2. Synopsis - *amended*

Study title:	An open-label, non-randomized, multi-center study to optimize image assessment and evaluate the efficacy and safety of florbetaben (ZK 6013443) positron emission tomography (PET) for detection/exclusion of cerebral amyloid beta in patients with probable Alzheimer's disease compared to healthy volunteers
EudraCT number:	2007-002256-42
Clinical phase:	Phase 2
Study objectives:	**Primary objective of Part A** To determine the sensitivity and specificity the independent visual assessment of florbetaben PET images (from the 90 to 110 min imaging window) in detecting/excluding cerebral beta-amyloid in patients with probable Alzheimer's disease (AD) compared to healthy volunteers (HVs). The *clinical diagnosis* - established by the onsite investigator – and based on internationally accepted, validated criteria and established after comprehensive clinical and neuro-psychiatric examination and examination of other relevant data served as the *Standard of Truth (SoT) in Part A*. **Primary objective of Part B** Although the primary objective of Parts A and B were identical, the method by which the diagnosis was established differed. To determine the sensitivity and specificity of the independent visual assessment of the florbetaben PET images (from the 90 to 110 min imaging window) in detecting/excluding cerebral beta-amyloid in patients with probable Alzheimer's disease (AD) compared to healthy volunteers (HVs). In Part B, the clinical diagnosis was established by an independent consensus panel (CP) of experts in dementia and – as in Part A- was based on internationally accepted diagnostic criteria established after comprehensive review of all available onsite clinical, neuropsychiatric and other relevant data. The *final CP diagnosis* served as the *SoT in Part B* and was made without knowledge of florbetaben PET scan findings. The onsite investigators also provided a clinical diagnosis (hereafter referred to as the 'onsite diagnosis') which served to track subjects' diagnoses during the course of recruitment and as a quality assurance check when compared to the CP diagnosis. For both Part A and B, the florbetaben PET scans were visually assessed by three independent nuclear physicians (and experts in PET neuroimaging). Images were assessed for the regional presence or absence of tracer uptake (which was assumed to correlate to the presence or absence of beta-amyloid deposition). The readers were blinded to all clinical findings, including the CP diagnosis. The readers analyzed the images in a blinded and consecutive order - independent of one another. To verify efficacious diagnostic performance in this exploratory phase 2 trial, a highly characterized collective of individuals expected to have a positive florbetaben scan (ie, subjects with probable AD) for calculation of sensitivity and those with expected 'negative' scans (ie, HVs) for calculation of specificity were required.

Study objectives (continued)	**Secondary objectives of Part A** To evaluate the usefulness of two additional imaging windows (ie, 45 to 60 min and 110 to 130 min) for the visual assessment based on sensitivity and specificity values obtained in these two imaging windows. To evaluate the proposed florbetaben visual assessment procedure and subsequent classification. To assess various quantitative florbetaben PET image analysis methods. To evaluate the safety and tolerability of a single dose of florbetaben in patients with probable AD and HVs. **Secondary objectives of Part B** To evaluate the usefulness of two additional imaging windows (ie, 45 to 60 min and 110 to 130 min) for the visual assessment based on sensitivity and specificity values obtained in these two imaging windows. To determine the sensitivity, specificity of both, volume of interest (VOI) and voxel-based quantitative image analysis in detecting/excluding cerebral beta-amyloid when compared to the independent CP diagnosis as the SoT. The quantitative measures of subjects in Part B were used to validate the quantitative image analysis methods derived in Part A independently concerning their discriminative ability in detecting/excluding cerebral beta-amyloid. To confirm the safety profile of a single dose of florbetaben in patients with probable AD and HVs.
Test drug:	*trans*-4-(*N*-methyl-amino)-4´-{}-stilbene
Name of radioactive drug substance **Name of drug product (ie, radioactive drug and formulation)**	Florbetaben (ZK 6013443) - hereafter referred to solely as florbetaben Material No.: 80940611 - hereafter referred to as Investigational Medicinal Product (IMP)
Dose:	**Part A** The administered florbetaben radioactive dose was 300 MBq (8.1 mCi) +/- 20% per injection. The specification of the mass dose of active drug substance was ≤5 µg/injection. **Part B** The administered florbetaben radioactive dose was 300 MBq (8.1 mCi) +/- 20% per injection. The specification of the mass dose of active drug substance was ≤50 µg/injection. The investigational medicinal product (IMP) was administered as a slow intravenous bolus injection (ie, 6 sec/ml) into a large vein (e.g. antecubital vein).
Route of administration:	Intravenous injection
Bulk batch number:	Not applicable. Patient data listings see Appendix 16.1.6
Duration of treatment:	Single administration of a diagnostic agent
Reference drug:	None
Dose:	--
Route of administration:	--
Bulk batch number:	--
Duration of treatment:	--
Indication:	Florbetaben PET imaging can detect cerebral beta-amyloid plaques/deposition in the brain.

