N=45)	Mild Cognitive Impairment (N=60)	Cognitively Normal (N=78)	p-value	P<0.05
Precuneus	1.54 (0.29)	1.31 (0.32)	1.13 (0.19)	P<0.0001	a, b, c
Frontal	1.28 (0.29)	1.09 (0.27)	0.96 (0.16)	P<0.0001	a, b, c
Temporal	1.42 (0.27)	1.22 (0.27)	1.10 (0.17)	P<0.0001	a, b, c
Parietal	1.24 (0.25)	1.09 (0.27)	0.95 (0.16)	P<0.0001	a, b, c
Anti cingulate	1.51 (0.34)	1.27 (0.31)	1.10 (0.19)	P<0.0001	a, b, c
Post cingulate	1.44 (0.27)	1.22 (0.28)	1.08 (0.16)	P<0.0001	a, b, c

Values provided are means (SD) unless otherwise noted.

p-value: test of overall association

^a AD vs CN

^b MCI vs. CN

^c AD vs. MCI

Table 4
Baseline neuropsychological test scores by amyloid classification across all diagnostic groups

	Majority Visual Rating			SUVR Cutpoint > 1.10		
	AB+ N=68	AB- N=115	P-Value	AB+ N=83	AB- N=100	P-Value
All subjects						
CDR-SB	3.57 (3.16)	1.06 (2.07)	<0.0001	3.16 (3.11)	1.03 (2.07)	<0.0001
Activities of Daily Living	67.75 (10.69)	75.25 (5.83)	<0.0001	68.89 (10.54)	75.43 (5.40)	<0.0001
Digit Symbol Substitution	27.13 (14.34)	44.56 (13.45)	<0.0001	30.07 (15.00)	44.73 (13.92)	<0.0001
WMS Delayed Recall	4.88 (5.25)	10.69 (4.97)	<0.0001	5.49 (5.37)	11.05 (4.86)	<0.0001
WMS Immediate Recall	6.78 (5.29)	12.29 (4.05)	<0.0001	7.34 (5.12)	12.65 (4.03)	<0.0001
Verbal Fluency Animals	13.37 (6.27)	17.86 (5.20)	<0.0001	13.88 (6.06)	18.07 (5.30)	<0.0001
Verbal Fluency Vegetables	8.93 (4.81)	12.54 (4.16)	<0.0001	9.34 (4.69)	12.70 (4.23)	<0.0001
ADAS-COG	16.46 (10.52)	7.05 (5.64)	<0.0001	14.95 (10.19)	6.89 (5.83)	<0.0001

Table 5
Baseline neuropsychological test scores by amyloid classification and diagnostic group

	Majority Visual Rating			SUVr Cutpoint > 1.10		
	AB+ N=11	AB- N=67	P- Value	AB+ N=18	AB- N=60	P- Value
Cognitively Normal						
CDR-SB	0.05 (0.15)	0.02 (0.10)	0.5256	0.03 (0.12)	0.03 (0.11)	0.9265
Activities of Daily Living	75.91 (2.34)	77.40 (1.44)	0.0049	76.83 (2.04)	77.30 (1.53)	0.2985
Digit Symbol Substitution	42.27 (7.42)	49.93 (9.83)	0.016	44.56 (7.46)	50.13 (10.17)	0.0343
WMS Delayed Recall	10.55 (4.18)	12.82 (3.60)	0.0611	10.61 (4.35)	13.07 (3.37)	0.0136
WMS Immediate Recall	11.91 (3.99)	14.01 (2.94)	0.0401	11.67 (3.38)	14.33 (2.85)	0.0013
Verbal Fluency Animals	20.09 (7.29)	19.90 (4.15)	0.8982	19.06 (6.58)	20.18 (3.93)	0.3703
Verbal Fluency Vegetables	14.18 (4.71)	13.94 (3.49)	0.8402	13.78 (4.45)	14.03 (3.41)	0.7962
ADAS-COG	6.00 (2.72)	4.49 (2.29)	0.052	6.17 (2.83)	4.27 (2.07)	0.0026
Mild Cognitive Impairment						
CDR-SB	1.74 (1.10)	1.46 (0.91)	0.2887	1.59 (1.07)	1.55 (0.92)	0.8555
Activities of Daily Living	72.30 (7.16)	75.38 (3.47)	0.0297	72.85 (6.75)	75.30 (3.64)	0.0781
Digit Symbol Substitution	34.78 (9.40)	40.84 (11.64)	0.0399	36.07 (9.61)	40.52 (12.06)	0.1261
WMS Delayed Recall	7.87 (4.82)	9.11 (4.44)	0.3134	8.07 (4.67)	9.09 (4.54)	0.3977
WMS Immediate Recall	9.91 (4.60)	10.84 (3.36)	0.373	9.96 (4.34)	10.91 (3.46)	0.3509
Verbal Fluency Animals	15.22 (4.52)	16.22 (4.37)	0.3993	15.26 (4.26)	16.30 (4.56)	0.3672
Verbal Fluency Vegetables	10.96 (3.50)	11.70 (3.64)	0.4361	10.78 (3.26)	11.94 (3.77)	0.2129
ADAS-COG	10.61 (4.49)	8.84 (5.27)	0.1863	9.96 (4.48)	9.15 (5.46)	0.5379
Clinically Diagnosed AD						
CDR-SB	5.96 (2.66)	6.05 (3.24)	0.9271	5.76 (2.65)	7.14 (3.35)	0.231
Activities of Daily Living	62.03 (11.22)	61.73 (9.88)	0.9368	62.32 (11.22)	60.00 (8.60)	0.6078
Digit Symbol Substitution	17.06 (10.96)	24.36 (15.62)	0.0916	18.95 (12.35)	18.29 (14.13)	0.8991
WMS Delayed Recall	1.03 (1.71)	3.00 (4.56)	0.0387	1.24 (1.92)	3.00 (5.51)	0.1228
WMS Immediate Recall	3.00 (2.73)	6.64 (5.10)	0.0039	3.42 (3.11)	6.43 (5.86)	0.0496

