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An *In Vivo* Evaluation of Cerebral Cortical Amyloid with [¹⁸F]Flutemetamol Using Positron Emission Tomography Compared with Parietal Biopsy Samples in Living Normal Pressure Hydrocephalus Patients

Dean F. Wong^{1,2,3,4}, Abhay R. Moghekar⁵, Daniele Rigamonti⁶, James R. Brašić¹, Olivier Rousset¹, William Willis¹, Chris Buckley⁷, Adrian Smith⁷, Beril Gok¹, Paul Sherwin⁸, and Igor D. Grachev⁸

¹The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, 601 N. Caroline St., JHOC Room 3245, Baltimore, MD, 21287-0807, USA

²Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA

³Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

⁴Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁵Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, USA

⁶Department of Neurosurgery, Johns Hopkins School of Medicine, Baltimore, MD, USA

⁷Medical Diagnostics, GE Healthcare, Amersham, HP7 9LL, UK

⁸Medical Diagnostics, GE Healthcare, Princeton, NJ, USA

Abstract

Purpose—The primary objectives of this study were to assess the safety of [¹⁸F]flutemetamol injection and determine the level of association between the quantitative estimates of brain uptake of [¹⁸F] flutemetamol and the quantitative immunohistochemical (IHC) estimates of amyloid levels in cerebral cortex biopsies obtained during shunt placement in patients with normal pressure hydrocephalus (NPH).

Procedures—Parietal lobe biopsies were obtained from 12 subjects (mean (SD), 71 (8.1) years), during shunt placement for NPH. Shunt procedures and biopsies were performed within 8 weeks after the positron emission tomography (PET) imaging, and followed by a computed tomography scan. The quantitative estimates of the brain uptake of [¹⁸F]flutemetamol (standard uptake value ratios (SUVs)) from the biopsy site, contralateral to the biopsy site, and composite were made from the analysis of PET images. The quantitative IHC levels of amyloid load were estimated using a monoclonal anti-amyloid β antibody, 4 G8 (in percent area), as the standard of truth ($N=8$, of which 5 had full histopathology staining). The primary analysis determined the level of

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Correspondence to: Dean Wong; dfwong@jhmi.edu.

Conflict of interest. Igor D. Grachev and Paul Sherwin work for GE Healthcare, Princeton, NJ and Chris Buckley and Adrian Smith work for GE Healthcare, Amersham, UK. Chris Buckley, Adrian Smith, Paul Sherwin, and Igor D Grachev are employees of GE Healthcare. There are no other conflicts of interest.

association between the SUVR (with cerebellum as the reference region) from the biopsy site, and the level of amyloid was determined from IHC estimates of amyloid in the biopsy sample.

Results— ^{18}F Flutemetamol injection was found to be well tolerated. The biopsied area well represented the amyloid deposition throughout the cortex in this small sample. The biopsy site SUVR was significantly correlated with the biopsy specimen amyloid β level (expressed as percent of biopsy specimen area staining with 4 G8). The full model was significant ($p=0.0174$). In the secondary efficacy analyses, contralateral (to biopsy site) and composite SUVR values correlated significantly with the percent of biopsy specimen staining for amyloid β based on 4 G8. Blinded visual ^{18}F flutemetamol image interpretations showed a sensitivity of 100 % and a specificity of 100 % with pathology reads staining for amyloid plaque with Bielschowsky and thioflavin S and overall pathology read. The results of the blinded reader agreement for ^{18}F flutemetamol PET showed full agreement among three readers.

Conclusions—PET imaging of NPH patients following the administration of ^{18}F flutemetamol injection was highly correlated with the presence of fibrillar amyloid β in subsequent cortical biopsy samples in this small sample. Administration of ^{18}F flutemetamol injection was well tolerated.

Keywords

Amyloid; Biopsy; Flutemetamol; NPH; Positron emission tomography

Introduction

Alzheimer disease (AD) is the most common and progressive of the dementing illnesses and is often unrecognized in its early stages [1]. Definitive diagnosis of AD requires tissue examination through biopsy or autopsy of the brain. Histopathologic hallmarks of the disease are neurofibrillary tangles, synapse loss, and neuritic amyloid β (A β) plaques, which correlate with neuron loss and cognitive decline in patients [2–5]. The presence of amyloid plaques in brain tissue is a microscopic hallmark of AD and, specifically, β -amyloid.