Diagnosis and main criteria for inclusion:	Study participants were HVs and patients diagnosed with probable AD. HVs were ≥ 55 years of age and had to have no evidence of cognitive impairment by medical history. The lack of cognitive impairment was also to be based on an MMSE which was ≥28 and a Clinical Dementia Rating (CDR) of 0. Patients with probable AD were ≥ 55 years of age and had to fulfill both the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition [DSMIV] criteria for dementia of Alzheimer's type and the National Institute of Neurological and Communicative Disorders and Stroke, Alzheimer's Disease and Related Disorders Association [NINCDS-ADRDA] criteria for probable AD. The Mini mental Status Examination (MMSE) score was between 18 and 26 and the CDR 0.5 or 1.0 or 2.0. In both subject groups, the presence of relevant cerebrovascular disease was ruled out by Magnetic Resonance Imaging (MRI). The mean age of the probable AD and HV cohorts were balanced. All HVs and all patients with probable AD were able to understand and comply with all study procedures.
Study design:	**<u>Part A</u>** This was a phase 2, open-label, multi-center, non-randomized single dose study to assess the safety and determine the point estimates sensitivity and specificity of the visual assessment of florbetaben PET imaging in detecting beta-amyloid plaque in the brain in patients with probable AD compared to HVs. All aspects related to image acquisition, processing and visual as well as quantitative evaluation were developed, validated, and optimized (where required.) **<u>Part B</u>** This was a phase 2, open-label, multi-center, non-randomized single dose study to assess the safety and to determine the diagnostic efficacy (ie, sensitivity and specificity) of the visual assessment of florbetaben amyloid-targeted PET imaging in detecting beta-amyloid plaque in the brain in patients with probable AD compared to HVs . Sensitivity and specificity of the visual assessment of the florbetaben PET images in detecting/excluding cerebral beta-amyloid for majority read were tested against pre-specified thresholds. Primary analysis was done for the 90-110 min. imaging window in the PPS population

Study design (continued)	**Part B (continued)** Each subject was required to visit the study center during the screening phase, on the florbetaben PET imaging day (baseline), and for 1 follow-up visit on the next day. A telephone follow-up visit was to be performed 7 days after florbetaben PET administration. During the screening visit, each subject and the caregiver were asked to provide written informed consent. During the screening phase (recommended duration of 8 weeks, with up to 12 weeks acceptable), the medical, neurological and surgical history, clinical assessments and a neuro-psychiatric evaluation were performed on all eligible subjects. Subjects were allowed to leave the center after all evaluations were completed (screening procedures could be performed during several visits to the clinic). During this period, an MRI of the brain was performed. During the florbetaben PET imaging day, all subjects received a single IV injection of IMP and scanning was performed from 45 to 60 min and from 90 to 130 min post-injection (p.i). Each subject was asked to return to the site for a follow-up visit (20 to 28 hours after IMP administration) and a telephone follow-up was performed 7 days thereafter. Safety was assessed during both follow-up visits. The association between Apolpoprotein (*APOE)* ε4 genotype and florbetaben PET scan results was also investigated.
Methodology:	The lack of cognitive impairment in HVs and the presence of dementia of Alzheimer's type in patients with probable AD was verified on the basis of comprehensive clinical, laboratory, MRI and neuro-psychiatric examination (including extensive psychometric testing). After administration of IMP, images were generated with a state of the art PET or PET/CT scanners. Images were visually assessed for the presence or absence of florbetaben uptake by the onsite nuclear physicians and as part of an independent, blinded read. Quantitative assessment of the images was also performed by an independent nuclear medicine expert.
Type of control:	**Part A** The results of PET imaging with florbetaben were compared to the clinical diagnosis (established by the onsite physician) as the SoT. The diagnostic performance of PET imaging with florbetaben was not compared to that of another imaging modality. **Part B** The results of PET imaging with florbetaben were compared to the clinical diagnosis (established by an independent CP of experts) as the SoT. The diagnostic performance of PET imaging with florbetaben was not compared to that of another imaging modality
Standard of Truth:	**Part A** The clinical diagnosis established by the investigators served as the SoT. **Part B** The final CP diagnosis as established by an independent CP served as the SoT.
Co-ordinating investigator:	Prof. Dr. Osama Sabri Department of Nuclear Medicine, University of Leipzig Hospital Stephanstr: 11, 04103 Leipzig, Germany
Investigator(s):	**Part A** 17 principal investigators (for details see section 16.1.4) **Part B** 22 principal investigators (for details see section 16.1.4)