	Majority Visual Rating			SUVR Cutpoint > 1.10		
	AB+ N=11	AB- N=67	P- Value	AB+ N=18	AB- N=60	P- Value
Cognitively Normal						
Verbal Fluency Animals	9.94 (4.52)	10.30 (5.62)	0.8356	10.35 (4.63)	8.29 (5.22)	0.2939
Verbal Fluency Vegetables	5.85 (3.21)	6.20 (3.68)	0.7725	6.14 (3.24)	4.86 (3.53)	0.3506
ADAS-COG	23.79 (9.67)	16.64 (8.44)	0.0335	22.66 (9.77)	18.71 (9.96)	0.3334

Table 6

Treatment-emergent adverse events (safety population)

Clinical Diagnostic Group	AD (n=45)	MCI (n=60)	HC (n=79)	Total (N=184)
System Organ Class Preferred Term				
Total number of adverse events	2	6	12	20
Number of subjects with at least one adverse event, No. (%)	2 (4.4%)	5 (8.3%)	10 (12.7%)	17 (9.2%)
Musculoskeletal and connective tissue disorders, No. (%)	1 (2.2%)	3 (5.0%)	0	4 (2.2%)
Back pain	0	1 (1.7%)	0	1 (0.5%)
Musculoskeletal pain	0	1 (1.7%)	0	1 (0.5%)
Musculoskeletal stiffness	1 (2.2%)	0	0	1 (0.5%)
Neck pain	0	1 (1.7%)	0	1 (0.5%)
General disorders and administration site conditions, No. (%)	0	0	3 (3.8%)	3 (1.6%)
Fatigue	0	0	1 (1.3%)	1 (0.5%)
Feeling cold	0	0	1 (1.3%)	1 (0.5%)
Infusion site rash	0	0	1 (1.3%)	1 (0.5%)
Injection site haemorrhage	0	0	1 (1.3%)	1 (0.5%)
Vessel puncture site haematoma	0	0	1 (1.3%)	1 (0.5%)
Gastrointestinal disorders, No. (%)	0	1 (1.7%)	1 (1.3%)	2 (1.1%)
Abdominal distension	0	1 (1.7%)	0	1 (0.5%)
Flatulence	0	0	1 (1.3%)	1 (0.5%)
Investigations, No. (%)	0	0	2 (2.5%)	2 (1.1%)
Blood pressure increased	0	0	1 (1.3%)	1 (0.5%)
White blood cell count increased	0	0	1 (1.3%)	1 (0.5%)
Nervous system disorders	0	2 (3.3%)	0	2 (1.1%)
Headache	0	2 (3.3%)	0	2 (1.1%)
Injury, poisoning and procedural complications, No. (%)	1 (2.2%)	0	0	1 (0.5%)
Upper limb fracture	1 (2.2%)	0	0	1 (0.5%)
Psychiatric disorders, No. (%)	0	0	1 (1.3%)	1 (0.5%)
Claustrophobia	0	0	1 (1.3%)	1 (0.5%)
Renal and urinary disorders, No. (%)	0	0	1 (1.3%)	1 (0.5%)
Haematuria	0	0	1 (1.3%)	1 (0.5%)
Skin and subcutaneous tissue disorders, No. (%)	0	0	1 (1.3%)	1 (0.5%)
Pruritus generalised	0	0	1 (1.3%)	1 (0.5%)
Vascular disorders, No. (%)	0	0	1 (1.3%)	1 (0.5%)
Flushing	0	0	1 (1.3%)	1 (0.5%)

Abbreviations: AD: Alzheimer's disease; HC: cognitively normal (healthy) controls; MCI: mild cognitive impairment

Note: Treatment-emergent adverse events were defined as adverse events that began or worsened in intensity or frequency at the time of or after the administration of study drug. At each level of summarization, a subject was counted once if the subject reported one or more events in a given level

of summarization. Adverse event terms were coded using Medical Dictionary for Regulatory Activities Version 10.1. Percentages were based on the number of subjects in each clinical diagnostic group.