Piramal | Life Sciences
knowledge action care

25.06.2015

BfArM
Kurt-Georg-Kiesinger-Alle 3
D-53175 Bonn

Betreff: Stellungnahme zum Mängelbericht bezüglich der Unterlagen 8B0301

Sehr geehrte Damen und Herren,

hiermit möchten wir gerne zu ihrem Mängelbericht vom 30.5.2015 Stellung nehmen:

- Alle in Amendment 5 des Studienprotokolls aufgeführten sekundären Endpunkte, die zum Zeitpunkt der Einreichung des Antrages zur Marktzulassung gültig waren sowie die daraus resultierenden Ergebnisse sind in der Synopse aufgeführt und nicht wie zuvor nur der primäre Endpunkt und der Endpunkt, der zur Zulassung in der EU geführt hat.
- Eine Schlussfolgerung wurde in die Synopse eingefügt

Wie mit Herrn Dr. Pohly telefonisch am 18.6.2015 besprochen erläutern wir im Folgenden kurz welcher sekundäre Endpunkt zur Zulassung in der EU geführt hat und wie dies in der Synopse berücksichtigt wurde:

Der dritte sekundäre Endpunkt ("*To determine the sensitivity and specificity of the visual assessment of florbetaben PET images on the subject level (according to the regional cortical tracer uptake [RCTU] and brain ß-amyloid plaque load [BAPL] rating scale) in detecting/excluding cerebral ß-amyloid compared to the onsite neuropathological diagnosis as the standard of truth (SOT)*") war essentiell für die Marktzulassung in der EU und ergab sich aus unserer Diskussion mit dem CHMP im Rahmen des Zulassungsverfahrens. Dies ist an entsprechender Stelle in der Synopse auch vermerkt und mit den Daten aus der SmPC unterlegt.

Wir hoffen, dass diese zusätzlichen Erläuterungen sowie die überarbeitete Synopse ihre Fragen und Hinweise angemessen beantworten und berücksichtigen. Die korrigierten Unterlagen wurden mit diesem Anschreiben fristgemäß über das PharmNet.Bund-Portal am 25.6.2015 eingereicht.

Wir verbleiben mit freundlichen Grüßen,

Mariana Lagos Quintana

Piramal Imaging GmbH
Tegeler Straße 6 – 7 13353 Berlin Deutschland
T +49 30 461 124 603 F +49 30 461 124 629
W www.piramal.com

An open-label, non-randomized study to evaluate the efficacy and safety of BAY 94-9172 (ZK 6013443) positron emission tomography (PET) imaging for detection / exclusion of cerebral β-amyloid when compared to postmortem pathology

Safety and efficacy of positron emission tomography (PET) imaging with BAY 94-9172 (ZK 6013443)

Test drug:	^{18}F-Florbetaben
Synonyms:	NeuraCeq, ZK 6013443, BAY 94-9172
Study number:	14595
Clinical study phase:	3
Study dates:	First subject, first visit: 25 NOV 2009 Last subject, last visit: 24 DEC 2013
Date of final study report:	30 SEP 2014
Sponsor:	Piramal Imaging SA, c/o Pascale Nguyen, Route de l'Ecole 13, 1753 Matran, Switzerland
Approval status of test drug:	European Union: approved via centralized procedure on 20.02.2014, marketing authorization number: EU/1/13/906/001 US: approved on 19.03.2014 South Korea: 19.12.2014
Marketing Authorization	Piramal Imaging Limited, Langstone Technology Park, Langstone Road, Havant, Hampshire PO9 1SA, United Kingdom

List of Abbreviations

AE	Adverse event
ARWMC	Age-related white matter changes
Aβ	Amyloid β
BAPL	Brain amyloid beta plaque load
BAY 94-9172	^{18}F-Florbetaben
BSS	Bielschowsky Silver Staining
CERAD	The consortium to establish a registry for Alzheimer's Disease
CRO	Clinical research organization
CSP	Clinical study protocol
DLB	Dementia with Lewy Bodies
FDA	Food and Drug Administration
FDG	^{18}F-Fluordesoxyglucose
GCP	Good Clinical Practice
HV	Healthy volunteer
IHC	Immunohistochemistry
IND	Investigational New Drug Application
MedRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
NDV	Non-demented volunteer
NINDS-AIREN	National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences
PET	Positron emission tomography
pi	Post injection
RCTB	Regional cortical tracer binding score
RCTU	Regional cortical tracer uptake
SAE	Serious adverse event
SAP	Statistical analysis plan
SOT	Standard of truth
SUV	Standardized Uptake Values
SUVR	Standard uptake value ratio
TEAE	Treatment emergent adverse event
VOI	Voxel of interest
ZK 6013443	^{18}F-Florbetaben

Study title:	An open-label, non-randomized study to evaluate the efficacy and safety of BAY 94-9172 (ZK 6013443) positron emission tomography (PET) imaging for detection/exclusion of cerebral β-amyloid when compared to postmortem histopathology
EudraCT number:	2009-012569-79
Clinical phase:	3
Study objectives:	**Primary objective:** To determine the sensitivity and specificity of the visual assessment of regional tracer uptake in the florbetaben (also referred to as BAY 94-9172) PET images compared to histological verification of the presence or absence of cerebral β-amyloid in the respective postmortem specimens as the standard of truth (SOT). *Note: The primary objective was analyzed as planned after collecting the first 32 brains in the initial study period. It refers to the regional level visual assessments regarding the 6 brain regions* **Secondary objectives:**[1] 1. To determine the sensitivity and specificity of the composite "whole brain" (per subject) regional visual assessment collapsed from the regional PET visual assessment results in detecting/excluding cerebral β-amyloid plaques based on the "whole brain" histopathological verification of the presence/absence of β-amyloid deposition (collapsed from the results of the regional histological findings from the Pathology Consensus Panel). 2. To determine the sensitivity and specificity of the quantitative assessment of regional tracer uptake in florbetaben PET images compared to histological verification of the presence or absence of cerebral β-amyloid in the respective postmortem specimens as the SOT. *Note: The two objectives above were analyzed as planned after collecting the first 32 brains in the initial study period. They refer to the regional level visual and quantitative assessments regarding the 6 brain regions* 3. To determine the sensitivity and specificity of the visual assessment of florbetaben PET images on the subject level

[1] Changes to all secondary objectives were made via Amendment 5.