Study center(s):	**Part A** 17 recruiting centers: Australia (3 centers), Germany (9 centers), Switzerland (1 center), Unites States (4 centers) **Part B** 22 study centers: Australia (3 centers), Germany (9 centers), Japan (3 centers), Switzerland (1 center), USA (6 centers)
Publication(s) based on the study (references):	Barthel et al, Lancet Neurol. 2011 MAY;10(5):424-435
Study period:	**Part A** First subject, first visit: 27 AUG 2008 Last subjects, last visit: 26 MAR 2009 **Part B** First subject, first visit: 01 OCT 2009 Last subjects, last visit: 30 NOV 2010
Number of subjects per treatment group:	**Part A** **Planned:** A total of 140 evaluable subjects (70 patients with probable AD and 70 HVs) were required for determination of the point estimates. To account for possible non-evaluable subjects a total of 166 subjects were planned to be recruited into the trial. **Analyzed:** Of 214 screened subjects, 150 (81 patients with probable AD and 69 HVs) were treated. Per protocol set (**PPS**): 78 patients with probable AD and 68 HVs Full analysis set (**FAS**) and Safety analysis set **(SAF**): 81 patients with probable AD and 69 HVs per set **Part B** Subjects who were treated in Part A could not be included in Part B. **Planned:** 119 evaluable subjects with probable AD (149 subjects assuming a 20% failure rate) and 98 evaluable HVs (123 HVs assuming a 20% failure rate) were required to be recruited. Statistical rationale given was to based on the results obtained in Part A. **Analyzed:** Of 392 screened subjects, 272 (147 patiens with probable AD and 125 HVs) were treated Per protocol set (**PPS**): 139 patients with probable AD and 116 HVs Full analysis set (**FAS**) and Safety analysis set **(SAF**): 147 patients with probable AD and 125 HVs per set

Criteria for evaluation **Efficacy:**	**<u>Part A</u>** **Primary efficacy variables:** Sensitivity and specificity of the visual assessment of the florbetaben PET images in detecting/excluding cerebral beta-amyloid in patients with probable AD compared to HVs. The 90 to 110 min imaging window and the average blinded reader approach were used to determine these variables. In addition, the **majority read approach** (which was the primary approach used in Part B of this study) was also used to determine sensitivity and specificity. Sensitivity was calculated from the data obtained from patients with probable AD, while specificity was calculated on the basis of results from HVs. The clinical diagnosis established by the onsite investigators served as the SoT. **<u>Part B</u>** **Primary efficacy variables**: Sensitivity and specificity of the visual assessment of the florbetaben PET images in detecting/excluding cerebral beta-amyloid in patients with probable AD compared to HVs. The 90 to 110 min imaging window and the majority read approach were used to determine these variables. Sensitivity was to be calculated from the data obtained from patients with probable AD, while specificity was calculated from HVs. The clinical diagnosis as established by an independent CP was the SoT.
Safety:	Adverse events, physical examination, vital signs, electrocardiogram, injection site monitoring, laboratory evaluation
Other:	Evaluation of pharmacogenomics

Statistical methods:	**Part A** The 95% confidence intervals were calculated for the sensitivity and specificity of the independent blinded read, as well as for the onsite assessment of the florbetaben PET images using normal approximation. This was done for each individual reader and as an average for the independent, blinded readers. Hypothesis testing was not performed. Descriptive, exploratory analysis of quantitative parameters (ie, Standard Uptake Values [SUVs] and Standard Uptake Value Ratios [SUVRs]). This included graphic demonstration and assessment of the effects of PET cameras on these parameters. Furthermore, a linear discriminant analysis of regional SUVR was performed to determine which regions of the brain provide the best discrimination between AD subjects and HVs. Post hoc "classification tree" and "random classification forests" data mining approaches for discrimination were also analyzed. Finally, the SUVs and SUVRs genenerated by two different MR-based, Volume of Interest (VOI) templates were compared using a model-based approach. As additional post-hoc evaluation of the visual assessment procedure, majority read of the three blinded readers for the modified threshold with BAPL 2 and 3 being considered "abnormal" was evaluated. Due to the two statistical approaches in this study (ie, Part A = exploratory part followed by Part B = confirmatory part), an interim analysis was performed after database lock of Part A. **Part B** The primary efficacy variables of the study were the sensitivity and specificity of the visual assessment of the tracer images in correctly differentiating between patients with probable AD and healthy volunteers (based on the presence or absence of tracer uptake) when compared to the clinical diagnosis as established by the independent CP as the SoT. The primary efficacy population consisted of patients with probable AD and healthy volunteers. A hypothesis was specified for both primary efficacy variables. For sensitivity, the null hypothesis was given as $H_{0,\,sens}$: $p \leq p_0$: = 0.65, the parameter p was representing sensitivity. For specificity, the null hypothesis was given as $H_{0,\,spec}$: $p \leq p_0$: = 0.75, the parameter p was representing specificity. The α-level was 0.025 (one-sided) for each of the two tests. The study was considered successful when both null-hypotheses could be rejected, ie, no adjustment for multiplicity was done. To have an overall study power of 80%, the single tests (ie, the test on sensitivity and the test on specificity) needed to be powered at 90%. The two-sided 95% confidence intervals were calculated for both the sensitivity and specificity based on the assessments of the majority read approach, yielding the result for the primary efficacy variables using normal approximation. With respect to the discriminative methods for quantitiative assessment estimated in Part A, in terms of sensitivity and specificity, these results were independently validated on the basis of the Part B data.