Currently, the only means to detect amyloid is by histopathologic analysis of brain tissue taken during a brain biopsy or autopsy, using immunohistochemical (IHC) reagents (based on antibodies to amyloid) [6] or histochemical (HC) dyes (such as thioflavin S) [7]. Noninvasive methods to detect amyloid may provide useful clinical information and could enable more appropriate selection of patients for clinical trials of novel therapies for disorders associated with cognitive impairment.

In vivo detection of A β amyloid plaques in the brain using positron emission tomography (PET) has been successful with ^{11}C -labeled compounds, and more recently, ^{18}F -based amyloid imaging agents, which can be produced commercially and delivered to clinical PET facilities, have been developed and are currently undergoing formal clinical trials.

The current study investigated the safety and efficacy of ^{18}F flutemetamol PET for the *in vivo* detection of amyloid in the brain, via the level of association between brain uptake of ^{18}F flutemetamol and levels of amyloid from histochemical analyses of biopsy samples. The biopsy samples were obtained during placement of intraventricular shunts in patients with normal pressure hydrocephalus (NPH). NPH is one cause of dementia, and investigating the level of amyloid in patients with NPH may help increase our understanding of the causes of dementia, in addition to helping validate the ability of ^{18}F flutemetamol to detect amyloid *in vivo*. We hypothesized that there would be a high level of association between the two measures.

Materials and Methods

Subjects, Eligibility, and Overall Study Design

Subjects scheduled for shunt placement for NPH were contacted from Johns Hopkins. Inclusion criteria were the following: 50 years of age, be in good health, and nonpregnant. Subjects were required to sign an informed consent, satisfy all entry criteria, undergo safety assessments, and undergo 3D magnetic resonance imaging (MRI).

Subjects were then administered with a flutemetamol (^{18}F) injection and underwent a PET scan. A follow-up phone call was made to assess adverse events (AEs). Within 8 weeks of the PET imaging, the shunt procedure and biopsy, followed by a computed tomography (CT) scan, were performed following Johns Hopkins University's (JHU) standard practice.

This was an open-label, single-center, noncomparative study. The protocol and the informed consent form were reviewed and approved by the Western Institutional Review Board before subjects were enrolled in the study.

PET Imaging

Radiosynthesis of [^{18}F]Flutemetamol—[^{18}F]Flutemetamol injection, a fluorine-18-labeled PET fibrillar amyloid β imaging agent, is prepared as a ready-to-inject sterile solution with a theoretical maximum mass of 20 μg total (radioactive and nonradioactive) flutemetamol per dose. The pH of the [^{18}F]flutemetamol injection was 7.2 to 7.4. The radiochemical purity was >90 %. [^{18}F] Flutemetamol injection was prepared at Cardinal Health PET Center (Beltsville, MD).

PET Scan Procedure—On the day of the PET imaging, each subject received an intravenous (i.v.) dose of [^{18}F]flutemetamol. The activity of a single administration of [^{18}F]flutemetamol was ~185 MBq (5 mCi) and given within ~40 s. The PET imaging was ~30 min, beginning ~90 min after administration (mean 90.45, SD 1.75 min) of [^{18}F]flutemetamol. Dynamic PET scans of the whole brain were obtained in a single field of view (FOV). The dynamic PET data consisted of six 5-min frames. Safety assessments were performed within 24 h of the imaging.

Imaging was performed using a GE Advance PET scanner (GE Medical Systems, Milwaukee, WI, USA) in 3D modes with a 14.875-cm axial FOV. Each PET scan was reconstructed to 35 transaxial images of 128 $\times$ 128 voxels by a back-projection algorithm using the manufacture-provided software correcting for attenuation, scatter, and dead time. Resulting resolution was approximately 6 mm at full width at half maximum [8].

Biopsy

The shunt procedure and consequent biopsy were performed under control of CT scans (before, during, and after shunt placement). The tissue (~14 mm³) was immersion-fixed in 10 % neutral buffered formalin for 7 days prior to paraffin embedding. Tissue sections were cut at 4 μm , stained with hematoxylin–eosin and modified Bielschowsky [9], and immunostained for β -amyloid. These tissue sections were examined by one independent neuropathologist at the clinical research organization (CRO) who was blinded to both imaging and clinical results. Silver stains were used to assess neuritic plaques and neurofibrillary tangles according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria [10]. The presence or absence of amyloid β was determined with the 4 G8. Tissue blocks were then sent to the CRO for quantitative and qualitative assessments.