(according to the regional cortical tracer uptake [RCTU] and brain β-amyloid plaque load [BAPL] rating scale) in detecting/excluding cerebral β-amyloid compared to the onsite neuropathological diagnosis as the standard of truth (SOT).

Note: This constitutes the analysis of all brain specimens that became available until study termination. The SOT used here is the histopathological verification according to CERAD criteria and is described as SOT 3 in the final study report. To increase clarity SOT 3 will be described as SOT in the whole synopsis. This analysis reflects the CHMP discussions and led to regulatory approval in the EU.

4. To evaluate the safety and tolerability of a single dose of florbetaben.
5. To perform an exploratory investigation of the association between the subject level visual assessment of florbetaben and FDG PET images for detecting the abnormalities in subjects with Alzheimer's disease (AD) compared to individuals with other types of dementia and/or without cognitive impairment. The on-site clinical diagnosis (if available) served as the reference standard.[2]
6. To investigate the association between the visual assessments of the regional tracer uptake apparent in florbetaben PET images and the regional glucose hypometabolism as seen in the FDG PET images in subjects with AD compared to individuals with other types of dementia and/or without cognitive impairment.

Note: FDG PET scans were available from 14 Japanese subjects that underwent also florbetaben PET and were descriptively analyzed in the study report. Given the low sample size they are not further described here..

Posthoc Analyses

Note: The following three exploratory endpoints were prepared as posthoc analyses generated during the assembly of the final study report.

- To determine sensitivity and specificity of the subject level composite SUVR calculated based on pathology results, which were available at the time point of analysis during the

[2] FDG PET imaging in Japan only was added (the last 2 bullets) in Amendment 1, Modification 1.

	review of the EU application for marketing authorization. • To determine sensitivity and specificity of the subject level composite SUVR calculated based on pathology results covering all available data. • To determine the subject level composite SUVRs by SOT for baseline and available follow-up scans.
Test drug:	^{18}F Florbetaben
Name of active ingredient(s):	trans-4-(N-methyl-amino)-4'-{2-[2-(2-[18F]-fluoro-ethoxy)-ethoxy]-ethoxy}-stilbene
Dose:	The applied florbetaben radioactive dose was 300 MBq (8.1 mCi) ± 20%. The mass dose of the ligand was ≤ 50 µg.
Route of administration:	Intravenous (iv) injection
Bulk batch number:	Since this is a radioactive tracer [^{18}F], almost all subjects received a different batch of study drug
Duration of treatment:	Single administration
Reference drug:	Not applicable
Indication:	Detection of β-amyloid in the brain
Diagnosis and main criteria for inclusion:	Subjects with a low probability of cerebral β-amyloid deposition (e.g. non-demented volunteers [NDVs]) and subjects with a high probability of β-amyloid deposition (e.g. subjects diagnosed with AD or Dementia with Lewy Bodies [DLB]) were included in the trial. Subjects were to be at least 21 years of age; of no child-bearing potential (if female); willing and able to lie down in magnetic resonance imaging (MRI) and PET scanners; and willing to donate their brain for postmortem examination in case of death.
Study design:	This was a Phase 3, open-label, multi-center, non-randomized, single dose study to assess the safety and efficacy of florbetaben. Efficacy was determined by the sensitivity and specificity of the regional uptake of florbetaben in the brain as depicted in the PET images compared to the presence or absence of β-amyloid in the respective brain histology specimens. During the florbetaben PET imaging visit, each subject received a single intravenous injection of the study drug and scanning was performed from 90 to 110 minutes post-injection (pi). Each subject was asked to return to the site for a follow-up visit 20 to 28 hours after study drug administration and a telephone contact occurred 7 days thereafter.

	All subjects whose health allowed and were willing returned yearly for repeat MRI and florbetaben PET scans (except those in Germany due to restrictions on allowed radiation exposure). During these follow-up visits, safety was assessed at the PET imaging visit, up to 20 to 28 hours after each florbetaben PET scan, and per telephone 7 days thereafter.
SOT:	For this study, the SOT for the primary endpoint was based on the histopathological results obtained from postmortem neurohistopathological examination of the brains of those individuals who died within the initial study period. The SOT for the different secondary endpoints varied and are described in more detail in the methods section.
Type of control:	The presence or absence of florbetaben uptake in the PET scan was compared to postmortem histopathology as the SOT and PET scan results from 10 young healthy volunteers (HVs) served as additional negative controls. For the primary efficacy analysis, the diagnostic performance of PET imaging with florbetaben was not compared to that of another imaging modality.
Coordinating investigator:	[redacted] Banner Sun Health Research Institute; Sun City, Arizona, United States