Study subjects

This clinical phase 2 study report presents the results of both Part A and Part B of the study. The efficacy results of each part are presented separately and the safety results are presented in a combined manner.

Part A

A total of 214 subjects (113 probable AD patients, 101 HVs) were screened in 17 study centers in 4 countries. Of these, 64 subjects (63 screening failures and 1 drop-out) were not included in the efficacy and safety analyses of this study. A total of 150 subjects (81 AD patients, 69 HVs) received a single injection of florbetaben (≤5 µg/injection) and were included in the safety (SAF) and full analysis set (FAS). Due to major protocol deviations, 146 subjects (78 AD patients, 68 HVs) were included in the per protocol set (PPS). Florbetaben injection was followed by PET scanning from 45-60 minutes, 90-110 minutes, and 110-130 minutes post injection.

There were no relevant differences between AD patients and HVs regarding the baseline and demographic parameters (both FAS and PPS). The mean age of the AD patients was 70.7 years (range: 55-86 years) and of the HVs was 68.2 years (range: 55-85 years). The vast majority of the subjects were White with 45 (55.6%) males and 36 (44.4%) females in the AD patient group and 30 (43.5%) males and 39 (56.5%) fermales in the HV group. All subjects met the inclusion criterion of at least 6 years of education.

All AD patients and HVs received a thorough neuro-psychiatric evaluation which included the Clinical Dementia Rating (CDR), and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test battery and other special cognitive tests. Among the AD patients, 29 (35.8%) had a CDR score of 0.5, 49 (60.5%) of 1.0 and 3 (3.7%) of 2.0. All HVs had a CDR score of zero. For the CERAD total score, AD patients showed a range from 59 – 130 (mean 87.9) and HVs from 112 – 155 (mean 138.1).

With regard to medical and surgical history, 78 (96.3%) AD patients had abnormal findings in the body system 'nervous system disorders' which were related to Alzheimer dementia, Alzheimer disease or dementia in general compared to 6 (8.7%) HVs who had nervous system disorders related to apoplex, Carpal tunnel syndrome, carotis stenosis, cognitive impairment, migraine, or recurrent headache.

Part B

A total of 392 subjects (204 AD patients, 188 HVs) were screened in 22 study centers in 5 countries. Of these, 272 subjects (118 screening failures and 2 drop-outs) were not included in the efficacy and safety analyses of this study. A total of 272 subjects (147 AD patients, 125 HVs) received a single injection of florbetaben (≤50 µg/injection) and were included in the safety (SAF) and full analysis set (FAS). Due to major protocol deviations, 257 subjects (139 AD patients, 118 HVs) were included in the Per Protocol Set (PPS). Florbetaben injection was followed by PET scanning from 45-60 minutes, 90-110 minutes, and 110-130 minutes post injection.

There were no relevant differences between AD patients and HVs regarding the baseline and demographic parameters. The mean age of the AD patients was 73.9 years (range: 56-92

years) and of the HVs was 70.7 years (range: 58-95 years). The vast majority of the subjects were White with 74 (50.3%) males and 73 (49.7%) females in the AD patient group and 73 (58.4%) males and 52 (41.6%) fermales in the HV group. All subjects met the inclusion criterion of at least 6 years of education.

All AD patients and HVs received a thorough neuro-psychiatric evaluation which included the Clinical Dementia Rating (CDR), and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test battery and other special cognitive tests. Among the AD patients, 60 (40.8%) had a CDR score of 0.5, 77 (52.4%) of 1.0 and 10 (6.8%) of 2.0. All HVs had a CDR score of zero. For the CERAD total score, AD patients showed a range from 49 – 130 (mean 88.0) and HVs from 124 – 156 (mean 138.0).