Safety

Safety was assessed during the course of the study following drug administration by monitoring the occurrence of all AEs, changes in the physical and the limited neurological examination, an ECG, clinical laboratory evaluation, and vital signs. A 24-h telephone follow-up for adverse event monitoring was then conducted.

Analyses

The safety population was defined as all subjects who received a dose of [¹⁸F]flutemetamol. The efficacy population was defined as all subjects who had a quantitative assessment of PET images and an amyloid measurement from the biopsy sample.

For each subject, the level of association between the degree of cortical uptake, determined from the quantitative assessment of PET images, and the levels of amyloid in the subject's biopsy sample, as determined from quantitative assessment of immunostains, were measured.

Image Analysis—Anonymized image data were transferred from JHU to the sponsor's Image Review Center in Oslo. The images were processed for Volume-of-Interest (VOI) analysis to assess the level of tracer uptake.

The [¹⁸F]flutemetamol PET image data were displayed in axial, sagittal, and coronal views. A Sokolov color table was applied to images of the parietal lobe, anterior cingulate, posterior cingulate, and precuneus, and the intensity of the image (as rendered by the color scale) was used to determine elevated tracer binding. Increased tracer (orange/red/pink) indicated an abnormal image. Blue/green/yellow indicated a normal image. If any one of the cortical regions (parietal, insula, lateral temporal, or striatal) had an elevated intensity, the image was rated as abnormal.

From the dynamic PET data, a 30-min summed image was derived and was spatially realigned with the subject's MRI. The postbiopsy CT was also spatially realigned with the MRI and information about the localization of the biopsy in the CT images was used to guide the definition of VOIs in the PET summed image. The target VOI analysis was completed in two ways: (1) a VOI sampling the region in the biopsy area and (2) a VOI sampling the same anatomical region on the contralateral side.

Standard uptake value ratio (SUVR) is a semiquantitative measure of tracer uptake, normalized for the mean uptake in a reference region. SUVR is defined as SUV_{VOI}/SUV_{REF} ; SUV is an integrated activity over a given time period per unit of injected dose and body mass. The injected dose and body mass variables cancel out in the SUVR calculations.

The primary endpoint was the SUVR at the site of the biopsy with cerebellum as the reference region. The secondary endpoints were as follows: (1) SUVR contralateral to the biopsy site with cerebellum as the reference region; (2) composite SUVR with cerebellum as the reference region, an average of SUVRs in the frontal cortex, anterior cingulate gyrus, posterior cingulate gyrus/precuneus, lateral temporal cortex, and parietal cortex. This average is used by a number of groups [11, 12] and allows assessment of overall amyloid load in the brain; (3) biopsy site SUVR based on the pons as the reference region; (4) SUVR contralateral to the biopsy site with pons as the reference region; (5) composite SUVR with pons as the reference region; and (6) classification of a subject's PET images as normal or abnormal based on the blinded evaluation of PET images by three independent readers trained in the evaluation of PET amyloid imaging.

The primary reference region for this study was the cerebellar cortex, but the pons was added as an alternative. Hemisphere choice was not an issue, as symmetrically placed VOIs in the left and right frontal cortices result in SUVRs that differ by only a few percentages [13].

Biopsy Tissue Analysis—The standard of truth for the brain amyloid level was quantitatively estimated using the percent area for each biopsy sample using IHC with a monoclonal antibody raised against amyloid (4 G8). Quantitative measurements of 4 G8 were made on Aperio scanned images according to the St. James University Hospital, Leeds pathology protocol [14]. The semiquantitative HC estimates of amyloid levels were estimated using thioflavin S and Bielschowsky silver stain by an independent neuropathologist at CRO.

