19 December 2013
EMA/5028/2014
Committee for Medicinal Products for Human Use (CHMP)

Neuraceq

Florbetaben (^{18}F)

Procedure No. EMEA/H/C/002553

Applicant: Piramal Imaging GmbH

Assessment report for an initial marketing authorisation application

Assessment report as adopted by the CHMP with all commercially confidential information deleted

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

Table of contents

List of abbreviations

[C-11]PiB	[C-11]-labeled Pittsburgh Compound-B
AAL	automated anatomical labeling (template)
AD	Alzheimer's disease
ADNI	Alzheimer's disease neuroimaging initiative
AE	Adverse Event
ANCOVA	analysis of covariance
ANOVA	Analysis of variance
APOE	genetic locus encoding for Apolipoprotein E
APP	amyloid precursor protein
ARPANSA	Australian Radiation Protection and Nuclear Safety Agency
Aβ	Amyloid Beta
BAPL	Brain beta-amyloid plaque load
BMI	Body Mass Index
Bq	Bequerel
Bq/cc	Bequerel/cubic centimeter
BR	blinded reader
CDR	Clinical Dementia Rating
CERAD	The Consortium to Establish a Registry for Alzheimer's Disease
CFR	Code of Federal Regulations
CI	confidence interval
COWAT	Controlled Oral Word Association Test
CP	consensus panel
CrCl	creatinine clearance
CSF	cerebrospinal fluid
CSP	Clinical Study Protocol
CSR	clinical study report
CSR	Clinical Study Report
CTN	Clinical Trial Notification
CVLT	California Verbal Learning Test
DEM	other dementia disabilities
DLB	dementia with Lewy bodies
DLW	Diffuse Lewy Body dementia
DNA	deoxyribonucleic acid
DS	Down's syndrome
DSM IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition,
DSQIID	dementia screening questionnaire in individuals with intellectual
DVR	Distribution Volume Ratio
ECG	electrocardiogram
eCRF	electronic case report form
ED	Effective Dose
EDC	Electronic Data Capture
EoS	end of synthesis
EU	European Union
FAS	full analysis set
FDA	Food and Drug Administration
FDG	fludeoxyglucose (18F)
FN	false negative
FP	false positive
FTD	fronto-temporal dementia
FTD	Frontotemporal Dementia
FTLD	fronto-temporal lobe degeneration (dementia)
GCP	Good Clinical Practice
HC(s)	healthy control (s)
HR	Heart Rate
HREC	Human Research Ethics Committee
HV	Healthy Volunteer
IASAP	integrated analysis statistical analysis plan
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMPACT	International Management Package for the Administration of Clinical

IND	Investigational New Drug
INN	International Nonproprietary Name
IQ	intelligence quotient
IRC	image review charter
ISS	Investigator Sponsored Study
IV	intravenous infusion
LCL	lower confidence limit
LDA	linear discriminant analysis
ldCT	Low Dose CT
LLI	Lower Large Intestine
LLN	Lower Limit of Normal Range
MBq	MegaBecquerel
MCI	mild cognitive impairment
Min	minute(s)
MIRD	Medical Internal Radiation Dose
mL	milliliter
MMSE	Mini Mental State Examination
MNI	Montreal Neurologic Institute
MRI	Magnetic Resonance Image
MRI	Magnetic Resonance Imaging
MRTM	multilinear reference tissue model
mSv	millisievert
NC	Normal Control
NDVs	non-demented volunteers (including HVs and NCs/HCs)
NHMRC	National Health and Medical Research Council
NHMRC	National Health and Medical Research Council and Stroke-Alzheimer's Disease and Related Disorder Association
NINDS-AIREN	National Institute of Neurological Disorders and Stroke – Association
NMRI	nuclear magnetic resonance imaging
OLINDA	Organ Level Internal Dose Assessment
p.i.	post injection
PD	Parkinson´s disease
PET	Positron Emission Tomography
PIB	Pittsburgh compound B
PK	pharmacokinetics
PMOD	PMOD technologies Zurich, Switzerland, imaging software
PoM	proof of mechanism
PPS	Per Protocol Set
Q	quartile
QC	quality control
RCI	Reliable change indices
RCTB	regional cortical tracer binding
RCTU	regional cortical tracer uptake
ROC	receiver operating characteristics
ROI	region of interest
RR	Respiratory Rate
SAE	Serious Adverse Events
SAP	Statistical Analysis Plan
SOC	System Organ Classes
SoR	standard of reference
SoT	standard of truth
SPECT	single photon emission computed tomography
SPM8	Statistical Parametric Mapping
SUV	Standardized Uptake Values
SUVR	standardized uptake value ratio
SUVR	SUV Ratio
Sv	Sievert
TAC	Time Activity Curve
TGA	Therapeutic Goods Administration
TN	true negative
TNS	Taylor Nelson Sofres
TP	true positive
UCL	upper confidence limit
ULN	Upper Limit of Normal Range

USA	United States of America
UT	upper threshold
VaD	Vascular dementia
VB	Volunteer for brain imaging
VOI	Volumes of interest
VSP	Valid for Analysis of Safety Population
VT	Volunteer for total body imaginA
WAIS	Wechsler Adult Intelligence Test

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Piramal Imaging GmbH submitted on 7 January 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Neuraceq, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 14 April 2011.

The applicant applied for the following indication:

"This medicinal product is for diagnostic use only.

Neuraceq is indicated for the detection of β-amyloid in the brain, thereby assisting in the differential diagnosis in adult patients who are being evaluated for Alzheimer's disease and other causes of cognitive decline."

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that florbetaben (^{18}F) was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/193/2011 on the granting of a product-specific waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance florbetaben (^{18}F) contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Scientific Advice

The applicant received Scientific Advice from the CHMP in 2008 and 2010. The Scientific Advice pertained to clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturers responsible for batch release

BV Cyclotron VU
De Boelelaan 1081
1081 Amsterdam
Netherlands

CIS BIO INTERNATIONAL - PARIS
14 rue de la Grange aux Belles
75010 PARIS
France

IBA MOLECULAR ITALY
c/o Ospedale San Gerardo dei Tintori
VIA PERGOLESI,33
20052
Monza
Italy

Alliance Medical Molecular Imaging Ltd.
Unit 19, Quadrum Park - Old Portsmouth Road GU3 1LU - Peasmarsh, Guildford
United Kingdom

MOLYPHARMA, S.A.
Pol. Ind. Conpisa, C/ Veguillas, 2 Nave 16, Ajalvir, Madrid, 28864, Spain

PET NET GmbH
Franz-Josef-Strauss-Allee 11
93053 Regensburg
Germany

Manufacturer responsible for import and batch release in the European Economic Area

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Concepcion Prieto Yerro

Co-Rapporteur: Kristina Dunder

CHMP Peer reviewer: Philippe Lechat

- The application was received by the EMA on 7 January 2013.
- The procedure started on 30 January 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 20 April 2013. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 22 April 2013.
- PRAC RMP advice and overview assessment report, adopted by PRAC on 16 May 2013.
- During the meeting on 30 May 2013, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 30 May 2013.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 23 August 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 26 September 2013 .
- PRAC RMP advice and assessment overview, adopted by PRAC on 10 October 2013.
- During the CHMP meeting on 24 October 2013, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 18 November 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 28 November 2013.
- The Rapporteurs circulated the Final Joint Assessment Report to all CHMP members on 15 December 2013.
- During the meeting on 16-19 December 2013, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Neuraceq.

2. Scientific discussion

2.1. Introduction

2.2. Quality aspects

2.2.1. Introduction

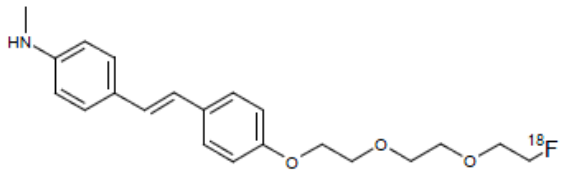
The finished product is a solution for injection presented in single dose vials that contains 1 to 10 mL of a solution of 300 MBq/mL of florbetaben (^{18}F) at the date and time of calibration.

Other ingredients are: Ascorbic acid, Ethanol anhydrous, Macrogol 400, Sodium ascorbate and Water for injections.

The product is available in colourless Type I glass vial, sealed with a chlorobutyl rubber stopper and aluminium seal.

2.2.2. Active substance

The chemical name of active substance florbetaben (^{18}F) is: 4-[(E)-2-(4-{2-[2-(2-[18F]fluoroethoxy)ethoxy]ethoxy}phenyl)vinyl]-N-methylaniline, its molecular formula is $C_{21}H_{26}{}^{18}FNO_3$ and it has the following structure:



The active substance is insoluble in water, practically insoluble in ethanol, slightly soluble in acetonitrile and soluble in dimethylformamide. Its PKa-value is 4.5±0.2 (using acetonitrile as cosolvent), LogP(o/w)=3.3 (octanol:buffer pH 7), it has not chiral molecular structure, hygroscopicity and polymorphism are not relevant for this active substance due to the fact that it is not isolated.

Manufacture

The active substance, florbetaben (^{18}F), is not isolated and it is synthesized in situ during the manufacture of the finished product using a non-radioactive chemical precursor as the key starting material. The manufacturing process is a continuous process without intermediates

The manufacturing process of PET radiopharmaceuticals uses characteristic automated and remote controlled apparatus called modules. They contain the necessary 'hardware' components to perform the synthesis

Adequate in-process controls are applied during the synthesis. The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

Adequate specifications and control methods for intermediate products, starting materials and reagents have been presented. Adequate specification of the IFP (Integrated Fluidic Processor) including inspection of absence of defects, functional testing, bioburden and endotoxins limits has been presented.

Relevant leachable test on the IFP have been initiated. Although the leachable study is still not completed, the control of the whole process guarantees the quality and safety of the product. However based on the available data, the CHMP recommends the Marketing authorisation holder (MAH) to provide results of the studies once they are available

Specification

The active substance florbetaben (^{18}F) is not isolated during the manufacturing of Neuraceq. Details of the specified limits are described on the product specification sections.

Appropriate specification for the main precursor, has been presented, and it includes tests for colour, identity (IR and HPLC), water (KF), assay (HPLC) and impurities (GC and HPLC).

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines.

Batch results are given for three commercial scale batches of precursor, and additional batches used for the clinical trials. All results comply with the specifications.

Stability

Not applicable. The active substance cannot be stored.

2.2.3. Finished medicinal product

Pharmaceutical development

The objective of the development was to get a parenteral formulation suitable for intravenous injection, protected against the radiolysis and convenient for patient administration.Thefinal formulation is optimised for the protection against radiolysis, to reduce the adsorption on sterilising filters, and also to enhance the solubility and water for injection. The formulations used at the different stages of the clinical development were described and the stage of the clinical trial in which they were used.

The parenteral finished product is sterilized by sterile filtration. This sterilization method was selected instead of heat sterilization because of the susceptibility of the drug substance and of ascorbic acid to decompose at higher temperatures. The microbiological safety of the manufacturing process is favoured by several process parameters and by use of harsh reaction conditions that limit the risk of microbial growth. Integrity testing of the sterile filter is performed post-filtration as process control and required for release of the drug product. Batches of florbetaben (^{18}F) solution for injection are subject to testing for sterility and endotoxins as part of the drug product specification.

Detailed information related to the container closer system such as studies on the closure integrity (physical and microbiological tests), declaration of compliance with relevant Ph.Eur, studies on compatibility of the vial and the stopper with the drug product, self-sealing test of the rubber closure and description of the pre-sterilisation of the vials and the stoppers was provided.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

The primary packaging is Type I glass vial, sealed with a chlorobutyl stopper and aluminum seal. The material complies with Ph Eur and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Adventitious agents

No excipients derived from animal or human origin have been used.

Manufacture of the product

By contrast to conventional pharmaceutical products, the manufacturing of PET tracers is not divided into separate manufacturing steps. The production of the active substance (chemical manufacturing) is directly followed by the manufacturing of the finished product (pharmaceutical manufacturing). The manufacture of the active substance takes place “in situ” during the manufacturing process of the finished product which is a continuous highly automated process which does not allow the isolation of the active substance florbetaben (^{18}F).

The manufacturing process can be described in five steps. The first three steps (production of the radionuclide 18F in a cyclotron, synthesis and purification) describe the manufacturing of the active

substance, while the last two steps (sterilising filtration and aseptic dispensing steps) describe the manufacturing of the drug product.

The process is considered to be a non-standard manufacturing process. Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this pharmaceutical form.

Product specification

The finished product release and shelf-life specifications include appropriate tests for this kind of pharmaceutical form: assay (radioactive concentration), clarity (visual inspection), radionuclidic identity and purity (half-life and gamma spectrometry) (Ph Eur), radiochemical identity(HPLC), chemical identity of florbetaben (HPLC), radiochemical purity (HPLC), chemical content of florbetaben and chemical impurities (HPLC), acetonitrile (GC), bacterial endotoxin (Ph Eur), sterile filter integrity and sterility (Ph Eur).

Batch analysis results are provided for 18 production scale batches manufactured in 6 different sites (3 each) confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing, although sterility test and the radionuclidic purity test for long lasting radionuclides are conducted after batch release (as usual for these type of products).

Stability of the product

Stability data of 3 commercial scale batches for florbetaben (^{18}F) solution for injection at temperature storage conditions of 5 °C, 25 °C and 40 °C (according ICH) are presented covering a storage period of 10 hours.
In addition stability data of 15 (5 sites, 3 batches each) commercial scale batches of florbetaben solution for injection at storage conditions of 25 °C are presented covering a storage period of 10 hours. All batches are packaged in the commercial packaging materials (Glass vial 15 mL colorless glass type 1 sealed with a chlorobutyl stopper and aluminium seal).

Stress tests at higher temperatures were not performed since the secondary container necessary for radioprotection reasons also has a temperature shielding effect.
Photo stability tests were not performed since the secondary container necessary for radioprotection reasons also protects the vial against light.

The results demonstrate that the finished product remains stable under long-term and accelerated conditions for the tested period of 10 hours.

All stability indicating parameters,: clarity, pH, radiochemical identity radiochemical purity, radiochemical impurities were tested according to the release test procedures and evaluated against

the specification. In addition, sterility test and radionuclidic purity test for long lasting radionuclides, performed after the end of shelf-life were also satisfactory.

Based on available stability data, the shelf-life as stated in the SmPC is acceptable.

2.2.4. Discussion on chemical, and pharmaceutical aspects

The quality of Neuraceq is adequately established. In general, satisfactory chemical and pharmaceutical documentation has been submitted for marketing authorisation. Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

Nevertheless, based on the available data, the CHMP recommends the Marketing authorisation holder (MAH) to complete the leachable test studies on the IFP (Integrated Fluidic Processor). In case of out of specification results occur, the MAH is reminded that such would need to be reported to the Agency and to the Rapporteurs

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

1.4. Non-clinical aspects

1.4.1. Introduction

1.4.2. Pharmacology

The primary pharmacodynamics studies submitted by the Applicant show that florbetaben had nanomolar affinity to synthetic $A\beta_{1-42}$ fibrils and AD brain homogenate. High levels of ^{3}H-florbetaben binding were found in cortical regions of AD patients showing the following rank order frontal cortex > parietal cortex > temporal cortex. No difference in binding between AD patients and control group was detected in the cerebellum, indicating that the cerebellum could be used as a reference region for PET image analysis. The ApoE ε4 status of the AD patients did not impact ^{3}H-florbetaben binding to AD brain homogenates.

Florbetaben binding to β-amyloid deposits correlated with β-amyloid specific IHC and Bielchowsky stain (silver staining). Florbetaben did not bind to tau NFT and α-synuclein positive lesions in cortical brain tissue sections of FTD and LBD patients, respectively.

In the secondary pharmacodynamics studies, florbetaben did not show significant binding to any receptor or transporter assayed.

Regarding safety pharmacology, the effects of florbetaben hydrochloride in central nervous system, cardiovascular and respiratory systems and renal function were evaluated. No treatment-related effects were found in any system, although some effects on the respiratory system were observed in the repeated-dose toxicity study in rats.

However, the cardiohemodynamics study was conducted in anesthetized dogs (A45856). Despite the preference for unanesthetized animals stated in the guideline CPMP/ICH/539/00 S7A, it is considered that the use of anesthetized animals for assessing cardiovascular effects of drugs is a valid approach, due to the highest sensitivity for detecting possible drug-induced effects and the use of more invasive techniques. In addition, the Applicant conducted an exploratory study in conscious dogs but without control group and with a very small number of animals. Therefore, the study A45856 is considered appropriate.

The lack of pharmacodynamic drug interaction studies is acceptable considering the low mass dose of ^{19}F-florbetaben hydrochloride given, its specific binding to beta-amyloid deposits and the lack of binding shown in the radioligand-binding assay including a 78-target panel of animal and human receptors, ion channels and transporters.

1.4.3. Pharmacokinetics

Pharmacokinetic studies

Pharmacokinetics of non-labeled ^{19}F-florbetaben were studied in vivo in Wistar rats and Beagle dogs after single intravenous administration. Data on pharmacokinetics after repeated administration were obtained from the sub-acute toxicity studies in dogs. The biodistribution of ^{18}F-florbetaben was studied in NMRI mice. In addition, in vitro studies were conducted to investigate plasma protein binding, blood cell/plasma partitioning and drug metabolism in several species including man, as well as the drug-drug interaction potential.

In vitro studies showed that plasma protein binding in human was high. Pharmacokinetic studies in rats after single intravenous administration were conducted. The compound was rapidly eliminated and the volume of distribution was high. No differences between male and female animals were observed.

A biodistribution study conducted in male mice showed a high initial brain uptake followed by a fast initial elimination of radioactivity from the brain. A slow elimination of the radioactivity from the blood was detected. The extrapolation of mouse data to human indicated that an infusion of 300 MBq of ^{18}F-florbetaben to a 70 kg adult patient will result in an effective dose of 3.7 mSv.

The in vivo PET study conducted in rhesus monkeys showed that the time of peak uptake of ^{18}F-florbetaben was between 1.8 and 2.6 minutes and that the radioactivity decreased rapidly in the brain.

Florbetaben is subject to two oxidative pathways resulting mainly in N-demethylation leading to the main metabolite M-1 and several minor polar metabolites. No significant differences were observed regarding biotransformation in vitro. After administration of either ^{18}F-florbetaben or ^{14}C-florbetaben to mice, the parent compound represented the majority of the radioactivity in plasma and brain in the initial moments, and at later time points, a polar fraction of metabolites with fluoroacetate as the major component, accounted for most of the radioactivity present in brain and plasma. Metabolite M-1 was found nearly at all time-points investigated.

In human, several CYP isoforms mediate the N-demethylation, whereas CYP3A4 contribute to formation of polar metabolites. Clinically, plasma levels are not expected to be highly variable due to involvement of polymorphic CYPs or to be significantly altered when inhibitors of single CYPs are coadministered to patients. Florbetaben was not able to inhibit any CYP isoform except CYP3A4. However, considering that the administered doses of ^{18}F-florbetaben to patients will be< 100 µg, there is no risk of clinically relevant drug-drug interactions through inhibition of CYP1A2, 2C8, 2C9, 2D6 or 3A4 by ^{18}F-florbetaben.

1.4.4. Toxicology

The formulation used in the non-clinical toxicity studies was different from the clinical one. The clinical formulation contained florbetaben as a free base and not florbetaben hydrochloride. Florbetaben

hydrochloride was used for technical reasons and the formulation containing 20% hydroxylpropyl-β-cyclodextrin was used in order to achieve high exposures, and this was considered acceptable.

Single dose toxicity

- *Single intravenous dose safety study of florbetaben hydrochloride in Sprague-Dawley rats with CNS safety pharmacology Functional Observational Battery (FOB) and 14 days recovery (A35108, GLP)*

The endpoints of this study were clinical observations, body weight, food consumption, a functional observation battery (FOB), hematology, serum chemistry and a full necropsy with determination of organ weights and histopathological evaluation of the complete set of organs.

Overall, there were no major findings associated with florbetaben hydrochloride treatment. The most apparent finding was the increased serum phosphorus in Day 3 females, and at the highest dose, a slight increase in mean erythrocyte cell volume (MCV) and mean erythrocyte cell hemoglobin (MCH) in male animals observed on day 3.

- *Acute intravenous toxicity study of florbetaben in rats (A35107, GLP)*

No mortalities were observed during the study. The test item did not produce any clinical signs of toxicity and produced no adverse effect on body weight gains. NOAEL > 100 µg/kg.

- *Acute intravenous toxicity study of florbetaben hydrochloride in the rat (A35105, GLP)*

No mortalities were recorded during the study. The test item at a dose of 0.4 mg/rat did not produce any clinical signs of toxicity, produced no effect on body weight gains, and had no effects on blood haematology during the study period. Histopathology of the heart tissues and liver did not show any test item-related effects. No test item related histopathology findings were observed in the other tissues.

NOAEL < 400 µg/kg.

- *Single intravenous dose toxicity study of florbetaben hydrochloride in New Zealand White rabbits followed by 14 days recovery (A35109, GLP)*

- No findings were associated with florbetaben hydrochloride treatment. NOAEL > 48.4 µg/kg (586.6 µg/m2).

- *Acute intravenous toxicity of florbetaben hydrochloride in the rabbit (A35106, GLP)*

No mortalities were observed during the study. The test item at doses up to 250 µg/kg did not produce any clinical signs of toxicity, produced no adverse effect on body weight gains, and had no adverse effects on blood haematology. There was no evidence of any gross necropsy effects. The test item produced no treatment-related histopathological effects on the heart.

NOAEL > 250 µg/kg.

The below table summarizes the abovementioned studies and their findings:

Study ID	Species/ Sex/Number/ Group	Dose/Route	NOAEL	Major findings
A35108	SD rats 5/sex/group	10, 50, 100 µg/kg, i.v.	females: 50-100 µg/kg males: 100 µg/kg	Increase in MCV, MCH, phosphorus.
A35107	SD rats 4/sex/group	50, 100 µg/kg, i.v.	> 100 µg/kg	Variability in troponin I levels.
A35105	SD rats 4/sex/group	>1633 µg/kg, i.v.	< 400 µg/kg	Increase in troponin I levels and liver weight.
A35109	NZW rabbits 3/sex/group	4.9, 24.4, 48.8 µg/kg, i.v.	> 48.8 µg/kg	No effects.
A35106	Male NZW rabbits 3-4/group	25, 250 µg/kg, i.v.	> 250 µg/kg	No effects.

Repeat dose toxicity

- *Repeated-dose toxicity study in male and female rats with daily intravenous injection over a period of 4 weeks followed by a recovery period of 4 weeks (A41484, GLP)*

The vehicle used in this study containing 20% hydroxylpropyl-β-cyclodextrin (HPBCD) caused histological alterations in kidney, urinary bladder, lung, small intestine and lymph nodes as well as injection site reactions with similar incidence and severity in dose groups and control group.

The extent of the changes in the kidneys and lung may have compromised the detection of minor histological findings related to the compound in these organs, but not the identification of distinct lesions.

NOAEL: 45 µg/kg.

- *Dose range finding study in Beagle dogs (A41386, non GLP)*

The effects of the compound were evaluated on the basis of clinical parameters (mortality, general observations, food consumption, body weight) as well as haematological, biochemical and coagulation measurements. No treatment-related effects were observed in this study, and the dose of 200 µg/kg florbetaben hydrochloride was considered to be a suitable high dose level for the pivotal toxicity studies with repeated daily dosing.

- *Repeated-dose toxicity study in male and female dogs with daily intravenous injection over a period of 4 weeks followed by a recovery period of 4 weeks (A42190, A45433 (TK), GLP)*

- In this study, florbetaben hydrochloride did not cause any treatment-related finding at any dose. NOAEL: 200 µg/kg.

The below table summarizes the repeat dose toxicity studies:

Study ID	Species/Sex/ Number/Group	Dose[a]/Route	Duration	NOEL/ NOAEL (µg/kg/day)	Major findings
A41484	Wistar rats 10/sex/group	45, 175, 700 µg/kg, i.v.	4 weeks	45	Irregular respiration at ≥ 175 µg/kg.
A41386	Beagle dogs 2/sex	50, 100, 200 µg/kg	10 days		No effects.
A42190	Beagle dogs 2-3/sex/group	12.5, 50, 200 µg/kg	4 weeks	200	No effects.

Toxicokinetics

In the following table the data of the PK study A48517 in rats with a single dose are included for comparison.

Study ID	Daily Dose[a] (µg/kg)	Cmax (µg/L)	Animal AUC(0-6) (µg.h/L)	Animal:Human Exposure Multiple Human AUC: 0.43[b]-0.48[c] µg·h/L
A48517 Rats (Day 1)	40.7	19.9	7.45	16-17
	158.4	83.1	32.9	69-77
	633.5	399	159.0	331-369
A45433 Dogs (Day 1)	12.5	9.73	2.56	5-6
	45.3	32.3	9.27	19-21
	181	148	45.4	95-105
A45433 Dogs (Day 25)	12.5	7.68	2.52	5-6
	45.3	27.8	10.5	22-24
	181	121	44.2	92-103

a: Dose of florbetaben as a free base

b: Data in Caucasian subjects at a dose of 50 µg per subject (clinical study A40922)

c: Data in Japanese subjects at a dose of 51.6-54.8 µg per subject (clinical study A42441)

The comparison of the TK data showed no evidence of sex-related differences in exposure or accumulation. The exposures achieved in the preclinical species are much higher than the exposures in humans and the safety factors are considered to be appropriate.

Genotoxicity:

Summary of the studies on genotoxicity:

Type of test/study ID/GLP	Test system	Concentrations/ Concentration range/ Metabolizing system	Results Positive/negative/equivocal
Gene mutations in bacteria A35112	Salmonella strains TA 1535, 1537, 98, 100, E. coli WP2 uvrA	15.6-500 µg/plate +/- S9	Negative.
Gene mutations in bacteria A41391	Salmonella strains TA 1535, 1537, 1538, 98, 100, E. coli WP2 uvrA	100-5000 µg/plate +/- S9	Negative.
Gene mutations in mammalian cells A41387	Human lymphocytes	5-50 µg/mL +/- S9	Negative.
Gene mutations in mammalian cells A40674	Human lymphocytes	5-100 µg/mL +/- S9	Negative.
Chromosomal aberrations in vivo A41703	Mouse, micronuclei in bone marrow	6.5-25 mg/kg +/- S9	Negative.

The Applicant conducted the recommended standard genotoxicity testing battery. No mutagenic effects have been detected for florbetaben hydrochloride in the in vitro or in vivo genotoxicity assays.

Carcinogenicity

Considering the absence of a genotoxic risk for florbetaben hydrochloride and the intended clinical use of florbetaben, and according to the Guideline on the need of carcinogenicity studies of pharmaceuticals (CPMP/ICH/140/95 S1A), no carcinogenicity studies are required for florbetaben.

Reproduction Toxicity

Studies on reproductive and developmental toxicity were not performed. Due to the low mass dose, no relevant effects on reproductive function related to chemical toxicity are expected. Developmental toxicity data were not collected, since the target population consists of men and mainly postmenopausal women. If a woman of childbearing potential is intended to be treated, pregnancy has to be excluded prior to treatment because of the nature of the clinical drug product including exposure to circulating radioactive drug.

Taking into account the intended clinical use of the drug in elder patients and that the drug is going to be administered on few occasions, no reproductive and developmental toxicity studies are considered necessary. In addition, the EMA Paediatric Committee granted a product-specific waiver for ^{18}F-florbetaben (EMEA-00I090-PIP01-11).

Local Tolerance studies

- *Local tolerance test of florbetaben hydrochloride in the rabbit after single intravenous administration into the uncongested vein of the ear and with single paravenous administration (A41268, GLP)*

- The undiluted formulation caused transient, slight to moderate local irritation, but no morphological damage, that was reversible by day 6. After rinsing of the vein, only minimal irritation was found on the first two days after treatment with the undiluted drug product, whereas the diluted formulation was tolerated without effects. The paravenous injection caused clear-cut moderate local irritation, with signs of recovery.

- *Local tolerance test in rabbits after single intravenous administration in the uncongested ear vein and after single paravenous administration at the hind leg (A52804, GLP)*

A single injection into the uncongested ear vein and a single paravenous injection of a nonradioactive formulation of florbetaben hydrochloride were tolerated with minor signs of local irritation.

- *Local tolerance test in the dog after single infusion into the uncongested vena cephalica antebrachii*

No compound-related effects were noted at the injections sites in the clinical observations as well as in the macroscopical and histological examinations after single injection of 10 mL of a formulation containing 5.0 µg/mL florbetaben hydrochloride.

Other toxicity studies

Studies on impurities

The drug product Florbetaben solution for injection may contain various impurities, mainly reaction-by products. Additional toxicity studies were conducted on the decayed clinical drug product in order to evaluate potential degradation products. Several impurities were tested in the standard in vitro genotoxicity testing battery.

1.4.5. Ecotoxicity / environmental risk assessment

Florbetaben is administered to patients a maximum i.v. dose of 50 µg/person. Typically, only one diagnostic administration is required for a patient.

Phase I assessment

$$PEC_{surfacewater} = \frac{DOSE_{ai} \times F_{pen}}{WASTEW_{inhab} \times D \times 100}$$

For florbetaben the following values are used:

DOSEai = 50 µg (maximum daily dose of the active ingredient)

Fpen = 1 (default)

WASTEWinhab = 200 $L \cdot inh^{-1} \cdot d^{-1}$ (default)

D = 10 (default, dilution factor).

The PECsurfacewater for florbetaben is 0.00025 µg/L, which is below the trigger value of 0.01 µg/L, and therefore no Phase II assessment is required.

- *PBT assessment*

The logkow was calculated by a HPLC method (OECD117) and the value was 3.3, below the trigger value of 4.5.

Summary of main study results

Substance (INN/Invented Name):			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD117	3.3	Not potential PBT
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}		B/not B
	BCF		B/not B
Persistence	DT50 or ready biodegradability		P/not P
Toxicity	NOEC or CMR		T/not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.00025	µg/L	< 0.01 threshold
Other concerns (e.g. chemical class)			No

Florbetaben PEC surfacewater value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5. Therefore florbetaben is not expected to pose a risk to the environment.

1.4.6. Discussion on non-clinical aspects

The primary pharmacodynamics studies submitted by the Applicant show that florbetaben binds to β-amyloid deposits and this binding correlated with β-amyloid specific IHC and Bielschowsky stain (silver staining). Florbetaben did not bind to tau NFT and α-synuclein positive lesions in cortical brain tissue sections of FTD and LBD patients, respectively. In the secondary pharmacodynamics studies, florbetaben did not show significant binding to any receptor or transporter assayed.

Regarding safety pharmacology, the effects of florbetaben hydrochloride in central nervous system, cardiovascular and respiratory systems and renal function were evaluated. No treatment-related effects were found in any system, although some effects on the respiratory system were observed in the repeated-dose toxicity study in rats.

The Applicant presented an extensive pharmacokinetics package. The Applicant has conducted pharmacokinetic studies in rats after single intravenous administration. The compound was rapidly eliminated and the volume of distribution was high. A biodistribution study conducted in male mice showed a high initial brain uptake followed by a fast initial elimination of radioactivity from the brain. Florbetaben is subject to two oxidative pathways resulting mainly in N-demethylation leading to the main metabolite M-1 and several minor polar metabolites. No significant differences were observed regarding biotransformation in vitro.

Florbetaben was not able to inhibit any CYP isoform in vitro except CYP3A4. However, considering that the administered doses of ^{18}F-florbetaben to patients will be < 100 µg, there is no risk of clinically relevant drug-drug interactions through inhibition of CYP1A2, 2C8, 2C9, 2D6 or 3A4 by ^{18}F-florbetaben.

The formulation used in the non-clinical toxicity studies was different from the clinical one, as the clinical formulation contained florbetaben as a free base and not florbetaben hydrochloride. According to the Applicant, florbetaben hydrochloride was used for technical reasons and the formulation containing 20% hydroxylpropyl-β-cyclodextrin was used in order to achieve high exposures, and this was accepted.

General toxicity studies with florbetaben hydrochloride were conducted in rats and rabbits (single dose) and in rats and dogs (repeated dose) by intravenous administration. In rats, alterations in respiration rate were found at doses ≥ 175 µg/kg, although these findings were not associated with any other clinical finding or morphological correlates. No adverse effects were observed in dogs at doses up to 200 µg/kg. The safety factors are considered to be appropriate. The genotoxicity studies did not show mutagenic effects.

The absence of standard parts of pharmacology (pharmacodynamic drug interaction), pharmacokinetics (placental transfer, excretion and pharmacokinetic drug interactions) and toxicology (reproductive toxicology and carcinogenicity) was acceptable as the drug product is intended as a single administration of a very low dose in elderly patients.

1.4.7. Conclusion on the non-clinical aspects

The non-clinical package presented by the Applicant was considered appropriate.

Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, single and repeated dose toxicity and genotoxicity.The potential toxicity of 28 days of repeated intravenous injections of florbetaben was tested in rats and dogs, and the NOAEL was found to be at least 20 times the maximum human dose.

Chronic studies and carcinogenicity studies have not been carried out, since the medicinal product is not intended for regular or continuous administration.

Studies on reproduction toxicity have not been performed.

1.5. Clinical aspects

1.5.1. Introduction

Florbetaben (^{18}F) is a novel radiopharmaceutical agent which has been developed for imaging β-amyloid (Aβ) in the human brain by PET. In vitro, Florbetaben (18F) binds with high affinity and specificity to Aβ aggregates in brain tissue homogenates from patients with AD.

The following indication was proposed by the applicant:
"This medicinal product is for diagnostic use only.
Neuraceq is indicated for the detection of β-amyloid in the brain, thereby assisting in the differential diagnosis in adult patients who are being evaluated for Alzheimer's disease and other causes of cognitive decline."

The proposed activity to be administered should not exceed 360 MBq and not fall below 240 MBq of florbetaben (^{18}F), administered intravenously as a slow bolus injection (6 sec/ml) without dilution. The administered volume can be up to 10 mL in order to provide the target activity of 300 MBq. A 15-20 minute image should be acquired from 45 to 130 minutes post injection.

Findings from 2 phase III studies (studies **14595** and **16034**) were presented as the key evidence that florbetaben (18F) allowed the visual detection of β-amyloid in the brain.

Additional evidence of efficacy from independent sources was presented as supportive:

- Two proof-of-mechanism Clinical Phase 1 studies: the first-in-man proof-of-mechanism investigator-sponsored Melbourne study (A42404) and the proof-of-mechanism Leipzig study (Study 310863)
- Four additional Clinical Phase 1 studies (Studies 91790, 311722, 312161, and 312043)
- Two supportive Clinical Phase 2 studies (Studies 14311 and 311741)
- A pooled analyses of the studies composing the florbetaben (18F) clinical development program

Populations studied in the clinical development program included European, US and Japanese healthy volunteers (HVs), subjects with MCI, pAD, other dementias, and non-demented or Down syndrome (DS) individuals.

Four additional phase 1 studies evaluated PK of florbetaben (18F): A35694, A42404, A40922 and A42441.