Study centers:	Austin and Repatriation Centre Nuclear Medicine and Centre for PET 145 Studley Road PO Box 5555 3084 Heidelberg Victoria Australia Hôpital Roger Salengro Neurologie B Rue du Professeur Emile Laine 59037 Lille France Hôpital Civil - Strasbourg Hôpitaux Universitaires Nouvel Hôpital Civil 1, place de l'Hôpital 67091 Strasbourg France Universitätsklinikum Leipzig AöR Klinik und Poliklinik für Nuklearmedizin Liebigstraße 18 04103 Leipzig Germany Forschungszentrum Jülich GmbH Institut für Neurowissenschaften und Biophysik (INB-3) / Medizin Wilhelm-Johnen-Straße 52425 Jülich Germany Tokyo Metropolitan Geriatric Hospital Neurology 35-2 Sakae-cho 173-0015 Itabashi-ku Tokyo Japan Mihara Memorial Hospital Neurology 366 Otamachi 372-0006 Isezaki Gunma Japan

Fukushimura Hospital
Internal Medicine
19-14 Azayamanaka Noyori-cho
441-8124 Toyohashi Aichi
Japan

Banner Sun Health Research Institute
10515 W. Santa Fe Drive
85351 Sun City Arizona
United States

Holy Name Medical Center
Institute for Clinical Research
718 Teaneck Road
07666 Teaneck New Jersey
United States

University of Texas Health Science Center
7703 Floyd Curl Drive
78229 San Antonio Texas
United States

Stanford University
Psychiatry Department
401 Quarry Road
94305 Stanford California
United States

University of South Florida
USF Health Byrd Alzheimer's Institute
4001 East Fletcher Avenue
33616 Tampa Florida
United States

University of Texas Southwestern Medical Center
James W. Aston Ambulatory Center
5303 Harry Hines Boulevard
U4.704
75390 Dallas Texas
United States

Hospital of the University of Pennsylvania
University of Pennsylvania Health System
3400 Spruce Street
19104 Philadelphia Pennsylvania
United States

Publication based on the study (references):	Sabri O; Sabbagh MM; Seibyl J; Barthel H; Akatsu H; Ouchi Y; Senda K; Murayama S; Ishii K; Takao M; Beach TG; Rowe CC; Leverenz JB; Ghetti B; Ironside JW; Catafau AM; Stephens AW; Mueller A; Koglin N; Hoffmann A; Roth K; Reininger C; Schulz-Schaeffer WJ. „Florbetaben PET imaging to detect amyloid beta plaques in Alzheimer disease: Phase 3 study.“, Alzheimer's & Dementia 2015 (in press, doi: 10.1016/j.jalz.2015.02.004.).
Study period:	**First subject, first visit:** 25 NOV 2009 **Last subject, last visit (for final report):** 24 DEC 2013
Number of subjects per treatment group:	• 253 subjects were screened for this study. • 218 subjects were enrolled to the study; whereby 216 were administered with florbetaben. • The Primary Efficacy analysis consisted of data from the first 41 subjects and reflects the analysis from the initial study period (tissue-scan matched regional analysis). • The Final Analysis Set (Whole Brain) consisted of data from 97 subjects whereby PET scans. • 74 subjects were included in the Posthoc data set covering all available subjects that were available at the respective time point of analysis during the review of the EU application for marketing authorization • The First Annual Repeat Injection included 91 subjects returning for first follow-up administration and PET-scan. • The second Annual Repeat Injection included 34 subjects returning for second follow-up administration and PET-scan.

Criteria for evaluation Efficacy:

Primary Objective

The sensitivity/specificity of the visual assessment were calculated based on the majority read assessment of regional tissue-scan matched tracer uptake. This result was derived from assessments by 3 independent readers for brain regions of a subject where a Standard of Truth (SOT) was available. The SOT for this analysis was established from a centralized histopathological determination of β-amyloid presence/absence based on both Bielschowsky silver and immunohistochemical staining by a consensus panel of neuropathology experts. Based on the PET images, a brain region was classified as "normal" or "abnormal" depending on the presence or absence of regional tracer uptake in the respective region. "Normal" therefore meant absence of β-amyloid and "abnormal" presence of β-amyloid. Sensitivity was defined as the percentage of abnormal brain regions from all regions where an SOT was available and the SOT was "β-amyloid present". Specificity was defined as the percentage of normal brain regions from all regions where an SOT was available and was "β-amyloid not present".

Secondary objectives

1. The composite “whole brain” regional assessment, which provided the secondary endpoints, was determined from the 6 pre-specified regions as follows:

   The highest score across the six pre-defined brain regions in the PET scan determined the composite “whole brain” regional result. That is if one region was scored "yes" for β-amyloid uptake this was the "composite score". The scan was negative for tracer uptake only if none of the regions are scored "yes".

   The 'highest' score from the Consensus Panel histopathological evaluation of the 6 predefined brain regions determined the composite “whole brain” regional histology result for this subject: If in any of the 6 regions β-amyloid plaques were evaluated as being 'present' at a clinico-pathologically relevant level; (either modest or frequent), the subject was determined as having clinico-pathologically relevant β-amyloid deposition in the brain. If in none of the regions the histopathological findings were assessed as being more than 'no' or 'sparse' β-amyloid plaques, the subject is scored as 'no β-amyloid present'.

2. Quantitative assessment of the florbetaben PET image data was performed by one experienced nuclear medicine expert

at the image core laboratory for the image data sets acquired from 90 to 110 minutes pi. The expert aligned the T1 weighted MRI scan and each PET frame using a standard mutual information algorithm. A VOI template was then applied to the aligned MRI for standardized regional brain sampling and was adjusted on the MRI for optimal fit to the respective individual neuroanatomy. After transfer of the adjusted VOIs to the PET study, for each VOI the Bq/cc brain tissue was extracted for calculation of the standard uptake values (SUVs). For each region, the SUV was derived from activity in a VOI determined as described above by taking into consideration study participant weight and dose injected. Acquired SUV data was then normalized to the cerebellar cortex resulting in a "region to cerebellar" ratio termed the SUVR ratio (SUVR).