With regard to medical and surgical history, 101 (68.7%) AD patients had abnormal findings in the body system 'nervous system disorders' which were related to Alzheimer dementia, Alzheimer disease or dementia in general compared to 21 (16.8%) HVs, who had nervous system disorders related to Carpal tunnel syndrome, migraine, or tremor.

Efficacy evaluation

Part A

In **Part A** of this study, two Beta-Amyloid Plaque Load (BAPL) algorithms (algorithm A and algorithm B) for assessing the normality/abnormality of beta-amyloid plaque load in the brain scans were used.
Using algorithm A (primary analysis), a brain scan of a subject with a BAPL score of "1" (without beta-amyloid plaque load) or "2" (with minor beta-amyloid plaque load) was considered normal and a brain scan with a BAPL score of "3" (with significant beta-amyloid plaque load) was considered abnormal. Using algorithm B (post hoc analysis), a brain scan of a subject with a BAPL score of "1" was considered normal and a brain scan with a BAPL score of "2" or "3" was considered abnormal.

The initial analysis of the primary efficacy variable was performed using algorithm A and the sensitivity and specificity calculated using the average blinded reader approach; the post hoc analysis of the primary efficacy variable was performed using algorithm B with efficacy calculated using the majority read approach.

Visual assessment

The primary efficacy variable was the average blinded reader results (based on the independent visual assessment of the florbetaben PET imaging window from 90 to 110 min post injection [p.i.]) for sensitivity and specificity in detecting/excluding cerebral beta-amyloid plaque load in the brain in patients with AD compared to HVs.

For the imaging window 90-110 min p.i., the results yielded an average blinded reader sensitivity of 74.79% (95% CI: 65.69% to 83.88%) and a specificity of 96.08% (CI: 91.85% to 100.30%) for the PPS population. The results for the FAS population were similar. The average blinded reader results for sensitivity and specificity of the imaging windows 45-60 min p.i. and 110-130 min p.i. (both secondary variables) are also provided in the table below.

Part A_Primary analysis: Sensitivity and specificity in beta-amyloid plaque load detection in the brain for imaging windows 90-110 min p.i. (PPS and FAS, primary variable), 45-60 min p.i. (PPS) and 110-130 min p.i. (PPS) by average reader

Blinded reader	TP	TP + FN	Sensitivity estimate [%]	Sensitivity, 95% CI [%]	TN	TN + FP	Specificity, estimate [%]	Specificity, 95% CI [%]
Average blinded reader 90-110 min (PPS)	58.33	78	74.79	65.69-83.88	65.33	68	96.08	91.85-100.30
Average blinded reader 90-110 min (FAS)	61.00	81	75.31	66.51-84.11	66.33	69	96.14	91.97-100.30
Average blinded reader 45-60 min (PPS)	57.67	78	73.93	64.88-82.98	64.33	68	94.61	90.36-98.85
Average blinded reader 110-130 min (PPS)	54.33	77	70.56	60.98-80.15	65.67	67	98.01	95.65-100.37

TN = True Negative, TP = True Positive

For the majority read using BAPL algorithm B for imaging window 90-110 min p.i., sensitivity was 79.49% (95% CI: 65.69% to 83.88%) and specificity was 96.08% (95% CI: 91.85% to 100.30%). Compared to the primary analysis for the average reader, sensitivity for the majority read was higher whereas specificity was lower. This algorithm was planned to be used for Part B and for all other studies (ie, the Down Syndrome Study 14311 and the phase 3 study 14595) in the development program.

Quantitiative assessment

- Regional brain SUVRs were considerably higher in patients with AD compared to HVs in all gray matter regions, particularly in the frontal, temporal, parietal, occipital, anterior and posterior cingulate cortices. Upon graphic demonstration of the means and variances it became apparent that the effect size between AD patients and HVs was larger with values generated using the 2nd MR-segmented SUV template when compared to those generated with 1st SUV template.

  Although no direct groupwise significance testing was performed, several methods were explored to determine the discriminative value of tracer uptake (as reflected by the SUVR) in the various brain VOIs:

- A stepwise linear discriminant analysis of regional SUVRs was performed to select a linear combination of non-white matter regions (eg SUVRs of prespecified regions) that best discriminates between AD patients and HVs. Here the two regions of the posterior cingulate and thalamus were found to be the best discrimator for the 90 to 110 min p.i imaging window. Using this discriminator together with the threshold for maximum accuracy (ie, 84.77 on the original scale), re-substitution estimates for sensitivity and specificity in differentiating between subjects with AD patients and HVs were 85% and 91%.