For each brain biopsy tissue sample, where the tissue block allowed, 50 serial sections were prepared at a nominal 5 μm (7 μm for Bielschowsky silver stain). Amyloid levels were determined by IHC (percent area using the monoclonal antibody 4 G8) for correlation analysis. To evaluate the sensitivity and specificity of the blinded visual read, semiquantitative HC using thioflavin S and Bielschowsky silver stain was dichotomized. For thioflavin S samples, each microscope slide (3 per biopsy) was assessed for amyloid plaque frequency using a CERAD score [15]. Neuritic plaque frequencies for up to three Bielschowsky slides per biopsy (five fields of view per slide) were scored as 0–3. Semiquantitative measures for thioflavin S and Bielschowsky were categorized by modified CERAD criteria as 0=no plaques, 1=sparse (1–5 plaques), 2=moderate (6–19 plaques), or 3=frequent (20+ plaques). The mean scores per slide were calculated and served as the regional thioflavin S or Bielschowsky silver score. The midpoint on this scale is 1.5; a mean score of >1.5 was considered abnormal and a mean score of ≤ 1.5 as normal. An overall pathology assessment of normal or abnormal was based upon examination of all three histological stains. A 4 G8 abnormality, with a normal score on Bielschowsky silver stain and thioflavin S, was considered normal. An abnormal Bielschowsky silver stain was considered abnormal regardless of the 4 G8 or thioflavin S results.

Semiquantification of neuritic plaques in gray matter using Bielschowsky stain is standard for AD diagnostics. Multiple stains were used to better understand the relationships between pathology and amyloid imaging endpoints and to improve diagnostic confidence of the classification of relatively limited brain tissue samples. Analysis of pathology data was performed by one independent neuropathologist blinded to both imaging and clinical results.

Analysis of Relationship Between Biopsy and PET Data—Statistical analyses were performed using SAS[®] software. The last preadministration observation was the baseline for calculating postadministration changes.

The primary analysis determined the level of association (Pearson correlation coefficient) between the SUVR (using the cerebellum as the reference region) from the biopsy site and the level of amyloid determined from IHC estimates of amyloid in the biopsy sample. The null hypothesis of no association between the PET image SUVR and the level of the amyloid determined from IHC estimates of amyloid in the biopsy sample was tested using a level of significance of 0.05. This relationship was also analyzed using a regression model with the PET image SUVR measure as the independent variable and the amyloid level from the biopsy sample as the dependent variable.

The regression model is shown below:

$$Y_i = \beta_0 + \beta_1 X_i + \varepsilon_i$$

where Y_i is the percent amyloid from IHC (4 G8) analysis of biopsy sample, β_0 is the intercept, β_1 is the slope, X_i is the PET image SUVR, and ε_i is the error.

The test statistics for determination of an association was the t test for significance of the coefficient for the SUVR (β_1) at the 0.05 level of significance. The β coefficient describes the slope associated with the biopsy results relative to the SUVR. The overall model fit was checked by the F statistics, associated p value, and R -squared.

While the biopsy region SUVR was the principal measurement of amyloid in this study, an average SUVR measurement also was made, which is a global tracer signal average, and was used to show how representative the biopsy region amyloid was of that present in the brain as a whole.

In addition to the quantitative SUVR analyses, the sensitivity and specificity of the binary image assessments were determined. The blinded visual assessment of the brain PET images of the patients administered with flutemetamol (^{18}F) injection was performed by three independent blinded readers trained in the evaluation of PET amyloid imaging. Each reader independently assessed each subject's PET image and then compared with the corresponding histopathological assessment of the subject's biopsy sample. Sensitivity and specificity was assessed for Bielschowsky Silver stain pathology, thioflavin S pathology, and overall pathology.

The analysis was provided for each of the three blinded image readers and for a majority read based on 2 readers' agreement. The interreader agreement and the intrareader reproducibility were established. A kappa coefficient with 95 % confidence interval was determined (1) for each reader comparison, (2) for a multiplicity coefficient for all readers, (3) for each reader compared to the consensus read, and (4) for each reader compared to their reread assessment for subject data. A cross tabulation of the determination of normal/abnormal by pathology assessment for Bielschowsky silver stain, thioflavin S, and overall assessed agreement of the different methods was conducted.