For the purpose of diagnosis, a single administration of florbetaben (18F) is needed and therefore the development program has been designed to assess the safety and efficacy of a single use.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

Tabular overview of clinical studies

Summary of Studies Included in the Summary of Clinical Efficacy and pharmacokinetics

Study number; study design; study site	Tracer mass dose specification; radioactivity	Age of subject population in years	Number of subjects per diagnosis	Evaluated post injection (minutes)	Report number	Integrated analysis pools	
						Efficacy	Safety
Proof-of-mechanism Phase 1 studies							
Investigator-sponsored study A42404: Safety, dosimetry, efficacy in HV and dementia subjects; Dynamic imaging Study site: Melbourne, Australia	< 5 µg tracer mass dose [a] 300 MBq	HV 60 to 85 AD 55 to 85	19 HV 15 AD 11 FTLD [d] 6 DLB 5 PD 4 VaD	90 to 120 135 to 165	A42404	No	No
Study 310863: **A35694:** Safety, dosimetry, efficacy in HV and dementia subjects Study site: Leipzig, Germany	≤ 5 µg tracer mass dose 300 MBq	HV ≥ 55 FTLD ≥ 50 AD ≥ 55	14 HV 10 AD 4 FTLD	70 to 90	A35694	No	Yes
Additional Phase 1 studies							
Study 311722: **A40922:** Safety, dosimetry in HV (2 parallel arms of florbetaben and vehicle) Study site: Leipzig, Germany	≤ 5 vs > 50 to ≤ 55 µg tracer mass dose [b] 300 MBq ±20%	≥ 55	24 HV	100 to 120	A40922	Yes	Yes
Study 91790: **A42441:** Safety, dosimetry in Japanese HV; (2 parallel arms of florbetaben and vehicle). Study site: Kobe, Japan	≤ 5 vs > 50 to ≤ 55 µg tracer mass dose [b] 300 MBq ±20%	≥ 55	24 HV	100 to 120	A42441	Yes	Yes
Study 312161: Efficacy in AD subjects and HV; (2-period cross-over with different tracer mass dose). Study site: Melbourne, Australia	≤ 5 vs > 50 to ≤ 55 µg tracer mass dose [b]	≥ 60	8 HV 8 AD	90 to 115 45 to 65	A41147	Yes	Yes

Study number; study design; study site	Tracer mass dose specification; radioactivity	Age of subject population in years	Number of subjects per diagnosis	Evaluated post injection (minutes)	Report number	Integrated analysis pools	
						Efficacy	Safety
Study 312043: Efficacy in MCI subjects Study site: Melbourne, Australia	250 MBq ≤ 5 µg tracer mass dose 300 MBq ±20%	≥ 60	45 MCI	90 to 110 45 to 60	A50622	No	Yes

(Contd): Table 1. Summary of Studies Included in the Summary of Clinical Efficacy and pharmacokinetics

Study number; study design; study site	Tracer mass dose specification; radioactivity	Age of subject population in years	Number of subjects per diagnosis	Evaluated post injection (minutes)	Report number	Integrated analysis pools	
						Efficacy	Safety
Phase 2 studies							
Study 14311: Single dose – efficacy, safety in DS and HV Study site: USA	≤ 50 µg tracer mass dose 300 MBq ±20%	HV: ≥ 21 to ≤ 40 DS: ≥ 40	70 HV 39 DS	100 to 120	A51672	Yes (HVs only)	Yes
Study 311741 Part A: Single dose – efficacy, safety in AD and HV Study sites: Australia, Europe, USA	≤ 5 µg tracer mass dose 300 MBq ±20%	≥ 55	69 HV 81 AD	90 to 110 45 to 60 110 to 130	A45264	Yes	Yes
Study 311741 Part B: Single dose – efficacy, safety in AD and HV Study sites: Australia, Europe, Japan, USA	≤ 50 µg tracer mass dose[c] 300 MBq ±20%	≥ 55	125 HV 147 AD	90 to 110 45 to 60 110 to 130	A45264	Yes	Yes
Pivotal Phase 3 study							
Study 14595: Single dose; efficacy, safety in AD and HV Study sites: Australia, Europe, Japan, USA	≤ 50 µg tracer mass dose; 300 MBq ±20%	HV ≥ 21 < 40 NDV, AD, DEM, DLB ≥ 21	32 NDV 11 HV 139 AD 31 DEM 5 DLB	90 to 110	A47592	No	Yes

(Contd): Table 1. Summary of Studies Included in the Summary of Clinical Efficacy and pharmacokinetics

Study number; study design; study site	Tracer mass dose specification; radioactivity	Age of subject population in years	Number of subjects per diagnosis	Evaluated post injection (minutes)	Report number	Integrated analysis pools	
						Efficacy	Safety
Pivotal Phase 3 non-interventional pooled read study							
Study 16034: 461 PET scans from the following Phase 1, Phase 2 and the pivotal Phase 3 studies were pooled and re-read by 5 blinded readers: A42404 91790 311722 312043 311741 Part B 14595 Study site: USA	Not applicable	≥ 21	4 VaD 12 FTLD/FTD 188 HV/NDV 182 AD 4 DEM 51 MCI 5 PD 10 DLB 3 Other [e] 3 no diagnosis [f]	90 to 110 100 to 120	PH-36928	Yes	No

[a] In this study 4/56 subjects received higher (> 5 µg) tracer mass dose.

[b] For these 3 studies the protocols required a 5 µg (low) and 55 µg (high) tracer mass dose. The actual higher mass dose range was applied by means of spiking" each dose with 50 µg before administration.

[c] Based on respective mean µg ± SD values administered, the actual tracer mass doses received by AD patients and HVs were similar in Parts A and B and were, in fact, even slightly lower in Part B. This is related to improved tracer synthesis during this phase of the study which resulted in increased yield and a drug product with more radioactivity per substance. Thus, less volume was required to achieve the target radioactivity of 300 MBq and with that, less tracer mass was administered.

ne out of a total of 12 FTLD subjects failed screening and did not receive drug (11 received drug).

ther = clinical Consensus Panel established a diagnosis other than dementia.

) diagnosis = the clinical Consensus Panel could not establish a diagnosis.

AD = Alzheimer disease; DEM = other dementia; DLB = dementia with Lewy bodies; DS = Down's syndrome; FTLD = fronto-temporal lobe dementia; HV = healthy volunteer (ie, normal control); NDV = non-demented volunteer; MBq = MegaBequerel; MCI = mild cognitive impairment; PD = Parkinson's Disease; p.i. = post injection; SUVR = standardized uptake value ratio; VaD = [v]ascular dementia; USA = United States of America.

Source: Module 5.3.4.1, A42404, A35694, A40922, A42441, A41147; Module 5.3.4.2, A50622; Module 5.3.5.2 A51672, A45264 and Module 5.3.5.1 A47592, PH-36928.

1.5.2. Pharmacokinetics

Florbetaben (also referred to as BAY 94-9172, ZK 6013443) is labeled with a radioactive isotope [^{18}F], which has a half-life of 110 minutes. Florbetaben is used for diagnostic purpose only, as a tracer in positron emission tomography (PET) imaging.

The dosing of florbetaben is, however, based on the radioactivity. A dose of 300 MBq has been selected for the use of florbetaben. Each patient receives the same radioactivity dose, whereas the administered mass dose may vary depending on the volume and the concentration of ^{18}F-florbetaben in the particular batch. As indicated above, florbetaben is produced at a high specific activity resulting in a mass dose (ie, the sum of ^{18}F and ^{19}F florbetaben) of equal or less than 3 µg/mL of solution. Depending on the time between manufacturing and administration to the patient, the volume to be injected in order to administer a radioactive dose of 300 MBq is between 0.5 and 10 mL, leading to a corresponding mass dose of less than 1 µg up to a maximum of 30 µg.

The Clinical Pharmacology program contains information on the pharmacokinetics of total ^{18}F-radioactivity in blood/plasma and urine and on pharmacokinetics of the unchanged tracer florbetaben in plasma. The pharmacokinetic investigations are considered as supportive data in the overall Clinical Pharmacology program as compared to safety and efficacy.

Pharmacokinetic data were obtained from four Phase 1 studies (A35694, A40922, A42404, and A42441) comprising overall 53 healthy volunteers (HV), 21 Alzheimer disease patients (AD) and 5 patients with frontotemporal lobe degeneration (FTLD). Experimental radio-HPLC analyses of collected plasma samples for pharmacokinetic evaluation were always performed at the clinical study site. Pharmacokinetics was evaluated using non-compartmental analysis (NCA) and is part of the Summary of Clinical Pharmacology.

Pharmacokinetics of florbetaben has not been investigated in patients with renal impairment and hepatic impairment. However, the effect of renal impairment on safety and efficacy was evaluated as part of a pooled analysis of Phase II-III data and as florbetaben is applied as a single low i.e. pharmacodynamically inactive dose, patients with mild to moderate hepatic impairment do therefore not require dose reduction.

Ethnic differences in the pharmacokinetics of florbetaben have been performed between Caucasian (study 311722, A40922) and Japanese population (study 91790, A42441).

No dedicated study on the effect of age and gender on the pharmacokinetics of florbetaben were performed. The target population of florbetaben is mainly elderly patients, this population relevantly contributed to the clinical study population for the characterization of safety, efficacy and pharmacokinetics of florbetaben.

Study Protocol number: A35694 (310863)

This study was an open-label, non-randomized, single centre study to evaluate the safety and clinical feasibility of a single dose of 300 MBq ZK 6013443 ([^{18}F]AV1) for the detection of cerebral Amyloid-β in Alzheimer disease patients (AD), healthy volunteers (HV) and frontotemporal lobe degeneration patients (FTLD) by Positron Emission Tomography.

Primary objective of this study was:

– Validation of BAY 94-9172 (ZK 6013443) as an in-vivo marker of cerebral β-amyloid deposition by comparison of the in vivo binding characteristics in AD, FTLD, and HV patients.

Secondary objectives of this study were:

– Evaluation of safety and tolerability of BAY 94-9172

– Determination of the radiation dosimetry and standard pharmacokinetic parameters for BAY 94-9172

– Development of a kinetic model in order to quantitatively describe regional cerebral β-amyloid load in the brain of AD patients and controls

Study Protocol number: A42404 (ISS study)

This was a Phase 1, single centre, open label, pilot study to evaluate the safety and usefulness of a single dose of [F-18]FAβ for the detection by PET of Aβ amyloid in the brain of patients with AD (15 subject), DLB (6 patients), VaD (4 patients), PD (5 patients), FTLD (12 patients) and age-matched Normal Control (NC; 19 patients). The study has been staged with progression through the following stages:

– Stage A: safety testing in 4 healthy volunteers with 1/100th and 1/10th of the dose to be used in subsequent stages. There was no imaging in this group.

– Stage B: performance of brain scans in 5 AD and 5 NC.

– Stage C: whole body imaging and calculation of radiation dosimetry in 3 normal volunteers as well as performance of brain scans in a larger number of NC or patients from the indications listed above.

The following radioactivity/mass doses were administered:

Stage	Patient Number	Mean dose
A	101 and 102 103 and 104	2.91 MBq/0.01µg (median 2.91/0.01) 34.90 MBq/0.04µg (median 34.90/0.04)
B	201-210	227.2 MBq/1.81µg (median 236.2/1.17)
C	301-327, 329-347	301.4 MBq/1.96µg (median 292.30/1.25)

Study Protocol number: A40922 (311722)

The study was performed as a single-blind, randomized, placebo-controlled, parallel-group study of single doses of florbetaben after intravenous application of two different specific radioactivities to 24 healthy volunteers (N= 9 verum, N= 3 placebo per group).

Primary objectives:

– Determine radiation dosimetry of florbetaben by using dynamic whole body imaging and the OLINDA software

– Safety and tolerability of florbetaben

Secondary objectives:

– Pharmacokinetics and metabolism of florbetaben

– Determination of cerebral florbetaben uptake by visual and quantitative analysis comparing injection of 300 MBq +/-20% using low specific radioactivity (i.e. with < 5 µg tracer mass) and high specific radioactivity (i.e. with > 50 to ≤ 55 µg tracer mass).

Study Protocol number: A42441 (91790)

The study was performed as a single-blind, randomized, placebo-controlled, parallel-group study of single doses of BAY 94-9172 after intravenous application of 2 different specific activities (mass doses) to 24 Japanese healthy subjects (N=9 verum, N=3 placebo per step).

Twenty–four (24) subjects received the study drug; 9 received BAY 94-9172 low mass dose, 9 received BAY 94-9172 high mass dose, and 6 received placebo.

Primary objectives

– Determine radiation dosimetry of BAY 94-9172 using dynamic whole body imaging and OLINDA software

– Evaluate safety and tolerability of BAY 94-9172

Secondary objectives

- Evaluate pharmacokinetics and metabolism of BAY 94 9172
- Determine cerebral BAY 94 9172 uptake by visual and quantitative analysis

Absorption

Bioavailability

No clinical studies investigating bioavailability were conducted. Florbetaben (^{18}F) is administered via intravenous injection and is therefore fully bioavailable.

- **Bioequivalence**

N/A

Influence of food

N/A

Distribution and dosimetry

Biodistribution and radiation dosimetry was investigated in the above 4 clinical studies (Study A42404, Study 310863/A35694, Study 91790/A42441 and Study 311722/A40922. As per the results from the studies, the organs that received the highest radioactivity were the gallbladder wall, the urinary bladder wall, the liver, the lower large intestine wall and the upper large intestine wall, the ovaries was the red marrow and the stomach wall.

- **SUV (standard uptake values) and SUVR (standard uptake values ratio)**

To assess the ability to distinguish between AD patients and healthy volunteers on the basis of regional tracer uptake, the regional radioactivity expressed as standard uptake values (SUVs) were determined. The SUV was derived from the activity concentration in a pre-specified cerebral region of interest (ROI), subject weight and dose injected. SUV values were normalized to SUVs of the cerebellar cortex, and presented as SUV ratios (SUVRs). SUVs and SUVRs for each ROI were calculated at several time points for image data sets acquired during four post injection (p.i.) intervals (0-90 min, 120-140 min, 180-200 min and 240-260 min p.i.).

Comparison of regional tracer uptake between AD patients and healthy volunteers SUVs and SUVRs were higher in AD patients in all neocortical regions. The differences in SUVR were most pronounced for the ROIs of the: lobus frontalis cortex, gyrus cingulum anterior, gyrus cingulum posterior and precunaeus and slightly higher in the left hemisphere compared to the right.

Elimination

Excretion

Study protocol 311722 (A40922)

The mean urinary excretion of total radioactivity, florbetaben and polar metabolites expressed as percent of dose is summarized in the below Table 12.

Table 12: Arithmetic mean (± SD) urinary excretion of total radioactivity, florbetaben and polar metabolite fraction expressed in percent of dose

Parameter	Total ^{18}F-radioactivity (% ID)	florbetaben (% ID)	Polar metabolite fraction (% ID)
Aex,ur (0-tlast)*	28.5 ± 5.81 (N=17)	--	--
Aex,ur 0-135 min	13.3 ± 3.36 (N=17)	0.188 ±0.0981 (N=14)	12.2±3.37 (N=15)
Aex,ur 135-240 min	5.09 ± 1.79 (N=17)	0.0337±0.0229 (N=9)	4.52±1.24 (N=15)

*t_{last} ranged between 5.0 h and 12.3 h

Study Protocol number: A42441 (91790)

In all subjects that were randomized to receive BAY 94-9172, urine was quantitatively collected up to 12 hours p.i.

The mean urinary excretion of total radioactivity, BAY 94-9172 and polar metabolites expressed as a percent of dose is summarized in Table 13.

Table 13: Mean urinary excretion of total radioactivity, BAY 94-9172 and polar metabolite fraction expressed as a percent of dose-Pharmacokinetic analysis set (N=9, if not specified otherwise)

$A_{ex,ur}$	Total ^{18}F-radioactivity (% ID)		BAY 94-9172 (% ID)		Polar metabolite fraction (% ID)	
	Low mass dose	High mass dose	Low mass dose	High mass dose	Low mass dose	High mass dose
0-135 min	19.5±7.9	17.4±6.1	0.117±0.038 (N=8)	0.111±0.044	18.7±7.6	16.6±5.8
135-305 min	5.3±2.9	8.2±3.6	n.d.	n.d.	5.0±2.7	7.9±3.4
305-435 min	3.7±1.1	4.9±0.6	-	-	-	-
435-720 min	6.2±4.9 (N=8)	5.2±1.3	-	-	-	-
0-720 min	33.1±7.2 (N=8)	35.7±5.1	-	-	-	-

Source: Table 64 to Table 67, Section 16.2.5
Values represent the arithmetic mean ± SD
n.d.: not detected

- **Metabolism and Clearance**

In vitro

Study report A56319: Identification of human CYP isoforms involved in the in vitro metabolism of ^{14}C-florbetaben.

In summary, florbetaben is a substrate of several CYP isoforms in human. Clinically, florbetaben plasma levels are not expected to be highly variable due to involvement of polymorphic CYPs (2D6, 2C19) or to be significantly altered when inhibitors of single CYPs are co-administered to patients.

Study report A41537: In Vitro metabolic profiling and species comparison in liver microsomes and hepatocytes.

In summary, in this in vitro study, no major species differences in the metabolism of ^{19}Fflorbetaben in liver microsomes were found in all species investigated. Following incubation with human hepatocytes, demethylation was the major pathway as well as glucuronidation as phase II reaction.

In vivo

In humans, florbetaben is predominantly metabolized to a polar metabolite fraction comprising presumably several components based on radio-HPLC analysis. A second, less hydrophilic metabolite was detected amounting to less than 10% of total radioactivity in plasma.

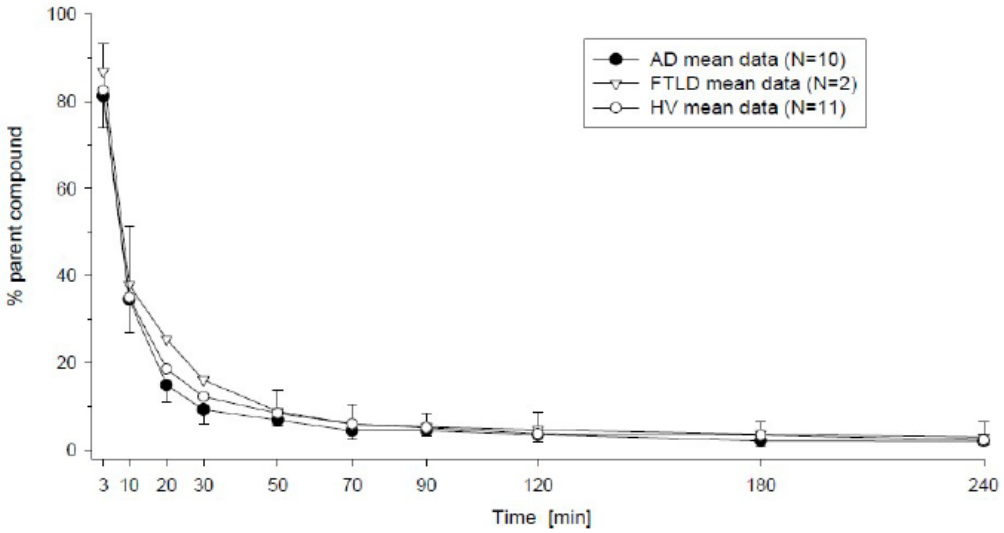
Preclinical investigations in animals suggest that ^{18}F-fluoroacetate is a main component of this polar metabolite fraction. ^{18}F-flouroacetate can enter the brain but it is assumed that F18-fluoroacetate will not bind to beta-amyloid and does not interfere with the specific detection of beta-amyloid deposits by florbetaben (A35694).

The metabolism data show that florbetaben is intensively cleared by metabolic oxidative reactions. The ^{18}F-labeled metabolites are not considered to impair the diagnostic evaluation by florbetaben.

Protocol number: 310863 (A35694)

Florbetaben was rapidly eliminated from plasma. At 10 min post injection only about 30 to 40% of non-metabolized florbetaben was detected in plasma on average and approx. 10 to 20% after 30 min post injection. No significant differences between patients and healthy volunteers were observed.

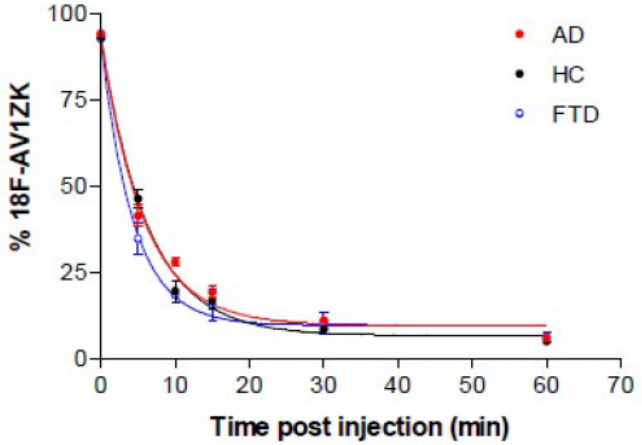
Figure 5: Percentage remaining of ZK 6013443 in plasma after administration of 300 MBq ZK 6013443 to Alzheimer disease patients (AD), frontotemporal lobe degeneration patients (FTLD) and healthy volunteers (HV)



Study Protocol number: A42404 (ISS study)

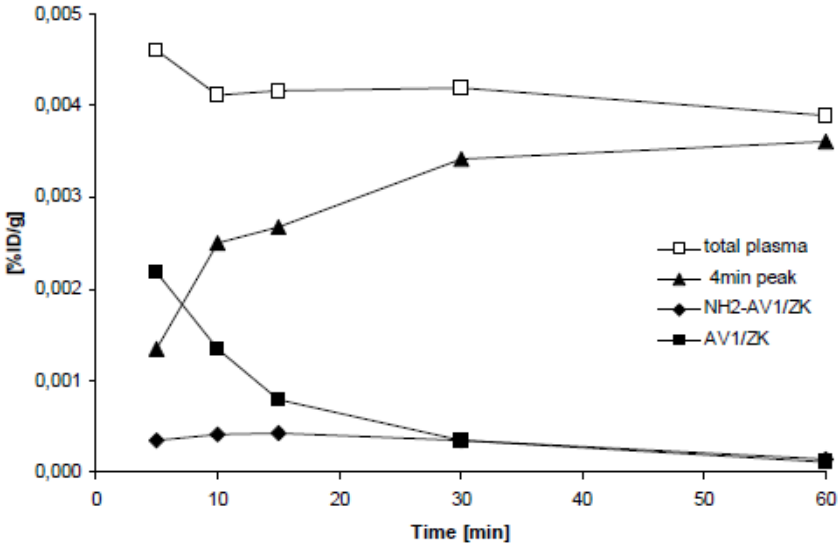
Metabolism of ^{18}F-AV1/ZK occurred rapidly to 3 or 4 major radioactive metabolites. Only 6% of the radioactivity in plasma was unchanged parent compound 60 minutes (see Figure 7 after injection of the study drug. The rate of metabolism was the same in healthy controls (4.6% ± 1.7%), Alzheimer's (5.9% ± 1.8%) and FTLD patients (6.2% ±1.9%).

Figure 7: Percentage of unchanged florbetaben in plasma samples. Values are the mean ± SD for AD patients (n=11), HV (n=7) and FTDL patients (n=2)



The major route of ^{18}F-AV1/ZK metabolism is through the formation of the polar metabolites (retention time 4.2 and 4.5 min).

Figure 9: Concentration time profiles of total radioactivity, 18F-AV1/ZK, the polar metabolite (4 min peak), and NH2-AV1/ZK in plasma following single intravenous bolus injection of approx. 300 MBq 18F-AV1/ZK to 4 Healthy Controls (B10, C01, C07, C14) and 4 Alzheimer's patients (B09, C02, C03, C04). Values are the mean of all subjects and calculated based on the integrated HPLC trace, expressed in %ID/g plasma (data are decay corrected).

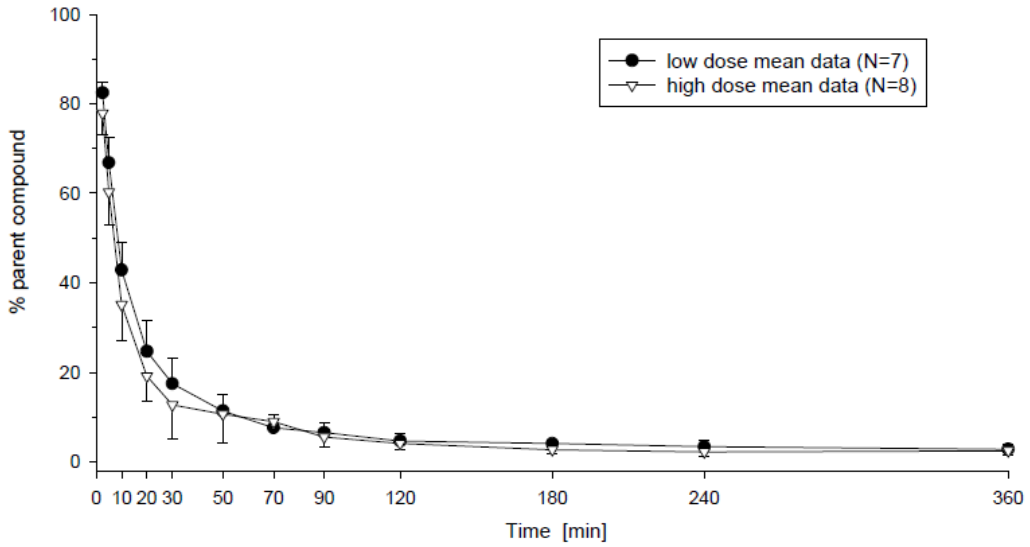


Study protocol 311722 (A40922)

At the first sampling time point (2.5 min), almost all radioactivity can be attributed to the parent compound florbetaben. Thereafter, radioactivity concentrations of florbetaben rapidly decrease in

plasma. At 30 min post injection, the relative contribution of florbetaben is around 10% of all integrated radioactivity.

Figure 10: Percentage remaining florbetaben in plasma after administration of 300 MBq florbetaben to healthy subjects with an actual mass tracer dose in the range of 0.2 - 1.1 µg (low dose) and 50 µg (high dose)



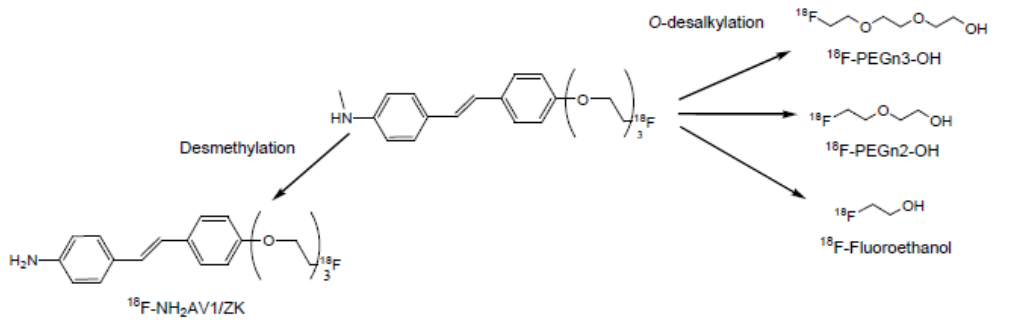
The polar metabolite fraction is the main ^{18}F-labelled component in plasma samples which was rapidly formed representing almost all ^{18}F-radioactivity in plasma from 30 min post injection onwards. In addition, low levels of the metabolite N-desmethyl florbetaben was detected amounting to about 10 % of total radioactivity at 30 min post injection.

Study protocol 91790 (A42441)

Metabolism proposed pathway

The structure of ^{18}F-AV1/ZK suggests 2 main metabolic pathways. The lack of accumulation of $_{18}$F-NH2-AV1/ZK over the 60 min period suggests that this metabolite might present an metabolic intermediate that is further degraded by cleavage of the PEG site chain similarily as proposed for ^{18}F-AV1/ZK.

Figure : Proposed metabolic pathways of 18F-AV1/ZK in humans



- **Inter-conversion**

N/A

Pharmacokinetics of metabolites

To explore the potential impact of florbetaben and its metabolites on beta-amyloid binding parameters, a model-based quantification of binding parameters from florbetaben PET was performed by two experts located at the investigational site (G. Becker, Nuclear Medicine Department, University of Leipzig, Germany) as well as by a further independent expert (M. Ichise, Columbia Kreitchman PET Center New York, USA).

The clinical PK data showed that the radiolabeled metabolites contributed significantly to the total radioactivity and preclinical experiments suggested that these metabolites can distribute into the brain. Consistent modeling results of the clinical PK could be obtained with the assumption that the metabolites enter the brain and contribute to the measured PET signal as well as with an absence of ^{18}F-labeled metabolites in the brain.

The binding parameters of florbetaben that were simulated with the model that did not consider the formation of radiolabeled metabolites correlated very well with those that accounted for it and all binding parameters discriminated effectively between beta-amyloid positive and negative scans. Therefore, the modelling data suggest that the metabolite did not influence the image results in any significant manner.

Dose proportionality and time dependencies

N/A

Special populations

Impaired renal function

Pharmacokinetics of florbetaben has not been investigated in patients with renal impairment during a dedicated clinical pharmacology study. However, influence of renal impaired function on safety and efficacy was evaluated as part of a pooled analysis of Phase II clinical data (study 91708 (311741) Part A and Part B, A45264).

CrCl did not show a relevant influence on the efficacy of florbetaben. The ANCOVA model for detection of differences in CrCl with age and results of the visual assessment could not detect differences, neither in patients nor in controls. Thus renal function did not influence the efficacy of florbetaben (A45264).

Similar results were found in study Part B (including 28% of AD patients with normal renal function and 47/ 21/ 3% of patients with mild/ moderate/ severe renal impairment, respectively) (A45264).

The pooled analysis of all Phase I - III data included 406 AD patients and 291 non-demented volunteers (≥ 55 years), and showed no relevant effect of renal function on the safety of florbetaben.

Impaired hepatic function

Pharmacokinetics of florbetaben have not been investigated in patients with hepatic impairment during a dedicated clinical pharmacology study. Florbetaben is metabolized by several CYP enzymes (see above) that are not exclusively expressed in the liver but also present in other organs.

Beside the liver, CYP2J2 is expressed in the intestine, lung and heart. Thus, in the case of hepatic impairment with reduced metabolic capacity, a compensatory extrahepatic metabolic capacity may be present. Considering furthermore, that florbetaben is applied as a single low microdose i.e. pharmacodynamically inactive dose, a clinically relevant effect with respect to safety and efficacy is not expected in patients with mild and moderate hepatic impairment and do therefore not require a dose reduction.

- **Age and Gender**

No dedicated study on the effect of age and gender on the pharmacokinetics of florbetaben were performed. Because of the specific indication for florbetaben (i.e. Alzheimer's disease), a population at the higher age range was inherent to the clinical development program.

Accordingly, pharmacokinetic data were obtained in an elderly population. In the PK studies, gender distribution was balanced (311722: 12 male, 12 female) or had a higher percentage of male subjects (310863: 75% male; 91790: 70% male). Influence of age and gender on PK parameters was not evaluated.

The pooled analysis of Phase I - III safety data included 872 subjects, with a mean age of 67.7±15.6 years (range: 21 to 98 years). The population was almost equally divided between males and females (54% vs 46%). There was no difference in safety across subject group by age or gender. There was a trend to higher SUVRs with increasing age, a finding which reflects the development of the disease over time. No difference in SUVR means between males and females was observed in either non-demented volunteers or AD subjects.

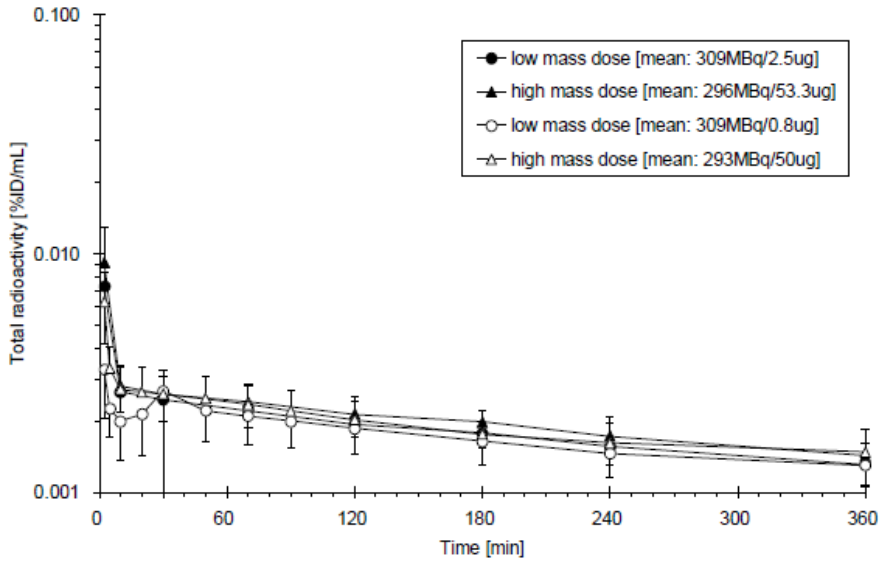
Data suggest that dose adjustment based on age and gender is not necessary.

- **Race**

Two clinical studies with identical designs were performed in Caucasian (A40922, Study 311722) and Japanese (A42441, Study 91790) healthy volunteers. In these studies, the PK of a single administration of 300 MBq florbetaben, with either a low (≤ 5 μg) or high (50 to 55 μg) tracer mass dose was analysed. Pharmacokinetic parameters were evaluated based on the total ^{18}F-radioactivity in plasma and urine followed by metabolite analysis using radio-HPLC. Both ethnic and tracer mass dose differences were evaluated.

The total ^{18}F-radioactivity concentration-time profiles and the area under the curve from time zero to the last data point ($AUC_{0\text{-}tlast}$) for Caucasian and Japanese populations are shown in Figure 13. The calculated dose normalized $AUC_{0\text{-}tlast}$ values were similar between the low and high dose as well as between Caucasian and Japanese subjects (see figure 14). Furthermore, no significant differences in the $AUC_{0\text{-}tlast}$ could be detected between Japanese and Caucasian population.

Figure 14: Total plasma ^{18}F-radioactivity in Caucasian (open) and Japanese (bold) population after single injection of nominal 300 MBq of florbetaben at a low (< 5 µg) or high (50-55 µg) mass dose (decay corrected)



Source: 5.3.4.1 A40922 (study 311722) and 5.3.4.1 A42441 (study 91790)

- **Children**

The pharmacokinetics in children was not studied as Florbetaben is not indicated for use in the pediatric population.

