17 January 2013
EMA/30808/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Amyvid

International non-proprietary name: florbetapir (^{18}F)

Procedure No. EMEA/H/C/002422

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature 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

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Acronym	Definition
AB	β-amyloid
AChE-I	Acetylcholinesterase inhibitor
ACRIN	American College of Radiology Imaging Network
AD	Alzheimer's Disease
ADAS	Alzheimer's Disease Assessment Scale
ADCS	Alzheimer's Disease Cooperative Study
ADL	Activities of Daily Living
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADNI-GO	Alzheimer's Disease Neuroimaging Initiative-Grand Opportunity
AE	Adverse Event
ALARA	As Low As Reasonably Achievable
BP	Binding Potential
CAA	Cerebral Amyloid Angiopathy
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CN	Cognitively Normal
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
CT	Computed Tomography
DAT	Dementia of the Alzheimer Type
DICOM	Digital Imaging and Communications in Medecine
DLB	Dementia with Lewy Bodies
DVD	Digital Versatile Device
DVR	Distribution Volume Ratio
ECG	Electrocardiogram
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
FDG	Fludeoxyglucose (^{18}F)
FTD	Frontotemporal Dementia
GE	General Electric
GI	Gastrointestinal
HC	Healthy Control
HPLC	High-Performance Liquid Chromatography
ID	Identity Document
IHC	Immunohistochemistry
IL	Illinois
IRB	Institutional Review Board
IV	Intravenous
KD	Dissociation Constant
LBD	Lewy Body Dementia
LC/MS	Liquid Chromatography-Mass Spectrometry
MAA	Marketing Authorization Application
MBq	Mega Becquerel
MCI	Mild Cognitive Impairment
MedDRA	Medical Dictionary for Regulatory Activities
MID	Multi-infarct Dementia
MMSE	Mini-mental State Examination
MRI	Magnetic Resonance Imaging
NAC	Non-attenuation Corrected
NC	Normal Controls

Acronym	Definition
NIA	National Institute on Aging
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NMDA	N-methyl-D-aspartic acid
NOAEL	No Observed Adverse Event Level
NP	Neuritic Plaques
NPV	Negative Predictive Value
ODD	Other Dementing Disorders
OHC	Old Healthy Control
PD	Parkinson's Disease
PET	Positron Emission Tomography
PIB	Pittsburgh Compound B
PIL	Product Information Leaflet
RAMLA	Row-Action Maximum-Likelihood algorithm
ROI	Region of Interest
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SHRI	Sun Health Research Institute
SmPC	Summary of Product Characteristics
SPC	Summary of Product Characteristics
SPECT	Single-photon emission computed tomography
SUV	Standard Uptake Value
SUVR	Standard Uptake Value Ratio
UK	United Kingdom
US	United States
YHC	Young Healthy Controls

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Eli Lilly Nederland B.V. submitted on 4 January 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Amyvid, 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 28 September 2010.

The applicant applied for the following indication: radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β-amyloid neuritic plaques in the brains of adult patients with cognitive impairment being evaluated for suspected Alzheimer's Disease (AD).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

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 tests or studies.

Information on Paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included an EMA Decision EMEA-001060-PIP01-10 on the granting of a product-specific waiver.

The EMA Paediatric Committee granted a waiver for (E)-4-(2-(6-(2-(2-(2-[18F] fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylbenzenamine on 14 January 2011 (EMEA-001060-PIP01-10) as there is no intended use in the paediatric population.

New active Substance status

The applicant requested the active substance florbetapir ^{18}F contained in the above medicinal product to be considered as a new active substance in itself.

Scientific Advice

The applicant received EMA Scientific Advice on questions on clinical development in 2010 (procedure number EMA/SAWP/201958/2010).

Licensing status

Amyvid was given a Marketing Authorisation in the USA on 6 April 2012.

1.2. Manufacturers

Manufacturers responsible for batch release

Advanced Accelerator Applications-Bethune
126 Rocade Sud
62660 Beuvry
France

Advanced Accelerator Applications (Italy). S.r.l.
Via Piero Maroncelli,40
47014 Meldola (FC)
Italy

Advanced Accelerator Applications-Saint Genis-Poully
20 Rue Diesel
01630 Saint Genis-Poully
France

Advanced Accelerator Applications Iberica
Avda Navarra 3-5 Pol. Ind. La Cuesta, Sector 3
50100 La Almunia de Dona Godina
Zaragoza
Spain

Cyclopharma Laboratories – Glisy
Allee Nautilus
80440 Glisy
France

Cyclopharma Laboratories – Toulouse Canceropole
Voie interne
31000 Toulouse-Langlade
France

PETNET solutions
Heathfield Way, Nottingham City Hospital, Gate 1
Hucknall Road
Nottingham
NG51PB United Kingdom

1.3. Steps taken for the assessment of the product

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

Rapporteur: **Harald Enzmann**

Co-Rapporteur: **Concepcion Prieto Yerro**

- The application was received by the EMA on 4 January 2012.
- The procedure started on 25 January 2012
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 16 April 2012. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 13 April 2012.
- During the meeting on 21-24 May 2012, 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 25 May 2012.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 20 July 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 31 August 2012 and 11 September 2012.
- During the CHMP meeting on 17-20 September 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 26 September 2012.
- The Rapporteurs circulated the Joint Assessment Reports on the applicant's responses to the List of Outstanding Issues to all CHMP members on 8 October 2012.
- The Rapporteurs circulated the updated Joint Assessment Reports to all CHMP members on 10 October 2012.
- During the meeting on 15-18 October 2012, 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 Amyvid on 18 October 2012.

2. Scientific discussion

2.1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, affecting approximately 3.7 to 5.1 million people in the European Union (EU). The current standard of diagnosis based on the current internationally accepted standardized clinical criteria, however, has only a sensitivity and specificity of approximately 81% and 70%, respectively (Knopman et al. 2001). This is a considerable error margin if measured against the gold standard of diagnosis of AD on the basis of pathology: this can be made only after the patients' death and includes autopsy histopathology (Mirra et al. 1991).

Diagnosis and treatment opportunities of AD have been hampered by the absence of reliable, non-invasive markers for its underlying pathology. Given the limitations regarding both accuracy of an AD diagnosis and early identification of AD, a reliable biomarker could increase the validity of a clinical

diagnosis of AD and reduce the frequency of false positive diagnoses by documenting the presence or absence of AD-associated pathological changes.