Results

Subjects

A total of 16 subjects were enrolled and 4 withdrew prior to administration of [^{18}F]flutemetamol. Twelve subjects were administered with [^{18}F]flutemetamol, were evaluable for safety, and completed the study through the 24-h follow-up. Due to an insufficient amount of tissue in the biopsy samples, no pathology assessment was performed for two subjects who were included in the safety population, but not in the efficacy population. In addition, two subjects did not have 4 G8 amyloid percent measurements for the same reason. Therefore, eight subjects were included in the efficacy analyses using 4 G8 results as an estimate of amyloid burden. Amyloid burden by Bielschowsky silver stain was evaluable in five subjects' biopsies (two subjects did not have a sufficient amount of tissue, one subject had white matter). Consequently, five of the eight subjects in the efficacy population were included in efficacy analyses using Bielschowsky silver stain result as an estimate of amyloid burden.

In the safety population, of the 12 subjects, 5 were female and the subjects' mean (SD) age in years was 71 (8.1). In the efficacy population, three subjects were female and the

subjects' mean (SD) age in years was 73 (8.1). The mean (SD) mini mental state examination score for the 12 subjects was 24.5 ($\pm$ 3.68).

Safety

There were no deaths or other significant AEs reported during this study. Changes in serum chemistry, hematology, and coagulation laboratory values from screening to postimaging were not clinically significant. Subjects were given an average [^{18}F]flutemetamol dose of 5.4 mCi $\pm$ 0.48 (SD), with a flutemetamol mass of 20 μg /dose.

Efficacy

The results for the blinded visual image interpretations compared to the reference standard are shown in Table 1. The blinded visual assessments of PET images by readers 1, 2, and 3 were associated with Bielschowsky silver stain, thioflavin S, and overall pathology reads, with 100 % sensitivity and 100 % specificity. There was complete agreement between the Bielschowsky and thioflavin S read. Five subject images were reread. The intrareader reproducibility of those reads was 100 % and 100 % interreader agreement.

The biopsy site SUVR was correlated with the biopsy specimen amyloid β level (expressed as percent of biopsy specimen area staining with 4 G8). The full model was significant ($R^2=0.64$, $p=0.0174$) (see Table 2, biopsy sample in Fig. 1). In the secondary efficacy analyses, regardless of the reference region, there were correlations between biopsy site SUVR and the biopsy specimen amyloid β level (see Table 2).

Association Between Biopsy Site SUVR and Composite SUVR

The association between biopsy site SUVR and the composite SUVR was highly significant ($r=0.91$, $p<0.05$) (Fig. 2), demonstrating that the amyloid burden in the biopsied parietal region was representative of amyloid deposition in the entire cortex. Mean regional SUVR for the 12 subjects who participated in the PET scan is shown in Fig. 3.

Discussion

The objectives of this study were to provide safety and efficacy data to support the evaluation of [^{18}F]flutemetamol as a diagnostic radiopharmaceutical indicated for the *in vivo* detection of amyloid in the brain. Safety assessments showed that single doses of [^{18}F]flutemetamol were well tolerated in NPH subjects. For PET imaging measurements using the cerebellum as the reference region, PET SUVR values in all brain regions evaluated correlated with HC-derived tissue amyloid β levels. Thus, cortical uptake of [^{18}F]flutemetamol in NPH reflected amyloid β brain content.

The level of association was similar whether the SUVR value was determined at the biopsy site or at a site contralateral to the biopsy site or whether an average value was based on multiple brain regions. Further, similar levels of correlation were observed when the pons was used as the reference region.

Despite the small number of subjects sampled ($N=10$, 8 of which had IHC and only 5 of those 8 had Bielschowsky staining), the current study showed a good correlation between cerebral cortical uptake of [^{18}F]flutemetamol and tissue levels of fibrillar amyloid β . This is remarkable, as only a single small cortical biopsy was collected.

The results of this study are comparable with those obtained in a previous study with [^{11}C]PiB, an amyloid imaging agent that is structurally similar to [^{18}F]flutemetamol [16]. In that study, 10 NPH subjects who had undergone a right frontal cortical biopsy 2 to 36

months earlier, underwent PET imaging after i.v. injection of [^{11}C]PiB. The right frontal cortical [^{11}C]PiB uptake values correlated with the number of amyloid β aggregates determined by HC staining with 4 G8. Four of the 10 cases had elevated cortical [^{11}C]PiB uptake, while 5 had elevated biopsy amyloid plaque levels with immunostaining. This served as the preliminary basis for the design of this study.