3. Sensitivity and specificity of the whole brain visual assessment were calculated. Any brain with a region classified as abnormal from PET imaging was to be classified as abnormal for the "whole brain" assessment. This result was derived from assessments by 3 independent readers for a subject where a Standard of Truth (SOT) was available. The SOT for this analysis was based on a histopathological assessment of the presence/absence of β-amyloid according to CERAD Criteria. The sensitivity was defined as the proportion of brains classified as abnormal from all brains where this SOT was available and was "β-amyloid present". The specificity was defined as the proportion of brains classified as normal from all brains where this SOT was available and was "β-amyloid not present".
4. Safety and tolerability of a single dose of florbetaben was assessed via analysis of serious adverse events

Posthoc Analyses

Subject level composite SUVRs (calculated as mean of SUVRs from the frontal, parietal, lateral temporal, anterior and posterior cingulate, and occipital cortices) by SOT are reported for subjects with brain tissue that was available at different time points. SUVR analysis was performed for baseline and available follow-up scans.

Safety:	The following safety variables were evaluated before and after administration of florbetaben: vital signs, injection site monitoring, physical examination, adverse event (AE) assessment, and concomitant medication assessment.
Other:	To allow possible comparison of markers based on gene expression profiling to the results of PET imaging, blood samples were taken from study participants who agreed to participate and stored for potential future use.
Statistical methods:	**Primary Outcome** Sensitivity and specificity for the primary outcome was reported for six different brain regions. Sensitivity was defined as the percentage of abnormal brain regions from all regions where an SOT was available and the SOT was "β-amyloid present". Specificity was defined as the percentage of normal brain regions from all regions where an SOT was available and was "β-amyloid not present". Percentage and confidence intervals are reported for all regions. In addition, the following hypotheses were formulated for sensitivity and specificity: H0,sens: sensitivity ≤ 0.6 vs. H1, sens: sensitivity > 0.6 H0,sens was to be rejected if the lower bound of the two-sided 95% CI is larger than 0.6. H0,spec: specificity ≤ 0.8 vs. H1, spec: specificity > 0.8 H0,spec was to be rejected if the lower bound of the two-sided 95% CI is larger than 0.8. **Secondary Outcome** Descriptive statistics were applied for secondary endpoints and no formal hypothesis testing was performed. **Posthoc Analyses** Descriptive statistics were applied for secondary endpoints and no formal hypothesis testing was performed.

RESULTS

Efficacy evaluation

Primary efficacy analysis

The aim was to determine the sensitivity and specificity of the majority read of visual assessment of tracer uptake compared to histological verification of the presence or absence of cerebral beta-amyloid in tissue-matched postmortem specimens.

Analysis Population Description

All participants included in the Primary Efficacy analysis were included in this analysis.

Reporting Groups Description

Sensitivity (Primary Efficacy Analysis): The full analysis set consisted of 31 subjects for whom both brain specimen with a valid Standard of Truth in at least one brain region and a florbetaben PET scan were available and 10 healthy controls for whom a florbetaben PET scan was available and whose SOT was considered β-amyloid negative by definition (results see table 1 and 2).

Specificity (Primary Efficacy Analysis): The full analysis set consisted of 31 subjects for whom both brain specimen with a valid Standard of Truth in at least one brain region and a florbetaben PET scan were available and 10 healthy controls for whom a florbetaben PET scan was available and whose SOT was considered β-amyloid negative by definition (results see table 1 and 3).

Table 1: Sensitivity and Specificity

	Sensitivity (Primary Efficacy Analysis)	Specificity (Primary Efficacy Analysis)
Number of Participants Analyzed	41	41
Sensitivity and Specificity of the Majority Read of Visual Assessment of Tracer Uptake Compared to Histological Verification of the Presence or Absence of Cerebral Beta-amyloid in Postmortem Specimens [units: percentage of regions]		

	Sensitivity (Primary Efficacy Analysis)	Specificity (Primary Efficacy Analysis)
Total	77.36	94.20
Frontal Cortex	85.71	95.00
Occipital Cortex	88.89	86.36
Hippocampus	57.14	100.00
Anterior Cingulate Cortex	90.00	85.71
Posterior Cingulate Cortex	81.82	94.44
Cerebellar Cortex	0.00	100.00

Table 2: Statistical Analysis 1 for Sensitivity of the Majority Read of Visual Assessment of Tracer Uptake Compared to Histological Verification of the Presence or Absence of Cerebral Beta-amyloid in Postmortem Specimens

Statistical Analysis Overview	Comparison Groups	Sensitivity (Primary Efficacy Analysis)
	Comments	For the primary analysis, point estimates together with normal-approximated, two sided 95% confidence intervals, were given for sensitivity in beta-amyloid detection based on the majority read. The following hypothesis was formulated for sensitivity: H0,sens: sensitivity ≤ 0.6 vs. H1, sens: sensitivity > 0.6 H0,sens was to be rejected if the lower bound of the two-sided 95% CI is larger than 0.6

	Non-Inferiority or Equivalence Analysis?	No
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Other [Sensitivity]
	Estimated Value	0.774
	Confidence Interval	(2-Sided) 95% 0.654 to 0.894
	Estimation Comments	Point estimate of sensitivity was calculated by the method of Rao and Scott. Variance for sensitivity is based on subjects that contribute at least one brain region, which is amyloid positive according to the SOT

Table 3: Statistical Analysis 2 for Specificity of the Majority Read of Visual Assessment of Tracer Uptake Compared to Histological Verification of the Presence or Absence of Cerebral Beta-amyloid in Postmortem Specimens