Pharmacokinetic interaction studies

In vitro

No drug-drug interaction studies have been performed in humans.
In vitro studies using human liver microsomes did not show inhibitory effects on CYP1A2, 2C8, 2C9, and 2D6 (IC50: >2 μM). CYP3A4 activity was moderately inhibited with IC_{50} values of 1.6 μM and 1.4 μM, respectively when midazolam and testosterone were applied as substrates. Preincubation (30 min) of ^{19}F-florbetaben with NADPH-supplemented human liver microsomes slightly decreased CYP3A4 activities compared to the co-incubation experiment as indicated by IC_{50} values of 1.0 μM on midazolam 1'-hydroxylation and 0.7 μM on testosterone 6β-hydroxylation, respectively. The observed inhibition of enzymes by florbetaben is not considered to be clinically relevant and no drug interaction with co-medications is expected because florbetaben is administered as a single microdose (less than 100 μg) and the observed mean maximum total plasma concentrations in all studies did not exceed 0.01 μM at the high mass dose of 50-55 μg (study 311722 and study 91790). This threshold is about 100-fold below the observed inhibitory IC_{50}-concentration of 1 μM (A56318).
A clinically relevant drug-drug interaction affecting the pharmacokinetics of florbetaben by co-administration of a CYP-dependent inhibitor can be excluded, because florbetaben is metabolized by several CYP enzymes and inhibition of one enzyme is unlikely to show any effect.
The induction potential of cytochrome P450 e.g CYP3A4, CYP1A2 by florbetaben in in vitro assays e.g. human hepatocytes was not investigated because florbetaben is intended for single use and relevant induction of CYP enzymes requires a continuous exposure with the inducer over several days. Thus, no clinically relevant induction will occur with florbetaben.
Induction of CYP3A4 or CYP1A2 by an inducer given as co-medication, will not result in clinically relevant changes in florbetaben concentrations because florbetaben is metabolized by several enzymes and the overall relative contribution of CYP3A4 and CYP1A2 is lower compared to CYP2J2 and CYP4F2.

1.5.3. Pharmacodynamics

Florbetaben (^{18}F) is a molecular imaging agent designed for PET imaging of β-amyloid in the human brain.
Florbetaben (^{18}F) is administered in doses no higher than 30 µg and does not have any detectable pharmacological activity.

Six phase I studies were performed to primarily or secondarily analyse the brain uptake and distribution of florbetaben (^{18}F) in probable AD (pAD) patients, HVs or other demented patients.

Mechanism of action

The company attempts florbetaben (^{18}F) as an in vivo tracer of Aß deposition in the brain. The fluor (^{18}F) isotope produces a positron signal that is detected by a PET scanner. The correlation of florbetaben (18F) binding to β-amyloid deposition was investigated *in vitro s*tudies (for further information, see the preclinical assessment report).

The binding target(s) of florbetaben (^{18}F) has not been clearly elucidated *in vivo* either in normal subjects or in targeted patients. In the pivotal study 14595 in end-of-life patients, whose cognitive impairment status was difficult to determine, correlation between the *in vivo* florbetaben ($_{18}$F) quantitative uptake in cortical grey matter and the β-amyloid burden averaged from six particular cortical regions was not assessed quantitatively by using correlation analysis.

Primary and Secondary pharmacology

At the low chemical concentrations present in Neuraceq, florbetaben (18F) does not have any detectable pharmacological activity.

Brain uptake and distribution of florbetaben (18F) was evaluated in five phase 1 studies mostly aimed to evaluate other clinical aspects (study A42404, 310863, 311722, 91790 and 312161). Only relevant information about their pharmacodynamics analyses and results are displayed hereinafter.

Study A42404

This *first-in-man* investigator-sponsored study was a single center, open-label, pilot study to validate florbetaben (18F) as a marker of brain amyloid by comparison of the in-vivo binding characteristics in different neurodegenerative dementias and HVs. The analysis set for efficacy included 15 old HVs, 15 AD with mild-moderate dementia, 11 FTLD, 6 DLB, 5 PD, and 4 VaD subjects.

PET images after administration of 300 MBq ± 20% florbetaben (18F) with < 5 µg tracer mass dose were visually evaluated in rainbow color scale, transverse orientation. The summed, dynamic frames of the florbetaben (18F) PET images were viewed from 45-120 minutes post injection (p.i.). One blinded nuclear Medicine physician provided a regional cortical tracer binding (RCTB) score in 8 pre-specific regions and a BAPL score per subject, using the scoring algorithm A of study 311741. Images were also quantitatively analysed after corregistration with MRI, and SUVR values were calculated. Selected regions were based on the previous experience with C11-PIB: frontal, parietal, lateral and medial temporal cortices, occipital cortex, caudate, posterior cingulate /precuneus cortex, and anterior cingulate gyrus.

The applicant states that the brain uptake kinetics of florbetaben (18F) tracer and the visual inspection of images from various time points post injection showed that the optimal imaging time window for florbetaben (18F) lies between 80 and 120 minutes p.i. The results showed no difference in the quality score between the 20 and 30 minutes duration images, while the 10 minutes images were visually inferior. However, the company currently acknowledges not having access to the raw data of study A42404 regarding image quality and diagnostic confidence of the compared alternatives in which optimal PET timing recommendations were based.

Results for the 20 minutes duration (90 - 110 minutes p.i.) are here summarised:

- All AD subjects showed extensive cortical florbetaben (18F) retention that was greater in the frontal and posterior cingulate cortex/precuneus cortex, and slightly less in the lateral temporal and parietal cortex. In the blinded reading, images from AD subjects were assessed as "probable AD" in all cases.
- Twelve (80%) of the HVs presented no cortical grey matter β-amyloid florbetaben (18F) uptake and their scans were clearly distinguishable from subjects with AD. However, three (20%) HVs were classified as having mild to diffuse cortical β-amyloid florbetaben (18F) uptake. One blinded reader rated the scans on two of these three subjects as being positive.
- All 5 PD, 3 of 4 VaD, 10 of 11 FTLD and 4 of 6 DLB subjects had low cortical florbetaben (18F) β-amyloid uptake. One FTLD subject showed mild frontal uptake of florbetaben (18F) β-amyloid, while 2 DLB subjects presented with cortical uptake similar in distribution to AD. However, when present, the degree of β-amyloid florbetaben (18F) uptake in the DLB subjects was generally lower to the degree observed in AD. One VaD subject presented with a PET scan indistinguishable from AD.

The sensitivity of florbetaben (18F) β-amyloid PET for the detection of AD vs HV by blinded visual reading of the images, at 90 - 110 minutes p.i. of florbetaben (18F) for 20 minutes, and with the standard of reference based on clinical criteria was assessed as 97% with a specificity of 88%.

The mean cortical SUVR for the 90 to 110 minutes period in AD subjects (2.02 ± 0.3) was significantly higher (P < 0.0001, effect size d = 3.2) than in the HVs (1.29 ± 0.2) (text table 7). Grey matter regions in the AD group were significantly higher (P < 0.05) than in HV group. Specific differences were observed between white and grey matter structures in HVs at 90-110 min p.i. florbetaben (18F) images.

Text Table 7: Regional standardized uptake value ratios (SUVR) for the 90 to 110 minute post injection imaging period

	NC (n=15)	PD (n=5)	DLB (n=6)	AD (n=15)	FTLD (n=11)	VaD (n=4)
Dorsolateral Prefrontal	1.23 ± 0.2	1.09 ± 0.1	1.24 ± 0.4	1.94 ± 0.3*	1.10 ± 0.2	1.43 ± 0.6
Ventrolateral Prefrontal	1.32 ± 0.2	1.08 ± 0.1	1.39 ± 0.4	2.11 ± 0.3*	1.18 ± 0.3	1.54 ± 0.6
Orbitofrontal	1.33 ± 0.2	1.11 ± 0.1	1.40 ± 0.3	2.08 ± 0.3*	1.21 ± 0.2	1.59 ± 0.8
Posterior Cingulate	1.26 ± 0.2	1.03 ± 0.0	1.44 ± 0.5	2.12 ± 0.4*	1.21 ± 0.3	1.52 ± 0.7
Anterior Cingulate	1.31 ± 0.2	0.90 ± 0.1	1.22 ± 0.3	2.06 ± 0.4*	1.20 ± 0.3	1.43 ± 1.0
Parietal Cortex	1.19 ± 0.2	1.12 ± 0.1	1.37 ± 0.4	1.94 ± 0.3*	1.07 ± 0.2	1.35 ± 0.3
Occipital Cortex	1.37 ± 0.2	1.41 ± 0.2	1.43 ± 0.2	1.84 ± 0.3*	1.27 ± 0.1	1.59 ± 0.3
Lateral Temporal Cortex	1.30 ± 0.2	1.15 ± 0.1	1.34 ± 0.2	2.05 ± 0.3*	1.19 ± 0.2	1.61 ± 0.6
Mesial Temporal Cortex	1.24 ± 0.1	1.14 ± 0.1	1.24 ± 0.2	1.47 ± 0.2*	1.17 ± 0.1	1.29 ± 0.2
Caudate Nuclei	1.34 ± 0.2	1.24 ± 0.1	1.53 ± 0.4	2.03 ± 0.5*	1.24 ± 0.2	1.48 ± 0.6
Putamen	1.31 ± 0.1	1.24 ± 0.1	1.48 ± 0.4	1.89 ± 0.4*	1.27 ± 0.2	1.40 ± 0.4
Thalamus	1.41 ± 0.2	1.21 ± 0.2	1.36 ± 0.1	1.66 ± 0.4	1.27 ± 0.2	1.30 ± 0.2
Midbrain	1.67 ± 0.2	1.88 ± 0.3	1.88 ± 0.2	1.85 ± 0.3	1.65 ± 0.3	1.97 ± 0.3
Pons	1.94 ± 0.2	2.06 ± 0.3	1.93 ± 0.1	2.02 ± 0.3	1.80 ± 0.2	2.10 ± 0.4
White Matter	1.97 ± 0.2	1.82 ± 0.2	1.84 ± 0.1	1.89 ± 0.5	1.80 ± 0.3	1.95 ± 0.2
Neocortical	1.29 ± 0.2	1.14 ± 0.1	1.35 ± 0.3	2.02 ± 0.3*	1.18 ± 0.1	1.51 ± 0.6
Effect size (d)		*1.1*	*0.2*	*3.2*	*0.6*	*0.5*

*Significantly different from NC (p<0.05). Method for definition of ROIs described in 8.3.1.1 and Figure 1.

There was scalp and facial activity detected in scans acquired at 90-110 min and 135-155 min p.i. The reason for this accumulation is unknown, but the applicant hypothesis that such activity may be due to accumulation of florbetaben (18F) or to any of its radioactive metabolites or to blood radioactivity.

Study 310863

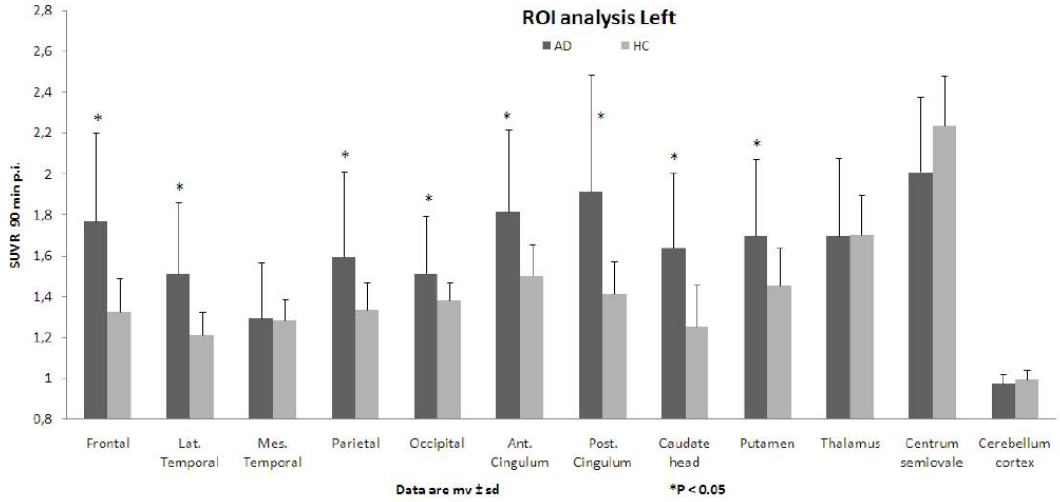
This *company*-sponsored *proof of mechanism* study was a single center, open-label, non-randomized study mainly to validate florbetaben (18F) as a marker of brain amyloid by comparison of the in-vivo binding characteristics in AD, FTLD and HVs. 10 subjects with mild to moderate AD (≥ 55 years of age), 10 age- and sex matched HVs, and 3 subjects with FTLD (≥ 50 years of age) were included in the efficacy assessment.

Florbetaben (18F) PET images after administration of 300 MBq ± 20% florbetaben (18F) (with ≤ 5 µg tracer mass dose) were visually evaluated in rainbow color scale, transverse orientation. The summed, dynamic frames of the florbetaben (18F) PET images were viewed from 70-90 minutes post injection (p.i.). The same 8 regions, as assessed in the Melbourne PoM study (A42404), were assessed by 3 blinded neuro-PET experts. The images were visually scored by a method that was not later implemented in phase 2 studies. Images were also quantitatively analysed after corregistration with MRI, and SUVR values were calculated.

The results of the independent assessment of images from AD subjects and from HVs, carried out by all three readers showed that there was total agreement between all three readers for 8 out of 10 AD subjects. The scans of 9 out of 10 HVs were classified by all three readers as normal. Sensitivity of florbetaben (18F) PET scan for differentiating between AD and HVs on the basis of tracer uptake pattern and with clinical criteria as the SoR was 0.87 (average of three readers; lower limit of the 95% CI = 0.67). The specificity for detection of a HV based on the lack of cortical tracer uptake was 0.90 (lower limit of 95% CI = 0.70). (There was a high inter-reader agreement between the three readers with a resulting kappa value of 0.94 for Readers 1 and 3, and Readers 2 and 3 while the kappa value for Readers 1 and 2 was 0.88. Two of the three readers attained a sensitivity and specificity of 90%, respectively.)

Calculated SUVRs were higher in AD subjects in all cortical regions and in the striatum.

Text Figure 7: SUVR by ROI, left hemisphere, 80-90 min frame – FAS/PPS



Study 311722

This was a single-blind, randomized, placebo (vehicle)-controlled, parallel-group, single center study aimed secondarily at determining cerebral florbetaben (18F) uptake by visual and quantitative analysis comparing injection of 300 MBq ± 20% using high specific radioactivity (i.e. with < 5 µg tracer mass) and) and low specific radioactivity (i.e. with > 50 to ≤ 55 µg tracer mass) in HVs.

A total of 24 HVs were randomized and received either florbetaben or placebo (vehicle) in two groups of 12 HVs each. Six HVs received vehicle, 9 subjects received low tracer mass dose, and 9 subjects received high tracer mass dose. Males and females were equally enrolled in the study. The overall mean age of the 24 HVs was 62.4 years and the average BMI was 26.1 kg/m2. The FAS included 18 subjects all randomized to the active drug (excluding the 6 vehicle subjects), who had sufficient

information available, to analyze the pharmacodynamics and PK data. The PPS included 17 subjects (1 subject was excluded due to a pelvic neoplasm which was diagnosed within this study and was considered a major protocol deviation).

The analysis of the visual PET image assessment was based on scans taken at 100-120 minutes p.i. read in color scale by 1 unblinded expert. Selected regions were similar to those in study A42404. All 17 subjects were visually assessed as showing a typical healthy pattern in cerebral PET imaging. One HV in the florbetaben (18F) low dose tracer mass group had abnormal findings in several cortical areas similar to the patterns observed in the AD subjects.

There were quantitative analyses of PET images after co-registration with MRI. Standardized uptake values (SUVs) and standardized uptake value ratios (SUVRs) from predetermined ROIs were based on the FAS of 18 subjects and done by two methods; the investigator (manual) analysis method and the automated analysis method applying the segmented template by the MNI. The cerebellar cortex showed the lowest tracer uptake in both groups. Other regions were found with low tracer binding (subcortical white matter, pons and cerebellar white matter). Calculated SUVRs were used to describe tracer uptake in other brain regions based on cerebellar cortex as reference region. The differences in SUVRs found between the tracer mass dose groups were without clinical relevance (see table 6). For the cerebral cortex-8-ROI (a combination of 8 selected cortical brain ROIs), the geometric means were 1.359 for florbetaben (18F) low and 1.369 for florbetaben (18F) high. The quotient florbetaben (18F) high / florbetaben (18F) low was 1.008 with a 95% CI from 0.896 to 1.133.

Table 6. SUVR for the 100-120 min p.i. imaging period for both mass-dose subgroups

Region of interest	Treatment group			n	Nmiss	Mean	SD	CI low	CI high	CV	Min	Median	Max
frontal cortex	AV1	low	<5µg	9	0	1,339	0,1703	1,227737	1,450263	12,723	1,17	1,29	1,65
	AV1	high	50-55µg	9	0	1,336	0,1304	1,250805	1,421195	9,762	1,19	1,316	1,58
temporal cortex	AV1	low	<5µg	9	0	1,317	0,1182	1,239776	1,394224	8,972	1,2	1,282	1,57
	AV1	high	50-55µg	9	0	1,303	0,1276	1,219635	1,386365	9,789	1,14	1,317	1,53
lateral temporal	AV1	low	<5µg	9	0	1,296	0,1401	1,204468	1,387532	10,809	1,17	1,25	1,61
	AV1	high	50-55µg	9	0	1,293	0,13	1,208067	1,377933	10,054	1,13	1,294	1,54
mesial temporal cortex	AV1	low	<5µg	9	0	1,34	0,0996	1,274928	1,405072	7,434	1,22	1,314	1,52
	AV1	high	50-55µg	9	0	1,316	0,1317	1,229956	1,402044	10,008	1,15	1,355	1,54
parietal cortex	AV1	low	<5µg	9	0	1,318	0,1667	1,209089	1,426911	12,649	1,19	1,252	1,7
	AV1	high	50-55µg	9	0	1,379	0,1685	1,268913	1,489087	12,22	1,18	1,303	1,63
occipital cortex	AV1	low	<5µg	9	0	1,369	0,1381	1,278775	1,459225	10,085	1,25	1,34	1,71
	AV1	high	50-55µg	9	0	1,375	0,1052	1,306269	1,443731	7,654	1,23	1,398	1,55
anterior cingulate	AV1	low	<5µg	9	0	1,444	0,175	1,329667	1,558333	12,116	1,29	1,395	1,81
	AV1	high	50-55µg	9	0	1,4	0,1669	1,290959	1,509041	11,916	1,17	1,401	1,65
posterior cingulate	AV1	low	<5µg	9	0	1,384	0,1764	1,268752	1,499248	12,749	1,17	1,335	1,78
	AV1	high	50-55µg	9	0	1,404	0,2067	1,268956	1,539044	14,717	1,14	1,382	1,76
caudate nucleus	AV1	low	<5µg	9	0	1,347	0,0963	1,284084	1,409916	7,146	1,26	1,321	1,56
	AV1	high	50-55µg	9	0	1,309	0,1147	1,234063	1,383937	8,765	1,1	1,294	1,49
putamen	AV1	low	<5µg	9	0	1,453	0,1812	1,334616	1,571384	12,467	1,19	1,45	1,85
	AV1	high	50-55µg	9	0	1,4	0,1787	1,283249	1,516751	12,765	1,11	1,405	1,67
thalamus	AV1	low	<5µg	9	0	1,32	0,161	1,214813	1,425187	12,199	1,11	1,331	1,59
	AV1	high	50-55µg	9	0	1,255	0,1362	1,166016	1,343984	10,854	1,06	1,276	1,44
gyrus rectus	AV1	low	<5µg	9	0	1,315	0,1745	1,200993	1,429007	13,264	1,14	1,295	1,61
	AV1	high	50-55µg	9	0	1,33	0,1883	1,206977	1,453023	14,157	1,08	1,358	1,65
orbitofrontal cortex	AV1	low	<5µg	9	0	1,348	0,1356	1,259408	1,436592	10,063	1,22	1,293	1,63
	AV1	high	50-55µg	9	0	1,324	0,1079	1,253505	1,394495	8,145	1,18	1,301	1,54
neocortex-7-ROI	AV1	low	<5µg	9	0	1,355	0,1443	1,260724	1,449276	10,65	1,19	1,312	1,64
	AV1	high	50-55µg	9	0	1,365	0,1679	1,255305	1,474695	12,299	1,15	1,36	1,68
neocortex-8-ROI	AV1	low	<5µg	9	0	1,366	0,1531	1,265975	1,466025	11,207	1,19	1,322	1,69
	AV1	high	50-55µg	9	0	1,379	0,1785	1,26238	1,49562	12,943	1,15	1,369	1,7

Study 91790

This study was conducted in Japanese HVs under a protocol similar in design to the European Union (EU) Phase 1 Study 311722. A total of 24 Japanese HVs aged ≥ 55 years received either florbetaben (18F) or placebo (vehicle) in two groups of 12 HVs (6 placebo volunteers, 9 HVs receiving low tracer mass and 9 HVs receiving high tracer mass).

There was no difference in visual PET scan reading at 100-120 min p.i. between subjects who received the low mass dose and the subjects who received the high mass dose, showing all them a typical healthy pattern. The differences in SUVRs found between the tracer mass dose groups were without clinical relevance.

Study 312161

This was a randomized, cross over, single-blinded, single center aimed to compare the results obtained by visual evaluation (primary objective) and by quantitative analysis (secondary objective) of PET images of the brain made with a target radioactivity of 250 MBq/≤ 5 µg (low) tracer mass with those made with a target radioactivity of 250 MBq/50–55 µg (high) tracer mass.

The study enrolled 16 subjects where 8 subjects with mild to moderate AD and 8 age-matched HCs were randomized to receive either treatment Sequence A (starting with 250 MBq florbetaben at the low tracer mass dose of ≤ 5 µg followed by 250 MBq florbetaben at the high tracer-mass dose of > 50 to ≤ 55 µg) or Sequence B (starting with the high tracer mass dose of > 50 to ≤ 55 µg followed by the low tracer mass dose of ≤ 5 µg). A wash-out period of at least 14 days with a time interval between the two injections of 14 to 42 days was utilized.

Florbetaben (18F) PET images were visually evaluated in rainbow color scale, transverse orientation. The summed, dynamic frames of the florbetaben (18F) PET images were viewed from the acquisition interval of 45 to 65 minutes and 90 to 115 minutes post injection (p.i.) Images were read by 3 blinded Nuclear Medicine physicians. The same 8 regions, as assessed in study 42404, were assessed. Each reader scored to obtain regional cortical tracer binding (RCTB) score in 8 pre-specific regions and a BAPL score per subject, using the scoring algorithm A of study 311741.

When the combined data from all three raters was used with the binominal (normal/abnormal) categorization, a consistent rating for both tracer mass doses was observed in 88% of all image pairs for the image acquisition interval 45 to 65 minutes, and in 92% for the image acquisition interval from 90 to 115 minutes. The CIs were 77% to 98%, and 82% to 100%, respectively.
The alternative binominal categorization (merging of score 2 and 3) showed slightly lower values. This latter categorization has since been adopted for the two Phase 2 clinical trials (Study 14311 and Study 311741) and the pivotal Phase 3 clinical trial (Study 14595).

The different treatments (florbetaben (18F) tracer high, low) did not result in significant differences in the SUVRs determined in some of the cortical regions studied, whereas the subgroups AD and HC show different uptake and differences in quantitative values (see Table 7). The geometric means of the ratios of SUVRs of florbetaben (18F) low / florbetaben (18F) high were 1.014 (CI: 0.982-1.047) for the cortex-8.

Table 7 Confidence intervals of the means for both mass-dose and disease subgroups

Region of interest	Subject group	Treatment		n	Nmiss	Mean	SD	CI low	CI high	CV	Min	Median	Max
frontal cortex	AD	AV1	low	7	0	1,912	0,3296	1,667829	2,156171	17,234	1,41	1,876	2,28
		AV1	high	7	0	1,881	0,297	1,660979	2,101021	15,79	1,37	1,883	2,25
	HC	AV1	low	8	0	1,568	0,2373	1,403559	1,732441	15,13	1,3	1,54	1,86
		AV1	high	8	0	1,544	0,2383	1,378867	1,709133	15,43	1,31	1,451	1,92
temporal cortex	AD	AV1	low	7	0	1,715	0,2557	1,525575	1,904425	14,907	1,29	1,756	1,98
		AV1	high	7	0	1,679	0,2296	1,50891	1,84909	13,672	1,25	1,681	1,95
	HC	AV1	low	8	0	1,479	0,2276	1,321281	1,636719	15,39	1,25	1,422	1,85
		AV1	high	8	0	1,457	0,2308	1,297064	1,616936	15,838	1,24	1,364	1,86
lateral temporal cortex	AD	AV1	low	7	0	1,79	0,2949	1,571535	2,008465	16,471	1,29	1,846	2,09
		AV1	high	7	0	1,76	0,265	1,563685	1,956315	15,062	1,26	1,792	2,06
	HC	AV1	low	8	0	1,481	0,2589	1,301591	1,660409	17,477	1,23	1,403	1,92
		AV1	high	8	0	1,463	0,2652	1,279226	1,646774	18,127	1,23	1,337	1,94
mesial temporal cortex	AD	AV1	low	7	0	1,517	0,1744	1,387803	1,646197	11,501	1,27	1,532	1,7
		AV1	high	7	0	1,47	0,1451	1,362508	1,577492	9,873	1,24	1,488	1,65
	HC	AV1	low	8	0	1,47	0,1511	1,365293	1,574707	10,274	1,3	1,466	1,68
		AV1	high	8	0	1,456	0,1516	1,350947	1,561053	10,413	1,27	1,435	1,68
parietal cortex	AD	AV1	low	7	0	1,839	0,3238	1,599126	2,078874	17,603	1,33	1,876	2,2
		AV1	high	7	0	1,805	0,2674	1,606907	2,003093	14,816	1,3	1,757	2,1
	HC	AV1	low	8	0	1,498	0,2832	1,301752	1,694248	18,9	1,2	1,429	1,85
		AV1	high	8	0	1,482	0,2908	1,280486	1,683514	19,621	1,22	1,338	1,89

occipital cortex	AD	AV1	low	7	0	1,695	0,2173	1,534022	1,855978	12,815	1,36	1,677	2,05
		AV1	high	7	0	1,652	0,1865	1,513839	1,790161	11,289	1,32	1,672	1,91
	HC	AV1	low	8	0	1,513	0,2738	1,323266	1,702734	18,094	1,25	1,456	1,97
		AV1	high	8	0	1,498	0,2884	1,298149	1,697851	19,256	1,25	1,379	1,99
anterior cingulate	AD	AV1	low	7	0	2,017	0,3714	1,741863	2,292137	18,415	1,45	2,085	2,5
		AV1	high	7	0	1,979	0,3263	1,737274	2,220726	16,49	1,44	1,908	2,5
	HC	AV1	low	8	0	1,638	0,2413	1,470788	1,805212	14,733	1,32	1,612	1,96
		AV1	high	8	0	1,622	0,247	1,450838	1,793162	15,224	1,37	1,584	2,03

Region of interest	Subject group	Treatment		n	Nmiss	Mean	SD	CI low	CI high	CV	Min	Median	Max
Posterior	AD	AV1	low	7	0	1,909	0,3380	1,658606	2,159394	17,705	1,40	1,789	2,35
cingulate		AV1	high	7	0	1,873	0,3077	1,645053	2,100947	16,422	1,36	1,886	2,27
	HC	AV1	low	8	0	1,611	0,2618	1,429582	1,792418	16,252	1,37	1,494	1,95
		AV1	high	8	0	1,604	0,2899	1,40311	1,80489	18,074	1,35	1,471	2,01
Caudate	AD	AV1	low	7	0	1,532	0,2668	1,334352	1,729648	17,413	1,21	1,583	2,01
nucleus		AV1	high	7	0	1,505	0,2481	1,321205	1,688795	16,483	1,16	1,503	1,98
	HC	AV1	low	8	0	1,405	0,1932	1,271119	1,538881	13,752	1,17	1,334	1,72
		AV1	high	8	0	1,393	0,2372	1,228629	1,557371	17,019	1,14	1,324	1,84
Putamen	AD	AV1	low	7	0	1,908	0,3600	1,641308	2,174692	18,866	1,32	1,932	2,39
		AV1	high	7	0	1,884	0,3475	1,626568	2,141432	18,445	1,30	1,877	2,39
	HC	AV1	low	8	0	1,673	0,1412	1,575153	1,770847	8,438	1,49	1,688	1,84
		AV1	high	8	0	1,632	0,1171	1,550854	1,713146	7,176	1,52	1,594	1,83
Thalamus	AD	AV1	low	7	0	1,291	0,1649	1,16884	1,41316	12,775	1,03	1,272	1,47
		AV1	high	7	0	1,263	0,1734	1,134543	1,391457	13,735	0,98	1,304	1,50
	HC	AV1	low	8	0	1,405	0,1087	1,329675	1,480325	7,737	1,27	1,391	1,60
		AV1	high	8	0	1,365	0,1017	1,294525	1,435475	7,451	1,26	1,352	1,59
Gyrus	AD	AV1	low	7	0	1,995	0,3577	1,730012	2,259988	17,925	1,43	2,080	2,46
rectus		AV1	high	7	0	1,978	0,3515	1,717605	2,238395	17,771	1,38	1,914	2,45
	HC	AV1	low	8	0	1,613	0,2843	1,41599	1,81001	17,624	1,30	1,565	2,00
		AV1	high	8	0	1,601	0,2759	1,409811	1,792189	17,236	1,29	1,522	2,02
Orbito-	AD	AV1	low	7	0	1,987	0,3752	1,709048	2,264952	18,880	1,44	1,935	2,44
frontal		AV1	high	7	0	1,962	0,3381	1,711532	2,212468	17,235	1,41	1,939	2,40
cortex	HC	AV1	low	8	0	1,655	0,2552	1,478155	1,831845	15,420	1,35	1,650	1,97

		AV1	high	8	0	1,636	0,2499	1,462828	1,809172	15,275	1,37	1,590	2,00
Neocortex-	AD	AV1	low	7	0	1,827	0,3042	1,601645	2,052355	16,650	1,34	1,771	2,21
7-ROI		AV1	high	7	0	1,791	0,2731	1,588685	1,993315	15,248	1,31	1,808	2,10
	HC	AV1	low	8	0	1,537	0,2359	1,37353	1,70047	15,350	1,30	1,469	1,91
		AV1	high	8	0	1,521	0,2468	1,349976	1,692024	16,227	1,29	1,428	1,94
Neocortex-	AD	AV1	low	7	0	1,851	0,312	1,619867	2,082133	16,851	1,36	1,773	2,26
8-ROI		AV1	high	7	0	1,815	0,2804	1,607277	2,022723	15,448	1,33	1,830	2,14
	HC	AV1	low	8	0	1,562	0,2443	1,392709	1,731291	15,642	1,32	1,480	1,93
		AV1	high	8	0	1,549	0,2602	1,368691	1,729309	16,795	1,31	1,444	1,96

In AD patients, cortical SUVR test–retest variability was 6.2% (range 0.6-12.2%, median 6.6%). In HC cortical SUVR test–retest variability was 2.9% (range 0.1-9.0%, median 2.8%). Tracer specific activity (high or low mass dose) had no effect on image quality or SUVR. The variability in the AD subjects was slightly higher than in the healthy controls.

- ***Pharmacodynamic interactions with other medicinal products or substances***

In vitro studies using human liver microsomes showed only a moderate inhibition of CYP 3A4 P450 enzymes. Because florbetaben is administered as a single microdose resulting in low plasma concentrations, no clinically relevant drug interaction with comedications is expected.

No *in vivo* pharmacodynamics drug-drug interaction studies have been performed with a number of drugs belonging to classes that may be frequently used by elderly patients.

1.5.4. Discussion on clinical pharmacology

PHARMACOKINETICS

In all studies, the pharmacokinetics of florbetaben was investigated by analysing the total 18F-radioactivity concentrations in blood and plasma. Determination of the total 18F radioactivity provides information about the distribution and elimination from plasma of all 18F-labeled compounds including the non-metabolized florbetaben and its 18F-labeled metabolites as well renal excretion of these components from the body. Selected plasma samples were analysed by radio-HPLC to determine the fraction of non-metabolized florbetaben in plasma and to use this information to estimate the main pharmacokinetics of florbetaben.

In the three clinical studies focusing on pharmacokinetics, similar pharmacokinetics of total 18F-radioactivity and florbetaben were observed with no major differences.

Maximum plasma concentrations of total 18F-radioactivity and florbetaben were reached within the first 1-2 minutes after end of infusion of 300 MBq of florbetaben as slow intravenous bolus over 90 seconds (0.02% ID/mL [study 310863], 0.003% ID/mL and 0.006% ID/mL for Low dose and High dose, respectively [study 311722] in Caucasian population and 0.007% ID/mL and 0.009% ID/mL for LD and HD, respectively in Japanese population [study 91790]). Total radioactivity concentration declined rapidly in plasma due to rapid distribution into tissues and metabolism. At 10 min p.i. radioactivity concentrations of 0.002% ID/mL [study 310863] 0.002% ID/mL (LD) and 0.003% ID/mL (HD) [study 311722] and 0.003% ID/mL (LD and HD [study 91790] were determined in plasma samples of all 3 studies, and after 4 hours post injection, no radioactivity could be quantified in plasma. There were no significant differences in the plasma concentration-time profiles of total radioactivity between AD, FTLD, and HV.
In vitro studies with ^{18}F-florbetaben showed that florbetaben is highly bound to plasma proteins with a fraction unbound (fu) of 1.6%. A blood/plasma ratio (B/P) of 0.44 was determined in human blood in the concentration range of 0.3 - 3.0 μM suggesting that florbetaben is moderately bound to red cells or taken up into blood cells (study A38277).

After intravenous bolus injection a ^{18}F-radioactivity concentration of 2-3% ID/L is achieved in arterial plasma 10 min after injection. Florbetaben is highly bound to plasma proteins (>98.5%).

Uptake of radioactivity in the brain is rapid: A maximum amount of approximately 6% of the administered radioactivity dose of florbetaben can be measured in the brain at 10-13 min after injection studies 310863 and study 311722 in Caucasian population and study 91790 in Japanese population. The amount of radioactivity in brain afterwards steadily declined to about 3.4% at 40 min and to about 2% at 2 h p.i. Florbetaben is eliminated from plasma of AD patients with a mean biological half-life of about 1 h. No radioactivity could be measured in blood at about 4 hours post injection.

The liver was the organ with highest uptake of total radioactivity. Maximum concentrations of about 17% of the radioactivity dose in the mean were measured 15 min after injection. Thereafter,

radioactivity declined slowly amounting to about 5.5% of administered radioactivity dose at 6 hr post injection (study 311722).

During injection and during the first few minutes post injection, total radioactivity concentration in plasma was almost completely represented by florbetaben. At the end of injection, C_{max} concentrations of florbetaben comprising ^{18}F- and ^{19}F-florbetaben correlated with the administered mass dose reaching mean values between 171 pmol/L (FTLD) to 514 pmol/L (HV) values (study 310863, dose range 0.28 - 2.84 µg), of about 50 pmol/L (study 311722, dose range 0.2-1.1 µg) and 365 pmol/L (study 91790, dose range 1.9-32 µg) at the low mass dose or approx. 6500 pmol/L (study 311722) and about 9600 pmol/L (study 91790) at the high mass dose (50-55 µg). No major differences in maximum florbetaben concentrations were observed between AD patients, FTLD patients and HV when normalized to the mass dose.

Plasma concentrations declined rapidly due to extensive distribution into tissues. A distribution volume at steady state (Vss) of about 50 L (study 310863), 196 L (LD) and 116 L (HD) (study 311722) and about 32 L (LD and HD) in study 91790 were determined with no relevant differences between AD, FTLD and HV (study 310863).