Recently, the use of AD pathology biomarkers has been included in the new consensus diagnostic guidelines for AD and mild cognitive impairment (MCI) proposed by the National Institute on Aging (NIA) and the Alzheimer's Association. Whereas the original NINCDS-ADRDA criteria (McKhann et al., 1984) assumed that AD is a clinical-pathological entity, the new criteria take into account that AD dementia is part of a continuum of clinical and biological phenomena (McKhann et al., 2011). Accordingly, in the revised NIA-Alzheimer's Association criteria, a semantic and conceptual distinction is made between the AD pathophysiological process (AD-P) and the clinical manifestation (AD-C) (Jack et al., 2011, Dubois et al., 2010). This distinction should be carefully considered when the role of biomarkers is evaluated.

Neither new diagnostic criteria nor potential biomarkers have yet been validated for diagnostic purposes in the context of AD. The best ways to diagnose, stage and follow AD premortem, are matters being actively debated in the scientific literature and consensus has not yet been reached. Controversy also exists on the validity of certain diagnosis referring to cognitive impairment in its pre-dementia stages. It is not yet settled if mild cognitive impairment (MCI), as an episodic memory impaired group, is an intermediate stage that a patient with AD will pass through before becoming demented. On the other hand, the concept of minimal cognitive impairment (as defined by the Petersen Criteria 2003) or the prodromal AD (as defined by Dubois Criteria 2007) reflects a different population.

Recently the CHMP published a number of qualification opinions on the use of biomarkers in the context of AD [1]. These are to be used solely to identify subjects with clinical diagnosis of predementia at increased risk of underlying AD neuropathology or to identify patients with clinical diagnosis of mild to moderate AD, for the purposes of enriching recruitment into clinical trials aimed at studying drugs potentially slowing the progression/conversion to (severe) AD dementia of the included patients, but not for its use as a diagnostic tool or as an outcome or longitudinal measure.

Aβ peptide, as β-amyloid fibrils, and neuritic β-amyloid plaques are a defining component of the neuropathological criteria for autopsy-based diagnosis of Alzheimer's disease. The amyloid cascade hypothesis suggests that accumulation of β-amyloid (Aβ) is the key pathological step in the pathogenesis of AD (Karran et al 2011). However, imperfect correlation between cognitive status and Aβ deposits in brain have been described (Golde at al., 2011), as amyloid deposition can occur as well in normal aging (Davis et al., 1999; Price et al., 1999; Knopman et al., 2003; Aizenstein et al., 2008) and amyloid pathology has been observed in autopsy brains of older persons without dementia (Bennett et al., 2006).

β-amyloid plaques may also be present in patients with MCI, with other dementias (dementia of Lewy Body, Parkinson disease dementia), Niemann-Pick disease type C, and severe brain injury.
This has led to the view that Aβ is only one of the factors that causes AD and that other non Aβ factors also contribute to AD (Pimplikar et al., 2009).

Indeed, pre-specified levels of age-related brain neuritic β-amyloid plaques 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). Although neuritic plaques are a common factor for the post-mortem definitive diagnosis of the disease, the diagnostic value of different brain β-amyloid plaque types (diffuse plaques with pre-amyloid, neuritic and cored), as well as of different β-amyloid isoforms/species (oligomeric, fibrillar or non-fibrillar) may well be different.

Both the degree of β-amyloid deposition, but also its neuroanatomical localization is obviously important for determination of β-amyloid-related pathology in the brain. The characteristic pattern of deposition for

[1] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000319.jsp&mid=WC0b01ac0580022bb0

different stages of typical AD is well-known (Braak et al. 1994). The regional pattern of β-amyloid accumulation is different in typical AD (Edison et al. 2007) compared to other pathological entities that present amyloid deposition.

Only a portion of patients with MCI progress to clinical AD dementia over 5-10 years (Petersen et al., 1999; Ritchie et al., 2001; Visser et al., 2006; Mitchell et al., 2009) and a recent meta-analysis concluded that most people with MCI will not progress to dementia even after 10 years of follow-up (Klunk et al., 2011). In a longitudinal study of 143 MCI cases followed for 4 or more years 43% developed clinical AD, 42% remained cognitively stable and 15% developed other dementias (mostly vascular) (Hansson et al., 2006). Two community-based studies have shown over one-third of patients diagnosed with MCI at baseline may return to normal cognition (Ganguli et al., 2004; Larrieu et al., 2002).

It is agreed that it would be of great value to be able to predict which patients, who upon comprehensive diagnostic testing are found to have cognitive impairment but are not demented and thus do not meet diagnostic criteria for AD (e.g. patients with MCI), are destined to progress to a clinical diagnosis of AD dementia. In this context there is an unmet medical need for non-invasive methods for assessing Aβ pathology in vivo.

About the product

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

PET was chosen as the most suitable imaging modality for imaging β-amyloid because of its superior resolution and sensitivity compared to other Nuclear Medicine techniques, and because of the promising results for PET imaging of Aβ reported in the literature (Klunk, 2004; Nordberg, 2004). Fluorine (^{18}F) was chosen over other positron-emitting isotopes because of its ease for incorporation into potential ligands and well established use in current PET clinical practice. Fluorine (^{18}F) has a longer radioactive half-life (110 minutes) than carbon (^{11}C) and regional preparation and shipping of fluorine (^{18}F) labelled doses is both possible and practical (as demonstrated by fludeoxyglucose (^{18}F)), thus making florbetapir (^{18}F) PET imaging of Aβ potentially available to imaging centres across the EU.

2.2. Quality aspects

2.2.1. Introduction

Amyvid solution for injection is a novel, targeted radiopharmaceutical agent which has been developed for imaging β-amyloid neuritic plaques in the human brain by PET.

The finished product is presented as solution for injection containing 800 MBq/ml of florbetapir (18F) and 1900 MBq/ml of florbetapir (18F) at the date and time of calibration. The composition is described in section 6.1. of the SmPC.

The product is available in clear Type I borosilicate glass vials with FluroTec-coated chlorobutyl elastomeric closures and aluminium overseals. Each vial is enclosed in a shielded container of appropriate thickness to minimise external radiation exposure.