Statistical Analysis Overview	Comparison Groups	Specificity (Primary Efficacy Analysis)
	Comments	For the primary analysis, point estimates together with normal-approximated, two sided 95% confidence intervals, were given for specificity in amyloid detection based on the majority read. The following hypothesis was formulated for specificity: H0,spec: specificity ≤ 0.8 vs. H1, spec: specificity > 0.8 H0,spec was to be rejected if the lower bound of the two-sided 95% CI is larger than 0.8
	Non-Inferiority or Equivalence Analysis?	No

	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Other [Specificity]
	Estimated Value	0.942
	Confidence Interval	(2-Sided) 95% 0.886 to 0.998
	Estimation Comments	Point estimate of specificity was calculated using the method of Rao and Scott. Variance for specificity is based on subjects that contribute at least one brain region, which is amyloid negative according to the SOT

Secondary efficacy analyses

1. To determine the sensitivity and specificity of the composite “whole brain” (per subject) regional visual assessment collapsed from the regional PET visual assessment results in detecting/excluding cerebral β-amyloid plaques based on the "whole brain" histopathological verification of the presence/absence of β-amyloid deposition (collapsed from the results of the regional histological findings from the Pathology Consensus Panel).

   Reporting Groups Description

   The analysis set consisted of 31 subjects for whom both brain specimen with a valid Standard of Truth in at least one brain region and a florbetaben PET scan were available and 10 healthy controls for whom a florbetaben PET scan was available and whose SOT was considered β-amyloid negative by definition.

   Results

   Sensitivity and specificity was analyzed of the whole brain regional visual assessment compared to the composite whole brain histopathological assessment. The sensitivity and specificity of the whole brain regional assessment were 86.96% (95% CI: 73.19 – 100.00%) and 88.89% (95% CI: 74.37% – 100.00%), respectively, for the majority read.

2. To determine the sensitivity and specificity of the quantitative assessment of regional tracer uptake in florbetaben PET images compared to histological verification of the presence or absence of cerebral β-amyloid in the respective postmortem specimens as the SOT.

   Reporting Groups Description

   The analysis set consisted of 31 subjects for whom both brain specimen with a valid Standard of Truth in at least one brain region and a florbetaben PET scan were available and 10 healthy controls for whom a florbetaben PET scan was available and whose SOT was considered β-amyloid negative by definition.

   Results

   The analysis of the SUVRs shows results comparable to the results of the analysis of the visual assessment. The SUVRs provided a good differentiation between positive and negative brain regions for Regions 1 (frontal), 2 (occipital cortex), 4 (anterior

cingulate), and 5 (posterior cingulate/precuneus). The region that gave the best differentiation in terms of highest Youden Index was Region 5 (posterior cingulate/precuneus).

3. To determine the sensitivity and specificity of the majority read "whole brain" visual assessment in detecting/excluding cerebral neuritic β-amyloid plaques compared with the histopathological verification according to CERAD criteria as SOT.

Reporting Groups Description

Full Analysis Set Final Clinical Study Report: The full analysis of the final clinical study report consisted of data from 97 subjects, including 87 subjects with brain specimens and 10 young healthy controls considered to be β-amyloid-negative as a valid Standard of Truth (SOT).

Results

Table 4: Measured Values

	Full Analysis Set Final Clinical Study Report
Number of Participants Analyzed	97
Sensitivity and Specificity of the Majority Read “Whole Brain” Visual Assessment in Detecting/Excluding Cerebral Neuritic β-amyloid Plaques Compared With the Histopathological Verification According to CERAD Criteria. [units: percentage of subjects] Number (95% Confidence Interval)	
Sensitivity	96.49 (91.71 to 100.00)
Specificity	85.00 (73.93 to 96.07)

Post-hoc analysis 1:

A post-hoc analysis according to secondary endpoint 3 was performed during the review of the EU application for marketing authorization. It comprised the analysis of data from 74 deceased subjects, who were available at the respective time point of analysis. The results using the majority read of three in-person trained readers compared with the histopathology SOT are included in the SmPC: sensitivity: 97.87 (95% CI: 93.75% - 100%); specificity 88.89 (95% CI: 77.03% - 100%) and were similar to the main results of the final data set.

Post-hoc analysis 2:

To determine sensitivity and specificity of the subject level composite SUVR calculated based on pathology results.

Reporting Groups Description

Sensitivity of Subject Level Composite SUVR by SOT: The analysis set consisted of data from 96 subjects, including 86 subjects with brain specimens and 10 young healthy controls considered to be β-amyloid-negative as a valid Standard of Truth (SOT). Sensitivity of subject level composite SUVR by SOT for baseline scans and last available scans are reported.

Specificity of Subject Level Composite SUVR by SOT: The analysis set consisted of data from 96 subjects, including 86 subjects with brain specimens and 10 young healthy controls considered to be β-amyloid-negative as a valid Standard of Truth. Specificity of subject level composite SUVR by SOT for baseline scans and last available scans are reported.

Table 5: Measured Values

	Sensitivity of Subject Level Composite SUVR by SOT	Specificity of Subject Level Composite SUVR by SOT
Number of Participants Analyzed	96	96
Sensitivity and Specificity of the Subject Level Composite SUVR Calculated Based on Pathology Results. [units: percentage of subjects]		
SOT BSS (initial period)	89	82
SOT BSS+IHC (initial period)	90	91

	Sensitivity of Subject Level Composite SUVR by SOT	Specificity of Subject Level Composite SUVR by SOT
SOT CERAD (initial period)	89	90
SOT BSS (last available scan)	88	85
SOT BSS+IHC (last available scan)	89	94
SOT CERAD (last available scan)	95	87

Abbreviations: BSS = Bielschowsky Silver Staining, IHC = Immunohistochemistry

Post-hoc analysis 3:

To determine the subject level composite SUVRs by SOT for baseline and available follow-up scans.