- As different PET cameras were used in this study, a possible camera effect might have occurred which could have influenced the mean of the distribution of SUV values. Two types of models were performed: one in which a homogeneous residual variance between patient and subject group is assumed, and another, in which a heterogeneous residual

variance was assumed for the patient group compared to the volunteer group. Based on the Bonferroni adjusted CI, for SUV no camera effect was significant. For the SUVR, also based on the Bonferroni adjusted 95% CI, the proportion of the effect of the Philips Gemini PET/CT compared to Siemens ECAT EXACT was significantly different from 100%, and, thus, a potential relevant effect could not be excluded.

- To compare the efficacy of the 1st SUV and the 2nd MR-segmented SUV templates, a Receiver Operating System (ROC) analysis was performed. The use of the second template led to an increase in the area under the curve (AUC) for all three imaging windows (with 95% CI excluding zero). This was consistent with the increase in effect size between AD patients and HVs in all gray matter regions when the 2nd template was used.
- For analysis of the effects of CrCl on quantitative assessment results, a significant linear relationship (ie, slope) between CrCl and posterior cingulate SUVR could not be identified, neither in AD patients (p-value: 0.7372) nor in HVs (p-value: 0.2825).

Part B

In **Part B** of this study, the primary efficacy variable was calculated using the brain beta-amyloid plaque load (BAPL) visual rating algorithm B ("1" = normal scan and "2 and 3" = abnormal scan). Furthermore, the majority reader approach was used for the primary efficacy calculation.

Visual assessment

The primary efficacy variable was the sensitivity and specificity for majority read (3 blinded readers) in the detection of beta-amyloid plaque load in the brain in the imaging window of 90-110 minutes after injection.

Please note that in Part B the clinical diagnosis as SoT was established by a Consensus Panel (CP). Thus, in addition to AD other diagnoses were possible. For the primary efficacy calculations, the population was restricted to the 2 subject groups "probable AD patients" and "healthy HVs" as assessed by the CP. The results for the PPS were 67.24% (95% CI: 58.70% to 75.78%) for sensitivity and 96.61% (95% CI: 93.34% to 99.88%) for specificity. The single blinded reader results for sensitivity were 71.55% for both BR 1 and BR 3 and 62.93% for BR 2. The results for specificity were 94.92% for BR1 and BR 3 and 97.46% for BR 2.

Part B_Primary analysis: Sensitivity and specificity (including normal approximated Confidene interval) in beta-amyloid plaque load detection in the brain for imaging window 90-110 min p.i. by majority read and single blinded readers- PPS

Blinded reader	TP	TP + FN	Sensitivity estimate [%]	Sensitivity, 95% CI [%]	TN	TN + FP	Specificity, estimate [%]	Specificity, 95% CI [%]
Majority Read	78	116	67.24	58.70 – 75.78	114	118	96.61	93.34 – 99.88
Reader 1	83	116	71.55	63.34 – 79.76	112	118	94.92	90.95 – 98.88
Reader 2	73	116	62.93	54.14 – 71.72	115	118	97.46	94.62 – 100.30
Reader 3	83	116	71.55	63.34 – 79.76	112	118	94.92	90.95 – 98.88

For sensitivity of the majority read, the Null hypothesis H_{01} could not be rejected as the lower level of the confidence interval was smaller than the fixed threshold of 65% (sensitivity was statistically not significant greater than 65%). For specificity of the majority read, the Null hypothesis H_{02} could be rejected as the lower level of the confidence interval was greater than fixed threshold of 75% (specificity was statistically significant greater than 75%). The overall Null hypothesis H_0 for the combined test could therefore not be rejected, as hypothesis H_{01} for sensitivity could not be rejected.

Quantitative assessemnt

- In Part B or the study only the 2nd MR-segmented template was used to generated the quantitative values. As in Part A, for all timepoints analyzed, the mean SUVRs in the gray matter regions were higher in the AD patients (CP) compared to the HVs. The median SUVR for the posterior cingulate region was highest both for the AD patients (CP) (1.882) and for the HVs (CP) (1.432) when comparing the median SUVRs between these 4 brain VOIs (AD patients (CP): frontal=1.668; lateral=1.647; parietal= 1.615; HVs (CP): frontal=1.254; lateral=1.256; parietal= 1.234. In Part A, several discriminatory analyses were performed. The rules obtained in Part A were applied to Part B data. The results are briefly summarized below:

  Using discriminators (posterior cingulate and thalamus) from Part A that resulted in in the highest accuracy (when choosing the treshold of 84.77) in the 90-110 minute imaging window in Part B, the corresponding sensitivity was 86.2% while the corresponding specificity was 68.9%.