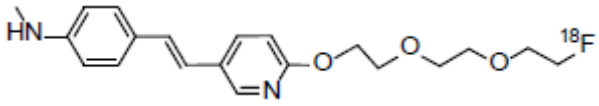
2.2.2. Active Substance

According to Guideline on Radiopharmaceuticals information on chemical precursors including those for synthesis of PET radiopharmaceuticals is presented in a separate section. Such section is submitted and

elaborated by the precursor (AV-105) manufacturer. The submitted data assures an adequate quality of this chemical precursor.

Florbetapir (18F) is a small organic chemical substance containing fluorine-18. The active substance is characterized by radiochemical identity, radiochemical purity, volumic activity, radionuclidic identity and radionuclidic purity.

The chemical name is (E)-4-(2-(6-(2-(2-(2-[18F]fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylaniline, alternative: Benzenamine, 4-[(1E)-2-[6-[2-[2-[2-(fluoro-18F)ethoxy]ethoxy]ethoxy]-3-pyridinyl]ethenyl]-N-methyl4-[(1E)-2-[6-[2-[2-[2-(fluoro-18F)ethoxy]ethoxy]ethoxy]-3-pyridinyl]ethenyl]-N-methyl- and has the following structural formula:



Florbetapir 18F, contains the radioactive isotope fluorine-18 (F-18). F-18 undergoes radioactive decay primarily through emission of a positively-charged beta particle having maximum and average energies of 635 and 249 keV respectively. The half-life of F-18 is 109.77 minutes.

Manufacture

The active substance (Florbetapir (18F)) is not isolated during the manufacturing process. Active substance and Finished Product (Florbetapir (18F) Solution for Injection) are manufactured in one continuous process. One manufacturing process is used at all manufacturing sites.

The Florbetapir 18F manufacturing process is standardized across all manufacturing sites, with minor changes at each site in order to be consistent with local manufacturing operations. However, because active substance and finished product are manufactured in one continuous process, process validation is performed on the active substance/finished product process together.

Specification

The active substance is not isolated during the manufacturing process. Therefore, information on specification is provided in the finished product section.

Stability

The active substance is not isolated during the manufacturing process. Therefore, information on stability is provided in the finished product section.

2.2.3. Finished Medicinal Product

Pharmaceutical Development

The formulation was developed to be compatible with intravenous administration, to contain simple, safe and European pharmacopoeial quality excipients, to solubilise the lipophilic active substance, to allow sterilization of the finished product by filtration and to protect the active substance from the effects of radiolysis.

Two volumic activities were developed, 1900 MBq/ml and 800 MBq/ml in order to obtain the maximum shelf-life and to ensure an injection volume of not less than 1 ml at times shortly after the end of synthesis.

Florbetapir (18F) Solution for Injection formulation contains simple and safe European pharmacopoeial excipients: ethanol, sodium ascorbate, and sodium chloride. Ethanol solubilises the active substance and the used concentration is suitable for intravenous injection, sodium ascorbate protects the active

substance from the effects of radiolysis and sodium chloride is used as isotonic agent. The aseptic steps of the manufacture of the finished product are the assembly of the intermediate and bulk product vials, the sterile filtration of the finished product and diluent and the dispensing of the quality control vials and multi-dose vials. Sterile filtration was chosen instead of terminal sterilization using an autoclave as the finished product is not stable to the conditions for terminal sterilization using autoclave.

The primary packaging proposed is Type I glass vials; a compatibility study was performed to evaluate the compatibility of Florbetapir 18F Solution for Injection with the container closure system.

Adventitious agents

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

Manufacture of the product

The active substance and the finished product (Florbetapir (18F) Solution for Injection) are manufactured in one continuous process in which the active substance is produced using a radiosynthesizer, formulated, and sterile filtered to form the finished product. The active substance (Florbetapir (18F)) is not isolated during the manufacturing process. However, the manufacturing steps comprise the manufacture of Florbetapir (18F) and its preparation as a solution in the finished product matrix. The steps in the finished Product manufacturing process begins in the radiosynthesiser with the last elution step of the active substance from solid phase purification using an excipient solution to gain the active substance in solution as an intermediate of the finished product and result in the finished product after dispensing in multi-dose Vials. The dispensing step is performed in a Class A environment, comprising sterile filtration of the intermediate solution and final formulation (dilution with sterile filtered diluent).

The manufacturing process has been validated by a number of studies for the major steps of the manufacturing process and has been demonstrated to be capable and to be able to reproducibly produce finished product of the intended quality. The in process controls are adequate for this radiopharmaceutical preparation.

The batch analysis data on 3 manufacturing batches at each manufacturing site shows that the product can be manufactured reproducibly according to the agreed finished product specification.

Product specification

The finished product prior to release specifications include appropriate tests for appearance, pH, volumic activity, radionuclidic identity by half life (radioactive half life), radionuclidic identity by gamma energy emission (MCA), radiochemical identity by comparison to external florbetapir F19 Reference standard (Radiometric and UV HPLC), radiochemical purity (radiometric, HPLC), radiochemical impurities (radiometric, HPLC), chemical impurities (UV-HPLC), florbetapir 19F concentration (UV-HPLC), assay of sodium ascorbate (reflectance photometry), assay of ethanol (GC), residual solvents (GC), cryptand 222 (colorimetry), filter integrity (13 mm for finished product and 33 mm filter for diluent), bacterial endotoxins (Ph Eur). The specifications post release are sterility (Ph Eur) and radionuclidic purity (gamma spectroscopy).

Batch analysis results confirm consistency and uniformity of manufacture and indicate that the process is capable and under control.

Stability of the product

Stability data were presented for 9 production scale batches stored at room temperature for up 10 hours in multi-dose vials manufactured at a manufacturing site within each contract manufacturing organization. Additional supporting stability studies were performed to assess the stability of florbetapir (18F) solution for Injection. These additional studies were: stability study of florbetapir (18F) solution for injection in upright and inverted vials at room temperature (25°C± 2 °C) and accelerated condition (40°C± 2 °C), stability study of florbetapir (18F) solution for injection in 10 ml and 50 ml vials at room temperature, stability study of florbetapir (18F) solution for injection stored as a whole batch volume, shipping stability study and stress stability (hydrogen peroxide, sodium hydroxide, high temperature (90°C) and full spectrum light). A stability study was also performed to evaluate the stability of florbetapir (18F) solution for injection at the lower concentration (800 MBq/mL) stored in multi-dose vials stored at room temperature. This study demonstrates that florbetapir (18F) solution for injection manufactured at the minimum concentration is stable for the defined shelf –life when stored at room temperature.