Reporting Groups Description

Initial Drug Administration: The analysis set consisted of data from 96 subjects, including 86 subjects with brain specimens and 10 young healthy controls considered to be β-amyloid-negative as a valid Standard of Truth (SOT). Descriptive statistics of subject level Composite SUVR by SOT for baseline scans and last available scans are reported.

1st Repeat Drug Administration: The analysis set consisted of data from 20 subjects with brain specimens available.

2nd Repeat Drug Administration: The analysis set consisted of data from 3 subjects with brain specimens available.

Table 6: Measured Values

	Initial Drug Administration	1st Repeat Drug Administration	2nd Repeat Drug Administration
Number of Participants Analyzed	96	20	3
Subject Level Composite SUVRs by SOT for Baseline and Available Follow-Up Scans [units: Standardized Uptake Value Ratio (SUVR)] Mean (Standard Deviation)			
SOT BSS (Aβ absent)	1.296 (0.232)	1.319 (0.414)	1.537 (NA) [1]

	Initial Drug Administration	1st Repeat Drug Administration	2nd Repeat Drug Administration
SOT BSS (Aβ present)	1.707 (0.276)	1.625 (0.250)	1.699 (0.046)
SOT BSS+IHC (Aβ absent)	1.237 (0.140)	1.175 (0.364)	1.537 (NA) [1]
SOT BSS+IHC (Aβ present)	1.714 (0.273)	1.632 (0.244)	1.699 (0.046)
SOT CERAD (Aβ absent)	1.263 (0.170)	1.260 (0.390)	NA (NA) [1]
SOT CERAD (Aβ present)	1.729 (0.270)	1.639 (0.236)	1.645 (0.099)

[1] Sample size too low

Safety evaluation

All treatment emergent adverse events were recorded within 7 days of the application or follow-up application of study drug. As this study was conducted in an end-of-life population, death occurring outside 7 day follow-up period after administration of the drug was not collected as part of the study treatment emergent SAE data (table 7) unless investigator considered the event to be related to drug administration or study procedure. Subjects with TEAEs within 7 days of the initial administration of florbetaben are reported for the initial drug administration, first repeat and second repeat injections.

Table 7: Serious Adverse Events

	Initial Drug Administration		1st Repeat Drug Administration		2nd Repeat Drug Administration	
	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events
Total	12/216 (5.56%)		0/91 (0%)		0/34 (0%)	
Cardiac disorders						
Cardiac Failure [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Gastrointestinal disorders						
Colitis [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Colon cancer [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
General disorders						
Heat stroke [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Oedema peripheral [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Hepatobiliary disorders						
Hepatic cancer metastatic [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Infections and infestations						
Pneumonia [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Pneumonia aspiration [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Injury, poisoning and procedural complications						

	Initial Drug Administration		1st Repeat Drug Administration		2nd Repeat Drug Administration	
	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events
Pubis fracture [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Musculoskeletal and connective tissue disorders						
Arthralgia [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Spinal compression fracture [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Nervous system disorders						
Convulsion [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Dementia, Alzheimers' type [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Frontotemporal dementia [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Psychiatric disorders						
Delirium [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0
Respiratory, thoracic and mediastinal disorders						
Respiratory failure [A] *	1/216 (0.46%)	1	0/91 (0%)	0	0/34 (0%)	0

*Indicates events were collected by non-systematic methods. [A] Term from vocabulary, MedDRA 16.1

Table 8: Other Adverse Events; Frequency Threshold Above Which Other Adverse Events are Reported: 1%

	Initial Drug Administration		1st Repeat Drug Administration		2nd Repeat Drug Administration	
	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events
Total	48/216 (22.22%)		22/91 (24.18%)		14/34 (41.18%)	
Gastrointestinal disorders						
Frequent bowel movements [A] *	1/216 (0.46%)	1	1/91 (1.1%)	1	0/34 (0%)	0
General disorders						
Injection site bruising [A] *	5/216 (2.31%)	5	0/91 (0%)	0	0/34 (0%)	0
Injection site erythema [A] *	4/216 (1.85%)	4	0/91 (0%)	0	1/34 (2.94%)	1
Injection site hematoma [A] *	10/216 (4.63%)	10	1/91 (1.1%)	1	0/34 (0%)	0
Injection site hemorrhage [A] *	1/216 (0.46%)	1	4/91 (4.4%)	4	2/34 (5.88%)	2
Injection site pain [A] *	4/216 (1.85%)	4	0/91 (0%)	0	0/34 (0%)	0
Puncture site reaction [A] *	7/216 (3.24%)	7	4/91 (4.4%)	4	0/34 (0%)	0
Pyrexia [A] *	7/216 (3.24%)	8	2/91 (2.2%)	2	0/34 (0%)	0
Vessel puncture site swelling [A] *	1/216 (0.46%)	1	1/91 (1.1%)	1	1/34 (2.94%)	1
Infections and infestations						
Cystitis [A] *	0/216 (0%)	0	0/91 (0%)	0	1/34 (2.94%)	1
Injury, poisoning and procedural complications						
Procedural hypertension [A] *	0/216 (0%)	0	0/91 (0%)	0	1/34 (2.94%)	1
Procedural pain [A] *	0/216 (0%)	0	1/91 (1.1%)	1	0/34 (0%)	0
Investigations						
Blood pressure increased [A] *	3/216 (1.39%)	3	1/91 (1.1%)	1	0/34 (0%)	0
Nervous system disorders						