Wolk et al. [17] also recently reported [^{18}F]flutemetamol PET findings in seven patients who had a prior right frontal cortical biopsy during ventriculoperitoneal placement for NPH [18]. They also found a correlation between [^{18}F]flutemetamol uptake and percentage of area of amyloid measured by a monoclonal antibody raised against amyloid. A regression model, including time from biopsy as a covariate, demonstrated a significant relationship ($p=0.011$) between [^{18}F]flutemetamol uptake and percent area of amyloid measured by a monoclonal antibody raised against amyloid (NAB228). Similar results were found with the amyloid-specific monoclonal antibody 4 G8 and the dye thioflavin S.

The current study had a few important differences from Wolk et al. [17]. The advantages of the current method were the following: First is the order of the biopsy relative to the PET scans, with the PET scans occurring after the biopsy in the work by Wolk et al. and first in the current study [17] study, which allowed a more accurate measurement of the imaged site in the current study. Second, in the current study, an independent blinded third party pathologist performed the biopsy read, whereas Wolk et al. [17] used a local pathologist. A potential disadvantage of the current study, however, is the much smaller biopsy size in the current study (14 mm^3) vs. the larger size (500 mm^3) used by Wolk et al. [17]. In spite of the differences in the study design (prospective in our study vs. Wolk's retrospective), timing between PET and biopsy (weeks in our study vs. months to years in Wolk et al. [17]), and the biopsy location (parietal cortex in our study vs. frontal cortex in Wolk's et al. [17]), both studies showed a good association between amyloid PET and neuropathology and demonstrated that the brain amyloidosis at the biopsy site appears to be representative for the entire cortex.

In the current study, the results for the comparison between blinded visual [^{18}F]flutemetamol image interpretation and the qualitative overall pathology read combining the interpretations of the 4 G8, Bielschowsky and thioflavin S stains showed 100 % sensitivity and specificity. This suggests that the results of [^{18}F]flutemetamol PET scanning reliably agrees with the presence of fibrillar amyloid β in the brain of living patients with NPH in this small sample.

Reader reproducibility between the blinded image readers was excellent, with perfect agreement between the three readers for the classification of 10 [^{18}F]flutemetamol scans, as well as perfect intrareader agreement upon partial reread.

Biopsy specimens with no or sparse levels of amyloid plaque were categorized as normal, and those with moderate or severe plaque loads of amyloid were categorized as abnormal according to the modified CERAD scale [15]. It is important to note that this amyloid-positive cutoff, based on the overall results with three stains, is meant to distinguish between amyloid-positive and amyloid-negative, independent of clinical diagnosis. The presence of fibrillar amyloid β is necessary, but not sufficient, for a diagnosis of AD to be made. However, the patterns of amyloid observed in NPH subjects classified as abnormal involved all cortical association areas and were consistent with the pattern of amyloid deposition typically observed in AD subjects.

A phase III study demonstrated that both visual interpretation of the florbetapir PET images and the mean cortical uptake correlate with presence and quantity of amyloid β pathology on

postmortem histopathological evaluations [19]. Clinically, both distribution volume ratios and standardized uptake value ratios of florbetapir PET also showed significant discrimination between AD patients and normal healthy controls [20]. Recently, *in vivo* quantitative estimates of brain uptake of [¹⁸F]flutemetamol have demonstrated close concordance with *in vivo* estimates of amyloid [17].

Conclusion

Despite the small sample size, this study shows 100 % sensitivity and specificity of [¹⁸F]flutemetamol PET for detection of fibrillar amyloid β deposits in the brain of living subjects with NPH. PET imaging of NPH patients following the administration of [¹⁸F]flutemetamol injection correlated with the presence of fibrillar amyloid β in subsequent cortical biopsy samples. Administration of [¹⁸F]flutemetamol injection was well tolerated.

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Fig. 1.

PET and pathology images for all subjects. Individual PET images with corresponding microscopic slides from biopsies (4 G8 stain). PET images are shown in sagittal and axial plane at the midlateral ventricular level. A *plus sign* at the *top left* of the sagittal image indicated abnormal majority PET read. Amyloid β HC (4 G8) is shown at $\times 100$ magnification for all biopsies. *NP* above the image indicates presence of neuritic plaques as determined by Bielschowsky silver stain (data not shown). A *gray X* indicates that insufficient cortical gray matter was available for Bielschowsky assessment.