The dose-normalized systemic exposure up to the last measured time point ($AUC_{0\text{-tlast/D}}$) was similar between the treatment groups AD, FTLD, HV (study 310683) with values between 0.0087 h/L (AD) and 0.01 h/L (FTLD) and 0.0094 (HV) and between studies. $AUC_{0\text{-tlast/D}}$ values of 0.0064 h/L (low mass dose, HV) and 0.0092 h/L (high mass dose, HV) were determined in study 311722 and 0.0059 h/L (low mass dose, HV) and 0.0075 h/L (high mass dose, HV) in the Japanese study 91790.

Florbetaben was rapidly eliminated from plasma with a calculated mean terminal half-life of about 1 h (study 310863) with no significant differences between AD, FTLD and HV and with a half-life of about 1 h and 20 min. in study 311722 (similar between low and high mass dose). The plasma clearance ranged between 86 L/h and 115 L/h with no significant differences between AD, FTLD and HV (study 310863), between low mass dose and high mass dose in Caucasian and Japanese population (study 311722 and study 91790). These values exceed the physiological liver plasma flow of 40 L/h indicating that Florbetaben can be considered to be a "High Clearance drug "(liver extraction ratio > 0.7).

In humans, florbetaben is predominantly metabolized to a polar metabolite fraction comprising presumably several components based on radio-HPLC analysis. A second, less hydrophilic metabolite was detected amounting to less than 10% of total radioactivity in plasma.

Preclinical investigations in animals suggest that ^{18}F-fluoroacetate is a main component of this polar metabolite fraction. ^{18}F-flouroacetate can enter the brain but it is assumed that F18-fluoroacetate will not bind to beta-amyloid and does not interfere with the specific detection of beta-amyloid deposits by florbetaben (A35694, section 16.1.12). In vitro and preclinical in vivo metabolic studies using radio-HPLC together with LC-MS/MS analysis indicate that the less hydrophilic metabolite represents N-desmethyl florbetaben. The contribution of N-desmethyl florbetaben to the specific signal is considered to be marginally due to the low occurrence in plasma.

In vitro CYP-phenotyping studies showed that florbetaben is metabolized by several CYP-dependent metabolic pathways with CYP1A1, CYP2J2, CYP4F2, CYP4F3b, and CYP4F12 as the main enzymes mediating the N-demethylation whereas CYP2J2 and CYP3A4 contribute predominantly to the formation of polar metabolites.

The metabolism data show that florbetaben is intensively cleared by metabolic oxidative reactions. The ^{18}F-labeled metabolites are not considered to impair the diagnostic evaluation by florbetaben.

Pharmacokinetics of florbetaben has not been investigated in patients with renal impairment during a dedicated clinical pharmacology study. However, the effect of renal impairment on safety and efficacy was evaluated as part of a pooled analysis of Phase II-III data.

Pharmacokinetics of florbetaben has not been investigated in patients with hepatic impairment during a dedicated clinical pharmacology study. However, florbetaben is applied as a single low i.e. pharmacodynamically inactive dose. Patients with mild to moderate hepatic impairment do therefore not require dose reduction.

In vitro studies using human liver microsomes showed only a moderate inhibition of CYP 3A4 P450 enzymes. Because florbetaben is administered as a single microdose resulting in low plasma concentrations, no clinically relevant drug interaction with co-medications is expected.

Because florbetaben is metabolized by several CYP-dependent pathways (study A56318), a drug-drug interaction by inhibition of the florbetaben metabolism by coadministration of an inhibitor was not expected. Therefore, no drug-drug interaction studies had been performed in humans.

The induction potential of cytochrome P450 enzymes e.g. CYP3A4, CYP1A2 by florbetaben in vitro assays e.g. human hepatocytes was not investigated, because florbetaben is intended for single use only and relevant induction of CYP enzymes requires a continuous exposure with the inducer over several days. Thus, no clinically relevant induction will be expected with florbetaben.

No ethnic differences in the pharmacokinetics of florbetaben between Caucasian (study 311722) and Japanese population (study 91790) were observed.

No dedicated study on the effect of age and gender on the pharmacokinetics of florbetaben were performed. However, because the target population of florbetaben is mainly elderly patients, this population relevantly contributed to the clinical study population for the characterization of safety, efficacy and pharmacokinetics of florbetaben.

PHARMACODYNAMICS

The Applicant has provided limited data on pharmacodynamics in this submission.

Florbetaben (^{18}F) does not have any detectable pharmacological activity.

Florbetaben (^{18}F) is a molecular imaging agent designed for PET imaging of β-amyloid in the human brain. The correlation of florbetaben (18F) binding to β-amyloid deposition was investigated in vitro studies. The binding target(s) of florbetaben (^{18}F) to particular β-amyloid structures or to other brain structures or receptors has not been clearly elucidated in vivo either in normal subjects or in targeted patients. In the pivotal study 14595 in end-of-life patients, whose cognitive impairment status was difficult to determine, quantitative correlation between the in vivo florbetaben ($_{18}$F) quantitative uptake in cortical grey matter and the β-amyloid burden averaged from six particular cortical regions was not assessed. Then, section 5.1. of the SmPC under subheading "Mechanism of action" has been modified as follows:
"Florbetaben (^{18}F) binds to β-amyloid neuritic plaques in the brain. In vitro, florbetaben (^{18}F) shows nanomolar binding affinity to synthetic β-amyloid fibrils and to AD brain homogenate. In addition, binding of florbetaben (^{18}F) to β-amyloid plaques in post-mortem AD brain sections was demonstrated by autoradiography and supported by immunohistochemistry and Bielschowsky stain, respectively. In vivo, quantitative correlation was not assessed in end-of-life patients between florbetaben (18F) uptake in cortical grey matter and the beta-amyloid deposition in autopsied samples. The in vivo binding of florbetaben (18F) to other β-amyloid structures or other brain structures or receptors remains unknown."

Florbetaben (18F) binds non-specifically to white matter, which is attributed to unspecific binding to the lipid-containing myelin sheath. This is observed both in normal and targeted patients (study A42404).To this regard, the following information has been included in section 5.2. of the SmPC:
"The biophysical basis of the white matter retention of florbetaben (^{18}F) in the living human brain cannot be definitively explained. It is hypothesized that unspecific binding of the radiopharmaceutical to the lipid-containing myelin sheath may contribute to white matter retention."—

Moreover, some extracerebral structures in the head (scalp and face) showed high uptake (radioactivity accumulation) *in vivo* in some cases unknowing if due to accumulation of florbetaben ($_{18}$F) or to any of its radioactive metabolites or to blood radioactivity. A wording is included in the SmPC. The SmPC has also been modified to include that non-specific uptake of florbetaben (18F) may appears sometimes in the midsagittal sinus due to the presence of tracer in the blood pool.

In the original SmPC it was recommended a time window of 45-130 min p.i. for 300 MBq florbetaben (18F) PET image acquisition with a duration of 20 minutes. This was based in the phase 1 study A42404 in which the florbetaben (18F) PET images were viewed from 45-120 minutes p.i. Despite the company currently acknowledges not having access to the raw data of study A42404 regarding image quality and diagnostic confidence of the compared alternatives of different image acquisition times, it was easy to differentiate pAD and HVs by visual PET scan reading of 8 particular brain regions (sensitivity of 97% and specificity of 88%) in a 20-min duration scan at 90 - 110 minutes p.i. of florbetaben (18F)). AD subjects showed extensive cortical florbetaben (18F) retention, while most (12 out of 15) HVs had only non-specific florbetaben (18F) uptake in the white matter without cortical grey matter uptake and the remaining 3 HVs had mild to diffuse cortical uptake. It was also easy to differentiate pAD and HVs by quantitative mean PET values in all regions of the cortex and striatal regions at that time point. Finally, the SmPC recommends acquisition of a 20-minute PET image starting at approximately 90 minutes after intravenous injection of florbetaben (18F).

Discrimination by visual and quantitative PET values was not successful for the comparison of AD and other non-AD neurodegenerative dementia subgroups in study A42404, with some overlapping in the quantitative uptake between AD and both DLB and VaD in a 20-min duration scan at 90 - 110 minutes p.i. of florbetaben (18F). All PD, VaD, FTLD and DLB subjects had low cortical uptake and no-specific uptake in the white matter, except 1 FTL subject who showed mild frontal uptake, 2 DLB subjects presenting with cortical uptake similar in distribution but generally lower in intensity to AD, and one VaD subject with a PET scan indistinguishable from AD. Therefore, the SmPC includes in section 4.4. under “Limitations” that other non-AD dementias (such as PD and DLB) might show cortical florbetaben (18F) retention.

Binding of florbetaben (18F) in the cerebellum cortex in all subgroups has not been detailed.
The pattern of quantitative PET uptake in HVs and AD subjects has been detailed in the SmPC as follows “*Healthy controls show relatively low levels of florbetaben (18F) retention in cortex. The highest level of uptake is in pons and other white matter regions. In AD subjects, cortical regions and striatal regions show significantly greater uptake compared to controls. In AD subjects, as in controls, there is high retention in pons and other white matter areas.*”

The company currently mentions about a recent retrospective study performed with images obtained from study 311741, part A, and diagnostic confidence in discriminating between AD patients and HVs was high (95%) for all the 20-min, 10-min and 5-min scans. This data are however obtained at a particular time-point window of 90 min p.i. and not to the broad recommended window at the SmPC of 45-130 min p.i.

The typical normal pattern of PET images was also shown in most HVs at 110-120 min p.i. of 300 MBq of florbetaben (18F) in study 311722, independently if using < 5 µg or > 50 to ≤ 55 µg tracer mass dose. The mean quantitative values of florbetaben (18F) PET uptake obtained for 8 particular cortical regions and any other brain region allow concluding that no statistical differences existed between both tracer mass doses.

No visual differences in florbetaben (18F) PET scans between low (≤ 5 microg) and high (50-55 microg) mass doses, for both pAD and age-matched HVs, were displayed at 8 particular regions in study 312161, even at 45 to 65 minutes or at 90 to 115 minutes post injection. The second time window showed better values. For some 8 particular cortical regions, it can be concluded that no statistical differences existed in the different mean quantitative florbetaben (18F) uptakes between both tracer mass doses either in AD or in HVs, whereas differences existed between disease subgroups (pAD and HVs). SUVRs were highly repeatable between test and retest image results for AD and HC subjects, in either mass group, indicating a high degree of test-retest reproducibility.

In all these three studies above the interpretation criteria of PET images was somehow similar to that used in phase 2 and phase 3 trials and the one proposed in clinical practice. Always the same brain regions were analyzed as well as the same visual and quantitative PET reading method was applied –

independent of disease subgroups, mass dose subgroups or time window subgroups- within each of these three early studies. Selected regions were based on the previous experience with 11C-PIB for the visual and quantitative PET reading. These regions were frontal, parietal, lateral and medial temporal cortices, occipital cortex, caudate, posterior cingulate /precuneus cortex, and anterior cingulate gyrus.

No in vivo pharmacodynamics drug-drug interaction studies have been performed.

1.5.5. Conclusions on clinical pharmacology

PHARMACOKINETICS

In the clinical studies focusing on pharmacokinetics, similar pharmacokinetics of total ^{18}F-radioactivity and florbetaben were observed with no major differences.

On average, 6% of the administered radioactivity dose distributed into the brain 10-13 min after injection (studies 310863 and study 311722 in Caucasian population and study 91790 in Japanese population). The amount of radioactivity in brain steadily declined to about 3.4% at 40 min and to about 2% at 2 h p.i. The liver was the organ with highest uptake of total radioactivity. Maximum concentrations of about 17% of the radioactivity dose in the mean were measured 15 min after injection. Thereafter, radioactivity declined slowly amounting to about 5.5% of administered radioactivity dose at 6 hr post injection.

Florbetaben was rapidly eliminated from plasma with a calculated mean terminal half-life of about 1 h (study 310863) with no significant differences between AD, FTLD and HV and with a half-life of about 1 h and 20 min. in study 311722 (similar between low and high mass dose). The plasma clearance ranged between 86 L/h and 115 L/h with no significant differences between AD, FTLD and HV (study 310863), between low mass dose and high mass dose in Caucasian and Japanese population (study 311722 and study 91790). These values exceed the physiological liver plasma flow of 40 L/h indicating that Florbetaben can be considered to be a "High Clearance drug "(liver extraction ratio > 0.7).

In the mean 28.5% of the administered radioactivity dose was renally excreted during a sampling period between 5.0 h and 12.3 h post injection. Almost all radioactivity recovered in urine was presented by the polar metabolites.

In humans, florbetaben is predominantly metabolized to a polar metabolite fraction comprising presumably several components based on radio-HPLC analysis. A second, less hydrophilic metabolite was detected amounting to less than 10% of total radioactivity in plasma.

In vitro CYP-phenotyping studies showed that florbetaben is metabolized by several CYPdependent metabolic pathways with CYP1A1, CYP2J2, CYP4F2, CYP4F3b, and CYP4F12 as the main enzymes mediating the N-demethylation whereas CYP2J2 and CYP3A4 contribute predominantly to the formation of polar metabolites.

PHARMACODYNAMICS

It was confirmed in vitro that florbetaben ($_{18}$F) binds to synthetic β-amyloid fibrils and β-amyloid plaques in human brain tissue. The *in vivo* binding of florbetaben (18F) to other β-amyloid structures or other brain structures or receptors remains unknown.

There is discrimination between pAD and HVs by visual florbetaben (18F) PET scan reading and quantitative uptake values in some brain regions in phase 1 studies; however, values might be overlapping in some individual cases. Discrimination between pAD and other non-AD dementias could not be concluded.

The initially recommended time window and duration of florbetaben (18F) PET scan (i.e. a 20-min duration scan at 45-130 minutes p.i. of florbetaben (18F)) cannot be justified on the basis of visual PET reading of study A42404. The influence of the tracer mass since is negligible for both, AD and HVs.

The quantitative correlation between the in vivo florbetaben (18F) quantitative uptake in cortical grey matter and the β-amyloid burden measured by histopathology was not assessed.

1.6. Clinical efficacy

1.6.1. Dose response study(ies)

The optimal dose was neither determined by specific studies nor adequately justified. Proof of mechanism was based on the detection of specific regional binding (^{18}F) of florbetaben to β-amyloid in the brain *in vivo* as determined in two Phase 1 studies: the Melbourne study (A42404) and the Leipzig study (310863). The aim of these studies was to provide initial evidence of the ability of florbetaben to differentiate between subjects with AD and HV on the basis of visual detection of ß-amyloid and quantitative PET results. Another objective was to select the most appropriate activity of florbetaben (^{18}F) to be used in the clinical study program. The lowest activity of 150 MBq provided suboptimal image quality for diagnostic evaluation while a simulated 450 MBq dose did not improve these endpoints compared to 300 MBq. Therefore, the 300 MBq dose was chosen for the pivotal efficacy and safety studies

The recommended activity of florbetaben (18F) is a single intravenous injection of 300 MBq ± 20% (i.e. a minimum of 240 MBq and a maximum of 360 MBq). Aside from the first 4 subjects included in study A42404, all subjects received 300 MBq ± 20%. Due to the cross-over design in study 312161 involving two study periods in two sequences, 250 MBq was used in order to meet the limits of maximum allowed radioactive dose exposure. The tracer mass dose was modified from ≤ 5 microg/injection used in earlier studies to ≤ 50 microg/injection in later studies.

The final formulation that is proposed for marketing is different from the one used in the single pivotal study. The company stated that the impact of such formulation change on efficacy was evaluated based on pre-clinical and supportive clinical data in addition to theoretical considerations without any detail. To date, the commercial formulation has been administered more than 181 times since January 2012 in the still ongoing autopsy study and other supportive trials.

1.6.2. Main study(ies)

The CHMP considers that, from the data provided by the company to support efficacy, there is a single pivotal study to base this application (i.e. 14595). Then, CPMP/EMA/2330/99, CPMP/EWP/1119/98/Rev and EMEA/CHMP/EWP/321180/2008 also apply. This was a multi-center, single-dose, open-label Phase III study.

Methods

Study Participants

Adult subjects willing to donate the brain and undergoing a MRI and PET scan were included.
Subjects were excluded if having severe cerebral macrovascular disease or brain tumor previously verified by MRI. Additionally, 10 young HVs were also recruited.

Treatments

Patients underwent screening visit before the administration of florbetaben (18F), including anatomic brain imaging and clinical history. MRI had to be performed within the previous 6 months before or simultaneously to PET image. A yearly repeated MRI brain scan and florbetaben (18F) PET imaging up to a 3-year follow-up was offered but not mandatorily, except for HVs.

Each subject received a single i.v. dose of 300 MBq ± 20% of florbetaben. PET imaging for 20 minutes was to start approximately at 90 minutes after the injection based on results of study A42404.

Florbetaben (18F) PET images were read by 3 blinded independent readers, who were previously in-person trained and qualified, and were also blinded to the standard of truth (SoT) results and all other information about the subject. A 20-min acquisition PET image was interpreted using a grey scale. Both visual and quantitative assessments were performed on the regional level data (for those brains for which there was histopathologic sampling) and on the subject level data (on all subjects regardless of whether there is histopathology). For exhaustive information about the PET scan reading method and the PET reader's training, see the day-80 and day-150 clinical assessment report.

The regional-level analysis involved co-registration with each subject's brain MRI image. Visual regional assessment considered six particular brain regions (middle frontal, occipital, hippocampus/inferior temporal, anterior cingulate, posterior cingulate and cerebellar cortex). Selection of regions was performed to include those with high and low likelihood to have amyloid plaques in the brain, and which could be directly compared versus histopathology through corregistration. The criteria to interpret images as normal or abnormal was "yes" or "no" in the regional one based on the cortical uptake versus the white matter uptake in a specific small predefined region. A composite "whole brain" regional visual assessment of those 6 regions was also performed.

A subject level visual PET scan assessment was performed. Readers had to determine the regional presence or absence of florbetaben (18F) tracer uptake in the non coregistered PET scan determining the Regional Cortical Tracer Binding score (RCTB = no, moderate or pronounced tracer uptake) in 4 predefined regions (frontal, posterior cingulate, lateral temporal and parietal cortex).

- *Table 12 Definitions of regional cortical tracer binding score (RCTB)*

Regional cortical tracer uptake score	**Condition for assessment**
1 (No tracer uptake)	Tracer uptake (i.e., signal intensity) in gray matter in the region is lower than in white matter.
2 (Moderate tracer uptake)	Smaller area(s) of tracer uptake equal to or higher than that present in white matter: extending beyond the white matter rim to the outer cortical margin involving the majority of the slices within the respective region.
3 (Pronounced tracer uptake)	A large confluent area of tracer uptake equal to or higher than that present in white matter extending beyond the white matter rim to the outer cortical margin and involving the entire region including the majority of slices within the respective region.

Note: For a score of tracer uptake in the cortex, the finding should have been present in the majority of the slices within the region in question

The readers will then determine the overall Brain Amyloid Beta Plaque Load (BAPL) score which was based on the highest RCTB score in one of the four brain regions. The BAPL was a 3-grade scoring system (without/moderate/pronounced) assessing beta-amyloid burden of the brain globally (see table below).

Table 7-12: Rules for the assessment of brain β-amyloid deposition by the independent blinded reader

Brain β-amyloid deposition score		Rules for brain β-amyloid deposition assessment
1	Without β-amyloid deposition	Regional cortical tracer uptake score 1 in each of the 4 brain regions 1, 2, 3, and 4
2	Scan with moderate β-amyloid deposition	Regional cortical tracer uptake score 2 in any or all of the 4 brain regions 1, 2, 3, and 4 and no score 3 in these 4 regions
3	Scan with pronounced β-amyloid deposition	Regional cortical tracer uptake score 3 at least in one of the brain regions 1, 2, 3, and 4

Note: BAPL was changed from brain β-amyloid plaque load (see Table 7-9) to brain β-amyloid deposition

For efficacy analyses of the subject-level PET scans, scans classified as BAPL 1 are considered normal (i.e. no beta-amyloid deposition) and those classified as BAPL 2 or BAPL 3 are considered abnormal (i.e. presence of pathological beta-amyloid deposition).

PET images were also interpreted quantitatively as SUVRs, after being co-registered with MRI, in 17 regions of the regional assessment. Cerebellar cortex was chosen as the optimal reference region for quantitation of PET images based on quantitative uptake values in study 311722. The 17 different regions were selected to cover mainly cortical regions with known presence of beta-amyloid as well as some white matter regions.

Pathologic evidence of beta amyloid deposition based on Bielschowsky silver stain (BSS) or immunohistochemistry with anti-amyloid antibody was defined as the SoT for this study. Brain tissue specimens were taken from 6 predefined cortical regions (middle frontal, occipital, hippocampus/inferior temporal, anterior cingulate, posterior cingulate/precuneus and cerebellar cortex) to include regions with high and low likelihood to have amyloid plaques in the brain, and which could be directly compared versus histology through co-registration. Pathology Consensus panel (CP) members are asked to answer the question **"Is beta-amyloid present in this region - yes or no?". This answer was the SOT.** In answering this question the CP members had to take into account the different methods (BSS and IHC for beta amyloid), the different forms of amyloid deposits (diffuse, neuritic, vascular) and the different frequencies and their relative impact. It should be a consensus opinion.

In addition to this categorical answer, the frequency score for post-mortem estimates of neuritic plaques of the CERAD criteria (Mirra et al. 1991) were used for interpretation criteria of the post-mortem estimates of both neuritic plaques measured by BSS, diffuse plaques measured by BSS, and beta-amyloid deposition (diffuse, neuritic, vascular) measured by IHC with anti-amyloid.

A "whole brain" histopathological verification was also obtained from collapsing the results of the regional histopathological findings from the Pathology CP.

A final onsite neuropathological diagnosis was also established classifying a patient as "beta-amyloid present/not" by using the following age-related neuritic plaque density (Mirra, 1991): a subject with a CERAD score B or C older than 75 years was considered as amyloid positive, a subject with a CERAD score of C and age of 50-75 years was considered as amyloid positive while those aged 50-75 years with a CERAD score of 0,A or B were considered to be amyloid-negative. Such density was established at the region of maximum involvement of different brain regions: those three of the CERAD criteria (middle frontal gyrus, superior and middle temporal gyri and inferior parietal lobe), hippocampus and other brain regions.

The onsite neuropathologist also histopathologically examined brains to establish if AD, DLB, PD or FTD were present according to international accepted criteria (i.e. NIA criteria for AD, CERAD

according to Mirra, Braak and Braak criteria for NFT, McKeith criteria for DLB, Gelb criteria for PD, FTD according to Cairns criteria). Therefore, the respective site was responsible to use the regions and methods/protocols they need for establishing the diagnosis according to these guidelines.

Objectives

Primary objective: to determine the sensitivity and specificity of the visual assessment of **regional** tracer uptake in the florbetaben (18F) PET images compared to histological verification of the presence or absence of cerebral beta-amyloid in the respective postmortem specimens.

Secondary objectives:

- To determine the sensitivity and specificity of the composite “whole brain” 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). To determine the sensitivity and specificity of the quantitative assessment of regional tracer uptake in florbetaben (18F) PET images compared to histological verification of the presence or absence of cerebral β-amyloid in the respective postmortem specimens.
- To determine the sensitivity and specificity of the visual assessment of florbetaben (18F) PET images on the subject level in detecting/excluding cerebral β-amyloid compared to the onsite neuropathological diagnosis. To perform an exploratory investigation of the association between the subject level visual assessment of florbetaben (18F) and fludeoxyglucose (18F) PET images for detecting the abnormalities in subjects with 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 (in Japan only).
- To determine agreement of blinded readers on the 3 methods of florbetaben (18F) PET reading

Outcomes/endpoints

The co-primary efficacy variables were the sensitivity and specificity of the visual assessment of **regional** tracer uptake in the florbetaben (18F) PET images in correctly differentiating between brain regions with and without beta-amyloid deposition. The 90 to 110 minutes imaging window and the majority read of the visual assessment by the 3 blinded readers were used to determine these variables over the 6 brain regions (of a particular subject).

Secondary efficacy variables were related to the comparison with the SoT (individual reader sensitivity and specificity of visual regional PET scan reading, quantitative PET uptake values and reader agreement) or with a SOR (various).

Sample size

Subjects would be followed until 30 histopathological specimens were to be recruited.

Randomisation

There was no randomized order to perform both recruitment and the imaging techniques. PET images were randomized in the reading sessions.

Blinding (masking)

No blinding of florbetaben (18F) administration was performed. PET readers for visual reading, and the one that performed quantitation, were blinded to post-mortem histopathology data as well as to the clinical diagnosis of the patient. The histopathology CP was blinded to both the clinical diagnosis and the PET scan result.

Statistical methods

This analysis was based on the first available 32 histopathological specimens. However, subjects recruited in the study and not deceased were to be followed up.

No imputation of missing data was planned with the exception of the rules for the definition of the majority read results and other exceptions given in the SAP.
Subjects were classified as NDV, AD subject, DLB subject, other dementia subject, HV, or unknown status, based on the onsite clinical diagnosis at study entry. This classification was called 'subject group'.
No interim analysis was planned.

Analysis sets

- Full analysis set (FAS): All subjects in whom any PET imaging with florbetaben (18F) was performed, with valid SoT for at least one brain region (within the particular subject), were included in the FAS. This analysis set also included the 10 HVs.

- Per protocol set (PPS): All subjects belonging to the FAS were evaluated for the presence of protocol deviations predefined as leading to non-evaluability of the subject for the primary efficacy variable. Such predefined protocol deviations could include, but were not restricted to no PET acquisition during the 90 to 110 minute imaging window.

Primary efficacy analysis:
The analysis of the primary efficacy variable was done for the PPS and FAS populations, with the PPS being considered the primary efficacy population.

As primary analysis, point estimates together with normal-approximated, two sided 95% confidence intervals (CIs) were given for sensitivity and specificity in beta-amyloid detection based on the majority read, derived from 3 individual assessments for regions (of a particular subject) where an SoT was available.

Amendment 4 introduced the following hypotheses for testing sensitivity and specificity:
H0,sens: sensitivity ≤ 0.6 vs. H1,sens: sensitivity > 0.6
H0,spec: specificity ≤ 0.8 vs. H1,spec: specificity > 0.8
H0,sens was to be rejected if the lower bound of the two-sided 95% CI is larger than 0.6.
H0,spec was to be rejected if the lower bound of the two-sided 95% CI is larger than 0.8.

Secondary efficacy analyses:
Kappa coefficients were computed across all three readers and between each pair of readers. The same statistical methodology was used either when the subject (i.e. “whole brain”) was the observational unit but also when a brain region was the observational unit.

For sensitivity and specificity of quantitative florbetaben (18F) PET assessments, ROC regions were used.

Post-hoc analyses:
After the originally planned analyses became available and the results were reviewed, the following additional post-hoc sensitivity analyses were conducted for the full analysis set.

- Sensitivity and specificity, analogous to the primary efficacy variable, by blinded read session
- Sensitivity and Specificity, analogous to the primary efficacy variable
  - excluding Region 3
  - excluding Region 6
  - excluding Regions 3 and 6
- Sensitivity and specificity, analogous to the primary efficacy variable, based on different SoTs
  - a brain region was considered to have ‘beta-amyloid present’, if the CP judged it as having a final rating of “moderate” or higher for neuritic or diffuse plaques based on the Bielschowsky silver staining
  - a brain region was considered to have ‘beta-amyloid present’, if the CP judged it as having a final rating of “moderate” or higher for neuritic plaques only based on the Bielschowsky silver staining
- Descriptive statistics of time from first study drug administration until death.

A potential high variability in standardized uptake value (SUV) due to the use of different scanners was acknowledged. In the pivotal autopsy study several standardization measures were introduced to attempt to control the most significant points causing such variability.

The assessment of the autopsy samples for the CERAD score (which was used as SoT for the post-hoc analysis) was performed according to the CERAD guidelines (Mirra, 1991). The pathologists were requested to look for amyloid deposition at least in the three neocortical regions “Middle frontal Gyrus“,”Superior and middle temporal gyrus”, and “inferior parietal lobule”. In addition, also the “Hippocampus” should be evaluated if needed. As the onsite-pathologists were requested to perform a detailed pathological diagnosis, additional regions of the brain were evaluated as part of their assessments for a final diagnosis.

At the end of the procedure the company provided additionally the results of a re-reading study.

This study included 82 autopsied patients from the pivotal autopsy study 14595 and 10 HVs considered amyloid-negative. The main objective was to assess the efficacy (sensitivity and specificity) of the visual assessment method proposed for clinical practice of florbetaben (18F) PET scans, by five new electronically trained readers (as it is intended to be used as part of the future training for users), in the detection of beta-amyloid neuritic plaques in the brain.

Subject-level sensitivity and specificity of florbetaben (18F) PET of those 82 autopsied patients excluding HVs, using as SoT the presence (moderate or frequent) or absence (none or sparse) of amyloid neuritic plaques as detected by BSS and scored by the histopathology consensus panel according to the CERAD criteria (except for the total regions to be assessed), was a secondary variable. Assessed regions are displayed in the table below:

Text Table 7-4: Regions of interest for determination of histopathology as Standard of Truth

Region number	Region name
1	Middle frontal gyrus (coronal plane)
2	Striate and parastriate areas of occipital cortex (coronal plane)
3	Hippocampus at the level of the lateral geniculate nucleus (coronal plane)
4	Anterior cingulate cortex (coronal plane)
5	Posterior cingulate cortex (coronal plane)
6	Cerebellar hemisphere directly adjacent to the cerebellar nuclei (sagittal plane)

If in any of the 6 regions neuritic beta-amyloid plaques were evaluated as being 'present' at a clinico-pathologically relevant level (either moderate or frequent), the subject was determined as having clinico-pathologically relevant neuritic beta-amyloid deposition in the brain. If in none of the regions the histopathological findings were assessed as being more than 'no' or 'sparse' neuritic beta-amyloid plaques, the subject was scored as 'no neuritic beta-amyloid present'.

In addition, the applicant extended the post-hoc analysis from n=74 brains to the available n=81 brains.

Results

Participant flow

Of the total 253 enrolled subjects, 216 received florbetaben (18F). The brains from only 32 brains were available for efficacy analyses at the original protocol. The brain from 1 of the remaining 32 subjects was not processed properly for pathological (SoT) evaluation (one major deviation).

Therefore, 31 subjects who died were included in the efficacy analyses (with clinical diagnosis of AD (n=22), DLB (n=1), other dementia (n=2) and 6 non-demented patients) together with 10 young HVs.

There were also other 4 subject with major and 174 subjects with minor protocol deviation. All 10 HVs included in the FAS seem to have systematically deviated from the protocol (minor deviation) and quantitative measures (i.e. SUVs) from their PET images on the regional-level quantitative analysis have not been obtained.
The company has performed a post-hoc analysis using the subject-level visual assessment of the 74 PET images from subjects, for whom histopathology is available. There were 57 subjects with probable AD, 9 subjects with other dementia (including 3 subjects with DLB) and 8 non-demented. There were no major protocol deviations.

Recruitment
No data provided.
Recruitment was originally intended mainly by way of brain bank initiatives. At amendment 2 (3-jun-10), recruitment could be done from several sources, including brain bank initiatives, hospices, private practices, and dementia initiatives.

Conduct of the study
The study started at 25-nov-09 and ended at 15-sep-11.
There were 5 amendments to the **Original Protocol**, dated 20 MAY 2009.
The most significant changes to the protocol resulting from these amendments concerned recruitment, statistical analysis, objectives and treatments.
The analysis was based on the first available 32 histopathological specimens. However, subjects recruited in the study and not deceased after these 32 histopathological specimens were available, are to be followed up for a maximum of 3 years (up to follow-up visits) after entry into the trial. As of August 1, 2013 the data base consisted of 74 brains.

Baseline data
Combining autopsied patients and HVs: 26 subjects (63.4%) were male. In addition, 26 subjects (63.4%) were white. The mean age is 68.5 years.

Numbers analysed
All of 216 subjects receiving florbetaben (18F) were imaged at the time of enrollment. 212 evaluable PET images were read by three blinded-readers in December 2011. 32 died and underwent brain autopsy at the original protocol; however, only 31 were considered evaluable for the standard-of-truth assessment. This sample was further enriched with 10 HV for whom a florbetaben (18F) PET scan was available and whose SoT was considered β-amyloid negative by definition.
The company has performed a post-hoc analysis using the subject-level visual assessment of the 74 PET images from subjects, for whom histopathology is available. Baseline florbetaben (18F) PET scan which resulted to be performed 329 days (+/- 272 days; range 75-972 days) before death. There were 15 subjects in whom florbetaben (18F) PET scan were repeated before death.

Outcomes and estimation
The final neuropathological diagnosis of all 31 subjects included in the FAS has been provided. There were 16 AD patients, 2 with DLB patients, 1 PD patient, 10 with other dementias and 2 normal.
In the post-hoc analysis, altogether 51 subjects had AD neurodegenerative pathology and 23 had non-AD neurodegenerative pathology as final diagnosis confirmed histopathologically by the onsite-pathologists. These non-AD subjects presented with Parkinson's disease (n=1), hippocampal sclerosis dementia (n=2), frontotemporal lobar dementia (n=2) or multi-system neuronal and glial tauopathy (n=1) or Pick's disease (n=1), cerebral amyloid angiopathy (n=1), mild age-related neurofibrillary degeneration (n=1), Lewy body disease (n=3), infarcts (n=4), motor neuron disease (n=2), senile dementia (n=2) and progressive supranuclear palsy (n=1).

PRIMARY EFFICACY ANALYSIS

The sensitivity and specificity of the majority read of the visual assessment of regional tracer uptake in the florbetaben (18F) PET images, in correctly differentiating between brain regions with and without β-amyloid deposition, was 77.4% (95% CI: 65.4% – 89.4%) and 94.2% (95% CI: 88.6% – 99.8%), respectively, for a total of 244 regions analysed. The success criteria for the co-primary endpoints of the study were to show that sensitivity was higher than the pre-specified threshold of 60% and that specificity was higher than the pre-specified threshold of 80%. As both thresholds were exceeded by the lower bounds of the respective 95% CIs, the co-primary endpoints (eg, goal) of the study were met.

The results for each of the 6 brain regions of interest, as well as the total, and for each of the single blinded readers, as well as the majority read, can be found in Table 9-1. There were 8 false-positive cases, and also 24 false-negative cases (13 out of the 24 resulted from regions 3 (hippocampus) and 6 (cerebellum)).