Samples in the stability studies were tested for appearance, pH, volumic activity, radiochemical identity radiochemical purity radiochemical impurities, chemical impurities, florbetapir 19F concentration, and assay of sodium ascorbate.

All studies support that Florbetapir (18F) Solution for Injection is stable for 10 hours.

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

2.2.4. Discussion on chemical, and pharmaceutical aspects

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.

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.

2.2.6. Recommendations for future quality development

Not applicable

2.3. Non-clinical aspects

2.3.1. Pharmacology

Binding of florbetapir ^{18}F to its intended target, β-amyloid, has been characterized in nonclinical studies on brain tissue sections and homogenates from human subjects with pathologically verified diagnoses of neurodegenerative diseases, including AD. The data obtained in these primary pharmacodynamic studies indicate that florbetapir ^{18}F selectively binds to and labels β-amyloid in human brain tissue and, furthermore, that the binding intensity of florbetapir ^{18}F is quantitatively correlated with the density of β-amyloid measured by standard neuropathological techniques. The Kd for the binding of florbetapir ^{18}F to its target, β-amyloid, was measured at 3.7± 0.3 nM. Extensive counter screening on known drug

receptors and binding sites has not identified any other binding site for florbetapir ^{18}F at up to 1000-fold higher concentrations.

There is accumulating evidence that soluble amyloid-β (Aβ) oligomers, rather than amyloid fibrils, are the principal pathogenic species in Alzheimer disease. It is not known if florbetapir (^{18}F) binds to different types of β-amyloid plaque other than neuritic plaque and to different forms of β-amyloid. However, taking into account that the binding of florbetapir (^{18}F) to neuritic Aβ plaque has been demonstrated by the applicant in preclinical in vitro studies, and considering the therapeutic indication, the knowledge of the florbetapir (^{18}F) binding to other different types of Aβ plaque may not be imperative.

The first safety pharmacology study consisted of the testing of florbetapir against a panel of 46 CNS and cardiovascular receptor binding sites from various species. florbetapir bound weakly to the rat peripheral benzodiazepine receptor and the rabbit vesicular monoamine transporter-2 (VMAT-2), although the affinity for these targets was 1000 times lower than for β-amyloid.

Further safety pharmacology evaluations of florbetapir ^{18}F did not reveal any undesired off-target activities. Standardized behavioural studies in rats did not reveal any effects up to and including the highest dose tested, which corresponded to 25 times the maximum human dose (MHD). Specific nonclinical *in vitro* tests on hERG channels and *in vivo* evaluations in dogs for potential adverse effects on the cardiovascular and respiratory system did not reveal any effects up to and including the highest dose level tested (100 times the MHD). Nonclinical radiation safety studies identified a radiation risk similar to that of the approved PET imaging agent ^{18}F-FDG. Pharmacodynamic drug-drug interaction studies conducted *in vitro* did not identify any drugs commonly used in the elderly or prescribed for AD patients, or any experimental AD drugs, which may be able to interfere with florbetapir ^{18}F binding to its intended target, β-amyloid.

No secondary pharmacodynamics evaluation is considered necessary.

2.3.2. Pharmacokinetics

Nonclinical studies on absorption were not conducted. Florbetapir ^{18}F is administered intravenously and is therefore 100% bioavailable.

Nonclinical pharmacokinetics and metabolism studies with florbetapir ^{18}F showed a profile suitable for its intended use as a single-dose IV injectable radiopharmaceutical. Following IV injection in mice and monkeys, florbetapir ^{18}F rapidly entered the brain and quickly washed out from the brain. It was swiftly transformed to two major desmethylated metabolites, which did not show any significant binding affinity to β-amyloid. Thus it is unlikely that metabolites of florbetapir ^{18}F will affect its binding to β-amyloid. *In vitro* metabolism studies using liver microsomes did not reveal any species- or gender-specific differences in metabolism of florbetapir ^{18}F.

No nonclinical data on the excretion of florbetapir ^{18}F were collected. Whole body distribution of ^{18}F from florbetapir ^{18}F injection was studied in humans via whole body PET imaging. The primary route of excretion is through the hepatobiliary system. Based on the short radioactive half-life and the low mass of florbetapir ^{18}F that is administered, the quantitation of ^{18}F collected in urine and faeces was considered impractical and of limited meaning.

2.3.3. Toxicology

The toxicity of florbetapir 19F (the non radioactive version of florbetapir ^{18}F) was evaluated in single and repeat-dose studies under GLP conditions.

Single dose toxicity

In the single dose study, conducted in rats, the animals received 50 and 100 times the MHD, respectively. Half of the animals were necropsied 2 days after dosing, and half of them were allowed to recover for 14 days. The study endpoints included clinical observations, ophthalmology, haematology, clinical chemistry, food consumption, body weight, gross pathology, and histopathology. There were no adverse effects observed in any of the groups. Based on all observations including histopathology, the no observable adverse effect level (NOAEL) was determined to be equal to or greater than the highest dose level tested, ie, ≥ 448 µg/kg or ≥ 100 times the intended MHD of 50 µg/70 kg.

Repeat dose toxicity

The potential toxicity of AV-45 after repeated dose was investigated in rats over 28 days. The animals (10 males/10 females in each dose group) were given 5, 12.5 and 25 times the MHD, respectively. 5 animals of each group were necropsied the day after the last dosing, the others were allowed to recover for 14 days. There were no adverse effects observed in any of the groups, so that the NOAEL in rats was determined to be be equal to or greater than the highest dose level tested, ie, ≥112 µg/kg/day for the 28- day repeated dose (≥ 25 times the MHD of 50 µg/70 kg.

Repeated administration of AV-45 was further investigated in a 14 day and a 28 day study in the dog.

In the 14 day study the dogs (6 males, 6 females in each dose group) received 5, 10 or 25 times the intended MDH. Half of the animals were necropsied the day after the last dose, the remaining ones were allowed to recover for 14 days. Since there was variability in the concentration of the formulated test article, the NOAEL was thus calculated based on the lowest results of the dose verification analysis and estimated to be ≥11.2 µg/kg/day corresponding to ≥8.7 times the MHD.