	Initial Drug Administration		1st Repeat Drug Administration		2nd Repeat Drug Administration	
	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events	Affected/At Risk (%)	# Events
Headache [A] *	3/216 (1.39%)	3	0/91 (0%)	0	0/34 (0%)	0
Respiratory, thoracic and mediastinal disorders						
Asthma [A] *	0/216 (0%)	0	0/91 (0%)	0	1/34 (2.94%)	1
Emphysema [A] *	0/216 (0%)	0	0/91 (0%)	0	1/34 (2.94%)	1
Skin and subcutaneous tissue disorders						
Erythema [A] *	1/216 (0.46%)	1	4/91 (4.4%)	5	3/34 (8.82%)	3
Haemorrhage subcutaneous [A] *	1/216 (0.46%)	1	3/91 (3.3%)	3	1/34 (2.94%)	1
Papule [A] *	0/216 (0%)	0	1/91 (1.1%)	1	0/34 (0%)	0
Rash [A] *	2/216 (0.93%)	3	1/91 (1.1%)	1	0/34 (0%)	0
Vascular disorders						
Haematoma [A] *	1/216 (0.46%)	1	5/91 (5.49%)	5	6/34 (17.65%)	6
Hypertension [A] *	5/216 (2.31%)	5	1/91 (1.1%)	1	1/34 (2.94%)	1
Hypotension [A] *	3/216 (1.39%)	3	0/91 (0%)	0	0/34 (0%)	0

*Indicates events were collected by non-systematic methods. [A] Term from vocabulary, MedDRA 16.1

Overall conclusions

The design of study 14595 served two different goals. The regional tissue-matched analysis with MRI co-registration was designed as a “target validation” study, aiming to provide pivotal support for the validity of florbetaben PET imaging to detect amyloid aggregates in precisely the same tissue as that examined by histopathology. The subject-level analysis of the florbetaben PET images without MRI co-registration was designed to provide pivotal support for the visual assessment methodology intended for clinical use. The subject level results of the final analysis, performed on all evaluable brains, were similar as compared to the results obtained in the initial study period. Adding more subjects in the analysis sets led to a narrowing of the confidence intervals.

The results of the trial confirm that florbetaben PET can detect β-amyloid in the brain during life with high sensitivity and specificity based on a direct regional comparison between scan and the respective postmortem specimen. This supports the usefulness of florbetaben PET as a biomarker for use in the detection of amyloid plaques associated with AD.

Furthermore, the visual assessment procedure and methodology proposed for clinical practice proved robust and reproducible with high accuracy.

The safety results revealed no trends or signs indicative of a safety concern and indicate that florbetaben is safe and well tolerated in subjects both with and without dementia. The overall safety profile thus remains favorable and no changes in the current risk-benefit assessment are indicated.

To conclude, florbetaben was shown to be safe and well tolerated in the population studied and the efficacy results indicate the potential of florbetaben PET as a valuable visual adjunct in the diagnostic algorithm of AD and other dementias.

Overview of Amendments to study 14595

There were six amendments to the original protocol dated May 20, 2009. The main modifications of these amendments are summarized below.

Amendment 1

Local amendment for Japan only.

- In Japan, in addition to evaluating the efficacy of BAY 94-9172 PET imaging compared to postmortem histopathology as the primary variables, it is intended to perform PET imaging with [^{18}F]-fluorodeoxyglucose (FDG) as an additional optional examination. This is planned because the Japanese regulatory authorities in general have been indicating that they would like to see comparative data with an additional, well established functional diagnostic imaging method.

- Changes to meet Japanese specific GCP requirements.

Amendment 2

- The duration of the screening period was increased to 8 weeks with 12 weeks acceptable to accommodate the specific characteristics of radiopharmaceutical manufacturing and changes in scheduling of patient visits by the investigator.

- Additional recruitment sources were added to the protocol.

- Exclusion criterion 6 was modified per discussions with the German Radiation Protection Board. According to the German Radiation Protection Law, subjects in Germany enrolled in this study who have a low probability of cerebral β-amyloid deposition in the brain are not allowed to receive more than 10 mSv of radiation within a 10 year period and therefore will only have the initial PET scan; the yearly follow-up PET scans are prohibited and will not be performed on these subjects.

- The time point regarding prior enrollment in this study or in another study involving an investigational pharmaceutical product was changed from 30 days prior to screening to 30 days prior to treatment in order to enroll subjects more rapidly.

- The use of an MRI performed up to 6 months prior to the florbetaben PET scan and meeting the technical requirements of the study was added to the protocol to reduce the burden on subjects.

- Conditions under which subjects could continue participation in the study if blood samples for the evaluation of biomarkers were not collected were added to the protocol.

- The description of the BAY 94-9172 injection procedure was modified to indicate that the study drug must be manually injected; power injectors are not permitted.

- The section “Autopsy and histopathology evaluation of brains” was extensively re-written to make it more general, to correct content, and to reference the Pathology Technical Manual.

- Statements were added to the protocol that the Boston Naming Test will not be used in this study. The owner of the Boston Naming Test, PRO-ED, Inc., does not allow use of the test in clinical studies by pharmaceutical companies.

- To ensure consistency with information in the IND submitted to the FDA, the description of the drug product characteristics in Text Table 1 and the drug product formulation in Text Table 2 were modified.

Amendment 3

- Change in the name of the Study Medical Expert and one of the Authors, as they were no longer with the company.

- Synopsis - Study Design - Change in the wording for clarity and better understanding (the first few words were misleading).

- The modification of the inclusion criterion for HVs was necessary, because the basis of the algorithm used in the computer assisted evaluation tool (CERAD Plus) is normative data, which were established on subject data covering an age range of 49 to 92 years. The normative data were transformed via CERAD Plus to generate a normal distribution for the age group between 49 to 92 years of age only. The HVs enrolled into the study are very young (21 to 40 years of age). Their age is far away from the normal distribution of the underlying normative data so that the algorithm of the CERAD Plus calculates "abnormal" z-scores although the HVs are absolutely cognitively healthy.