Table 9-1: Primary Analysis: Sensitivity and specificity (including normal approximated CI) in amyloid beta plaque load detection in the brain, by blinded reader (including majority read) (full analysis set)

Region	Method key item	TP	TP+FN	Sensitivity [%]	95% Sens. LCL [%]	95% Sens. UCL [%]	TN	TN+FP	Specificity [%]	95% Spec. LCL [%]	95% Spec. UCL [%]
Total	**Maj. read**	**82**	**106**	**77.36**	**65.35**	**89.37**	**130**	**138**	**94.20**	**88.57**	**99.84**
	Reader 1	85	106	80.19	67.39	92.99	125	138	90.58	83.60	97.55
	Reader 2	86	106	81.13	72.14	90.12	126	138	91.30	84.58	98.03
	Reader 3	63	106	59.43	46.81	72.06	127	138	92.03	85.36	98.70
Region 1	**Maj. read**	**18**	**21**	**85.71**			**19**	**20**	**95.00**		
	Reader 1	18	21	85.71			17	20	85.00		
	Reader 2	20	21	95.24			18	20	90.00		
	Reader 3	14	21	66.67			18	20	90.00		
Region 2	**Maj. read**	**16**	**18**	**88.89**			**19**	**22**	**86.36**		
	Reader 1	16	18	88.89			19	22	86.36		
	Reader 2	18	18	100.00			17	22	77.27		
	Reader 3	11	18	61.11			19	22	86.36		
Region 3	**Maj. read**	**12**	**21**	**57.14**			**20**	**20**	**100.00**		
	Reader 1	16	21	76.19			18	20	90.00		
	Reader 2	11	21	52.38			20	20	100.00		
	Reader 3	7	21	33.33			20	20	100.00		
Region 4	**Maj. read**	**18**	**20**	**90.00**			**18**	**21**	**85.71**		
	Reader 1	18	20	90.00			18	21	85.71		
	Reader 2	19	20	95.00			18	21	85.71		
	Reader 3	15	20	75.00			18	21	85.71		
Region 5	**Maj. read**	**18**	**22**	**81.82**			**17**	**18**	**94.44**		
	Reader 1	17	22	77.27			16	18	88.89		
	Reader 2	18	22	81.82			16	18	88.89		
	Reader 3	16	22	72.73			17	18	94.44		
Region 6	**Maj. read**	**0**	**4**	**0.00**			**37**	**37**	**100.00**		
	Reader 1	0	4	0.00			37	37	100.00		
	Reader 2	0	4	0.00			37	37	100.00		
	Reader 3	0	4	0.00			35	37	94.59		

Abbreviations: BR = blinded reader; CI = confidence interval; FN = false negative; FP = false positive; LCL = lower confidence level; TP = true positive, TN = true negative; UCL = upper confidence level

Source: Table 14.2.1/2

SECONDARY EFFICACY ANALYSES

1. Secondary efficacy variables related to the comparison with the SoT were:

a) *Individual reader sensitivity and specificity of brain uptake of florbetaben (18F) regarding the visual assessment results of PET images over the 6 brain regions based on each single blinded reader* (table 9-1 above)

b) *Quantitative parameters SUV and SUVR for each of the 6 brain regions defined*
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 (1.77) was Region 5 (posterior cingulate/precuneus). With an SUVR threshold of 1.171, this gave a sensitivity of 77% and a specificity of 100% (see Table 9-2). In line with the visual assessment results, with an SUVR threshold of 0.932 providing a sensitivity of 67% and a specificity of only 60%, the region that provided little to no differentiation also in terms of lowest Youden Index (1.27) was Region 3 (hippocampus).

c) *Agreement of blinded readers:* Substantial agreement (kappa=0.658) was observed across all readers, with similar values for each reader pair.

1. The following secondary efficacy variables were related to a SoR:

a) *Composite "whole brain" sensitivity and specificity (for the majority read and for each reader) of the regional visual assessment based on the composite "whole brain" regional histopathological findings (derived from SOT):* The sensitivity and specificity of the whole brain regional assessment compared to the SoR were 86.96% (95% CI: 73.19 – 100.00%) and 88.89% (95% CI: 74.37% – 100.00%), respectively, for the majority read.

b) *Subject level sensitivity and specificity (for the majority read and for each reader) of the visual assessment of baseline florbetaben (18F) PET images (according to the RCTU/BAPL scoring) as compared to the onsite neuropathological diagnosis of amyloid-present/amyloid-absent according to the CERAD criteria:*

a. The sensitivity and specificity of the subject level visual assessments compared to the 'on-site' SoR were 100% (95% CI: 80.5 – 100%) and 85.7% (95% CI: 67.4% – 100%), respectively, for the majority read of the first 31 patients who died excluding HVs. Considering also the 10 HVs, 17 patients were classified as beta-amyloid while there was no beta-amyloid in 24 cases. No false-negative and 2 false-positive cases existed (in a patient with focal atrophy and a patient with moderate amounts of neuritic plaques and frequent diffuse amyloid plaques).

b. In the post-hoc analysis of 74 patients who died, excluding the 10 HVs, the sensitivity and specificity was as shown in table 5. 47 patients were classified as beta-amyloid present while there was no beta-amyloid in 27 cases. For the majority read, 1 false-negative and 3 false-positive cases existed (1 case of progressive supranuclear palsy, 1 with infarct and 1 with Dementia with Lewy bodies).

Table 5 Subject level sensitivity and specificity of the visual assessment of florbetaben PET images as compared to onsite neuropathological assessment, by reader (including majority read), FAS of study 14595 and post-hoc analysis with additional brains; without Healthy volunteers

		Study 14595 (n=31)			Post-Hoc (n=74)		
		Estimate [%]	95% LCL [%]	95% UCL [%]	Estimate [%]	95% LCL [%]	95% UCL [%]
Sensitivity	**Maj Read**	**100.00**	80.49	100.00	**97.87**	93.75	100.00
	Reader 1	100.00	80.49	100.00	97.87	93.75	100.00
	Reader 2	100.00	80.49	100.00	100.00	92.45	100.00
	Reader 3	100.00	80.49	100.00	97.87	93.75	100.00
Specificity	**Maj Read**	**85.71**	67.4	100.00	**88.89**	77.03	100.00
	Reader 1	85.71	67.4	100.00	88.89	77.03	100.00
	Reader 2	85.71	67.4	100.00	85.19	71.79	98.59
	Reader 3	78.57	57.1	100.00	85.19	71.79	98.50

c) *Agreement of blinded readers, both based on the composite "whole brain" visual assessment and on the subject level (RCTU / BAPL) assessment:* Across all 3 readers, substantial agreement (kappa=0.764) was achieved for the "whole brain" visual assessment, with similar values for each reader pair. Across all 3 readers, almost perfect agreement (kappa=0.870) was achieved for reads based on subject level, with similar values for each reader pair.

ANCILLARY ANALYSES

Table 5 displays the sensitivity, specificity and 95% CI of the visual assessment of regional tracer uptake in the florbetaben (18F) PET images for the following analyses: without the hippocampus (Region 3), without the cerebellar cortex (Region 6) and without Regions 3 and 6, and Bielschowsky silver stain only, neuritic plaques only.

Table 5. Sensitivity and specificity of amyloid beta plaque load detection

Efficacy endpoints	% Sensitivity	95% CI	% Specificity	95% CI
Primary (silver stain and immunohistochemistry)	77.4	65.4 – 89.2	94.2	88.6 – 99.8
Without region 3	82.4	70.2 – 94.6	93.2	86.7 – 99.8
Without region 6	80.4	67.9 – 92.9	92.1	84.3 – 99.9
Without regions 3 and 6	86.4	73.4 – 99.4	90.1	80.5 – 99.8
Silver stain Neuritic and diffuse plaques	81.2	70.3 – 92.1	94.4	89.0 – 99.8
Silver stain Neuritic plaque only	85.9	75.3 – 96.5	86.1	77.6 – 94.7

Summary of main efficacy results

The tabular view of the study results is presented below:

Summary of study 14595

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		
Study identifier	Histopathology Study 14595	
Design	It was designed to determine the sensitivity and specificity of the visual assessment of regional tracer uptake in the florbetaben (18F) 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).	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	
Hypotheses	There were two hypotheses formulated, one hypothesis for sensitivity and one hypothesis for specificity. The lower bounds of the 2-sided 95% confidence intervals for sensitivity and specificity in β-amyloid detection based on the majority read being greater than 0.6 and 0.8, respectively.	
Treatments groups	A total of 218 subjects were assigned to treatment with the following clinical diagnoses: 139 probable AD subjects, 31 other dementia subjects, 5 DLB subjects and 32 NDVs. Those who were willing to donate their brain after death and undergo both a PET and an MRI scan were eligible. The sample was enriched with 11 HVs.	Participants received a radioactive dose of 300 MBq (8.1 mCi) ± 20%. The mass dose of the ligand was ≤ 50 μg. Imaging took place from 90 to 110 minutes post injection.
Endpoints and definitions	The co-primary endpoints of the study were the sensitivity and specificity of the visual assessment of regional tracer uptake in the florbetaben (18F) PET images in correctly differentiating between brain regions with and without β-amyloid deposition.	
Database lock	All those recruited patients until at least 30 histological specimens were available.	
Results and Analysis		
Analysis description	**Primary Analysis**	
Analysis population	From 216 subjects who were treated with florbetaben (18F), those 31 who provided an evaluable brain specimen (with clinical diagnosis as probable 22 AD subjects, 1 DLB subject, 2 other dementia subjects, and 6 NDVs) and 10 HVs, were included in the full analysis set (FAS) and the per protocol set (PPS) that were identical for efficacy analysis	
Descriptive statistics	**Statistical analysis**: The co-primary efficacy variables of the study were evaluated using the majority results of the 3 independent blinded readers. The 95% confidence intervals were calculated taking within brain dependencies into account using normal approximation.	

	Participant flow: A total of 253 subjects were screened for the study. The study included 218 subjects that were assigned to receive treatment. The primary objective of this study required postmortem brain specimens. A total of 34 subjects died, only 32 brains were available for efficacy analyses, and the brain from 1 of the remaining 32 subjects was not processed properly for pathological evaluation. Therefore, 31 subjects who died were included in the analyses. An additional sample of 10 HV were included in the study. There were some protocol deviations.	
Effect estimate per comparison	Co-primary Endpoint (Sensitivity and specificity)	The sensitivity and specificity of the visual assessment of regional tracer uptake in the florbetaben (18F) PET images, in correctly differentiating between brain regions with and without β-amyloid deposition for the 6 brain regions of interest, for the majority read was 77.4% (95% CI: 65.4% – 89.4%) and 94.2% (95% CI: 88.6% – 99.8%), respectively.
Analysis description	For Secondary analyses see “clinical assessment”	

Analysis performed across trials (pooled analyses AND meta-analysis)

The company presents pooled analyses of efficacy of the studies composing the florbetaben (18F) clinical development program. No meta-analysis was presented.

Data were generated on the basis of two originally planned subsets of studies, Pool 1 and Pool 2.

Pool 1 included one Phase 1 study, Study 312161 (91794) and the two Phase 2 studies: Study 14311 and Study 311741 (91708) Part A and Part B. Pool 1 evaluated the sensitivity and specificity of the visual assessment of florbetaben (18F) PET scan results from the various studies in which independent blinded reads were performed. The studies included in Pool 1 utilized both a different blinded reader training and visual assessment methodology than is currently proposed for florbetaben (18F) PET in clinical practice. Furthermore, the SoRs/SoT differed among studies in Pool 1, in that the determination of sensitivity and specificity in the Phase 1 and Phase 2 studies were based on the clinical diagnosis of either AD vs HV, or of Down syndrome (DS) vs HV, whereas the histopathology was the SoT for the pivotal Phase 3 study. Thus, pooling such data is not justified from a scientific point of view.

Pool 2 includes three Phase 1 studies: Study 311722 (91707), Study 91790, and Study 312161 (91794); and the two Phase 2 studies: Study 14311 and Study 311741 (91708) Part A, and Part B. The integrated efficacy analysis Pool 2 provides descriptive statistics related to regions of interest (ROI)-based Standardized Uptake Value Ratios (SUVRs) across the various subject groups (using the cerebellar cortex as reference region) and generated upon application of an MRI-segmented template.

Of the total 560 subjects in the Pool 2 integrated analysis FAS, 316 were NDVs (70 subjects < 55 years; 246 subjects ≥ 55 years) and 244 were AD subjects. A good differentiation between subjects with AD and HVs could be verified on the basis of the results of the pooled analysis of regional SUVRs across studies. The 5 key cortical regions provided excellent differentiation between NDVs and ADs.

Standard uptake values (SUV) was determined for subpopulations and presented by age, geographical region, gender and race. Data on subpopulations focused on the key cortical brain regions (the frontal cortex, the lateral temporal cortex, the parietal cortex, the anterior cingulate cortex and the posterior cingulate cortex) which showed increasing SUVR arithmetic means. A trend to higher SUVR with increasing age was observed. No difference in SUVR means between males and females was observed in either NDVs or AD subjects. There was no observed difference in SUVR means between Caucasian and Black NDVs < 55 years in any of the key cortical gray matter regions, however there were few

Black NDVs < 55 (n = 11)restricting the reliability and predictability for this subpopulation. In general, there was good differentiation between NDVs ≥ 55 years and AD subjects across Australia, Europe, Japan, and the USA. Across all geographic regions, subjects had higher SUVR values in the AD subjects than in the NDVs in cortical gray matter regions. NDVs ≥ 55 years in Japan had slightly lower SUVRs than the NDVs ≥ 55 years in the other geographic regions, for both the arithmetic and geometric means.

For the two groups (NDV and AD) and for each ROI, separate models using the variables study and age have been used. From the analysis of covariance, it could be concluded that the mean values for the different ROIs are different between the studies (separately for NDVs and AD subjects), and that the influence of the factor age is similar in all studies included in this analysis.

The pooled analysis of all Phase I – III data included 406 AD patients and 291 non-demented volunteers (⩾ 55 years). In the group of AD patients, 8% had normal renal function; 35% had mild renal impairment, 19% moderate renal impairment and 1% had severe renal impairment (missing data: 38%). The respective percentages in the group of non-demented volunteers were 24%, 51%, 14% and < 1%, respectively (missing data: 11%). The pooled analysis of subjects showed no relevant effect of renal function on the safety of florbetaben.

Clinical studies in special populations

No studies in special populations were conducted. With respect to subpopulation factors such as age, geographical region, gender and race see section analysis performed across trials below. The use of florbetaben (18F) in children cannot be recommended, and it is not expected.

Influence of renal impaired function on safety and efficacy was evaluated as part of a pooled analysis of Phase II clinical data (study 311741 Part A and Part B). In summary, reductions in renal function were inherent in the target study population and did not influence the safety and efficacy of florbetaben. Dose adjustments in patients with renal impairment are not necessary.

- In study 311741 Part A, 32% of AD patients (54% of healthy control volunteers) had normal renal function (CrCl ≥ 90 mL/min) at baseline. 62% of AD patients (41% of HV) had mild renal impairment (CrCl 60-89 mL/min), 6% of AD patients (6% of HV) had moderate renal impairment (CrCl 30-59 mL/min). Subjects with severe renal impairment (CrCl 15-29 mL/min) were not included in that study. As expected, renal function decreased with age both in AD patients and healthy control volunteers. In addition, baseline creatinine clearance was found to be lower in Alzheimer patients (n=81) compared to the healthy volunteer control group (n=69) (mean ± SD: 72.2 ± 15.8 mL/min vs. 81.9 ± 22.2 mL/min, p<0.01). The difference was consistently seen across all age groups. CrCl did not show a relevant influence on the efficacy of florbetaben.
- Similar results were found in study 311741 Part B (including 28% of AD patients with normal renal function and 47/ 21/ 3% of patients with mild/ moderate/ severe renal impairment, respectively).
- For the results in the pooled analysis see section above.

Supportive study(ies)

Study 14311

This was an open-label, non-randomised and non-controlled phase 2 study.

During the florbetaben (18F) PET imaging day, 39 individuals with DS (≥40 years) and 70 young HVs (≥21 and 40 years) received a single IV injection of 300 MBq of florbetaben (18F) with mass dose ≤50 micrograms. The mean ages of the DS subjects and HVs were 46.3 years and 27.7 years, respectively. The majority of subjects in the study were Caucasian. A total of 21 (53.8%) males and 18 (46.2%) females were in the DS group and 28 (40%) males and 42 (60.0%) females were in the HV group.

PET scanning was performed from 100-120 minutes post injection. Images were assessed for the regional presence or absence of tracer uptake as part of an independent blinded read. PET imaging interpretation (colour, image orientation, regions to be assessed, criteria to interprete regions and the whole brain) was similar to that used in part B of study 311741.

The results of PET imaging with florbetaben (18F) were compared to the clinical diagnosis as the SoR. The sensitivity and specificity of florbetaben in the detection of β-amyloid in the brains of the DS subjects when compared to HVs was based on the visual assessment of the BAPL score by three blinded readers. The BAPL score was based on the compilation of the RCTB score of the 4 brain regions, ie, frontal cortex, posterior cingulate cortex, lateral temporal cortex, and parietal cortex. An abnormal scan in a DS subject was a match for sensitivity and a normal scan in an HV subject was a match for specificity.

Sensitivity of the visual assessment of the florbetaben (18F) PET images in detecting/excluding cerebral beta-amyloid in individuals with DS compared to HVs, based on the majority read of visual assessment of the BAPL score by three blinded readers, was lower than expected with 46.2% (95%CI: 30.1-62.8%). The estimated reader sensitivity was lowest for reader 2 (23.1%; 95%CI: 11.1-39.3%) when compared to that of reader 2 (56.4%; 95%CI: 39.6-72.2%) and reader 3 (51.3%; 95%CI: 34.8-67.6%). Subsequent subanalysis revealed a trend towards higher sensitivity values in subjects >46 yrs (the median age): 72.2% with 38.9% for reader 1 and 88.9% and 77.8% for readers 2 and 3. In a post-hoc analysis, the sensitivity for the 10 subjects ≥ 50 yrs of age was 90% with the individual reader sensitivities of 60% (reader 1), 100% (reader 2) and 90% (reader 3).

In the HVs, the majority read specificity was 100% and the individual reader specificity estimates were 100% (95%CI: 94.9-100%) for reader 1, 97.1% (95%CI: 90.1-99.7%) for reader 2, and 94.3% (95%CI: 86-98.4%) for reader 3.

The inter-reader agreement was 80.7% (kappa of 0.55) in the DS and HV combined.

For DS with visually abnormal scans, the mean SUVR for the frontal cortex for example, was 1.396 compared to 1.146 in the HVs. Upon linear regression analysis, in subjects with DS, a significant ($p<0.0001$) correlation between SUVRs (for all regions) with age was verified.

Study 311741

This was a multi-center, open-label, non-randomised, phase 2 study.

It was primarily aimed to determine the sensitivity and specificity of the independent visual assessment of florbetaben (18F) PET images (from the 90 to 110 min imaging window) in detecting/excluding cerebral beta-amyloid in patients with probable AD compared to HVs. Although the primary objective of Parts A and B were identical, the method by which the diagnosis was established differed. It was clinical diagnosis by the onsite investigator (in part A) and an independent consensus panel (CP) of 3 experts in dementia (in part B).

Participants were age-matched HVs and patients diagnosed with pAD and mild-moderate dementia.
Part A: 146 subjects (78 AD patients, 68 HVs) were included in the PPS
Part B: 257 subjects (139 AD patients, 118 HVs) were included in PPS.

During the florbetaben (18F) PET imaging day, all subjects received a single IV injection of 300 ± 20% MBq per injection with ≤5 µg/injection (part A) or ≤50 µg/injection (part B). Scanning was performed in 5-min frames at 45-60 min, 90-110 min, and 110-130 min p.i. Quantitative assessment of the images was also performed by an independent nuclear medicine expert.

Blinded visual reading was performed on rainbow colour scale. They were 8 regions (frontal, posterior cingulate, lateral temporal, parietal, occipital, caudate nucleus, mesial temporal and anterior cingulate cortex) assessed in the transaxial orientation. Only the last four regions were not included in the BAPL rule. Each of the 3 blinded readers visually scored each region to obtain a RCTB as follows:

Table 1-7: Regional cortical tracer binding algorithm

Regional cortical tracer binding score		Condition for assessment
1	No binding	Tracer binding in region lower than in white matter
2	Minor binding	Few discrete cortical spots in region with tracer binding similar to white matter; activity extends beyond the white matter to the outer cortical margins
3	Pronounced binding	[a]At least one larger area in region with tracer binding equal to or higher than white matter

a. Adapted from PoM study A42404 but modified between Part A and Part B of Study 311741.
Source: Module 5.3.5.2, A45264, Text Table 7-9

The blinder reader had to classify brain BAPL on the basis of RCTL score.

In **Part A** of this study, two BAPL algorithms (algorithm A and algorithm B) for assessing the normality/abnormality of beta-amyloid plaque load in the brain scans were used (figure 1-3).
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.

In **Part B**, the same RCTB and BAPL scoring procedures were done but only using the algorithm B.

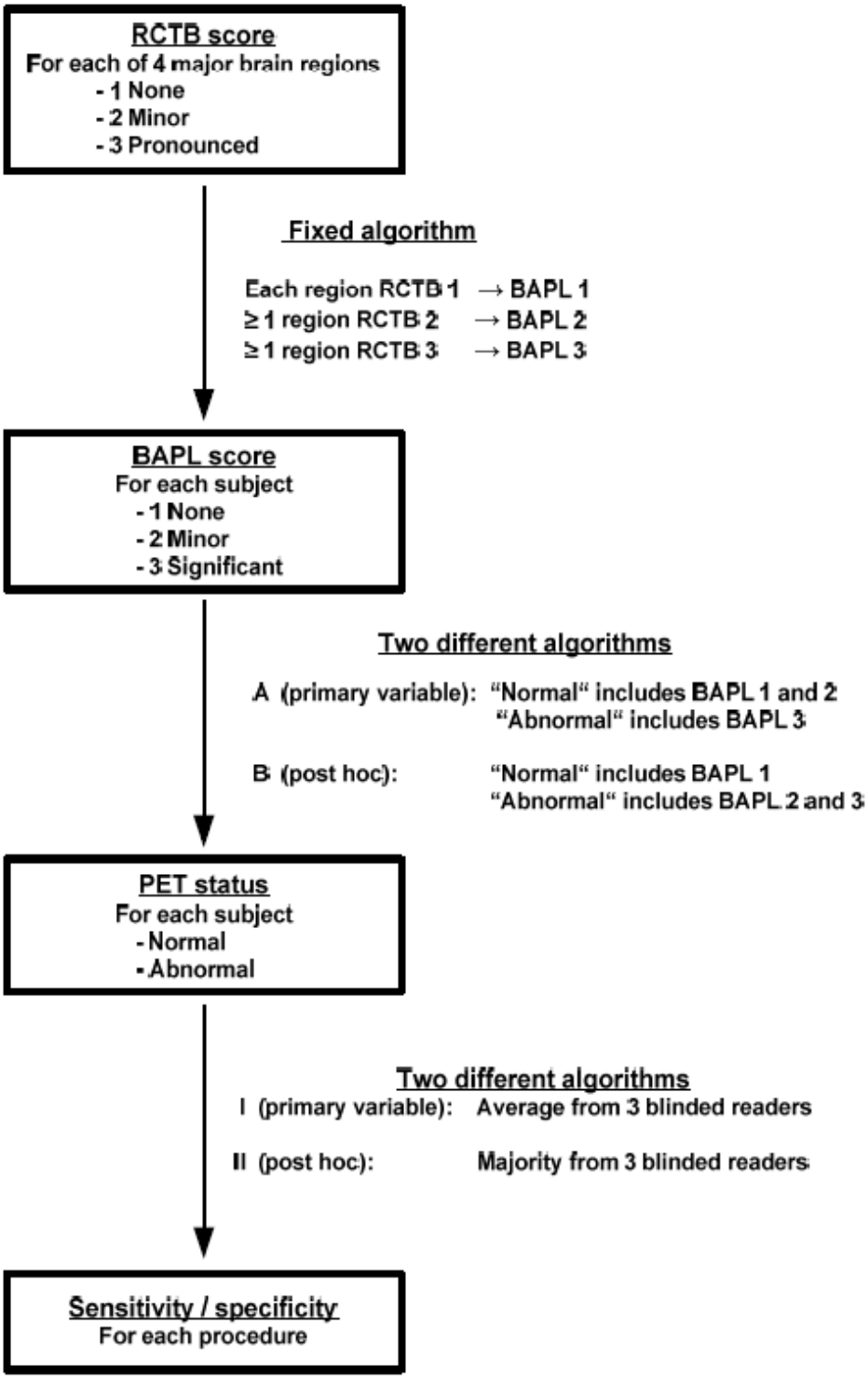


Figure 1-3: Schematic representation of variations in the determination of the sensitivity and specificity (Study 311741 Part A)

Source: Module 5.3.5.2, A45264, Text Figure 9-1.

Part A

The average blinded reader sensitivity of 74.8% (95% CI: 65.7% to 83.9%) and a specificity of 96.1% (CI: 91.8% to 100.3%) in detecting/excluding cerebral BAPL in the brain in patients with AD (set up by the onsite investigator) compared to HVs based on the independent visual assessment of the florbetaben (18F) PET using BAPL algorithm A for imaging window 90-110 min p.i.. 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 similar to the results of the imaging window 90-110 min p.i.: 73.9% and 94.6%, respectively, for the 45-60 min p.i. and 70.6% and 98%, respectively, for the 110-130 min p.i.

Using algorithm B and the majority reader approach a sensitivity of 79.5% (CI: 68.8% to 87.8%) and a specificity of 91.2% (CI: 81.8% to 96.7%) was obtained at 90-110 min p.i. At 45-60 min p.i. values were 80.8% and 89.7%, respectively. For the 110-130 min p.i.,78.2% and 89.7%, respectively.

Part B

The majority blinded reader sensitivity of 67.2% (95% CI: 57.9% to 75.7%) and a specificity of 96.6% (95% CI: 91.6% to 99.1%) in detecting/excluding cerebral beta-amyloid plaque load in the brain in patients with AD (as assessed by the CP) compared to HVs based on the independent visual assessment of the florbetaben (18F) PET using BAPL algorithm B for imaging window 90-110 min p.i.. As only the success criterion of specificity (i.e. statistically was greater than 75%) was met, but not that for sensitivity (i.e. sensitivity was statistically not significantly greater than 75%) the study could not be considered successful. At 45-60 min p.i., sensitivity and specificity for the majority read was 71.6% and 94.1% respectively. They were 73% and 96.6%, respectively, for the 110-130 min p.i.

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.

The inter-reader agreement across all 5 readers was 0.864.

Part A and B

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.

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.

No camera effect was significant for SUV, and a potential relevant effect could not be excluded for SUVR. In part B, several camera effects were significant for SUV data and no camera effect was significant for SUVRs.

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 in part A, neither in AD patients (p-value: 0.7372) nor in HVs (p-value: 0.2825). In part B, data did not indicate that within patients with AD or with HV there was a difference in CrCl those with negative versus (vs) those with positive scans (assessed by majority read for the 90 to 110 min p.i. imaging window).

Study 312043

This was a single-center, open-label, non-randomised, phase 1 study to investigate whether BAY 94-9172 PET has any ability to distinguish subjects with MCI progressing to AD from those with MCI not progressing to AD. A total of 45 subjects with MCI ≥ 60 yrs were included.

Baseline clinical, MRI and florbetaben (18F) PET scan were performed. Clinical assessment was repeated at 6, 12, 18 and 24 months and neuropsychology tests were repeated at 6, 12 and 24 months. MRI and PET scan was repeated at 12 and 24 months. The classification at two years was performed by a neurologist blind to the florbetaben PET scan results and hippocampal volumes but with access to MRI images and cognitive test scores. Clinical diagnosis of probable AD was made according to

the NINCDS-ADRDA criteria (McKhann 1984). Diagnoses for non-AD dementia were consistent with published criteria (Hauw 1994, Neary 1998, McKeith 1996).

All subjects received an i.v. dose of total activity amounting to 300 MBq (8.1 mCi) ± 20% (of no more than 5 µg). Blinded visual assessment of PET images were performed in rainbow color scale, using RCTB score and BAPL score were to those in part B of study 311741. BAPL score was assessed for each of the two imaging time windows (45-60 and 90-110 min p.i.). Quantitative assessment was performed for the images acquired from 90-110 min p.i. from 17 brain regions, similarly as in the pivotal study.

A total of 45 subjects (63.4%) were treated at least once and were part of the full analysis set (FAS). A total of 36 (50.7%) subjects completed the study. Majority of subjects were Caucasian (95.6%) and almost two-thirds were male (64.4%). Mean baseline age was 72.7±6.5 yrs.

The primary variable used to assess efficacy was the cortical SUVR. At all time points the neocortical SUVR was higher in subjects who progressed to AD than in subjects who did not progress to AD. In the FAS, the neocortical SUVR (as a measure of florbetaben (^{18}F) binding) was higher in subjects who progressed to AD (baseline: 1.726 ± 0.227, 12 months: 1.738 ± 0.236, 24 months: 1.796 ± 0.296) than in subjects who did not progress to AD (baseline: 1.430 ± 0.252, 12 months: 1.442 ± 0.270, 24 months: 1.439± 0.219) at all time points. Based on a Wilcoxon Mann-Whitney test (baseline p-value: 0.0001, 12 months p-value:0.0011, 24 months p-value: 0.0008) and on a t-test (baseline p-value: 0.0002, 12 months p-value: 0.0008, 24 months p-value: 0.0002), there was a notable difference between the neocortical SUVR in subjects who progressed to AD compared to those who did not progress.

Regarding visual assessment of PET image data, a BAPL score was assessed for each imaging window. The number and percentage of normal and abnormal scans based on the BAPL score by subject group, time point and imaging window is given Text Table 9-12 (FAS).

Text Table 9-12: Number and percentage of normal and abnormal scans based on the BAPL score by subject group, time point and imaging window – FAS

Time key second level	Time key third level		Subject group: not progressed to AD	Subject group: progressed to AD
Baseline	45 min	n	26 (100.0%)	19 (100.0%)
		PET assessment		
		normal	15 (57.7%)	2 (10.5%)
		abnormal	11 (42.3%)	17 (89.5%)
	90 min	n	26 (100.0%)	19 (100.0%)
		PET assessment		
		normal	16 (61.5%)	0
		abnormal	10 (38.5%)	19 (100.0%)
12 months	45 min	n	24 (100.0%)	17 (100.0%)
		PET assessment		
		normal	16 (66.7%)	0
		abnormal	8 (33.3%)	17 (100.0%)
	90 min	n	24 (100.0%)	17 (100.0%)
		PET assessment		
		normal	14 (58.3%)	1 (5.9%)
		abnormal	10 (41.7%)	16 (94.1%)
24 months	45 min	n		14 (100.0%)
		PET assessment	22 (100.0%)	
		normal	14 (63.6%)	0
		abnormal	8 (36.4%)	14 (100.0%)
	90 min	n	22 (100.0%)	14 (100.0%)
		PET assessment		
		normal	14 (63.6%)	2 (14.3%)
		abnormal	8 (36.4%)	12 (85.7%)

AD Alzheimer's disease
Source: Table 14.2.2/20

Study 16034

This non-interventional "study was performed primarily to assess the inter-reader agreement of the visual assessment results of florbetaben (18F) PET scans pooled from various florbetaben (18F) clinical studies after training new readers via a computer (web)-based training tool.

A total of 507 images, which include 461 subject images plus 46 (10%) repeat images for a re-read, were selected from the images provided in four Phase 1 studies, the Phase 2 Study 311741 Part B, and the pivotal Phase 3 Study 14595. These PET scan images were pooled, and randomly assigned for consecutive, blinded visual assessment by 5 independent readers. To reflect the future study population, the pooled images included cases with various forms of clinically diagnosed dementia such as probable/possible mild to moderate AD, FTLD, VaD, and DLB as well as cases from clinically non-demented subjects, eg, MCI, young (< 40 years) and elderly (> 55 years) cognitively normal HVs, as well as from subjects from the Phase 3 histopathology study. The latter one included 54 subjects (including clinically demented and non-demented subjects) who died and were autopsied by 19 MAY 2012, and had an evaluable PET scan. The data were enriched with scans from 10 young HVs who served as negative controls (without autopsy).

Table 1-3: Prior clinical studies from which images were drawn for this study

Diagnosis / Studies	A42404[a]	311722	91790	312043	311741	14595	Total
Alzheimer's dementia[b]	10 (2)[f]	-	-	-	131 (17)	41 (2)	182 (21)
HV[c]	10 (2)	17	18 (1)		125 (11)	18 (1)	188 (15)
MCI[d]	-	-	-	45 (5)	6	-	51 (5)
DLB[e]	6 (1)	-	-	-	2	2 (1)	10 (2)
FTLD	11	-	-	-	1	-	12
PD	5	-	-	-	-	-	5
VaD	4	-	-	-	-	-	4
DEM	-	-	-	-	-	3	3
Other[g]	-	-	-	-	3 (2)	-	3 (2)
CP diagnosis not available[g]	-	-	-	-	3 (1)	-	3 (1)
	46 (5)	17	18 (1)	45 (5)	271 (31)	64 (4)	461 (46)[5]

[a] A42404 is the report number for the investigator-sponsored study (no Sponsor study number was assigned)
[b] AD = combination of probable AD + possible AD;
[c] HV = combination of HV = non-demented volunteers + Healthy negative controls;
[d] MCI = combination of MCI + amnestic MCI + non-amnestic MCI;
[e] DLB = combined DLB + possible DLB;
[f] Numbers in parenthesis indicate repeat image re-reads (total 46);
[g] "other" and "not available" = these scans were used only for the primary and intra-reader endpoints, but not for the secondary endpoint analysis.
AD = Alzheimer's dementia; HV = healthy volunteers; MCI = mild cognitive impairment;
DLB = dementia with Levy bodies; FTLD = fronto-temporal lobe dementia; PD = Parkinson's disease;
VaD = vascular dementia; DEM = other dementia; Other = clinical Consensus Panel (CP) established a diagnosis other than dementia; not available = the clinical CP could not establish a diagnosis.
Source: Module 5.3.5.1, PH-36928, Appendix 8 and Appendix 6

The primary endpoint was inter-reader agreement, to be measured on a binomial scale on the subject level using kappa-values across all 5 blinded readers according to Fleiss. The hypothesis that had to be tested is H_0, inter: κinter ≤ 0.6 vs. H_1, inter: κinter > 0.6. Inter-individual kappa value for all 10 reader pairs and the determination of the intra-individual kappa values as coprimary endpoint, based on the re-reads separately for all 5 blinded readers, were assessed.

The applicant also assessed the sensitivity and specificity of florbetaben (18F) PET in detecting the presence/absence of β-amyloid deposition in the following sub-populations:

d) The PET scans of subjects in the Phase 3 Study 14595 for whom a histopathological specimen was available were assessed using the respective histopathological findings as the SoT. This SOT used for establishing sensitivity and specificity of subject-level visual florbetaben (18) PET assessement in the autopsy subpopulation in study 16034 (54 subjects) importantly differs from the SOT for similar assessment in the autopsy study 14595 (original data and post-hoc analysis). The SOT does not match the CERAD criteria (Mirra et al. 1991). It was constructed by using the consensus histopathology panel assessments of the six brain regions for every subject included in this analysis. A brain region was considered to have 'relevant beta-amyloid present', if the CP of neuropathology experts judged it as having a final rating of "moderate" or "frequent" for neuritic or diffuse beta-amyloid plaques based on the Bielschowsky silver staining. If in any of the 6 regions beta-amyloid plaques were evaluated as being 'present' at a clinico-pathologically relevant level (either moderate or frequent), the subject was determined as having clinicopathologically relevant beta-amyloid deposition in the brain on a subject-level.

   The company currently presents additional data comparing the results of PET scan reading in study 16034 with the onsite pathology CERAD assessment collected for all subjects (same as used for the post-hoc analysis) instead that the original SOT.

- The combined hypotheses to be tested were rejected if the lower limits of the 95% confidence intervals for sensitivity and specificity are higher than the thresholds of 0.6 and 0.7 respectively for at least 3 out of the 5 blinded readers.
- Sensitivity and specificity was assessed with the CP clinical diagnosis as the SoR in images from Part B of the 311741 study, for each of the 5 blinded readers. Here, subjects with a CP confirmed clinical diagnosis of AD served as β-amyloid-positive, and CP confirmed cognitively normal HVs as negative controls.