In the 28 day study the dog (6 males, 6 females of each dose group) were given 8.7 and 25 times the MHD, respectively. Half of the animals were sacrificed at the end of the treatment, the others after a recovery period of 14 day. There were no test article-related changes in any of the parameters evaluated during the study, except of some statistically significant but clinically minor increases in monocytes, fibrinogen, and globulin observed after 2 or 4 weeks of treatment in both sexes. They did not correlate with inflammatory processes by light microscopy and did not show a consistent dose- or time-relationship. They were, thus, not considered clinically relevant. In this study the NOAEL was determined to be equal or greater than 25 times the MHD.

Carcinogenicity

No studies on carcinogenicity have been performed with florbetapir, which is considered acceptable regarding the intended diagnostic use.

Genotoxicity

Potential genotoxicity was tested in both *in vitro* and *in vivo* assays. Bacterial reverse mutation assay results showed positive responses in 2 out of 5 tested strains. The HPL chromosomal aberration assay showed no statistically significant test-article–related increases in the percent of cells with structural aberrations after 3 hours of exposure, but a statistically significant positive result was seen after 22 hours of exposure. In the *in vivo* micronucleus assay, AV-45 produced no evidence of genotoxicity when administered at doses up to the highest practically-achievable dose (83 times the MHD) for 3 consecutive days. The different results in the *in vitro* bacterial mutation and chromosome aberration assays and the *in vivo* micronucleus study are likely related to differences in the exposure conditions encountered by the target cells in the different test systems. *In vivo*, AV-45 is cleared rapidly; however, the *in vitro*

experiments employ static, prolonged exposure of cells to high concentrations of the test article. The 3-hour incubation is substantially longer than the in vivo exposure, due to the rapid clearance of florbetapir ^{18}F from circulation, providing an adequate margin of safety with respect to potential genotoxicity.

AV-45 is positive in vitro in bacteria for mutation induction and in mammalian cells for clastogenic damage. It was negative in the in vivo test in rats. Exposure in vivo is low as no signs of toxicity were seen at the highest dose. Given that florbetapir (^{18}F) is expected to be administered in single doses at very low quantities and its short circulating half-time, the genotoxicity risk for this product is considered to be low.

Reproduction Toxicity

It was not considered necessary to undertake reproductive toxicology studies for florbetapir, because of the target population of elderly male or post-menopausal females patients, additionally a single dose of this rapidly excreted molecule will be administered, and exposure of reproductive organs is limited.
It is extremely unlikely that a pregnant woman would undergo a scan with AV-45, given that the intended population is primarily aged >50 years. However, appropriate statements are included in the SmPC, based on the recommendations in the CHMP core SmPC for radiopharmaceuticals.

Toxicokinetic data

In the clinical studies there are not metabolites at exposures >10% of total drug-related exposures, with metabolites present in circulation at levels of ~4.6% of the original injected 18F dose after the first five minutes following injection, with each individual metabolite present at <2% of the original injected dose. Therefore, no additional nonclinical characterization is necessary.

Other toxicity studies

No other toxicity studies eg in juvenile animals or on immunotoxicity have been conducted. This is acceptable because of the intended clinical use and the results of the pharmacological and toxicological investigations.

2.3.4. Ecotoxicity/environmental risk assessment

Summary of main study results

Substance (INN/Invented Name): Florbetapir (^{18}F)			
CAS-number (if available): 956103-76-7			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	pH metric method	**3.36 (neutral species)**	Potential PBT **No**
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	**< 4.5**	**No conclusion on P**
	BCF	Not Tested (NT)	Not Applicable (N/A)
Persistence	DT50 or ready biodegradability	NT	N/A
Toxicity	NOEC or CMR	NT	N/A
PBT-statement :	**A PBT assessment has not been conducted as in the screening procedure the log K_{ow} is below 4.5**		

Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	**2.5×10^{-4}**	µg/L			> 0.01 threshold **N**
Other concerns (e.g. chemical class)					**N**
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	NT	NT			Exposure is less than Phase I limit – therefore Ph II evaluation not required.
Ready Biodegradability Test	NT	NT			As above
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	NT	NT			As above
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	NT	NT	NT	NT	Exposure is less than Phase I limit – therefore Ph II evaluation not required.
Daphnia sp. Reproduction Test	NT	NT	NT	NT	As above
Fish, Early Life Stage Toxicity Test/*Species*	NT	NT	NT	NT	As above
Activated Sludge, Respiration Inhibition Test	NT	NT	NT	NT	As above
Phase IIb Studies					
Bioaccumulation	NT	NT	NT	NT	As above
Aerobic and anaerobic transformation in soil	NT	NT	NT	NT	As above
Soil Micro organisms: Nitrogen Transformation Test	NT	NT	NT	NT	As above
Terrestrial Plants, Growth Test/*Species*	NT	NT	NT	NT	As above
Earthworm, Acute Toxicity Tests	NT	NT	NT	NT	As above
Collembola, Reproduction Test	NT	NT	NT	NT	As above
Sediment dwelling organism	NT	NT	NT	NT	As above

The applicant has provided an environmental risk assessment according to Phase I of EMEA/CHMP/SWP/4447/00. The PEC surface water according to the guideline is calculated to be 2.5 x 10-4 µg/L. As this value is below the action limit a Phase II environmental risk assessment has not been conducted. The logKow is given as 3.36. Measurements at a range of different pH values were used to calculate the log Kow for the neutral species. This value is representative for the environmentally relevant pH range. According to EMEA/CHMP/SWP/ERA/4447/00 in general a study according to OECD 107 or 122 would be required, however, due to the very low predicted exposure and the special nature of the active ingredient the presented information is considered acceptable.

2.3.5. Discussion on non-clinical aspects

The non-clinical pharmacology, safety pharmacology, pharmacokinetic and toxicology studies conducted with florbetapir ^{18}F and its non radioactive analog florbetapir ^{19}F (or AV-45) have shown that florbetapir ^{18}F binds with high affinity and selectivity to its intended target, the β-amyloid plaques in the brain of patients with Alzheimer's disease and has a high margin of safety in animal toxicological studies. It meets the pharmacokinetic and pharmacodynamic requirements for a PET pharmaceutical for the visualisation of β-amyloid plaques.

The absence of substantial parts of classical parts of pharmacology and toxicology (eg toxicokinetics, reproduction toxicology, carcinogenicity) is justified because of the intented clinical use of florbetapir ^{18}F: the administration of a single very low dose in elderly patients.