- The models used to assess for the effects of camera type on SUVs and SUVRs were also applied to the data generated in Part B. Based on the Bonferroni adjusted confidence interval, several camera effects (Philips Gemini, Philips Allegro and SET-3000 SHIMADZU GCT/X) were significant for SUV data. However, for SUVRs no camera effect was significant. In addition also the point estimates were very close to 100%. For the reporting of results usually SUVRs and not SUVs are chosen. Therefore the overall conclusion is that there is no camera effect on the quantitative measurements.

- As in Part A, the impact of eGFR on the efficacy of visual assessment and of age was again calculated by descriptive statistics and by majority read for the 90 to 110 min p.i. imaging window. The data did not indicate that within patients with AD or with HV there was a difference in CrCl those with with negative versus (vs) those with positive scans. Confidence intervals for the point estimates for an estimated linear relationship (“slope”) of eGFR with posterior cingulate SUVR (and corrected for effects for age and gender) were calculated. The results yielded no statistical evidence of a linear relationship between CrCl and posterior cingulate. The difference in slope for AD patients minus the slope obtained for HVs was -8.848, 95% CI: [-27.468; 9.772]. Thus, no statistical difference was found between AD patients and HVs in the context of a linear relationship of CRCl to posterior cingulate.

Safety evaluation

- Adverse events

Part A

A total of 23 (15.3%) subjects reported 28 treatment-emergent adverse events (TEAEs), 9 (11.1%) AD patients with 9 TEAEs and 14 (20.3%) HVs with 19 TEAEs. There were no deaths, no TESAEs, and no study drug discontinuations due to AEs during the study. Most TEAEs were mild and resolved during the study period. There were 2 pre-treatment SAEs reported in 2 subjects (patient identification numbers [PIDs] 100070022 and 100030013). One incidence of lower arm fracture syncope and incidence of cardiac arythmia, both with hospitalization and both of which resolved. No relationship to study drug or conduct was documented.

A total of 5 subjects (3.3%) experienced 5 study drug-related TEAEs, 2 (2.5%) AD patients with 2 drug-related TEAEs (increase in blood pressure, tremor of both hands) and 3 (4.3%) HVs with 3 drug-related TEAEs (fatigue, feeling hot in upper back, injection site erythema).

The most frequently reported TEAEs, according to MedDRA SOC classification, were general disorders and administration site conditions (mainly erythema and/>or hematoma) in 9 (6.0%) subjects (3 AD patients and 6 HVs), nervous system disorders in 6 (4.0%) subjects (one AD patient and 5 HVs) and vascular disorders in 4 (2.7%), subjects (2 AD patients, 2 HVs).

Part B

A total of 66 (24.3%) subjects reported 91 TEAEs, 40 (27.2%) AD patients with 54 TEAEs and 26 (20.8%) HVs with 37 TEAEs. There were no deaths, no TESAEs, and no study drug discontinuations due to adverse events (AEs) during the study. The majority of TEAEs were mild and resolved during the study period. Two subjects (PIDs 400029003 and 1540049002) complained of pre-treatment SAEs, one HV experienced moderate chest pain which resolved and clear cell endometrial cancer was discovered in another HV. No relationship to study drug or conduct was documented.

A total of 15 subjects (5.5%) experienced 15 study drug-related TEAEs, 8 (5.4%) AD patients with 8 drug-related TEAEs and 7 (5.6%) HVs with 7 drug-related TEAEs. Most TEAEs (reported in n= 6 cases each) were injection site irritation/hematoma and injection site pain/warmth.

The most frequently reported TEAEs, according to MedDRA SOC classification, were general disorders and administration site conditions (mainly erythema and/or hematoma) in 31 (11.4%) subjects (21 AD patients and 10 HVs), investigations (preferred terms were mainly laboratory parameters) in 12 (4.4%) subjects (8 AD patients and 4 HVs) and vascular disorders in 6 (2.2%) subjects (3 AD patients and 3 HVs).

Part A and Part B

- Clinical laboratory evaluation

  Group mean laboratory values for serum biochemistry, hematology, and urinalysis data showed general stability over time with only small variations from median values in both Part A and Part B. No substantial and/or consistant change from baseline to follow-up were observed, and overall no trends indicative of safety concerns were noted.

  According to the investigator, abnormalities in laboratory parameters were documented as TEAEs in Part A in 2 AD patients and in Part B in 7 AD patients and 2 HVs. All treatment-emergent AEs were mild in intensity; one AE (Part B, transient increase in serum creatinine) was deemed to be study drug-related.