5 naive readers trained by an electronic based training program performed were to visually assess the RCTU score on a subject-basis as it was detailed in the pivotal study after amendment 4 (and which is proposed for clinical use and described in the SmPC) at a controlled central reading facility.

The inter-reader agreement of visual PET scan readings yielded a value of 0.787 (95% CI: 0.750 – 0.824) across all five readers, which exceeded the pre-specified kappa value threshold of 0.6 (for the lower bound of the two sided 95% CI). The inter-reader agreement was high in all reader pairs, the highest being 0.865 (95% CI: 0.819 – 0.911) between readers 1 and 3 and the lowest was 0.677 (95% CI: 0.609 – 0.744) between readers 2 and 5. The highest intra-individual kappa value was of 0.957 (95% CI: 0.872 – 1.041) for reader 4 and the lowest being 0.823 (95% CI: 0.657 – 0.989) for reader 1.

Inter-reader reproducibility from the autopsy population in study 16034 was 0.69. Intra-reader agreement in the autopsied population in study 16034 cannot be provided since only 4 patients were assessed for such objective.

The sensitivity and specificity data to detect β-amyloid on a subject level with histopathology as the SoT using PET scan images, from the 55 (only 54 were included in the read since one was not evaluable) autopsy cases enriched with the images of 10 healthy volunteers from Study 14595, are detailed in Table 9-3.

Text Table 9-3: Sensitivity and specificity (including normal approximated CI) of the visual assessment of florbetaben PET scans compared to histopathology as Standard of Truth (full analysis set) – Exact 95% confidence limits

Method key item	No. of TP	No. of TP + FN	Sensitivity [%]	Sensitivity 95% LCL [%]	Sensitivity 95% UCL [%]*	No. of TN	No. of TN + FP	Specificity estimate [%]	Specificity 95% LCL [%]	Specificity 95% UCL [%]*
BR 1	36	40	90.00	76.64	97.21	20	24	83.33	62.62	95.27
BR 2	36	40	90.00	76.34	97.21	15	24	62.50	40.59	81.20
BR 3	35	40	87.50	73.20	95.81	18	24	75.00	53.29	90.23
BR 4	35	40	87.50	73.20	95.81	19	24	79.17	57.85	92.87
BR 5	31	40	77.50	61.55	89.16	22	24	91.67	73.00	98.97

BR --- Blinded Reader

The sensitivity and specificity data to detect β-amyloid on a subject level with histopathology as the SoT using PET scan images from the 54 autopsy cases (*excluding* images of the healthy volunteers from Study 14595) was identical to that shown in Text Table 9-3 when the HVs were excluded from analysis (see Table 9-4).

Text Table 9-4: Sensitivity and specificity (including normal approximated CI) of the visual assessment of florbetaben PET scans compared to histopathology as Standard of Truth, excluding the healthy volunteers (full analysis set) – Exact 95% confidence limits

Method key item	No. of TP	No. of TP + FN	Sensitivity [%]	Sensitivity 95% LCL [%]	Sensitivity 95% UCL [%]*	No. of TN	No. of TN + FP	Specificity estimate [%]	Specificity 95% LCL [%]	Specificity 95% UCL [%]*
BR 1	36	40	90.00	76.34	97.21	10	14	71.43	41.90	91.61
BR 2	36	40	90.00	76.34	97.21	9	14	64.29	35.14	87.24
BR 3	35	40	87.50	73.20	95.81	10	14	71.43	41.90	91.61
BR 4	35	40	87.50	73.20	95.81	9	14	62.49	35.14	87.24
BR 5	31	40	77.50	61.55	89.16	12	14	85.71	57.19	98.22

BR --- Blinded Reader

The company currently presents additional data comparing the results of PET scan reading in study 16034 with the onsite pathology assessment collected for all subjects (same as used for the post-hoc analysis) instead that the original SOT.

Table 21 Subject level sensitivity and specificity of the visual assessment of florbetaben PET images as compared to onsite neuropathological assessment, by reader (including majority read), histopathology subset of 16034 with 54 available subjects; including 10 Healthy volunteers

		Study 16034 (n=54+10 HVs)		
		Estimate [%]	95% LCL [%]	95% UCL [%]
Sensitivity	**Maj Read**	**100.0**	89.4	100.0
	Reader 1	100.0	89.4	100.0
	Reader 2	100.0	89.4	100.0
	Reader 3	100.0	89.4	100.0
	Reader 4	97.0	91.1	100.0
	Reader 5	87.9	76.7	99.0
Specificity	**Maj Read**	**80.7**	66.7	94.6
	Reader 1	77.4	62.7	92.1
	Reader 2	61.3	44.1	78.4
	Reader 3	74.2	58.8	89.6
	Reader 4	74.2	58.8	89.6
	Reader 5	87.1	75.3	98.9

Table 22 Subject level sensitivity and specificity of the visual assessment of florbetaben PET images as compared to onsite neuropathological assessment, by reader (including majority read), histopathology subset of 16034 with 54 available subjects; without Healthy volunteers

			Study 16034 (n=54)		
			Estimate [%]	95% LCL [%]	95% UCL [%]
Sensitivity	**Maj Read**		**100.0**	89.4	100.0
		Reader 1	100.0	89.4	100.0
		Reader 2	100.0	89.4	100.0
		Reader 3	100.0	89.4	100.0
		Reader 4	97.0	91.1	100.0
		Reader 5	87.9	76.7	99.0
Specificity	**Maj Read**		**71.4**	52.1	90.8
		Reader 1	66.7	46.5	86.8
		Reader 2	61.9	41.1	82.7
		Reader 3	71.4	52.1	90.8
		Reader 4	61.9	41.1	82.7
		Reader 5	81.0	64.2	97.8

The sensitivity and specificity data of florbetaben (18F) PET scans with the Consensus Panel clinical diagnosis as the Standard of Reference using the images from 236 subjects from Phase 2 Study 311741 (Part B) ranged from approximately 69%-81% for the sensitivity, while the specificity was between 82% and 95% (table 9-5).

Text Table 9-5: Sensitivity and specificity (including normal approximated CI) of the visual assessment of florbetaben PET scans compared to Clinical Consensus Panel as Standard of Reference (full analysis set) – Exact 95% confidence limits

Method key item	No. of TP	No. of TP + FN	Sensitivity [%]	Sensitivity 95% LCL [%]	Sensitivity 95% UCL [%]*	No. of TN	No. of TN + FP	Specificity estimate [%]	Specificity 95% LCL [%]	Specificity 95% UCL [%]*
BR 1	91	116	78.45	69.85	85.54	107	120	89.17	82.19	94.10
BR 2	94	116	81.03	72.71	87.72	98	120	81.67	73.57	88.14
BR 3	90	116	77,59	68.91	84.81	104	120	88.67	79.25	92.18
BR 4	91	116	78.45	69.85	85.54	107	120	89.17	82.19	94.10
BR 5	80	116	66.97	57.71	77.23	114	120	95.00	89.44	98.14

BR --- Blinded Reader

The inter-reader agreement across all 5 readers in the histopathology subgroup was good with a kappa coefficient of 0.69 (95% CI: 0.54 – 0.83). In the MCI subgroup, the inter-reader agreement across all 5 readers was very high with a kappa coefficient of 0.82 (95% CI: 0.73 – 0.91). In the subgroup of probable Ads and HVs recruited in study 311741 part B, it was 0.81 (95% CI: 0.77-0.86).

1.6.3. Discussion on clinical efficacy

The company contends that the clinical efficacy of PET with florbetaben (18F) has been demonstrated by the findings in 2 key studies (14595 and 16034), supported by 6 phase 1 (A42404, 310863, 311722, 91790, 312161 and 312043), 2 phase 2 studies (311741 and 14311) and pooled analyses. The phase III study 16034 which does not recruit patients itself cannot be considered pivotal.

Therefore, there is only one pivotal study (i.e. 14595) to base this application, and then the points to consider for submitting a single pivotal study in support of marketing authorization should be followed (CPMP/EMA/2330/99). The "Guideline on Clinical Evaluation of Diagnostic Agents" (CPMP/EWP/1119/98/Rev 1) and Appendix 1 on Imaging Agents (EMEA/CHMP/EWP/321180/2008) also applies.

The proposed indication is as follows:
"This medicinal product is for diagnostic use only.
Neuraceq is indicated for the detection of β-amyloid in the brain, thereby assisting in the differential diagnosis in adult patients who are being evaluated for Alzheimer's disease and other causes of cognitive decline."

The SmPC recommends a dose of 300 MBq of florbetaben (^{18}F), not exceeding 360 MBq and not falling below 240 MBq of florbetaben (^{18}F), administered intravenously as a slow bolus injection (6 sec/ml) without dilution. The administered volume is up to of 10 mL in order to provide the target activity of 300 MBq. The company originally intended that image acquisition was a 15-20 minute images from 45 to 130 minutes postinjection.

Design and conduct of clinical studies
Florbetaben (^{18}F) is intended for registration for a pathological indication (i.e. with PET imaging for the detection of beta-amyloid in the brain).

The company is also focusing the use of florbetaben (^{18}F) for a concrete diagnostic paradigm (i.e. assisting in the differential diagnosis in adult patients who are being evaluated for AD and other causes of cognitive decline).

For the validation of florbetaben (^{18}F) as an imaging PET agent, two stages have to be considered:
a) An initial phase in which it is established how well (the relevant types of) brain β–amyloid deposition can be visualized and quantified by florbetaben (^{18}F) in the relevant areas
b) Further phase(s) where the efforts are aimed at demonstrating which particular practical purpose(s) the imaging is useful for and how this is achieved, i.e. for a particular use in clinical practice.

PIVOTAL STUDY 14595
Both phases were attempted in the pivotal study 14595 in which the visual detection of beta-amyloid in the brain on PET images was assessed versus histopathology of autopsied samples as a SOT. This approach using autopsy data as standard or truth is justified as it can demonstrate that the results obtained with the investigational diagnostic agent are valid for estimation of plaque detection (even though not for diagnosis of a particular disease).

The clinical use of PET with florbetaben (^{18}F) will rely on its accurate quantitative and topographic assessment of beta amyloid accumulation in the brain. Then, relevant quantitative correlation in quantity and topography of PET images with autopsy specimens should be first demonstrated. Study 14595 did not quantify such correlation.

The clinical value of the estimation (quantity and topography) of beta amyloid deposition in brain is still a matter of discussion at the scientific community. If any (i.e. for designation of a patient as beta amyloid positive/negative or for a definitive diagnosis/exclusion of a specific beta amyloid pathology) any in vivo reliable method of estimation of beta amyloid deposition has to demonstrate its sensitivity/specificity for the particular clinical setting and population in which it is intended. In this sense, the pivotal study 14595 primarily aimed at demonstrating sensitivity and specificity of the amyloid burden in 6 predefined cerebral regions on a region-basis on florbetaben (^{18}F) PET images after particular co-registration with MRI (evaluated by three blinded readers on a binary visual scale as normal/abnormal) versus the quantitative measurement of amyloid deposition at pathology.

A secondary analysis of the pivotal study referred to the composite "whole brain" assessment of PET images – a composite of the 6 pre-defined regions assessed as primary analysis- vs histopathology. This was an attempt to evaluate the "whole brain" and not individual regions.

Additionally, a so-called "subject level" analysis of PET images without co-registration with MRI for estimation of beta-amyloid deposition was also performed (this is the PET reading method to be used in clinical practice). The pivotal study secondarily assessed sensitivity and specificity of subject-level visual PET reading, for classifying a patient as "beta-amyloid present" or "beta-amyloid absent" by using the age-related neuritic plaque density of the CERAD criteria but including more regions than those assessed in the CERAD criteria. This is stated in section 5.1. of the SmPC.

The company a post-hoc analysis for the subject-based visual florbetaben (18F) PET assessment including all those subjects autopsied up to August 1st, 2013 (n=74).

The impact on diagnostic thinking or on patient management of PET with florbetaben (^{18}F) in the intended population and clinical context was not assessed.

Participants underwent a MRI prior or simultaneous to PET to allow appropriate anatomic location of the PET signal, which is of paramount importance in patients with cerebral atrophy that makes PET image interpretation difficult.

Inter-reader variability was also calculated. Intra-reader variability was designed to be assessed in only 10% of patients which, in this study, is a very small sample size for any conclusion to be solid.

Subjects in the confirmatory trial are not representative of the population in whom this diagnostic agent is intended to be used in routine clinical practice (i.e. "adult patients who are being evaluated for AD and other causes of cognitive decline"). They were mainly adults willing to donate the brain and undergoing a MRI and PET scan. Neither a particular cognitive status nor a probability of beta-amyloid deposition was mentioned among the inclusion criteria.

The clinical diagnosis of dementia subtype (if any) in the recruited patients is likely inaccurate as settled only on the basis of previous clinical history and an optional very brief neurological examination, without mandatorily performing other routine laboratory tests to exclude the presence of either significant white matter disease or other non-neurodegenerative dementias. Moreover, such dementia status referred to the time of baseline PET imaging and it might have changed within the time interval between PET and autopsy. Indeed, subjects recruited from brain bank initiatives might have higher probability of familiar AD forms.

The choice of the recruited population for the primary analysis has an impact on the external validity. Although the diagnostic utility in the intended clinical population has to be established, a different population may be justifiable to be recruited if allow assessing the correlation of PET versus histopathology within a reasonable timeframe. In the case of florbetaben (18F), the inclusion/exclusion criteria without a predefined life expectancy do not allow to conclude if autopsies were conducted in a reasonable timeframe after the screening and PET scan. However, the pivotal study was designed to repeat PET scan annually (not mandatory) and therefore the timeframe between PET and autopsy would be 12 months as the maximum.

There were 5 major protocol deviations in the autopsy study, but patients were excluded from the analysis and none in the post-hoc analysis. And, for the 74 autopsied patients included in the post-hoc analysis of the subject-level visual PET assessment, all the protocol deviations are considered minor and not affecting efficacy.

None of the 3 visual PET scan reading methods (on a region level, a "whole brain composite", and on a subject level) was previously optimized for in-vivo detection of brain beta amyloid deposition.

However, they were decided by a step-by-step refinement of the visual assessment methodology during the florbetaben development process.

The visual PET scan reading method on a region basis, and the derived composite "whole brain" method, outlined with a polygon (thanks to the corregistration with MRI) the brain region to be assessed. This is not expected to occur in clinical practice unless PET is mandatorily co-registered with a MRI of the patient before reading.

The visual PET scan reading method on a subject basis, introduced by amendment 5, is closed to the one proposed in the latest proposed SmPC for clinical practice. It is on a subject-level basis and in non-co-registered PET images. It condensed a cortical tracer uptake score in 4 predefined regions in florbetaben (18F) PET scan (without corregistration with MRI) into a single 3-grade scoring system (no, moderate or pronounced tracer uptake) to assess beta-amyloid burden of the brain globally. A global interpretation of the brain was achieved by using the highest score in one of the four brain regions to qualify the scan as without/minor/significant beta amyloid plaque load (the so-called BAPL score). It is transformed into a binary outcome as "beta-amyloid positive" (for scores 2 or 3) or "beta-amyloid negative" (for score 1) to calculate sensitivity and specificity of florbetaben (18F) PET scan. The company acknowledges that the differentiation of the amount of PET tracer uptake (and thereby amyloid deposition) as moderate or pronounced has no marked clinical consequences and was intended for further checking of the disease progression.

PET images were visually interpreted by independent blinded readers, who were in-person trained in the interpretation of such images.

Quantitation of PET images was also performed.

The SOT was a consensus opinion of 3 readers who had to take into account different forms of amyloid deposits (diffuse, neuritic, vascular) measured by either BSS or IHC, with their different frequencies and their relative impact. Both BSS and IHC techniques can be considered well-accepted for evaluating and measuring beta amyloid deposition in the brain post-mortem. However, the frequency score used for measuring the different amyloid deposits was neither previously standardized nor validated. The SOT also suffered from changes by protocol's amendment and from adaptations during the conduct of the study. SOT was therefore not standard for all patients, and has not been justified as of clinical relevance**.** Anyway, the SOT refers to the regional-based visual assessment of florbetaben (18F) PET scan and not to the subject-level basis analysis which is the crucial issue for clinical practice.

The applicant assures that the onsite neuropathologist assessed the autopsy samples evaluating presence/absence of neuritic amyloid plaques according to the CERAD criteria for subject-level sensitivity and specificity calculation in study 14595 (with n=31 subjects, in the analysis of one of secondary endpoints), and that the same method was then extended for the 74 subjects included in the post-hoc analysis. However, although the applicant states that "Neuritic plaque density was determined according to CERAD criteria (Mirra, 1991)", no further confirmation of the assessed regions was initially provided. To this regard, the CERAD criteria used the neuritic plaque density in the most heavily affected neocortical region (based on section with the maximum involvement among middle frontal gyrus, inferior parietal lobule and superior and middle temporal gyri). The applicant has recently clarified that neuritic plaque density was established at the region of maximum involvement of different brain regions: those three of the CERAD criteria (middle frontal gyrus, superior and middle temporal gyri and inferior parietal lobe), hippocampus and other brain regions. This was included in the SmPC.

The original protocol has been amended five times. Amendments 4 and 5 were the more important ones as they introduced changes in critical aspects of the trial. The timing of the amendments was also critical because were approved after recruitment was initiated. Moreover, last amendment was introduced once recruitment had finished and the SoT results were obtained for all 31 collected brains.

The changes introduced to the protocol were as important as the formulation of the main statistical hypotheses for the co-primary endpoints of sensitivity and specificity as well as the definition of the majority read of PET images (amendment 4). Last amendment modified the secondary objectives adding a "whole brain composite" endpoint and deleting the subject level comparison with the onsite clinical diagnosis. The training and visual scoring procedure for florbetaben (18F) subject level assessment was also modified changing the focus of the visual assessment from a "whole scan" assessment to regional anatomical assessment. These changes could have had a crucial impact on the whole trial result.

Statistical methods in the SAP were also changed to reflect the amendments that were introduced. Originally the protocol was designed to estimate sensitivity and specificity rather than to test any hypothesis around these endpoints. Amendment 4 moved from this estimative aim to a hypothesis testing approach, formulating the null hypotheses for the co-primary endpoints to be lower or equal to 0.6 and 0.8 for sensitivity and specificity of the regional majority read respectively. The hypotheses were rejected if lower limits of the 95% confidence interval were larger than the values at the null hypothesis.

The success criterion (the lower bound of the 2-sided 95% exact confidence interval for sensitivity and specificity being greater than a priori threshold of 60% and 80%, respectively) seems little demanding and has not been justified as of clinical relevance. Furthermore, in previous national scientific advice meetings held in 2011 between the applicant and Health Authorities in the United Kingdom (UK) and Spain it was discussed that the main usage of the tracer was seen to be ruling out rather than verifying AD. In such a scenario, maximisation of sensitivity rather than specificity should have been used in the formulation of statistical hypothesis.

As the company stated that there was a 3-year follow-up of the recruited patients not deceased after the first 32 histopathological autopsies, the CHMP requested at day 120 data obtained from all recruited patients that had already been autopsied by means of a well-designed re-reading study. Instead, the company currently presents a post-hoc analysis of sensitivity and specificity of the visual PET reading vs histopathology in all autopsied patients from the pivotal study until August 1st, 2013 (n=74). Since PET images of all 212 recruited participants were altogether read in a single session by similar blinded readers and were already available for all patients before autopsies, the post-hoc analysis might be an appropriate alternative to the requested re-reading of the images.

However, it might have happened that, while the data used for the post-hoc analysis was that from the PET scan at entry (resulted to be performed 329 days (+/- 272 days; range 75-972 days) before death), the patient might have a later PET scan before autopsy since a yearly repeated florbetaben (18F) PET imaging up to the 3-year follow-up was offered to the participants (but not mandatorily). In fact, there were 15 subjects in whom florbetaben (18F) PET scan were repeated before death. The applicant has not provided with their subject-basis result of the subsequent (second or third) florbetaben (18F) PET scans performed before autopsy. Therefore, it cannot be concluded that the baseline PET scan and subsequent PET scans had no different results. The most valuable PET scan data to be compared with autopsy results are those of the closer PET scan result to death (which may differ about 400 days from the previous one). However, the post-hoc analysis took advantage that initial baseline PET scan had already been read, and therefore reading of subsequent PET scans are not available.

Despite previous advice received by the company that highly recommended a patient-based rather than a region-based analysis for the primary analysis, the primary endpoint in the pivotal study is based on regional reading rather than subject-level readings of PET images particularly co-registered with MRI. This selection is problematic because it is expected to observe highly correlated reading patterns of regions within the same subject. By using randomization with images of single brain regions one at a time regardless which subject, instead of assessing the images of all brain regions of a subject as batch, the within-brain correlation effect was minimized.

The sample size for the study was calculated to demonstrate simultaneously a minimum sensitivity and specificity in a per-region analysis, assuming very optimistic initial values for both co-primary endpoints (e.g. sensitivity and specificity equal to 90%) and without taking into account within brain correlations. Data from previous studies could have well informed the design of the pivotal study by providing estimates of within brain intra-class correlations to aid in these calculations. Although the study has reached the success criteria in the per-region analysis, the sample size is deemed insufficient to draw firm conclusions in a per-subject basis.

The study included 10 HVs to enrich the sample used to estimate specificity. It has been empirically demonstrated that using HV to assess the accuracy of a test significantly overestimates the diagnostic performance of the test. Furthermore, including HVs in the analysis set makes the population to be very different for the intended to use population. In the same line, the study also has included one region that is assumed with known low probability of demonstrating significant β-amyloid deposition in an AD patient (region 6, Cerebellar hemisphere directly adjacent to the cerebellar nuclei). Including this region in the primary efficacy analysis could surely have positively biased specificity estimation.

The company has received previous advices from EMA and FDA regarding the questionable value of including HVs in their confirmatory Phase 3 trial. EMA critiqued the inclusion of young HVs in the pivotal study, as a means to assure recruitment of at least some regions negative for β-amyloid deposition, since the study results may be biased towards high specificity for detection of β-amyloid deposition. The company was also advised to only include HV as a form of sensitivity analysis being the primary analysis performed without HV individuals. The company performed an analysis excluding the HV in from the sample and the results of this post-hoc analysis confirmed the high sensitivity and specificity of the product.

The quantitative correlation between *in vivo* quantitative values of brain uptake of florbetaben (18F) and quantitative levels of amyloid deposition at autopsy has been neither calculated nor demonstrated**.** The approach used to this quantitative analysis of SUV was based on selecting optimal cut-off points to dichotomize SUV as normal and abnormal using ROC curves and summarize the results using sensitivity and specificity accuracy indices, instead of using more comprehensive quantitative statistical methods to assess the correlation (i.e. multi-level mixed effects models). This quantitative correlation could have been easily obtained for both neuritic plaques and also diffuse plaques since both plaque types were visualized and counted by researchers using BSS. In addition, researchers using immunohistochemistry measured total β-amyloid using anti-amyloid antibody that stained beta-amyloid found in both neuritic and diffuse plaques and in vascular amyloid.

Secondary efficacy analysis assessing the agreement of blinded readers was performed using kappa-statistics. This approach is deemed correct when the subject (i.e. “whole brain”) was the observational unit. However, when a brain region was the observational unit, i.e. where one subject contributed more than one observational unit, the correlation between regions should be taken into account in the analysis. By using randomization with images of single brain regions one at a time regardless which subject, instead of assessing the images of all brain regions of a subject as batch, the within-brain correlation effect was minimized.

POOLED ANALYSES

The company presents pooled analyses of the studies composing the florbetaben (18F) clinical development programme, as supportive. However, these analyses involved a mixture of populations, different SOTs/SORs, different PET reader training methods, different number of readers and different primary objectives. Therefore, the pooled analyses do not provide any additional accurate information of efficacy of PET with florbetaben (18F) to the efficacy results of the individual studies.

When assessing the quantitative uptake of florbetaben (18F) PET scan (SUVR) in non-demented volunteers aged < 55 years and ≥ 55 years and AD subjects, the pooled analysis has arisen a marked age effect. This effect could have a decisive impact in the quantitative analysis of the SUVR

(secondary outcome in the pivotal study 14595). Such analysis should consider age as a confounder. This is critical when specificity is analysed in the pivotal study. Around half of the participants providing information to compute specificity were HV younger than 40 years. Unfortunately, a protocol violation occurred in all this HV (protocol violation listing) which makes impossible to know the quantitative results of these PET images.

STUDY 14311

Enrolled subjects in this supportive study do neither encompass the overall anticipated population in which florbetaben (^{18}F) will be performed nor even in whom the product is requested to be indicated. Indeed, the type and frequency of beta-amyloid deposition in DS might be different from that in the intended population. The company studied DS individuals as a surrogate for the intended population (i.e. AD patients). However, it is unclear if both-amyloid deposition and the radiopharmaceutical behavior is similar in both entities. As a construct to show that in other "plaque producing" conditions the tracer under investigation also labels beta amyloid in patients without doing so in matched controls, DS can be considered as useful supportive evidence. Its value for estimation the clinical diagnostic performance in patients with potential AD appears limited.

In the DS group aged at least 40, and considering that virtually all DS individuals develop amyloid pathology over at that age, the expected sensitivity higher than 70% is little demanding.

Study 311741

The Company intends the role of florbetaben (^{18}F) to assist in the differential diagnosis in adults who are being evaluated for AD and other causes of cognitive decline. There is very limited clinical data to address the issue coming from the supportive study 311741.

This study was primarily aimed to evaluate the sensitivity and specificity of a visual interpretation of florbetaben (^{18}F) PET images (from the 90 to 110 min imaging window after administration of 300 MBq) in detecting/excluding cerebral beta-amyloid in patients with probable AD compared to HVs versus the clinical diagnosis.

Enrolled subjects in this supportive study do not encompass the overall anticipated population in which florbetaben (^{18}F) were recruited. Indeed, not all tests standardized for management of patients suspected of AD (e.g. blood tests, …) were followed to confirm the absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive cognitive impairment. Moreover, the clinical diagnosis is not an appropriate SoT to support claims for AD diagnosis due to its well-known lack of diagnostic performance. For all these reasons, results from study 331741 cannot reliably be extrapolated to the proposed indication for use. Furthermore, the study was designed to include HV to estimate specificity. It has been empirically demonstrated that diagnostic accuracy studies designed to include HV tend to overestimate the diagnostic performance of the test being evaluated. Thus, these case-control studies cannot proportionate reliable estimations of the accuracy indices.

STUDY 312043

The company presents this supportive study recruiting exclusively patients with MCI of non-obvious cause based on predefined criteria. Definitive conclusions regarding the relative progression to AD over a 2-year period of normal PET and abnormal PET populations of MCI might attempt to support a prognosis indication, which is not the intended use requested for florbetaben (^{18}F) at the moment.

Sensitivity and specificity of visual PET reading for detection of AD and the conversion rate to AD or to normal were assessed. Inter-reader and intra-reader reproducibility for the visual interpretation of florbetaben (18F) PET were not assessed.

STUDY 16034

The Applicant has chosen the electronic training programme to be available to physicians post-approval. The clinical development of florbetaben (18F) includes this phase 3 trial attempting to

evaluate efficacy of florbetaben (18F) PET images after electronic training of PET readers. No patients were recruited and florbetaben (18F) PET image sets were selected from 6 previous florbetaben (18F) clinical trials in subjects with histopathological confirmation of their brain amyloid status, HVs, subjects with clinically AD or other dementias and subjects with aMCI.

The visual PET scan reading method used in this study is a subject level method (yielding a result of normal or abnormal for each subject after assessing 5 pre-defined regions) and the Applicant states that in general is identical to the one that is intended for clinical practice.

The methodology of the pivotal study was changed in this study. Primary endpoint of the pivotal study was relegated to a secondary one due to the small sample size (which was contrarily higher than in the pivotal study). The SOT was also changed without any justification. The success criterion was asymmetric as in the pivotal study but less demanding (i.e. success criterion for specificity was to be higher than 70% instead than 80% as in the pivotal study).

Validation of the electronic programme in study 16034 was attempted in this study combining different populations, using different statistical analyses and different reference/truth standards. For this study, only sensitivity and specificity from the population subgroup matching that of the autopsy study population (including 10 HVs), the pAD/HVs in study 311741 part B and the MCI subgroup from study 312043, compared to the corresponding values in the actual studies, were taken into account. For the overall sample, evaluation of sensitivity and specificity mixing different populations diagnosed by different SoTs raise concerns about potential biases of the results. Indeed, the prevalence of beta-amyloid deposition will differ among different populations. For reproducibility, analyses were of interest for the whole sample, and the three mentioned population subgroups.

The intra-reader agreement the population subgroup matching that of the autopsy study in the present study cannot be provided since only 4 patients were included for such objective in study 16034.

The SOT used for establishing sensitivity and specificity of subject-level visual florbetaben (18) PET assessment in the autopsy subpopulation in study 16034 (54 subjects) importantly differs from the SOT for similar assessment in the autopsy study 14595 (original data and post-hoc analysis). The SOT does not match the CERAD criteria (Mirra et al. 1991). It was constructed by using the consensus histopathology panel assessments of the six brain regions for every subject included in this analysis. A brain region was considered to have ‘relevant beta-amyloid present’, if the CP of neuropathology experts judged it as having a final rating of “moderate” or “frequent” for neuritic or diffuse beta-amyloid plaques based on the Bielschowsky silver staining. If in any of the 6 regions beta-amyloid plaques were evaluated as being 'present' at a clinico-pathologically relevant level (either moderate or frequent), the subject was determined as having clinicopathologically relevant beta-amyloid deposition in the brain on a subject-level.

The company currently presents additional data comparing the results of PET scan reading in study 16034 with the onsite pathology assessment collected for all subjects (same as used for the post-hoc analysis).

Other issues related to the clinical development programme

Concerning interpretation of PET images, two distinct methods have been used during the development programme: binary visual (on a region level, a “whole body” composite, or on a subject level) and a quantitative one. However, the applicant has not justified why the particular visual method of PET scan reading has been chosen for recommendation on the SmPC, and was chosen for the pivotal and some supportive studies, instead of the quantitative one which was also performed in most trials if not all.

The visual reading criteria of florbetaben (^{18}F) PET scans as positive or negative has inherent difficulties and is a real challenge. First of all it has the difficulty of reading PET images of the brain, and secondly the difficulty derived from the intrinsic characteristics of a PET amyloid tracer. The criterion for positivity in the visual method is looking at loss of reduction of radioactive signal intensity

between white matter (with invariably high uptake) and grey matter (with either no radiopharmaceutical uptake (if normal) or some level of uptake (if abnormal)). Traditional scanners in use today often lack the fine volumetric resolution and high-contrast ratio required to precisely differentiate between grey and white matter. Because grey and white matters are interlaced in such a compact way, and also due to the short width of the grey matter (about 5 mm), distinguishing both can be challenging. So can accurate interpretation of florbetaben (^{18}F) PET scans based on visual assessment in cases where the uptake in grey matter is insufficient (as for example in borderline cases with insufficient intensity of amyloid deposition, or in cases with reduction of grey matter width as in brain atrophy).

Statistics improve the accuracy of diagnosis beyond that attainable by a human observer who relies on a familiarity with image appearances in both health and disease. The strength of this approach is that, no a priori hypothesis is required about which locations may be affected and the whole volume is automatically analysed. Comprehensive packages are available for statistical comparison of brain perfusion SPECT. The differences between the normal database and the test subjects are expressed and, to help to interpret the differences, functional images are displayed. They may allow for a single subject diagnosis.

A methodology to distinguish white matter and grey matter in PET scans and to quantify the intensity of amyloid uptake in grey matter is important and is potentially achievable nowadays in clinical practice. The company already used a quantitative PET reading methodology in the clinical programme. For all this, the company should develop and validate a quantitative reading PET methodology based on their product.

Technical problems in the scan itself (noise or oversmoothing) or in brain anatomy (levels of atrophy) can affect the anatomical location of the gray matter/white matter border and are important to be considered in the interpretation of a florbetaben (^{18}F) PET scan. MRI or CT scans may be helpful for discerning anatomy in cases in which atrophy or a low quality scan complicate the PET-only image interpretation. The CHMP highly encourage the use of a co-registered recent MRI image or CT scan for the visual interpretation of florbetaben (^{18}F) PET scans, particularly in those cases in whom there is uncertainty about the location of grey matter and grey/white matter border in the PET scan. For all this, the following paragraphs have been included in section 4.2. and 4.4. of the SmPC, respectively.

"*Neuraceq images should only be interpreted by readers trained in the interpretation of PET images with florbetaben (18F). A recent co-registered computed tomography (CT) scan or magnetic resonance (MR) imaging of the patient to get a fused PET-CT or PET-MR image is recommended in cases of uncertainty about the location of grey matter and of the grey/white matter border in the PET scan (see section 4.4. Image interpretation)."*

"Some scans may be difficult to interpret due to image noise, atrophy with a thinned cortical ribbon, or image blur, which could lead to interpretation errors. For cases in which there is uncertainty about the location of grey matter and of the grey/white matter border on the PET scan, and a co-registered recent CT or MR image is available, the interpreter should examine the fused PET-CT or PET-MR image to clarify the relationship of the PET radioactivity and the grey matter anatomy."

Since false-positive cases have already been detected in the subject-level visual PET assessment of the 31 autopsied patients in the FAS population (in a patient with focal atrophy and a patient with frequent diffuse amyloid plaques), and in the post-hoc analysis (1 case of progressive supranuclear palsy, 1 subject with infarct and 1 with Dementia with Lewy bodies), section 4.4. of the SmPC mentions that *"image interpretation errors in the estimation of brain beta-amyoid deposition, including false positive {and false negative}, have been observed."*

Florbetaben (18F) is to be prescribed by physicians skilled in the clinical management of neurodegenerative disorders. Another requirement is that PET scans be read blinded to clinical data as done in autopsy studies. It might acceptable since the clinical status of the patient might influence the

image interpretation if atrophy is suspected. This is included in the SmPC although it will be hard to be fulfilled considering that Nuclear Medicine physicians usually review clinical data to interpret brain SPECT/PECT images.

Due to the difficulties for visual qualitative interpretation of florbetaben (^{18}F) PET scans, it is mandatory to complete an appropriate reader training prior to routine clinical image interpretation. Two distinct methods have been used during the clinical development: an in-person training (in study 14595) and the electronic training (in study 16034). The company clarifies that in the pivotal study 14595 and in study 16304, similar PET reader training was implemented except for the fact that it was in-person and electronic, respectively. This implies that some differences may exist since, at least, in the in-person training the reader could ask the trainer any doubt which he/she may have while it is not possible for the electronic training.