2.3.6. Conclusion on the non-clinical aspects

The potential toxicity of florbetapir ^{18}F was tested in rats with single acute doses up to 100 fold the maximum human dose (50 µg for a 70 kg person) and up to 28 days of repeated doses up to 25 times the maximum human dose in beagle dogs. In none of these studies significant adverse effects were observed with regard to clinical observation, weight changes, clinical chemistry, gross pathology and histopathology. In each rat and dog study conducted, the NOAEL was determined to be equal or higher than the highest dose.

Florbetapir is positive in vitro in bacteria for mutation induction and in mammalian cells for clastogenic damage. It was negative in the in vivo test in rats. Exposure in vivo is low as no signs of toxicity were seen at the highest dose. However considering the maximum therapeutic dose of 50 µg the highest dose tested in vivo with 372 µg/kg/d seems to be high enough to provide a sufficient level of safety.

No reproductive and developmental toxicity or carcinogenicity evaluations were conducted, given the intended single-dose use of the drug product and the age of the patient group in which it will be used.

2.4. Clinical aspects

GCP

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

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

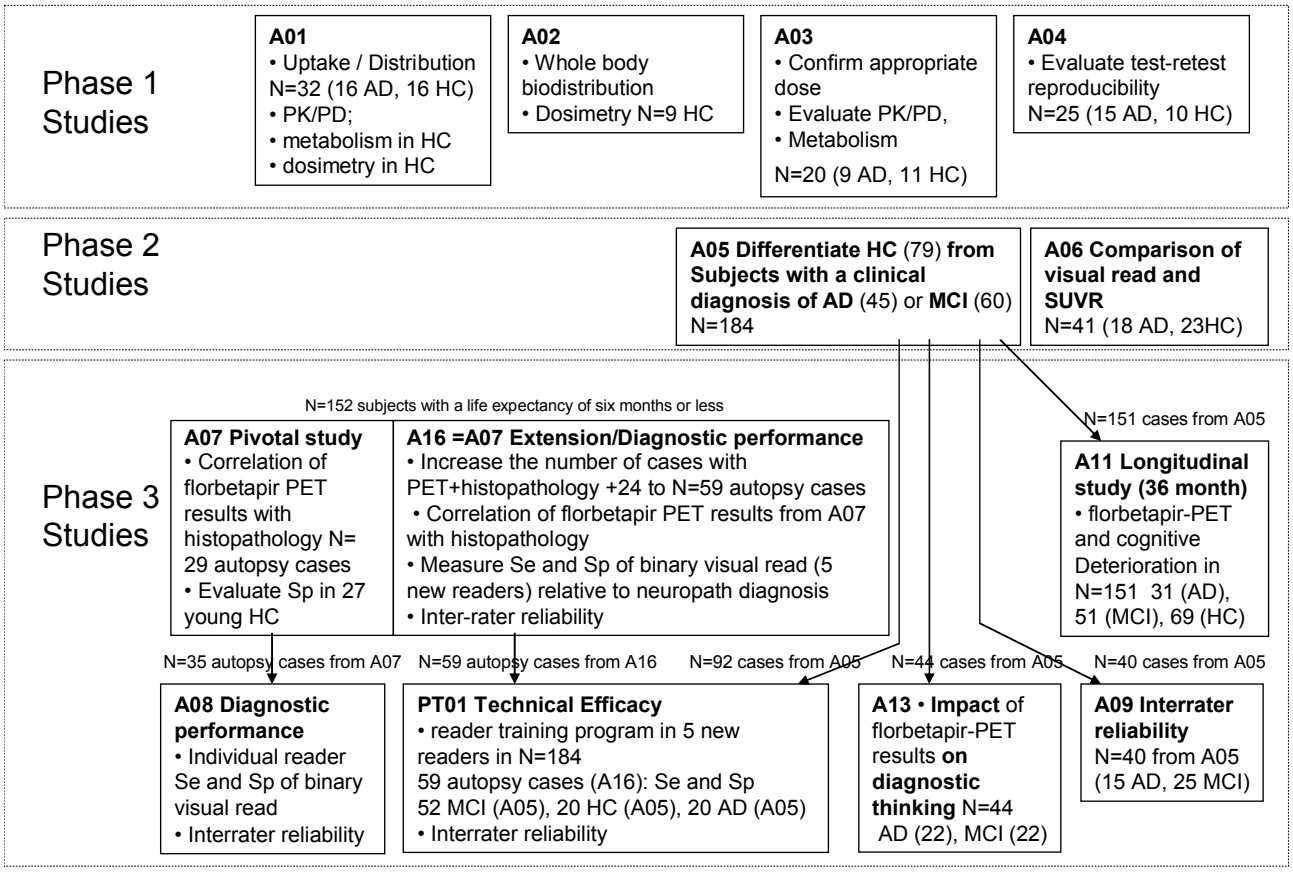
2.4.1. Introduction

The development programme of florbetapir (^{18}F) focused on three main areas of investigation: correlation of florbetapir (^{18}F) PET image uptake with histopathology assays at autopsy, diagnostic performance, and impact on diagnostic thinking. The number of patients exposed to at least one dose of florbetapir (^{18}F) during six clinical trials was 496 subjects. The clinical trial programme conducted by the company included 13 completed clinical studies:

- Four phase 1 studies (**A01**, **A02**, **A03**, **A04**) evaluating radiation exposure and dosimetry, tracer time course, optimal dose, and test-retest reliability.
- One phase 2 study (**A05**) evaluating the efficacy (diagnostic performance) to differentiate healthy controls from subjects with a clinical diagnosis of AD or MCI

- Two pivotal phase 3 studies (**A07**, **A16**) confirmatively evaluating the efficacy (diagnostic performance) and safety using histopathology as the standard of truth. The remaining six studies (**A11**, **A13, A08, A09, A06 and PT01**) enrolled no new patients, but consisted of extended follow-up of patients or re-analysis/re-read of images from subjects enrolled and imaged in the other seven studies.