- Electrocardiogram (ECG)

  As reflection of the frequent cardio-vascular co-morbidity observed in the elderly populations involved, in both Part A and B, a little over half of the subjects had abnormal ECG findings at baseline, with the most frequently reported abnormalities being atrial fibrillation and AV-block (1st degree). No significant effects of florbetaben administration on heart rate, rate ratio (RR) interval, PQ interval, QRS duration, QT interval and QTc intervals according to Bazett as well as to Fridericia were detected in Part A and Part B.
  Overall, the administration of florbetaben did not appear to adversely prolong or shorten atrial depolarisation and there was no detectable effect on ventricular depolarisation and repolarisation. In Part B, ECG changes were deemed by the investigator to be TEAEs in 3 HVs, one subject with QT-prolongation (>500ms) by pre-exisiting AV-Block (1st degree), one subject with transient sinus bradycardia (< 40 bpm) and one case of t-wave inversion.
  Overall, no trends in changes from baseline indicative of a safety concern were observed for any of the measured ECG parameters and no signs indicating a consistent systematic change due to the study drug were seen. All 3 events were reported to be mild and resolved during the study period.

- Vital signs

  Group mean values for vital signs showed stability over time when compared to baseline and changes in baseline were similar in AD patients and HVs in both Part A and Part B. Two TEAEs were related to changes in vital signs. One AD patient and one HV (each with a history of hypertension) experienced transient increases in blood pressure during the PET scanning procedure. The events were not deemed by the investigator to be related to study drug and resolved during the study period. In general, no significant signs and/or trends indicative of a safety concern were apparent in either Part A or Part B of the study.

- Injection site monitoring

  In injection site changes were documented as TEAEs in 9 subjects in Part A and in 17 subjects Part B. In the majority of cases, these TEAEs were deemed by the investigator to be related to study conduct (ie, intravenous injection). For study drug related TEAEs related to the injection site, in Part A 2 events and in Part B 8 of 12 events were deemed

by the investigator to also be related to study conduct. Thus the changes reported can be considered to be the result of the placement of an indwelling catheter with subsequent intravenous injection rather than local irritation caused by the injection of florbetaben.

To conclude, with only 20 drug-related TEAEs in 422 subjects (all of mild or moderate intensity) and with no trends or signals indicative of a safety concern in any of the above safety parameters measured, florbetaben can be considered to be a safe and well-tolerated radio-pharmaceutical in the population tested

Other evaluations

As a part of the safety variable analysis, the evaluation of the pharmacogenomics was performed. The main objective of the pharmacogenetic analysis was to investigate the association between *APOE* genotype and florbetaben PET scan results in HVs and AD patients.

Part A: The association between the *APOE* genotype and florbetaben PET scan was investigated using the onsite diagnosis.

Part B: The pharmacogenetic analysis of Part B was designed to confirm the pharmacogenetic results of Part A (which used the onsite diagnosis as SoT).

Overall conclusions

The findings of this global, multi-center phase 2 study suggest that florbetaben PET imaging can make a substantial contribution to improving differential diagnosis of AD and in dementia. Image acquisition was possible over a long time period and both visual and quantitative image analysis proved practical and sufficiently robust for use in multiple cameras. In particular, the specificity was high in both Part A and Part B. This indicates that florbetaben is a valid tool for ruling out AD in a subject without memory impairment. While the sensitivity in Part A with 79.5% (post hoc analysis) was in line with expectations, the corresponding sensitivity in Part B with 67.2% was notably lower. As we believe that the Part B results were due to blinded reader bias, a re-read of the PET data is planned. The visual assessment results of Part B elucidated the effect that reader training and methodology can have on visual grading and prompted an intense re-analysis of training and visual assessment techniques. On the basis of 3 test reads using 3 sets of readers, modified reader training and refined visual assessment methodology have since been developed for use in future studies. The VOI-based quantitative analysis of florbetaben regional uptake - expressed as SUVR and generated using an operator-independent and standardized technique - provided good differentiation between the 2 cohorts. The presence of a positive florbetaben PET scan was associated with a higher preponderance of *APOE* ε4 genotype, findings that support florbetaben tracer uptake to be the direct reflection of the *in vivo* deposition of beta-amyloid in the brain. Finally, we have shown the radio-tracer to be safe and well-tolerated in the population studied. These results provide a basis for the further clinical development of the tracer and underline the potential of florbetaben PET imaging as a valuable visual adjunct in the diagnostic algorithm of dementia.

Report Amendment 1:

For Part A and Part B, listing 1 in Appendix 16.1.6 Batch numbers has been corrected (listing 16.1.6/1 Part A and listing 16.1.6/1 Part B). These were editorial changes which had no impact on the conclusion of the results of this study. Therefore, a new signature from the co-ordinating investigator was not regarded necessary.