The electronic version is the methodology proposed in clinical practice. The company clarifies that the electronic training programme used during florbetaben (18F) clinical development matches the one that will be available to physicians post-approval. The Applicant has provided with the electronic training version. It was handicapped by lack of qualification of readers after reading and a low number of cases. Moreover, some training cases presented with drawbacks which might falsely influence the image interpretation: images very noisy or very smooth, images of low quality and others. These drawbacks should be addressed in the training programme. The electronic training programme should be improved as follows:

- Relevant information on florbetaben (18F), including the approved indication according to the SmPC, limitations on its use, interpretation errors, safety information and the results of the phase 3 autopsy should be at least included.
- The material should include florbetaben (18F) PET demonstration cases with correct PET scan interpretation by an experienced reader, florbetaben (18F) PET scans for self-assessment, and a self-qualification procedure to be offered to each trainee. Training should include a sufficient number of clearly positive and negative cases as well as intermediate level cases. Cases should be histopathologically confirmed, if possible.
- Expertise and qualification of trainers in the electronic training should be ensured.
- The following aspects should be incorporated:
    - that the visual PET scan reading should be made in a grey scale but not also in an inverse grey scale.
    - The pattern of tracer uptake in persons not suffering from AD should be eliminated
    - Any mention to 11C-PIB or (18F) fludesoxyglucose should be deleted as they are not approved radiopharmaceuticals.
- Any slide referred to research applications should be deleted (i.e. slides 8/17, 12/17 and 16/17 of module 2.1) to avoid confusing users in clinical practice. Slide 8/17 includes still non-validated criteria. Slide 11/17 and 16/17 refer to potential applications which have not been demonstrated yet.
- Slide 12/17 of clinical applications should be modified to include the approved indication but deleting any potential indication which is still neither approved nor concluded.
- Drawbacks which might falsely influence the image interpretation in some training cases (images very noisy or very smooth, images of low quality and others) should be addressed in the training programme since they are not acceptable in clinical practice.

Since residual tracer in the superior sagittal sinus is sometimes observed as incidental finding, as mentioned in the training, this has been included in the non-specific uptake of SmPC.

The company is advised to provide high quality training in all countries where florbetaben (^{18}F) is available, and that the training materials will be available in electronic programmes in clinical practice, and that the different channels will deliver a similar electronic-based method, and that such method is similar to that provided in CD and the one in study 16034.

The final visual assessment method (which is currently proposed in the SmPC and the one in the electronic training) includes visualisation of PET images using grey scale or inverse grey scale.

However, the pivotal study 14595 involved PET reading only on grey scale. Therefore, the inverse grey scale has been removed from the electronic training programme.

The optimal dose of florbetaben (18F) PET images was not determined by specific studies or adequately justified. The company proposed the dose of 300 MBq ± 20% of florbetaben (18F). The initially recommended optimal time window and duration scan at the SmPC (i.e. a 20-min duration scan at 45-130 minutes p.i. of florbetaben (18F)) cannot be justified on the basis of visual PET reading of study A42404. In the pivotal autopsy study, PET imaging for 20 minutes was to start approximately at 90 minutes after the injection based on results of study A42404. Therefore, the time window and duration scan at the SmPC has been modified in agreement with parameters used in the pivotal study.

Efficacy data and additional analyses

Results are discussed hereinafter on the basis of fulfillment of the "Guideline on Clinical Evaluation of Diagnostic Agents" (CPMP/EWP/1119/98/Rev 1) and Appendix 1 on Imaging Agents (EMEA/CHMP/EWP/321180/2008).

DIAGNOSTIC PERFORMANCE

The pivotal study 14595 was focused on evaluating the sensitivity and specificity of visual PET scan reading by 3 independent blinded readers, using the post-mortem histopathological estimates of beta amyloid deposition as SOT. This approach using autopsy data as SOT is justified as it can demonstrate that the results obtained with the investigational diagnostic agent are valid for estimation of plaque detection.

From 216 subjects who underwent florbetaben (18F) PET imaging, both 10 HVs and the first 31 subjects who died and underwent brain autopsy were considered evaluable for the SOT assessment (FAS). Apart from the HVs, the remaining participants do not represent the intended population for clinical use of this radiopharmaceutical in clinical practice: they were older (more than 80 years of mean, and 62-97 of age range), and they already presented with clinical AD at study entry for long time (mean of 69.6 months; range:17-300) or as much as 18.2% of them (n=4) had a family history of AD. Moreover, the cognitive impairment of the FAS which was likely set up inaccurately as discussed before, may be different from that of the intended population considering that 10 HVs (24.4%), 22 AD patients (53.7%), 2 with other dementias (5%), 1 patient with DLB (2.5%) and 6 non-demented volunteers (15%) were included. It remains unknown how many recruited subjects actually had MCI or no cognitive impairment at study entry.

This study has some important methodological drawbacks and limitations (see comments aforementioned in "Design and conduct of clinical studies").

When the study was stopped, it was found that actual values (mean and 95%CI) of sensitivity and specificity of the majority read of the visual assessment of regional tracer uptake in the co-registered florbetaben (18F) PET images, in correctly differentiating between brain regions with and without β-amyloid deposition, was 77.4% (95% CI: 65.4%–89.4%) and 94.2% (95% CI: 88.6%–99.8%), respectively, for a total of 244 regions analysed. Because the lower bound of the 2-sided 95% confidence interval was >60% for sensitivity and >80% for specificity (the predefined criteria for study success), the primary objective of the study was met. There were 8 false-positive cases, and also 24 false-negative cases (13 out of the 24 resulted from regions 3 (hippocampus) and 6 (cerebellum)).

Overall, sensitivities and specificities of the visual assessment of regional tracer uptake in the florbetaben (18F) PET images, in correctly differentiating between brain regions with and without β-amyloid deposition, were equal than those for the majority for reader 1: 80% (95% CI: 67%-93%) and 91% (95% CI: 84%-98%), respectively. So were for reader 2: sensitivity of 81% (95% CI: 72%-90%) and specificity of 91 (95% CI: 85%-98%). However, reader 3 showed sensitivity as low as 59% (95% CI: 47%-72%), and specificity of 92% (95% CI: 85%-99%). Sensitivities of the majority read also varies significantly between brain regions (0% region 6, 57.1% region 3, 81.8% region 5, 85.7% region 1, 88.9% region 2 and 90% region 4).

Using the composite “whole brain” for florbetaben (18F) PET and for autopsy, derived from combining results of the 6 particular regions used for the primary objective, increased sensitivity but decreased specificity for the majority read were obtained: 87% (95% CI: 73.2 – 100%) and 88.9% (95% CI: 74.4% – 100%), respectively. For this analysis, 95% CI were computed using a normal approximation. When the more appropriate exact confidence interval limits are computed, lower limits of the 95% CI are 66.4% and 65.3% for sensitivity and specificity, respectively.

Using the subject level assessment of non-co-registered florbetaben (18F) PET images (which is the one recommended in clinical practice), determined secondarily in the pivotal study in the first available 31 autopsied patients excluding HVs versus the onsite neuropathological diagnosis (until September 2011), the sensitivity and specificity of the majority read were: 100% (95% CI: 80.5% – 100%) and 85.7% (95% CI: 67.4% – 100%), respectively. Therefore, the visual subject-level method partially met the study hypotheses set up as the lower limit of the 95% confidence interval of sensitivity had to be higher than 0.6 and for specificity higher than 0.8. Considering also the 10 HVs, 17 patients were classified as beta-amyloid while there was no beta-amyloid in 24 cases. No false-negative and 2 false-positive cases existed (in a patient with focal atrophy and a patient with moderate amounts of neuritic plaques and frequent diffuse amyloid plaques).

In the post-hoc analysis, the sensitivity and specificity of the majority read of the visual subject-level assessment of florbetaben (18F) PET versus histopathology in the 74 subjects of study 14595 who had already died and be autopsied by August 1st, 2013 was 97.9% (95%CI: 93.8%-100%) and 88.9% (95%CI: 77%-100%). The confidence intervals were narrower than in the original analysis. The previously mentioned hypothesis in the original pivotal study was again met for sensitivity but not for specificity. In this sense, the crucial factor for the beta-amyloid PET imaging is achieving a reasonable level of sensitivity on a subject-level basis to exclude the presence of relevant beta-amyloid deposition in order to be able to exclude AD. 47 patients were classified as beta-amyloid present while there was no beta-amyloid in 27 cases. For the majority read, 1 false-negative and 3 false-positive cases existed (1 case of progressive supranuclear palsy, 1 with infarct and 1 with Dementia with Lewy bodies).

Study 14311 has limited value to draw firm conclusions on the diagnostic performance of visual PET scan reading in the intended population, as discussed previously. Moreover, the expected sensitivity of the visual assessment of the florbetaben (18F) PET images in detecting cerebral beta-amyloid in individuals with DS compared to HVs was far to be reached neither by the majority read nor by individual readers, expect in some subanalyses of DS aged at least 46 or 50. Specificity in young HVs was higher than expected (at least 94.3%).

Study 311741 represents a clinical scenario where amyloid imaging (both visual and quantitative PET scan reading) is tested to confirm the clinical diagnosis at baseline. The algorithm B was chosen in the first study part (part A) and tried to be validated at the second one (part B). When comparing pAD and HVs, the average sensitivity of visual florbetaben (18F) PET (from the 90 to 110 min imaging window) images was low (74.8%) with a high specificity (96.1%) versus the clinical diagnosis by the onsite investigator and considering positive PET scan if at least 1 out of 8 predefined regions presented with pronounced (not minor) beta amyloid deposition (part A of the study).

If using the majority read and considering a PET scan as positive if at least 1 out of 8 predefined regions presented with minor or pronounced beta amyloid deposition instead, sensitivity slightly increased (79.5%) but specificity decreased (91.2%) in that population of study part A. In an attempt to validate this approach in the study part B recruiting another sample of also pAD and HV, the majority CP reading did not allow meeting the success criteria (i.e. sensitivity higher than 65% and specificity higher than 75%).

	Agreement with clinical diagnosis (n=139 AD patients, 118 HVs)
Sensitivity	67.2% (58.7%-75.8%)
Specificity	96.6% (93.3%-99.9%)
Positive likelihood ratio	19.8 (7.5. – 52.4)
Negative likelihood ratio	0.34 (0.26 – 0.44)

As shown, as much as 32.8% of subjects with clinical diagnosis of probable AD with mild/moderate dementia (according to NINCS-ADRDA criteria) were considered negative after visual PET scan reading by the majority of 3 independent blinded readers in a CP. It is slightly higher than the expected prevalence of amyloid-negative individuals in a clinically diagnosed AD population based on some literature reports of the false positive rate of clinically-diagnosed AD subjects versus autopsy (about 20%, Lim et al. 1999, Pearl et al. 1997).

Only 3.3% of older HVs showed a positive PET scan. This observation is inconsistent with literature reports that 13% to 45% of apparently cognitively HVs subjects have significant beta amyloid pathology at autopsy (Hulette et al. 1998, Davis et al. 1999, Price et al. 1999, Schimitt et al. 2000; knopman et al. 2003, Aizenstein et al. 2008).

In Study 312043 , on visual baseline PET reading for detection of MCI versus the baseline clinical diagnosis, based on the BAPL score on subject basis on the 90-110 min florbetaben (18F) PET image, there were 29 MCI patients with a positive PET scan while 16 MCI patients had a negative scan. At the 45-60 time window, values were worst. The observation that 64.4% of MCI subjects were positive by florbetaben (18F)-PET scan is consistent with the autopsy literature that shows 33% to 62% of MCI subjects are positive at post-mortem examination (Bennett, 2005; Petersen, 2006).

At the 24-month follow-up (see table below), the assessment of progression from MCI to AD was done for all 45 subjects although only 36 were performed a third florbetaben (18F) scan at the follow-up:

- 19 out of the 45 (42.2%) converted from MCI at baseline to AD 24 months later, independently if they had a positive or a negative florbetaben (^{18}F) PET scan. The remaining subjects, i.e. 26 (57.8%), kept stable or regressed to cognitively normal
- Out of the total of 29 MCI subjects who had a positive PET scan, 19 (65.5%) were classified clinically as converted to clinical AD after 24 months.
- Based on the CHMP's calculations: sensitivity of florbetaben (18F) scan to show the MCI conversion rate to AD in 19 converters was 100%, specificity in 26 non-converters was 61.5% (95% CI: 42.8%-80.2%), positive likelihood ratio was 2.60 (95% CI: 1.60-4.23) and negative likelihood ratio was 0 (0.06-0.84). The design of this study does not allow estimating the risk of MCI progression to clinical AD.

		MCI unchanged plus MCI to CN	MCI to AD	Number of subjects
Month 24	Subjects with baseline PET positive, No (%)	10 (34.5%)	19 (65.5%)	29
	Subjects with baseline PET negative, No (%)	16 (100%)	0 (0%)	16

The corresponding cut-off values (ROC curve defined) and resulting sensitivity, specificity, positive and negative predictive values demonstrated that the SUVR (in the study using a threshold of cortical

SUVR=1.4) showed a good ability to distinguish subjects that progress to AD from those who do not over a 2-year period.

In study 16034 the diagnostic performance (sensitivity and specificity) of the visual interpretation of PET images, on a subject basis and considering the CP majority read, versus histopathology in the 54 subjects of study 14595 who had already died and be autopsied by 19 may 2012 was lower (sensitivity range: 77.5% to 90% and specificity range: 62.5%-85.5% -excluding HVs) than in the pivotal study (sensitivity: 100% (95% CI: 80.5 – 100%) and specificity: 85.7% (95% CI: 67.4% – 100%) when the same subgroup population was assessed by different readers with different training methods. On the analysis on a subject basis, the combined hypothesis that sensitivity is smaller or equal to 0.6 and specificity was smaller or equal to 0.7 could only be rejected for one out of the 5 readers, why the goal to reject this hypothesis for at least 3 out of the 5 readers was not met. Two reasons why the goal to reject the hypothesis failed have been provided. The small number of beta-amyloid negative subjects (as well as false positives) has an influence on the calculated specificity with a larger confidence interval and 95% LCI lower than for a larger sample size. The brains of the end-of-life patients can be expected to include more frequently structural abnormalities (thinner cortex layer, spill-over effects from white matter with false positive visual read assessments as a result). As for another beta-amyloid PET tracer approved for the same indication, CT- or MRI-co-registrations may be applied to enhance accuracy of visual readings in defined cortical brain regions.

There were 6 false-positive cases and 5 false-negative. False-positive cases occurred in a young HV, cases with low grey matter/white matter contrast, marked atrophy, intense patient motion and heterogeneous pattern of uptake in the same lobe. False-negative cases occurred in cases with moderate diffuse plaques.

The company currently presents additional data comparing the results of PET scan reading in study 16034 with the onsite pathology assessment collected for all subjects (same as used for the post-hoc analysis). For the majority read, sensitivity and specificity are 100% (95%IC: 89.4-100%) and 71.4% (95%IC: 52.1-90.8%), respectively.

The values in the population from study 311741 part B were slightly different that those obtained in the individual study: sensitivity (69%-81% versus 62.9%-71.6% in the original study) and specificity (82%-95% versus 94.9%-97.5% in the original study). In the MCI subjects from study 312043, the sensitivity and specificity for the conversion rate to AD over 24 months were slightly higher to those obtained in the individual study: sensitivity (89%-95% versus 85.7% in the original study) and specificity (65%-77% versus 63.6% in the original study).

The differences between this study and the individual ones might be a consequence of the formal training (electronic vs in person), of the selection process of candidates to participate as readers in the study and, in the autopsied population, of the section of a different SOT.

TECHNICAL PERFORMANCE AND PRACTICABILITY

Image quality

Data on image quality from the phase 2 and 3 trials have not been provided. The company should elucidate if image quality was an endpoint assessed in phase 2 or 3 trials. Data was obtained in the phase 1 study A42404 (see section “Pharmacodynamics”).

Reader confidence

Data on reader confidence from the phase 2 and 3 trials have not been provided. The company should elucidate if reader confidence was an endpoint assessed in phase 2 or 3 trials. Data was obtained in the phase 1 study A42404 (see section “Pharmacodynamics”).

Inter and intra-reader concordance

In the pivotal study, the agreement of blinded readers across the 6 brain regions measured by kappa coefficient across all 3 readers was substantial (kappa=0.658). However, this analysis is inadequate

because it has not taken into account the within subject correlation observed among regions. Although agreement across regions between pairs of readers is similar among the three different pairs of readers, this agreement varies greatly in some regions. For example, for region 6, kappa coefficients range from -0.025 for readers 1 and 3 to 1.00 for readers 1 and 2.

Inter-reader agreement computed in the study 14311 in DS and HV subjects was only moderate (kappa=0.55) and remained as such in the various analyses performed. The intra-reader agreement was substantial (kappa=1.00) for 2 of the three readers and poor in the third reader.

In the electronic training study, inter-reader agreement for the whole sample combining various diseases was higher than expected: 0.787 (CI: 0.750 – 0.824) across all five readers (the lowest one being 0.677 (95% CI: 0.609 – 0.744) between readers 2 and 5). The intra-reader agreement was established in a low sample size of 0.957 (95% CI: 0.872 – 1.041) for reader 4 and the lowest being 0.823 (95% CI: 0.657 – 0.989) for reader 1. Inter-reader agreement of visual assessment on subject basis of the florbetaben (18F) PET scan for the autopsy population subgroup (kappa=0.69) were lower to those obtained in the autopsy study (kappa=0.87). The company justifies the difference in part due to the different sample analysed (n=64 in study 16034 vs 41 in 14595) and partly to their different training programme (electronic in 16034 vs in-person in 14595), which is somehow worrisome since it is the training method proposed in clinical practice. Inter-reader agreement of visual assessment on subject basis of the florbetaben (18F) PET scan for the population subgroup of pAD/HVs recruited in study 311741 part B was similar to the values obtained in the original study (0.81 vs 0.86, respectively) .

Test-retest reproducibility

Data on test-retest reproducilibity from the phase 2 and 3 trials have not been provided. The company should elucidate if test-retest was an endpoint assessed in phase 2 or 3 trials. Only some data exist from phase 1 study 312161 (see section "Pharmacodynamics").

IMPACT ON DIAGNOSTIC THINKING AND/OR PATIENT MANAGEMENT

The guideline on clinical evaluation of diagnostic agents (CPMP/EWP/1119/98/Rev. 1) establishes as a requirement on study data for new diagnostic agents, that "……relevant impact on diagnostic thinking and/or patient management in the appropriate clinical context should be demonstrated, if therapeutic consequences of the diagnosis obtained with a new agent are not obvious ….." **.** This applies to florbetaben (^{18}F), although this has not been demonstrated for this diagnostic agent. The company is recommended to perform a study to assess the impact on diagnostic thinking and patient management.

INDICATION

The original wording of the indication refers to

"This medicinal product is for diagnostic use only.

Neuraceq is indicated for the detection of β-amyloid in the brain, thereby assisting in the differential diagnosis in adult patients who are being evaluated for Alzheimer's disease and other causes of cognitive decline."

The first statement (i.e. "*is indicated for the detection of β-amyloid in the brain*") should be approached by the results of technical and diagnostic performances of florbetaben (18F) PET scan reading to estimate the beta amyloid deposition, as assessed in a pivotal study and various supportive studies, which were previously discussed in this report. What an abnormal or normal represents in terms of level of a particular beta amyloid structure should be detailed in the wording. In this sense, CHMP acknowledged that none to sparse neuritic plaque density in the area with maximal neuritic plaque density measured on sections of frontal, temporal or parietal cortex, is incompatible with a definitive diagnosis of AD.

On the other hand, it is already known that moderate to frequent amount of neuritic plaques is present in patients with AD, but also other types of neurologic conditions as well as in older people with normal

cognition. Therefore, this amount per se (even in autopsy or in a positive beta-amyloid PET scan) does not establish a diagnosis of AD. To this regard, the amyloid cascade hypothesis suggests that accumulation of Aβ is the key pathological step in the pathogenesis of AD. However, Aβ deposition is not the only factor of AD. For that reason, pre-specified levels of age-related brain neuritic β-amyloid plaque at autopsy should be integrated with the presence of a clinical history of dementia to arrive at a diagnostic level of certainty with regard to AD (Mirra et al. 1991). β-amyloid plaques may also be present in cognitively normal elderly, patients with MCI, with other dementias (dementia of Lewy Body, Parkinson disease dementia), Niemann-Pick disease type C and severe brain injury.

The wording continues that this radiopharmaceutical is for "*assisting in the differential diagnosis in adult patients who are being evaluated for Alzheimer's disease and other causes of cognitive decline.".* This is worrisome since it might be interpreted as if the product is intended for many different subjects who might be evaluated for AD or cognitive impairment, even those pre-symptomatic familiar AD cases. Therefore, the intended population should be appropriately defined as adult patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment.

In this corrected intended population, the radiopharmaceutical intends to assist in the differential diagnosis. That implies not only that subjects with cognitive impairment might be differentiate from those with dementia (there are no data on the value of florbetaben (18F) for such approach) but also to differentiate among different types of dementia (only few comparative data on brain uptake phase 1 study A42404) or to differentiate among different types of cognitive impairment (no efficacy data provided with florbetaben (18F)).

Florbetaben (18F) PET scan should be used as an adjunct to other diagnostic evaluations, and not as a stand-alone test. As this product cannot replace to clinical assessment of patients, the indication should be reworded from the very beginning to state that florbetaben (18F) should be used in conjunction with clinical assessment.

The safety and efficacy of florbetaben (18F) have not been established for predicting the development of dementia or other neurological conditions, or for monitoring response to therapies". This is crucial since it may trigger decisions that patients with clinical features of AD may not be treated on the basis of these findings.

Since this radiopharmaceutical has not demonstrated efficacy for any particular diagnostic objective of a real validated internationally-recognised disease, as usually a radiopharmaceutical is granted, limitations of the radiopharmaceutical are of major interest and have been included in the indication wording as a cross-reference to the full information stated in section 4.4. of the SmPC."

Taking all this into account the following revised indication is accepted:
"This medicinal product is for diagnostic use only.
Neuraceq is a radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β-amyloid neuritic plaque density in the brains of adult patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment. Neuraceq should be used in conjunction with a clinical evaluation.
A negative scan indicates sparse or no plaques, which is not consistent with a diagnosis of AD. For the limitations in the interpretation of a positive scan, see sections 4.4 and 5.1."

with the following additional wording in section 4.4.
"Limitations of use
A positive scan does not independently establish a diagnosis of AD or other cognitive disorder since neuritic plaque deposition in grey matter may be present in asymptomatic elderly and some neurodegenerative dementias (Alzheimer's disease, Lewy body dementia, Parkinson's disease dementia).
For the limitations of use in patients with mild cognitive impairment (MCI), see section 5.1.

The efficacy of Neuraceq for predicting development of AD or monitoring response to therapy has not been established (see section 5.1)."

and including in section 5.1. the results of study 312043 recruiting subjects clinically diagnosed as MCI who underwent baseline florbetaben (18F) PET scans and were followed for 24 months to evaluate the relationship between florbetaben (18F) imaging and changes in diagnostic status. The diagnostic performance values of florbetaben (18F) for detection of MCI versus clinical diagnosis at baseline, and for the conversion rate from MCI to clinical AD at follow-up should be included.

EFFICACY IN SUBPOPULATIONS
The black subpopulation was not sufficiently represented in the clinical studies restricting the conclusions on potential differences in the diagnostic performance results based on ethnicity. Although differences in clinical presentations (frequency or survival, of AD and other types of dementia) has been suggested in different patient populations with different ethnic backgrounds in the literature, there is no evidence for differences in the presence or number of plaques, tangles, Lewy bodies, infarcts, or amyloid angiopathy, amyloid plaques and NFTs in neuropathologically confirmed AD among African American and white subjects of similar clinical dementia severity.

No efficacy subanalysis has been presented for the use of florbetaben (^{18}F) in patients with atypical presentations of AD (asymmetric, frontal variants, posterior cortical degeneration and a single positive abnormal region).

The paediatric use of this radiopharmaceutical has neither been assessed nor expected. A product-specific waiver for paediatric studies was granted.

The company provided with the results of the re-reading study. This study included 82 autopsied patients from the pivotal autopsy study 14595 and 10 HVs considered amyloid-negative. The main objective was to assess the efficacy (sensitivity and specificity) of the visual assessment method proposed for clinical practice of florbetaben (18F) PET scans, by five new electronically trained readers (as it is intended to be used as part of the future training for users), in the detection of beta-amyloid neuritic plaques in the brain.

Subject-level sensitivity and specificity of florbetaben (18F) PET of those 82 autopsied patients excluding HVs, using as SoT the presence (moderate or frequent) or absence (none or sparse) of amyloid neuritic plaques as detected by BSS and scored by the histopathology consensus panel according to the CERAD criteria (except for the total regions to be assessed), was a secondary variable. Actual assessed regions are displayed in the table below:

Text Table 7-4: Regions of interest for determination of histopathology as Standard of Truth

Region number	Region name
1	Middle frontal gyrus (coronal plane)
2	Striate and parastriate areas of occipital cortex (coronal plane)
3	Hippocampus at the level of the lateral geniculate nucleus (coronal plane)
4	Anterior cingulate cortex (coronal plane)
5	Posterior cingulate cortex (coronal plane)
6	Cerebellar hemisphere directly adjacent to the cerebellar nuclei (sagittal plane)

If in any of the 6 regions neuritic beta-amyloid plaques were evaluated as being 'present' at a clinico-pathologically relevant level (either moderate or frequent), the subject was determined as having clinico-pathologically relevant neuritic beta-amyloid deposition in the brain. If in none of the regions

the histopathological findings were assessed as being more than 'no' or 'sparse' neuritic beta-amyloid plaques, the subject was scored as 'no neuritic beta-amyloid present'.

Sensitivity and specificity results were successfully met with the same 3/5 readers exceeding the pre-specified threshold (i.e. the lower limit of the 95% confidence interval for sensitivity and specificity were higher than the thresholds of 0.6 and 0.5 respectively for at least 3 out of the 5 blinded readers) (see table 9-4 below). However, two readers had very low specificity.

Text Table 9-4: Sensitivity and specificity (including normal approximated CI) of the visual assessment of FBB PET scans compared to histopathology (neuritic plaques detected by BSS(CERAD)) as SoT, excluding the healthy volunteers (FAS)

Blinded Reader	No. of TP	No. of TP + FN	Sensitivity [%]	Sensitivity 95% LCL [%]	Sensitivity 95% UCL [%]*	No. of TN	No. of TN + FP	Specificity estimate [%]	Specificity 95% LCL [%]	Specificity 95% UCL [%]*
Blinded Reader 1	49	52	94.23	87.89	100.00	24	30	80.00	65.69	94.31
Blinded Reader 2	51	52	98.08	94.34	100.00	14	30	46.67	28.81	64.52
Blinded Reader 3	47	52	90.38	82.37	98.40	24	30	80.00	65.69	94.31
Blinded Reader 4	50	52	96.15	90.93	100.00	23	30	76.67	61.53	91.80
Blinded Reader 5	52	52	100.00	93.15	100.00	17	30	56.67	38.93	74.40

Note: TP = True positive / FN = False negative / TN = True negative / FN = False negative; LCL = lower confidence limit; UCL = upper confidence limit; CI = confidence interval; SoT = Standard of Truth
Note: * if the approximative confidence limit was >100% it was set to 100%
If sensitivity or specificity was 100%, the exact binomial distribution was used to calculate the 95% confidence intervals
Source: Table 14.2.3/2

In addition, the applicant extended the post-hoc analysis presented in the response to the day120 list of questions from n=74 brains to the currently available n=81 brains (Table 2). The high sensitivity of Neuraceq was confirmed in this analysis. The majority sensitivity for the three readers in the new post-hoc analysis was 96.3 % (ranging from 97.9%-100%) and the specificity was 88.9% (ranging from 85.2%-88.9%). In addition, no difference to the analysis with n=74 brains was observed. The specificity is unchanged, as no new beta-amyloid negative brains were included in the additional 7 brains.

Table 2 Subject level sensitivity and specificity of the visual assessment of florbetaben PET images as compared to onsite neuropathological assessment, by reader (including majority read), FAS of study 14595 and post-hoc analysis with additional brains; without Healthy volunteers

		Post-Hoc (n=74)			Post-Hoc (n=81)		
		Estimate [%]	Estimate [%]	Estimate [%]	Estimate [%]	95% LCL [%]	95% UCL [%]
Sensitivity	**Maj read**	**97.87**	**93.87**	100.00	**96.30**	91.26	100.0
	Reader 1	97.87	93.75	100.00	94.44	88.33	100.0
	Reader 2	100.00	92.45	100.00	98.15	94.55	100.0
	Reader 3	97.87	93.75	100.00	92.30	91.26	100.0
Specificity	**Maj read**	**88.89**	**77.03**	100.00	**88.89**	77.03	100.00
	Reader 1	88.89	77.03	100.00	88.89	77.03	100.00
	Reader 2	85.19	71.79	98.59	85.19	71.79	98.59
	Reader 3	85.19	71.79	98.50	85.19	71.79	98.50

1.6.4. Conclusions on the clinical efficacy

The clinical efficacy of the visual reading of (18F) florbetaben PET images is sufficiently supported by the original results from study 14595, and the post-hoc analysis in which the pivotal study 14595 expanded to include the additional patients. A consistent high sensitivity (>80%), and an increased specificity (95%LCI: 67%; n=31) in a larger patient population group (95% LCI: 77%; n=74) was demonstrated in the results when analysed without the additional 10 HVs. Sensitivity in the analyses exceeded 93% in the post-hoc (n=74 brains) analyses for all three readers.

Sensitivity and specificity to estimate beta-amyloid deposition of florbetaben (^{18}F) was further investigated in one additional study, in which a different set of 5 electronically-trained blinded readers interpreted images from 54 subjects followed to autopsy in the pivotal study. The histopathology criteria did not match the CERAD criteria. The results were lower than the results obtained in the pivotal trial: a sensitivity range between 77.5% to 90% and specificity range between 62.5-85.7%. Inter-rater agreement using Fleiss' kappa values ranged from 0.68 to 0.87. Comparing the results of PET scan reading with the histopathology assessment collected for all subjects (same as used for the original pivotal study and its post-hoc analysis), the majority read sensitivity and specificity were 100% (95%CI: 89.4-100%) and 71.4% (95%CI: 52.1-90.8%), respectively.

The analysis of the results from the "New Read Study" provided additional supportive data and made this subset comparable to the post-hoc analysis data of study 14595, using in-person trained readers.

Correlation of PET versus histopathology was not quantitatively measured. The impact of florbetaben (18F) PET on diagnostic thinking and/or patient management was not adequately assessed and that is why the company would be encouraged to perform a study addressing this issue. Additionally it would be advisable that the company continue to develop and validate a quantitative PET reading methodology based on their product.
Notwithstanding the limitations, discussed in the different sections of this report, it can be concluded that a consistent high sensitivity and specificity in the target patient population group was demonstrated for florbetaben (18F).

1.7. Clinical safety

The primary safety analysis is based on the comprehensive integration of data from all subjects exposed to any amount of florbetaben (18F) in all studies of the clinical development programme (see table 1 in section 3.1.), with some exceptions. The investigator-sponsored phase 1 study (Report A42404) is not included in the pooled safety analysis due to the study design.
It is not stated if there are ongoing florbetaben (18F) studies with safety assessment beside the pivotal autopsy study.

Patient exposure
The pooled integrated analysis of safety is based on 8 studies: 5 Phase 1 studies, 2 Phase 2 studies (of which one study comprises two consecutive independent parts), and a single pivotal Phase 3 study (excluding the 24 subjects discussed in Addendum report PH-36927) (see table 1-19).

Table 1-19: Number of subjects in the integrated safety pool by study

Protocol number	Assigned to study drug		Received study drug		Administration of study drug[b]	
	Florbetaben N = 878 N (%)	Vehicle N = 13 N (%)	Florbetaben N = 872 N (%)	Vehicle N = 12 N (%)	Florbetaben N = 978 N (%)	Vehicle N = 12 N (%)
310863	29 (3.3)	0	28 (3.2)	0	28 (2.9)	0
311722	18 (2.1)	6 (46.2)	18 (2.1)	6 (50.0)	18 (1.8)	6 (50.0)
91790	18 (2.1)	7 (53.8)	18 (2.1)	6 (50.0)	18 (1.8)	6 (50.0)
312161[a]	16 (1.8)	0	16 (1.8)	0	32 (3.3)	0
312043[a]	45 (5.1)	0	45 (5.2)	0	122 (12.5)	0
14311	109 (12.2)	0	109 (12.5)	0	109 (11.1)	0
311741	425 (48.4)	0	422 (48.4)	0	422 (43.1)	0
14595[a,b]	218 (24.5)	0	216 (24.8)	0	229 (23.4)	0

[a] Subjects with multiple treatment periods (Studies 14595, 312043, 312161) are analyzed by period (administration of study drug). Thus, the number of analyzed subjects (administrations of study drug) is higher than the number of subjects who received study drug.
[b] Study data analysis is ongoing.
N = number of subjects
Source: Module 5.3.5.3, Integrated Safety Analysis Part 1, Table 1.1.

Of the 884 subjects included in the integrated analysis pool, 872 subjects received 978 florbetaben (18F) administrations and 12 subjects received vehicle. Few subjects received vehicle (N = 12 doses) or florbetaben (18F) tracer mass dose > 10 microg/injection (N = 46 doses) when compared with the number of subjects receiving a tracer mass dose of ≤ 10 microg/injection (N = 932 doses).

The actual mean radioactivity applied to all subjects treated with florbetaben (18F) was 297.34 ±24.74 MBq (ranging from 169.49 to 374.29). The subjects who received the > 10 microg/injection received 279.17 ± 25.51 MBq and subjects who received ≤ 10 microg/injection received 298.24 ± 24.37 MBq. The mean florbetaben (18F) dose for the total population receiving the drug was 3.30± 9.51 microg/injection, with a mean of 43.54 ± 13.43 microg/injection for subjects receiving the tracer mass dose > 10 microg/injection and a mean of 1.30 ± 1.36 microg/injection for subjects receiving the tracer mass dose ≤ 10 microg/injection.

The final formulation that is proposed for marketing is different from the one used in the single pivotal study. As of April 10, 2013, in the phase 3 study 14595 florbetaben was injected 256 times in 216 patients using the development formulation. Until April 10, 2013, 65 florbetaben injections were done with the new commercial formulation in 60 patients.

The majority of studies included subjects with AD, DS, DEM, or MCI, as well as HVs and NDVs. All HV and NDV subjects from the studies included in the integrated pool are combined into an overall NDV category for analysis as they are all non-demented volunteers.

The mean age of the population was 67.7 ± 15.6 years (range: 21 to 98 years); 70.5% of the subjects (698/990) were ≥ 65 years old. Most study drug administrations were to Caucasian (83.4%, 826/990). More study drug administrations occurred at European sites (41.2%, 408/990), followed by the USA (24.7%, 245/990), Australia (20.3%, 201/990), and Japan (13.7%, 136/990). The population was almost equally divided between males and females (54.2% vs 45.8%, respectively). The mean weight was 71.4 ± 16.0 kg, with a mean body mass index (BMI) of 25.6 ± 4.76 kg/m2. Most study drug administrations in the overall safety population were to subjects with eGFR values of > 60 mL/minute (60.7%, 601/990).

In the total subject pool, 87.9% subjects had taken at least one prior or concomitant medication at study start. The most common drugs taken as prior medication were phsychoanaleptics (462 subjects, 46.7%), analgesics (342 subjects, 34.5%), lipid modifying agents (320 subjects, 32.3%), stomatological preparations (315 subjects, 31.8%) and topical products for joint and muscular pain (314 subjects, 31.7%).

Adverse events

In total, 383 adverse events (AEs) were reported: 18 were reported by 10 of 12 subjects administered vehicle (83.3%), and 365 AEs were reported for the 249 (25.5%) of the 978 subjects receiving florbetaben (18F) – 27 for the 46 subjects being administered mass dose < 10 micro/injection and 338 reported for the 932 subjects administered mass dose ≤10 microg/injection.