Overview of the development program of florbetapir (^{18}F)



Tabular overview of clinical studies

Study ID	Drug Dose, Route, and Frequency	Study Objectives	No. of Subjects Entered and Cohort Populations	Inclusion Criteria
Pivotal Clinical Efficacy Studies				
A07	370 MBq, IV, single dose	• Correlate brain PET imaging of Aβ plaque (semiquantitative read) with histopathology (IHC) at autopsy (success criterion rho>0) and evaluate safety • Evaluate specificity of brain PET imaging of Aβ plaque (visual read) in young healthy controls (success criterion specificity>90%)	Total no. of subjects entered: 226 Autopsy Cohort: 152 entered; 35 completed and are 29 in the efficacy group Specificity Cohort: 74 entered and 47 are in the primary efficacy group(47 non-ApoE4, 22 ApoE4, and 5 unknown)	Autopsy Cohort: Age ≥18 years Life expectancy ≤ 6 months Specificity Cohort: Age < 40 years Cognitively normal

Study ID	Drug Dose, Route, and Frequency	Study Objectives	No. of Subjects Entered and Cohort Populations	Inclusion Criteria
A16	None. Dosing was in Study A07	Increase the number of cases with PET-histopathology comparisons 1. Correlate brain florbetapir (^{18}F) PET imaging (semiquantitative reads from A07) with histopathology (IHC) (success criterion rho>0) 2. Measure sensitivity and specificity of binary visual PET read (5 new readers) relative to Aβ neuritic plaque density (primary hypothesis: majority read >80% sensitive and specific) 3. Measure inter-rater reliability	All subjects from A07 Autopsy Cohort still living at the close of A07 were followed in the next 12 months. Total number of autopsy cases evaluated: 59 46 deceased < 1 year post scan 13 deceased >1 year post scan	Enrolment as per above for A07 Autopsy Cohort
Supportive Clinical Efficacy Studies				
A05	370 MBq, IV, single dose	Differentiate healthy controls from subjects with a clinical diagnosis of AD or MCI by florbetapir (^{18}F) PET (binary visual and quantitative reads), and evaluate safety	No. of subjects entered: 184 • 45 AD • 60 MCI • 79 HC	Age ≥ 50 years AD: NINCDS probable AD MMSE: 10 – 24 MCI: CDR 0.5, normal ADL, not demented, <12 months of evolution, MMSE > 24 of non-obvious case HC: cognitively normal, MMSE 29 or 30
A11	None. Dosing was in A05	Relationship between brain florbetapir (^{18}F) PET imaging (binary visual and quantitative reads as performed in Study A05) and changes in cognition, and diagnostic status (18-month interim analysis and preliminary results of 36-month analysis)	Total number of subjects entered: 151 from Study A05 • 31 AD • 51 MCI • 69 HC	Per Study A05, all subjects willing to return for continued 3-year follow-up
A13	None Dosed in A05	Impact of florbetapir (^{18}F)PET result (binary visual read as performed in Study A05) on diagnostic thinking by three independent expert clinicians	Total number of cases: 44 from Study A05 A05 clinical diagnosis: 22 AD, 22 MCI	Cases selected from A05 to include • All A05 AD with Aβ- PET (n=11) • 11 Aβ+ AD • 11 Aβ-, 11 Aβ+ MCI • Where possible matched for age, education, site
Image Read Evaluation Studies				
PT01	None Dosing was in A07 or A05	Evaluate computerized self-study reader training program (no in person contact) in 5 new readers: • Inter-rater reliability in A16 & A05 cases • Sensitivity and Specificity of binary visual PET read (5 new readers) relative to Aβ neuritic plaque density of individual raters in A16 cases • Intra-rater read-reread reliability	Total number of cases evaluated: 184 • 59 autopsy cases (A16) • 52 MCI (A05) • 20 HC (A05) • 20 AD (A05) 33 cases, including 20 MCI were re-read for intra-rater reliability	As per A05 and A07.

Study ID	Drug Dose, Route, and Frequency	Study Objectives	No. of Subjects Entered and Cohort Populations	Inclusion Criteria
A08	None. Dosing was in A07	• Individual reader Sensitivity and Specificity of binary visual PET read relative to Aβ neuritic plaque density • Interrater reliability (overall Fleiss kappa and reader to reader kappa)	All 35 subjects who came to autopsy in Study A07	As per A07.
A09	None. Dosing was in Study A05	Preliminary evaluation of interrater reliability in 7 raters with binary PET read training methods	Total number of cases: 40 from Study A05 A05 clinical diagnosis: 15 AD, 25 MCI	Random selection of 15 AD and 25 MCI images from Study A05
A06	None. Dosing was in Studies A01 and A03	To compare visual read and SUVR values taken at 30 – 40 minutes versus 50 – 60 minutes post-dose of brain florbetapir (^{18}F) PET	41 cases (18 AD and 23 cognitively-normal subjects)	As per Studies A01 and A03
Phase 1 Studies				
A01	370 MBq , IV, single dose	To compare uptake and distribution of study drug in AD and HC; PK/PD; metabolism, safety; and dosimetry in HC	32 (16 AD, 16 HC)	Age $\geq$ 50 years NINCDS prob AD or HC MMSE: AD, 10 – 24, HC, ≥ 29
A02	370 MBq , IV, single dose	To evaluate whole body biodistribution and dosimetry	9 HC	Age 18-85 years
A03	111 MBq (N = 9) and 370 MBq (N = 11), IV, single dose	To confirm the appropriate dose for future studies and evaluate PK/PD, metabolism and safety	20 (9 AD, 11 HC)	Age (AD $\geq$ 50, HC 35 – 55 years) NINCDS prob AD or HC MMSE (AD, 10 – 24, HC, ≥ 29)
A04	370 MBq , IV, 2 doses within 4 weeks	To evaluate test-retest reproducibility of florbetapir (^{18}F) PET imagingin HC and AD and safety. Slow vs fast IV.	25 (15 AD, 10 HC)	Age (AD $\geq$ 50 years, HC 35 – 55 years) NINCDS prob AD or HC MMSE (AD, 10 – 24, HC, ≥ 29)

Abbreviations: AD, Alzheimer's disease; HC, healthy controls; HV, healthy volunteers; IV, intravenous; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; NINCDS, National Institute of Neurological and Communication Disorders; PK, pharmacokinetic

2.4.2. Pharmacokinetics

Absorption, Distribution, Elimination

Following intravenous administration florbetapir (^{18}F) is rapidly cleared and metabolized so that only around 5% of the original dose is present in circulation at 5 minutes: ^{18}F is distributed primarily to the liver and brain, approximately 1% of is present at 20 minutes and less than 0.5% by 45 minutes post-administration.