Fig. 2.
Association between biopsy site SUVR and composite SUVR.

Fig. 3.
Mean regional standard uptake value ratios (SUVr) for all 12 subjects who participated in the PET scan.

Association between blinded visual assessment and Bielschowsky silver stain, thioflavin S, and qualitative overall pathology read—efficacy population

Table 1

Reader	Blinded visual assessment	Qualitative Bielschowsky silver stain pathology read		Qualitative thioflavin S pathology read		Qualitative overall pathology read		Sensitivity % (95 % CI)	Specificity % (95 % CI)
		Abnormal (N=2)	Normal (N=3)	Abnormal (N=2)	Normal (N=8)	Abnormal (N=2)	Normal (N=8)		
Reader 1	Abnormal	2 (100)	0	2 (100)	0	2 (100)	0	Bielschowsky: 100 (16, 100) Thioflavin S: 100 (16, 100) Overall: 100 (16, 100)	Bielschowsky: 100 (29, 100) Thioflavin S: 100.0 (63, 100) Overall: 100 (63, 100)
	Normal	0	3 (100)	0	8 (100)	0	8 (100)		
Reader 2	Abnormal	2 (100)	0	2 (100)	0	2 (100)	0	Bielschowsky: 100 (16, 100) Thioflavin S: 100 (16, 96) Overall: 100 (16, 100)	Bielschowsky: 100 (29, 100) Thioflavin S: 100 (63, 100) Overall: 100 (63, 100)
	Normal	0	3 (100)	0	8 (100)	0	8 (100)		
Reader 3	Abnormal	2 (100)	0	2 (100)	0	2 (100)	0	Bielschowsky: 100 (16, 100) Thioflavin S: 100 (16, 100) Overall: 100 (16, 100)	Bielschowsky: 100 (29, 100) Thioflavin S: 100 (63, 100) Overall: 100 (63, 100)
	Normal	0	3 (100)	0	8 (100)	0	8 (100)		
Majority read	Abnormal	2 (100)	0	2 (100)	0	2 (100)	0	Bielschowsky: 100 (16, 100) Thioflavin S: 67 (22, 96) Overall: 100 (40, 100)	Bielschowsky: 100 (29, 100) Thioflavin S: 100 (66, 100) Overall: 100 (72, 100)
	Normal	0	3 (100)	0	8 (100)	0	8 (100)		

Relationship between PET and biopsy data. Biopsy site, contralateral, and average SUVR values, with cerebellum and pons as the reference regions, correlated with the percent of biopsy specimen staining for amyloid β based on 4 G8. SUVR-PONS standard uptake value ratio using pons as the reference region

Table 2

Standard of truth (SOT)									
SUVR-CER from PET imaging									
IHC 4 G8	Biopsy site	Contralateral to biopsy site				Average region			
	% area of amyloid	% area of amyloid		% area of amyloid		% area of amyloid			
	<i>p</i> value	<i>R</i> ²	<i>p</i> value	<i>R</i> ²	<i>p</i> value	<i>R</i> ²	<i>p</i> value	<i>R</i> ²	
	0.0174	0.64	0.0107	0.69	0.0025	0.81			
	Pearson correlation coefficient		Pearson correlation coefficient		Pearson correlation coefficient				
	Parameter estimate	<i>p</i> value	Parameter estimate	<i>p</i> value	Parameter estimate	<i>p</i> value	Parameter estimate	<i>p</i> value	
SUVR-PONS from PET imaging									
IHC 4 G8	Biopsy site	Contralateral to biopsy site				Composite region			
	% area of amyloid	% area of amyloid		% area of amyloid		% area of amyloid			
	<i>p</i> value	<i>R</i> ²	<i>p</i> value	<i>R</i> ²	<i>p</i> value	<i>R</i> ²	<i>p</i> value	<i>R</i> ²	
	0.0244	0.60	0.0190	0.63	0.0051	0.76			
	Pearson correlation coefficient		Pearson correlation coefficient		Pearson correlation coefficient				
	Parameter estimate	<i>p</i> value	Parameter estimate	<i>p</i> value	Parameter estimate	<i>p</i> value	Parameter estimate	<i>p</i> value	
	0.773	0.0244	0.793	0.0190	0.869	0.0051			0.0051