Most subject reports of AEs following florbetaben (18F) administration were considered mild (215 of 249) or moderate (31/249) in intensity, with only 3 subjects reporting (3/249) severe AEs (Subject 14595/200040024, respiratory failure; Subject 312042/000400123, injection site pain; Subject312043/000400124, injection site pain). Ten of the 12 subjects administered vehicle had AEs reported, with 9 of them being reported as mild and one reported as moderate in intensity.

82 (8.4%) out of the 978 subjects administered florbetaben (18F), and 8 (66.7%) out of the 12 subjects administered vehicle experienced drug-related AEs. Most of these events were injection site pain (37 florbetaben (18F)-administered and 4 vehicle-administered subjects), injection site irritation (12 florbetaben (18F)-administered and 2 vehicle-administered subjects), and headache (7 florbetaben (18F)-administered subjects). Drug-related AEs occurred most often (63) during the first 30 minutes after injection of florbetaben (18F), and 8 of 18 AEs after injection of vehicle. All drug-related AEs are displayed in table 2-5 by relationship to florbetaben (18F) use and primary SOC.

Table 2-5: Number of subjects with study drug-related TEAEs by primary SOC and PT

System Organ Class Preferred Term	Total florbetaben experience N = 978 n (%)	Tracer mass dose > 10 µg/inj N = 46 n (%)	Tracer mass dose ≤ 10 µg/inj N = 932 n (%)	Vehicle N = 12 n (%)	Overall total N = 990 n (%)
Number of subjects with any drug-related TEAE	**82 (8.4)**	**11 (23.9)**	**71 (7.6)**	**8 (66.7)**	**90 (9.1)**
Gastrointestinal disorders					
Diarrhea	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Nausea	1 (0.1)	0	1 (0.1)	0	1 (0.1)
General disorders and administration site conditions					
Application site erythema	2 (0.2)	0	2 (0.2)	0	2 (0.2)
Catheter site pain	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Fatigue	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Feeling hot	1 (0.1)	0	1 (0.1)	1 (8.3)	2 (0.2)
Injection site discomfort	2 (0.2)	1 (2.2)	1 (0.1)	1 (8.3)	3 (0.3)
Injection site erythema	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Injection site hematoma	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Injection site irritation	12 (1.2)	2 (4.3)	10 (1.1)	2 (16.7)	14 (1.4)
Injection site pain	37 (3.8)	8 (17.4)	29 (3.1)	4 (33.3)	41 (4.1)
Injection site warmth	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Puncture site reaction	3 (0.3)	0	3 (0.3)	0	3 (0.3)
Pyrexia	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Vessel puncture site pain	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Hepatobiliary disorders					
Hepatic function abnormal	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Investigations					
Blood creatinine increased	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Musculoskeletal and connective tissue disorders					
Limb discomfort	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Pain in extremity	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Nervous system disorders					
Burning sensation	3 (0.3)	0	3 (0.3)	0	3 (0.3)
Headache	7 (0.7)	0	7 (0.8)	0	7 (0.7)
Neuralgia	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Tremor	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Skin and subcutaneous disorders					
Hyperhydrosis	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Rash	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Toxic skin eruption	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Vascular disorders					
Flushing	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Hematoma	1 (0.1)	0	1 (0.1)	0	1 (0.1)
Hypotension	1 (0.1)	0	1 (0.1)	0	1 (0.1)

N = number of subjects; n = number of subjects with TEAE/N; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event(s)
For this integrated analysis, MedDRA version 14.1 was used.
Source: Module 5.3.5.3, Integrated Safety Analysis Part 1, Table 3.2.10.

There were also 134 subjects with AEs that the investigator judged to be study conduct-related not drug related. Of these AES, 106 subjects reported injection site reactions (pain, hematoma, irritation and erythema), 9 headache and 16 reported hematoma.

Section 4.8. of the SmPC states that the most frequently observed adverse drug reaction in subjects receiving Neuraceq is injection site pain (3.9% of subjects).

The applicant has presented in a table the different types of local reactions reported for florbetaben with the Preferred Terms according to MedDRA. The most common Treatment Emergent AEs were “injection site pain”, “injection site haematoma”, “injection site irritation” and “injection site erythema”. The reported AEs and ADRs were classified as mild or moderate and resolved in general without sequelae within a few hours after administration of florbetaben. To reduce the risk for local

reactions, the draft SmPC section 4.2 states that Neuraceq should be administered by intravenous slow bolus injection followed by a flush of 10 ml of 0.9 % sodium chloride. In addition, it is stated in 4.2 that the injection of florbetaben (^{18}F) must be intravenous in order to avoid irritation as a result of local extravasation. 'Injection site pain' and 'injection site irritation' are included as important identified risks in the RMP.

The respective adverse reaction profile for the new (commercial) formulation and the development formulation: only 2 ADRs (3.1%) were observed (post PET procedure hypertension / inflammatory papule) with the new formulation and 15 ADRs (5.9%) were observed with the development formulation. There was no hint suggesting that the local tolerability of Florbetaben suffered with the modifications which resulted in the new formulation. None of the ADRs in both groups was a serious ADR.

Serious adverse event / deaths / other significant events

A single death was reported with no causal relation to study drug or study conduct.

Only 3 SAEs were reported. These SAEs included one DEM subject who experienced respiratory failure (Subject 14595/200040024, tracer mass dose ≤ 10 microg/injection), one MCI subject who experienced hemiparesis (Subject 312043/000400130, tracer mass dose ≤ 10 microg/injection), and one subject in the NDV ≥ 55 years group who was diagnosed with a malignant neoplasm via the PET scan on the day of injection (Subject 311722/000000017, tracer mass dose > 10 microg/injection). These were all considered not related to either drug treatment or study procedures. Two of the subjects had their SAEs considered moderate in intensity (the neoplasm malignant and the hemiparesis) and one subject had an SAE considered severe (the respiratory failure).

Laboratory findings

Clinical laboratory investigations (electrolites, coagulations, hepatobiliary and renal parameters) and vital signs were evaluated. The changes from baseline in those parameters were neither considered to be of clinical concern nor drug-related.

Of the 978 subjects receiving florbetaben, 619 subjects had ECG data available for evalution and 25.0% (4/12) had ECGs showing changes of clinical relevance from baseline. No major differences were observed in the mean change in HR from baseline across subject groups. No trends in QRS interval and PQ interval were noted between the subject groups and their respective treatment doses. There is no specific effect of florbetaben (18F) on QT/QTc interval. Changes were small and without clinical significance.

Safety in special populations

AEs were evaluated by the study subpopulations of gender, age, race, region, and baseline eGFR values.

There was no difference in safety across subject group neither by gender, by age (in the range of 21-98) nor by race. Neither difference in safety was detected by geographic region. The black subpopulation was not sufficiently represented in the clinical studies restricting the conclusions on potential differences in the safety results based on ethnicity.

The PK in patients with renal impairment has not been evaluated. However, based on clinical Phase 2 (Study 311741) data including patients with renal impairment, the safey of florbetaben (18F) was not different in this population compared to patients with normal renal function.

No analysis of subjects with altered hepatic function (i.e. elevated serum liver enzymes) has been performed to check any discernible differences in AEs or lab values versus those with normal-range hepatic parameters. However, no safety effects of florbetaben (18F) are expected in impaired hepatic function different than in normal hepatic function population.

In patients with either altered renal function or impaired hepatic function, the higher irradiation in the body caused by slower hepatic and/or renal clearance of the radiopharmaceutical itself or their radioactive metabolites should be taken into account and will be reflected in the SmPC in the sense that careful consideration of the activity to be administered is required.

Florbetaben (18F) is not expected to be used in women of child-bearing potential.

Florbetaben (18F) is not indicated for use in the pediatric (age 18 years and below) population.

Safety related to drug-drug interactions and other interactions

No clinical studies to investigate interactions that may affect safety have been performed for florbetaben (18F). As explained in the section of clinical pharmacology, because florbetaben is administered as a single microdose resulting in low plasma concentrations, no clinically relevant drug interaction with comedications is expected.

Discontinuation due to adverse events

No AE occurred during study dosing that could lead to interruption or discontinuation of study medication.

Dosimetry and radiation protection

Dosimetry analysis was performed in 4 of the safety studies, the investigator-sponsored trial, Study 310863, Study 311721, and Study 91790. The studyA42404 studied the safety of a single dose of 18Flabeled florbetaben (18F) with dosimetry evaluated in 3 HVs. The mean effective dose was found to be 14.70±1.40 microSv/MBq. Study 310863 studied the safety of a single dose of florbetaben (18F) with dosimetry evaluated in 3 HVs. The mean effective dose was found to be 14.3±1.1 microSv/MBq.

Study 91790 and study 311722 evaluated the biodistribution, radiation exposure, and effective dose of florbetaben (18F) in 18 healthy Japanese subjects and 17 healthy Caucasian subjects, respectively. The mean effective dose was found to be 26.90±1.59 microSv/MBq and 19.30±1.40 microSv/MBq, respectively. The mass dose difference (< 5 microg or > 50 to ≤ 55 microg for 300 MBq of florbetaben (18F)) did not affect the biodistribution of florbetaben (18F) and the resulting exposure to radiation. No significant difference in radiation exposure or effective dose was found for the Caucasian subjects of Study 311722 when compared with both earlier studies.

In the SmPC, detailed organ doses for all organs, as calculated using OLINDA software from Caucasian healthy volunteers (n=17), are listed in Table 7. Dosimetry calculations were adapted to the adult model (with a body weight of 70 kg).

Table 7 Estimated radiation absorbed doses from intravenous injection of Neuraceq to Caucasian subjects

Organ	Dose absorbed per activity administered [mGy/MBq]
Adrenal	0.0130
Brain	0.0125
Breasts	0.0074
Gallbladder	0.137
Gastrointestinal tract	
Lower large intestine	0.0351
Small intestine	0.0314
Stomach	0.0116
Upper large intestine	0.0382
Heart	0.0139
Kidneys	0.0238
Liver	0.0386
Lungs	0.0148
Muscles	0.00948
Ovaries	0.0156
Pancreas	0.0139
Red marrow	0.0122
Osteogenic cells	0.0148
Skin	0.00689
Spleen	0.0102
Testes	0.00913
Thymus	0.00892
Thyroid	0.00842
Bladder	0.0695
Uterus	0.0163
Remaining organs	0.0110
Effective Dose (mSv/MBq)	**0.0193**

The effective dose resulting from the administration of a maximal recommended activity of 360 MBq dose for an adult weighing 70 kg is about 7.0 mSv. If a CT scan is simultaneously performed as part of the PET procedure, exposure to ionising radiation will increase in an amount dependent on the settings used in the CT acquisition. For an administered activity of 380 MBq the typical radiation dose to the target organ (brain) is 4.5 mGy.

For an administered activity of 360 MBq the typical radiation doses delivered to the critical organs, gallbladder, urinary bladder, upper large intestine wall, lower large intestine wall, small intestine and liver are 49.3 mGy, 25.0 mGy, 13.8 mGy, 12.6 mGy, 11.3 mGy and 13.9 mGy, respectively.

Frequent bladder emptying should be encouraged after dosing to minimize radiation exposure.

The SmPC does not include specifications related to radiation protection in the context of manipulation and elimination of the radiopharmaceutical by healthcare professionals, and radiation protection for the family body mass.

Post marketing experience

N/A

1.7.1. Discussion on clinical safety

The overall number of patients exposed to florbetaben (18F) in the clinical trials sponsored by the company is small (n= 872), particularly considering the prevalence of AD in the general population. No post-marketing data is available since no marketing authorization had been issued in the world until the start date of this application procedure. The company clarifies that safety data from all patients in the autopsy study who had already been performed a baseline florbetaben (18F) PET scan were included in the assessment.

The majority of subjects in the clinical development program received the dose recommended in clinical practice (i.e. 300 MBq ± 20%). Most of patients received a mass dose of <10 microg/injection. The majority of studies included subjects with AD, DS, DEM, or MCI, as well as HVs and NDVs. Most patients were under prior or concomitant medications.

The final formulation that is proposed for marketing is different from the one used in the single pivotal study. Adverse reactions with both formulations are not serious, and are of somehow similar probability. There was no hint suggesting that the local tolerability of Florbetaben suffered with the modifications which resulted in the new formulation.

Florbetaben (18F) was generally well tolerated. Related AEs were reported in 82 subjects (8.4%). Injection site pain was a common related AEs (n=37). Other related AES occurring in less than 1% subjects were injection site irritation (n=12) and headache (n=7). The company has fully listed all related AEs and their frequency in the safety database, and section 4.8. of the SmPC was modified accordingly.

Only few serious adverse events have been reported (n=3), and none was drug-related. No deaths were attributed to florbetaben (18F).

The changes from baseline in clinical laboratory investigations and vital signs were not considered to be of clinical concern. The company details 4 cases showing a clinical relevant ECG change from baseline following administration of placebo. No clinically relevant increases in QT intervals post administration were observed in any of the four patients. There were some cases in study 311722 and study 91790 of minor or borderline abnormalities in ECG parameters as well as cases of abnormal ECG diagnosis. Most of them were already present at baseline or screening, and those new were observed only in single volunteers at isolated time points and represent minor abnormalities or borderline changes which gave no indication of any possible pattern of treatment-emergent ECG abnormalities.

No immunological events have occurred.

The differences in safety in any subpopulation related to gender, age (in the range of 21-98), race and region are not considered to be of clinical significance. The black subpopulation was not sufficiently represented in the clinical studies restricting the conclusions on potential differences in the safety results based on ethnicity. No analyses were performed in subpopulations related to the concomitant use of AD medications; however, no clinically relevant drug interaction with comedications is expected.

Safety of florbetaben (18F) was not different in patients with renal impairment compared to patients with normal renal function. No analysis of subjects with altered hepatic function (i.e. elevated serum liver enzymes) has been performed. In patients with either altered renal function or impaired hepatic function, the higher irradiation in the body caused by slower hepatic and/or renal clearance of the radiopharmaceutical itself or their radioactive metabolites should be taken into account and will be reflected in the SmPC in the sense that careful consideration of the activity to be administered is required.

Florbetaben (18F) is not expected to be used in women of child-bearing potential.

Florbetaben (18F) is not indicated for use in the pediatric (age 18 years and below) population.

No clinical studies to investigate interactions that may affect safety have been performed for florbetaben (18F). No clinical studies to investigate interactions that may affect safety have been performed for florbetaben (18F). Because florbetaben is administered as a single microdose resulting in low plasma concentrations, no clinically relevant drug interaction with comedications is expected.

The human radiation dosimetry of florbetaben (18F) yields an effective dose of 0.0193 mSv/MBq. It is in the range of other approved radiopharmaceuticals.

Specifications related to radiation protection in the context of manipulation and elimination of the radiopharmaceutical by healthcare professionals, and radiation protection for the family, are appropriate to be included in the SmPC and in accordance with those approved for other fluorine (18F) radiopharmaceuticals.

As of April 10, 2013, in the pivotal autopsy study a total number of 216 patients underwent a first exposure, 82 of these patients had passed their second exposure and 23 patients had received a third PET-scan with Florbetaben injection. Therefore, a total of 321 florbetaben exposures are recorded in the phase 3 trial, which is still ongoing.

Repeat doses of florbetaben were also administered in the study 312043. Up to three doses were administered with an interval of one year between injections. The estimated effective radiation dose of one injection of florbetaben 300 MBq in this study was 4.80 (3.8-5.8) mSv which was below the maximum yearly radiation exposure recommended by the local authorities in Australia where the study was conducted and in the range of other approved radiopharmaceuticals. The effective dose for florbetaben is 0.0193 mSv/MBq. The effective dose resulting from the administration of a maximal recommended activity of 360 MBq dose for an adult weighing 70 kg is about 7.0 mSv. Neuraceq is primarily intended for single use; however if indicated in selected patients a repeat investigation is considered possible provided that there is a sufficient time interval between investigations.

The specifications of use of this radiopharmaceutical in pregnancy and lactation are to be drafted in line with the EMA core SmPC for radiopharmaceuticals. Florbetaben (18F) is not expected to be used in women of child-bearing potential.

The paediatric use of florbetaben (18F) cannot be recommended, and is not expected. A full waiver to perform paediatric investigations was granted.

1.7.2. Conclusions on the clinical safety

Florbetaben (18F) has been studied in a limited number of patients (safety population of completed clinical trials n=872). Overall, there were no significant safety signals identified with florbetaben (18F) PET imaging.

The paediatric use of florbetaben (18F) is not recommended. The use in patients with impaired renal function or impaired hepatic renal function should be prescribed after careful consideration of the higher irradiation to those patients.

Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

1.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 1.2, the PRAC considers by consensus that the risk management system for Florbetaben (18F) (Neuraceq) in the detection of beta-amyloid in the brain, assisting in the differential diagnosis of dementia, is acceptable. However the Applicant is required to consider the comments to the PASS protocols detailed in Section 4.

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

The applicant identified the following safety concerns in the RMP:

Table 2.1 Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	· Injection site pain · Injection site irritation · Carcinogenic and hereditary effects
Important potential risks	· Reactions due to ethanol content of formulation · Injection site extravasation · Hypersensitivity · PET scan interpretation errors · Off-label use
Missing information	· Safety in patients with impaired renal function · Safety in patients with impaired hepatic function · Drug-drug interaction (interaction with disulfiram)

The PRAC agreed.

- **Pharmacovigilance plans**

Table 2.2: Ongoing and planned studies in the PhV development plan

Activity/Study title (type of activity, study title [if known] category 1-3)*	Objectives	Safety concerns addressed	Status Planned, started,	Date for submission of interim or final reports (planned or actual)
Post-authorisation safety study (PAS-1): Effectiveness of Neuraceq reader training and risk minimisation measures (FBB-01_02_13)	To investigate the effectiveness of educational material for PET scan readers and the quality of trained reading (based on validated scans); Examine precision of PIL reading	PET scan interpretation errors Labeling (quality of special warnings and precautions for use)	Planned; first draft of study protocol submitted with revised RMP version 1.2 Nov. 2013 Planned start of study: Q1 / 2015	Interim report planned for Q1 / 2016. Final study report planned for Q1 / 2018.
Ongoing Phase III Trial: 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	Validation of PET imaging method Extension of Safety Profile	Rare adverse drug reactions Risks in specific subgroups of patients	Ongoing (start of trial 2009) Planned study termination: end of 2013	Final study report planned for end of 2014
Post-authorisation safety study (PAS-2): Usage pattern and safety profile of Neuraceq (FBB-01_03_13)	Extension of Safety Profile Usage of Florbetaben (^{18}F) including off label use	Rare adverse drug reactions Risks in specific subgroups of patients	Planned; first draft of study protocol submitted with revised RMP v 1.2, dated Nov 2013 Planned start of study: Q3 / 2014	Interim reports planned for Q1 / 2015, 2016, 2017 and 2018. Final study report planned for Q1 / 2020

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The PRAC also considered that the studies in the post-authorisation development plan are sufficient to monitor the effectiveness of the risk minimisation measures.

- **Risk minimisation measures**

- **Table 2.3:** Summary table of Risk Minimisation Measures

Safety concern	**Routine risk minimisation measures**	**Additional risk minimisation measures**
Injection site pain Injection site irritation	Proposed text in SmPC Method of administration in section 4.2: Neuraceq should not be diluted. The dose is administered by intravenous slow bolus injection (6 sec/mL) followed by a flush of approximately 10 mL of sodium chloride 9 mg/mL (0.9%) solution for injection to ensure full delivery of the dose. See section 12. The injection of florbetaben ($_{18}$F) must be intravenous in order to avoid irradiation as a result of local extravasation, as well as imaging artefacts.	None
Carcinogenic and hereditary risk	Proposed text in SmPC Special warnings and precautions for use in section 4.4: For each patient, the radiation exposure must be justified by the likely benefit. The activity administered should, in every case, be as low as reasonably achievable to obtain the required diagnostic information.	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Injection site extravasation	Proposed text in SmPC Method of administration in section 4.2: Neuraceq should not be diluted. The dose is administered by intravenous slow bolus injection (6 sec/mL) followed by a flush of approximately 10 mL of sodium chloride 9 mg/mL (0.9%) solution for injection to ensure full delivery of the dose. See section 12. The injection of florbetaben (^{18}F) must be intravenous in order to avoid irradiation as a result of local extravasation, as well as imaging artefacts	None
Hypersensitivity	Proposed text in SmPC Contraindications in section 4.3 Hypersensitivity to the active substance or to any of the excipients listed in section 6.1 (ascorbic acid, ethanol anhydrous, macrogol 400, sodium ascorbate) Package leaflet in section 2. Neuraceq must not be used if you are allergic to florbetaben (^{18}F) or any of the other ingredients of this medicine (listed in section 6).	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
PET scan interpretation errors	Proposed text in SmPC Special warnings and precautions for use in section 4.4 Neuraceq images should only be interpreted by readers trained in the interpretation of PET images with florbetaben (^{18}F). A negative scan indicates sparse or no cortical β -amyloid plaques. A positive scan indicates moderate to frequent β -amyloid-plaques. Image interpretation errors in the estimation of brain β -amyloid plaques, including false negatives and false positives, have been observed.	Submission of educational material Training of PET scan readers to avoid false interpretation and subsequent inappropriate treatment of patients Assessment of PET scan readers' training results (in a comparable manner as planned for the PAS-1 study)
Off-label use	Proposed text in SmPC Therapeutic use in section 4.1) Neuraceq is a radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β -amyloid neuritic plaque density in the brains of adult patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment. Neuraceq should be used in conjunction with a clinical evaluation. Package leaflet in section 2. Neuraceq is not intended for use in children and adolescents below the age of 18 years old.	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Patients with renal impairment	Proposed text in SmPC Special warnings and precautions for use in section 4.4 Renal impairment and hepatic impairment Careful consideration of the benefit risk ratio in these patients is required since an increased radiation exposure is possible. Florbetaben (^{18}F) is excreted primarily through the hepatobiliary system and patients with hepatic impairment have the potential of increased radiation exposure. See section 4.2 Pharmacokinetic propertiesin section 5.2.: Renal/hepatic impairment The pharmacokinetics in patients with renal or hepatic impairment has not been characterized	None
Patients with hepatic impairment	Proposed text in SmPC Special warnings and precautions for use in section 4.4 Renal impairment and hepatic impairment Careful consideration of the benefit risk ratio in these patients is required since an increased radiation exposure is possible. Florbetaben (^{18}F) is excreted primarily through the hepatobiliary system and patients with hepatic impairment have the potential of increased radiation exposure. See section 4.2 Pharmacokinetic propertiesin section 5.2.: Renal/hepatic impairment The pharmacokinetics in patients	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Drug-drug interactions (disulfiram)	No proposed text in SmPC	None

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

The CHMP endorsed this advice without changes.

1.9. Significance, Non-Conformity of paediatric studies

- N/A

1.10. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2. Benefit-Risk Balance

Benefits

Beneficial effects

Florbetaben (^{18}F) is a novel radiopharmaceutical which has been developed for imaging β-amyloid deposition by PET in the brain of adults who are being evaluated for AD and other causes of cognitive decline. The definitive diagnosis of AD can only be confirmed post-mortem by detection of pre-specified levels of age-related brain β-amyloid neuritic plaque density at autopsy in the presence of clinical history of dementia. The antemortem initial diagnosis of AD in demented patients relies solely on well-accepted standardised clinical criteria with limited sensitivity and specificity that allow only diagnosing patients as with "probable AD". This clearly shows the high need for better diagnostic procedures for AD in vivo.

Until recently, no method for detection of β-amyloid deposition in the brain during life was approved for diagnostic purposes. In the case where a PET radiopharmaceutical is used for in-vivo imaging β-amyloid neuritic plaques in the human brain in patients who are being evaluated for AD and other causes of cognitive impairment, a negative scan is not consistent with a diagnosis of AD, and a positive PET scan does not independently establish a diagnosis of AD or other cognitive disorder, since neuritic plaque deposition in grey matter may be present in asymptomatic elderly and some neurodegenerative dementias.

For florbetaben (18F), the diagnostic performance was evaluated versus the histopathological diagnosis at autopsy of the β-amyloid deposition in the pivotal study 14595, and versus the clinical diagnosis in two supportive studies.

Diagnostic performance was the primary focus of the pivotal efficacy study 14595 (n=216, 206 autopsy cohort; 10 young healthy controls), which investigated the relationship between uptake on the PET image and the underlying true amyloid levels determined by post-mortem histopathology. Results from the co-primary analyses for the first 31 autopsied patients achieved the pre-specified study

objectives: sensitivity (77.4%; 95%CI: 65.4%–89.4%) and specificity (94.2%; 95%CI: 88.6%–99.8%). The ability to correctly differentiate between brain regions with and without β-amyloid deposition by using visual assessments of regional florbetabene (18F) uptake in co-registered PET based on autopsy data thus clearly exceeded the respective target value of 60% and 80%.

Considering the visual subject-level assessment of non-co-registered florbetaben (18F) PET images (which is the one recommended in clinical practice), sensitivity (100%, 95%CI: 80.5 – 100%) and specificity (85.7%; 95%CI: 67.4% – 100%) of the majority read versus the neuropathological diagnosis clearly exceeded the target value for sensitivity in the first 31 autopsied patients. So did in a post-hoc analysis of the first 74 autopsied patients from the pivotal study: sensitivity (97.9% (95%CI: 93.8%-100%) and specificity (88.9% (95%CI: 77%-100%).

Florbetaben (18F) likely estimates beta-amyloid deposition, and therefore contributes additional information that is not yet included in the current clinical diagnostic standard of AD. In the current status of lack of reliable and validated biomarkers and the non-feasibility of biopsies, this kind of accurate information on amyloid burden is not available by any other approach apart from PET radiopharmaceuticals for beta-amyloid imaging (which, if achieving a reasonable level of sensitivity on a subject-level basis, can exclude a diagnosis of AD). Moreover, information on amyloid burden is made available for the physician at a time when this information may still be useful for patients' management decisions (i.e. prior to autopsy).

Uncertainty in the knowledge about the beneficial effects.

The dossier has not clearly elucidated in vivo how and which of the different types of beta-amyloid and other relevant structures are traced or not traced by florbetaben (18F) in the brain, to assess how useful/misleading tracing them might be for efficacy of the radiopharmaceutical in the proposed indication. In the pivotal autopsy study, correlation between in vivo florbetaben (18F) uptake values and the quantitative levels of amyloid deposition at autopsy was not assessed quantitatively.

Florbetaben (18F) is not a stand-alone diagnostic tool, and needs to be used in conjunction with a clinical evaluation.

Florbetaben (^{18}F) is proposed not only as a marker of β-amyloid deposition in the brain, but also for diagnostic purposes of AD. This objective is pursued in few supportive studies. In those studies, sensitivity and specificity of the visual reading of florbetaben (18F) PET uptake in probable AD and MCI subjects are handicapped by the limitations of the clinical diagnostic criteria used as standard. In supportive studies, the value of florbetaben (18F) was not specifically assessed for the differential diagnosis of either probable AD versus other dementia subtypes, or MCI versus other causes of cognitive impairment.

In addition, the company does not present compelling evidence from longitudinal long-term phase III trials in the intended population to confirm that florbetaben (^{18}F) allows either confirmation of AD pathology (whatever it means) or exclusion of AD in combination with other diagnostic evaluations. This would include differential diagnosis between AD and the most common non-AD dementias likely to be confused with AD (including Lewy body dementia, vascular dementia and some cases of frontotemporal dementia), and diagnosis of AD in those circumstances of particular uncertainty such as early stages of the disease or atypical presentations (asymmetric, frontal variants, posterior cortical degeneration, and a single positive abnormal region).

Intensive research is currently ongoing to move the diagnosis of AD earlier in life. So recently, MCI was proposed as a nosological entity in elderly patients with mild cognitive deficits but without the complete picture of dementia. It is not settled yet if MCI as an episodic memory impaired group is an intermediate stage that a patient with AD will pass through before becoming demented. For florbetaben (18F), very limited data were submitted in MCI patients (only 45 MCI patients from study

312043 assessed for their conversion rate to probable AD over a short 2-year follow-up period), and no data for predicting development of AD or monitoring response to therapy have been provided.

Actual impact of florbetaben (18F) PET on diagnostic thinking and/or patient management has not been demonstrated. Further to this, it remains unclear whether a tangible benefit for the patients can be expected from a change in patient management. Nowadays, in the absence of treatments to stop or revert AD:

- The clinical benefit to the patient brought by early AD diagnosis is unclear
- In false negative cases, omitting/delaying treatment is not crucial, and florbetaben (^{18}F) would not avoid other diagnostic tests (e.g. MRI, CT, blood tests, etc.) since they are generally performed before PET to exclude non-neurodegenerative dementias when suspicious of AD exists.

Risks

Unfavourable effects

As the exposure to florbetaben (18F) is limited, there were no significant safety signals identified with florbetaben (18F) PET imaging. From clinical studies, the most frequently reported treatment related adverse events were all related to time for Neuraceq dose administration as a single iv bolus injection. Accordingly, injection site pain, injection site irritation and injection site erythema were all reported as common. In response to the D120 LoQ, the applicant has discussed the potential local irritating effect of florbetaben based on the TEAEs and ADRs reported. Section 4.2 of the SmPC has been amended with instructions aimed to reduce the risk for local reactions.

Exposure to florbetaben (^{18}F) implies radiation exposure of the patient. It is in the range of other approved radiopharmaceuticals.

Uncertainty in the knowledge about the unfavourable effects

False positive findings in the detection of beta amyloid deposition, with the possible consequence of a wrong diagnosis of MCI/AD (and its consequences), cannot be excluded. The impact of reader's subjectivity on the subjective interpretation of florbetaben (18F) PET images was obvious even in the controlled setting of the clinical trials, and could not be completely eliminated by training. It is unknown what extent of either inter-reader variability or of individual readers with a high rate of wrong readings must be expected in a "real world setting" regardless of any training provided. Similarly, it is unclear to what extent any reached success of the reader's training in the clinical trials may be representative for the effect of a training of users post-marketing. Whereas the radiation exposure appears acceptable, a further minimization might be possible. However, as dose-finding studies of florbetaben (18F) were not performed, it is not clear whether a lower dose might be used with the same imaging quality.

Benefit-risk balance

Importance of favourable and unfavourable effects

As a non-invasive method for visualization and neuroanatomical localization of β-amyloid deposition in the brain antemortem, the potential value of florbetaben (^{18}F) in the diagnostic approach of AD (for differential diagnosis, early diagnosis, other diagnostic purposes) is of paramount importance. However, it is still unknown to which type of amyloid deposition florbetaben (18F) refers to.

With no relevant adverse events, and since therapeutic consequences of the diagnosis of labelling brain beta-amyloid are not obvious, in the absence of both disease modifying treatment in any non-degenerative dementia syndrome and treatments for early intervention to prevent widespread and irreversible neuropathological changes, early misdiagnosis of MCI/AD due to false positive readings of florbetaben (18F) PET images could easily create serious personal/social problems worse than moderately postponing the diagnosis.

Benefit-risk balance

Discussion on the benefit-risk balance

Until recently, histopathological methods in autopsied samples were the only available tool to estimate neuritic plaque density in the brain. The availability of a radiopharmaceutical which could provide estimation on that when the patient is still alive is considered a significant improvement in the diagnostic procedures for adult patients who are being evaluated for AD and other causes of cognitive impairment. In this sense, the presence of none to scarce β-amyloid neuritic plaque density in the brain is not compatible with a diagnosis of AD; however, the opposite is not true since there might be β-amyloid deposition in the brain in asymptomatic elderly and other neurodegenerative demented patients.

The sensitivity and specificity of florbetaben (^{18}F) in detecting the presence of β-amyloid deposition in the brain were convincingly demonstrated by the results from study 14595, the results from supportive study 16034, and the post-hoc analysis in which the pivotal study 14595 expanded to include the additional patients, even though initially there were a number of limitations identified in the primary analysis of the pivotal study 14595. The company performed a re-reading ("New Read Study") of the pivotal autopsy study, with new readers trained by the electronic training. The PET scan reading results were compared to the onsite pathology assessment collected for the 81 subjects. This analysis provided additional supportive data and made this subset comparable to the post-hoc analysis data of study 14595, using in-person trained readers.

Regardless of the fact that it was not shown which type of amyloid deposition was being visualised, and that the correlation between *in vivo* quantitative values of brain uptake of florbetaben (18F) and levels of amyloid deposition at autopsy were not assessed, the available data supported the claim that the radiopharmaceutical can reliably detect the presence or absence of β-amyloid in the intended population (the latter being of paramount importance for the clinical practice).

Unfortunately it was not shown how a change in the diagnosis after florbetaben (18F) PET scan result could lead to either an altered treatment strategy, or other measures translating in a tangible clinical benefit for the patients. For that reason it was recommended that the company assess the impact of florbetaben (18F) PET on diagnostic thinking and patient management.
The risk of false positive readings still remains an issue, which may result in the wrong diagnosis of AD. It should be highlighted that florbetaben (^{18}F) is to be used under "restricted" conditions, as described in the SmPC, and that the acquired PET scans are to be perceived as an additional diagnostic tool rather than the source of medical truth in the diagnostic process.
The safety profile of florbetaben (18F) was reassuring, despite of the relatively low numbers of the available safety database, with the most common adverse events being related to injection site reactions.
Taking into account all of the above, the CHMP considers that the benefit-risk balance of florbetaben (^{18}F) to be used for PET imaging of β-amyloid neuritic plaque density in the brains of adult patients with cognitive impairment, who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment, is positive.

3. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Neuraceq in the following indication:

"This medicinal product is for diagnostic use only.

Neuraceq is a radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β-amyloid neuritic plaque density in the brains of adult patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment. Neuraceq should be used in conjunction with a clinical evaluation.

A negative scan indicates sparse or no plaques, which is not consistent with a diagnosis of AD. For the limitations in the interpretation of a positive scan, see sections 4.4 and 5.1."

is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

Additional risk minimisation measures

Prior to launch in each Member State the Marketing Authorisation Holder (MAH) shall agree the final educational programme with the National Competent Authority.

The MAH shall ensure that, following discussion and agreement with the National Competent Authority in each Member State where NEURACEQ is marketed, at launch and after launch, all physicians who

are expected to use NEURACEQ have access to a training programme in order to ensure accurate and reliable interpretation of the PET images

The training programme should contain the following key elements:

- Information on amyloid pathology in Alzheimer's disease;
- Relevant information on NEURACEQ as an β-amyloid PET tracer, including the approved indication according to the SmPC, limitations of NEURACEQ use, interpretation errors, safety information and the results of clinical trials informing on the diagnostic use of NEURACEQ;
- Review of the PET reading criteria, including method of image review, criteria for interpretation, and images demonstrating the read methodology;
- The training material should include NEURACEQ PET demonstration cases with correct PET scan interpretation by an experienced reader NEURACEQ-PET scans for self-assessment and a self-qualification procedure to be offered to each trainee. Training should include a sufficient number of clearly positive and negative cases as well as intermediate level cases. Cases should be histopathologically confirmed, if possible.
- Expertise and qualification of trainers should be ensured.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

N/A

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that florbetaben (^{18}F) is qualified as a new active substance.