- Subjects with major cerebrovascular disease fulfilling the NINDS-AIREN criteria for vascular dementia and with an ARWMC score > 2 were excluded since the morphological changes occurring in this condition can lead to regional alterations in PET tracer uptake in the brain due to perfusion defects. This could pose difficulties in interpretation of the PET scans by the blinded readers as they do not have access to the structural anatomical information provided by a brain MRI. Furthermore the perfusion defects present in multi-stroke disease could prevent efficacious co-localization between the PET scan and the post mortem histopathological specimen.

- The wording in the exclusion criterion #7 was misleading and did not clearly specify the severity of the critical illness that was meant. The purpose of this exclusion criterion was to exclude subjects with unstable circulatory system as they would need intense surveillance, require resuscitation, and/or die in the PET suite/scanner. The current wording in the protocol resulted in numerous inquiries from investigators and was a potential recruitment barrier. In

particular, as many of the subjects recruited into the trial are critically ill, although cardiovascularly stable. Therefore, the exclusion criterion was reworded for clarity.

- Yearly follow-up visits (amended) for clarity and better understanding.

- Editorial change in Table 4 (section 7.2.5): Addition of missing "Xs" for the required examinations (for medical/surgical history, documentation of diagnostic tests outside this study, MRI brain scan, neuropsychometric tests, and body weight) in the annual follow-up visits, which were erroneously left out in the previous protocol versions.

Amendment 4

- A new inclusion criterion (no. 8) was added. In addition to the overall time constraints involved in gathering autopsy data, recruitment of patients and/or their family members into the trial has been a major challenge. To meet this challenge the team has opened as many centers globally as feasible, tried to support and motivate doctors and their teams, and accommodate patients and their family members as far as possible. Transportation to and from the investigative center and hotel accommodation (if required) is offered and voluntary yearly neuro-psychiatric, PET and MR examinations are provided to all subjects not deceased. Finally, the residual brain tissue is provided to the brain bank initiatives at all participating hospitals after the required specimens have been collected. In spite of the above efforts recruitment has been challenging. In view of the lack of ethical concerns it has, thus, been decided to allow subjects who have participated in (and completed) previous florbetaben trials to be eligible for the 14595 study. In order to avoid unnecessary exposure of the subject to additional radiation and/or procedural risk or discomfort, the florbetaben PET and MRI imaging procedures do not have to be repeated if they were performed within one year of entering the trial.

- Statistical evaluation has been revised according to the recommendation made by FDA during the Type C meeting relating to the study-specific SAP.

- Minor changes to the protocol were made.

Amendment 5

- After discussions with the regulatory authorities and with experts in the field, the secondary endpoints have been modified and further refined. First a "whole brain composite" endpoint was added to provide a subject level comparison between the PET scan results and histopathology. This subject level "composite" score (collapsed from the results of the regional PET or histological assessment of the blinded readers/Pathology Consensus Panel) is derived from the highest score in any of the 6 regions, both in the PET scan and in the respective post mortem specimen. The endpoints regarding the regional quantitative and subject level PET scan assessment have been maintained. Lastly, because the onsite clinical

diagnosis has often not been established in the subject collective involved, the subject level comparison with the onsite clinical diagnosis was deleted as a secondary endpoint.

- The current formulation does not lend itself to commercial manufacturing, which requires higher yields and radioactivity concentrations to allow filling of an appropriate number of doses per batch, combined with a proper shelf-life to ensure a broad access for a large number of subjects. Up-scaling the manufacturing to the commercially required scale led to a greater sensitivity of florbetaben to radiolysis. The formulation, therefore, had to be adapted to protect the tracer against such radiolysis. This was done by increasing the overall amount of scavenger (ascorbic acid) in the solution.

- Following an audit of a major US center, questions arose regarding: a) the dose administration of florbetaben, b) the procedure for "assent" in subjects who were not able to consent. For clarity a few facts/definitions were added to the CSP.

- The results of the independent blinded visual assessment of the Phase 2 Part B study greatly favored specificity at the expense of sensitivity. Further analysis of the data, both in house and with the help or external experts indicated that an improvement of the reader training and refinement of the visual scoring procedure were necessary. Thus, three "test" reads of a standardized image data set" (with images stemming from both Parts A and B of the phase 2 study) were performed with subsequent consensus analysis after each of the reads. This iterative process resulted in an improved training method which yielded an accuracy of 95% for test reads 2 and 3. The process also led to a more detailed definition of the 3 categories in the regional score. To better reflect the interaction between florbetaben and β-amyloid in the brain, the regional score was renamed to Regional Cortical Tracer Uptake score (RCTU) [from Regional Cortical Tracer Binding (RCTB)] and for the 3-point subject level Brain Amyloid Plaque (BAPL) score the term "β-amyloid plaque load" was replaced with "β-amyloid deposition." In addition, a gray color scale is used as the display mode for the subject level assessment by the independent readers.

- Clarification that the 3-year follow-up was not mandatory.

- Several other (additional) minor editorial changes.

Amendment 6

- Name and address of sponsor was changed to reflect sponsor take-over from Bayer HealthCare AG to Piramal Imaging.

- Name and email contact of sponsor's medical expert was changed.

- Name and contact details of submission support to radiation protection boards was changed for Germany.

- Change of subsection “Actions and reporting obligations in case of serious adverse events” within section Serious adverse events to reflect that CRO is not working with local drug safety managers but has a centralized approach

- Name of contract research organization was included in section 10.2 Monitoring.

Interruption and premature termination

Study 14595 prematurely terminated October 18, 2013, since it was decided, that the scientific goals of the study were achieved and further data collection were not expected to change the results. Health Authorities were informed about the early termination of the study on October 18, 2013 and investigators on November 13, 2013. Due to regulatory requirements, the study was terminated in Japan, December 24, 2013.