As the quantity of florbetapir in the blood during the terminal phase is too low to be accurately measured, and due to rapid clearance, the terminal half-life has not been calculated. The primary route of clearance from circulation is through the liver, followed by GI excretion. A low amount of ^{18}F (< 5%) accumulates in the urinary bladder in the form of more polar metabolites of florbetapir (^{18}F) and is eliminated in the urine.

Dose proportionality and time dependencies

The stable brain SUVR image results in AD patients that were seen in Studies A01 and A03 after 30 minutes following dose administration are consistent with the lack of effect of the very low levels of metabolites in the circulation over the imaging period of 30-90 minutes post-injection. The radiation dosimetry is similar to approved PET drugs like fludeoxyglucose (^{18}F) and is not substantially affected by patient weight. The radioactive half-life of (^{18}F) is approximately 110 minutes. The biological half-life of florbetapir has not been determined.

Special populations

Due to rapid brain uptake of florbetapir (^{18}F) and rapid clearance of the compound from circulation and the microdose applied, the omission of pharmacokinetic studies in special populations with hepatic or renal impairment is justified. However the respective standard text from the Radiopharmaceuticals Core SmPC is included in the SmPC for safety reasons. No use is expected in children.

2.4.3. Pharmacodynamics

Florbetapir (^{18}F) is administered in doses no higher than 50 µg and does not have any detectable pharmacological activity.

In a dose comparison study, the brain uptake and distribution of florbetapir (^{18}F) was investigated in 2 different dose groups (111 MBq and 370 MBq). Based on the improvement in the visual image quality ratings and acceptable radiation dose observed, a dose of 370 MBq was chosen as the standard dose for clinical application, and this was the dose level selected for use in all subsequent clinical studies.

The time course of florbetapir (^{18}F) brain uptake was investigated in two studies in which the standardized uptake value ratio of target cortical brain regions versus cerebellum (SUVR) was greater in subjects with AD compared with cognitively healthy control subjects (HC). The average cortical to cerebellar SUVR values showed a continual substantial increase from time zero through 30 minutes post-administration, with only small changes thereafter, reaching asymptote by 50 minutes. There were no clinically significant differences in SUVR for images acquired at time points between 30 and 90 minutes post-injection for AD and HC subjects, and this is therefore the supposed optimal time window for image acquisition.

The correlation of florbetapir (^{18}F) binding to β-amyloid deposition was investigated in three *in vitro* studies in brain tissue from 64 subjects with a pathological diagnosis of AD, other neurological diseases, or healthy control subjects. It was confirmed that florbetapir (^{18}F) binds to and labels β-amyloid in human brain tissue. There was also a correlation between florbetapir (^{18}F) binding measured by autoradiography and the density of β-amyloid measured by immunohistochemical assays.

In the pivotal study A16 in 59 end-of-life patients whose cognitive impairment status was not accurately settled, a positive correlation (ρ=0.76; 95%CI:0.62 to 0.85) was demonstrated between the *in vivo* florbetapir (^{18}F) uptake in cortical grey matter (the mean of the 5-point semiquantitative visual PET rating measured by three independent readers) and the total β-amyloid burden averaged from six particular cortical regions (anterior cingulate and frontal cortex, temporal cortex, posterior cingulate, precuneus and lateral parietal cortex) using 4G8 anti-amyloid antibody that stains β-amyloid found in both neuritic and diffuse plaques.

The binding of florbetapir (^{18}F) to other β-amyloid structures (such as cored, vascular or soluble) or to other brain structures (neurofibrillary tangles, other amyloid structures, etc.) has not been assessed in vivo.

^{18}F *in vivo* uptake (radioactivity accumulation) was found in some extracerebral structures in the head (scalp, salivary glands, muscles and cranial bone) in some cases of the electronic training programme (case 103, 108, 112, 113 and others). The reason for that extracerebral uptake (if due to accumulation of florbetapir (^{18}F) or to any of its radioactive metabolites or to blood radioactivity) is unknown but the company has stated that residual ^{18}F blood activity at the time of the scan may contribute to the signal. Uptake in these structures might be located in the same transverse slice in which cortical uptake should be interpreted in florbetapir (^{18}F) PET images, and then might interfere with image interpretation. Therefore, a wording in the SmPC was proposed to reflect this.

It was demonstrated that there is β-amyloid deposition in the frontal lobe and a comparison of a florbetapir (^{18}F) PET scan with a Pittsburgh compound B scan clearly shows the same distribution pattern in the frontal lobe with both compounds.

The observed residual activity found in white matter is considered to be linked to the regional cerebral blood flow in white matter, which is likely to contribute to slower radiotracer washout from the white matter relative to the grey matter. Even in healthy control subjects a characteristic white matter pattern of the PET image is observed. However, the grey matter retention is at least threefold higher than the white matter retention on the PET images of subjects with AD pathology, and this is the rationale for the visual binary read methodology.

For the time being, the binary method rating method of florbetapir-PET imaging is considered to be robust in different clinical settings but the CHMP proposed to develop the quantitative method further.

As cerebral amyloid angiopathy primarily shows up in the occipital lobe, which is one of the lowest neocortical sites of Aβ radiotracer retention in subjects with AD pathology interference with cerebral amyloid angiopathy is not suspected.

A wording was included in the SmPC section 5.2:

Healthy controls show relatively low levels of florbetapir (^{18}F) retention in cortex and cerebellum. Regional analyses show slightly higher levels of retention in the caudate, putamen and hippocampus. The highest level of uptake is in regions mainly composed of white matter (pons and centrum semiovale). In AD subjects, cortical regions and putamen show significantly greater uptake compared to controls. In AD subjects, as in controls, there is low retention in cerebellum and hippocampus and high retention in pons and centrum semiovale.

The biophysical basis of the white matter retention of florbetapir (18F) in the living human brain cannot be definitively explained. It is hypothesized that slower clearance of the radiopharmaceutical may contribute to white matter retention since regional cerebral blood flow in white matter is less than half of that of cortex. Uptake has also been identified in some cases in extracerebral structures such as scalp, salivary glands, muscles and cranial bone.
The reason for this uptake is unknown, but may be due to accumulation of florbetapir (18F) or to any of its radioactive metabolites or to blood radioactivity.

Given that the maximum concentration of cold florbetapir (^{19}F) in the average human brain is about 100 fold less than the target concentration of labeled florbetapir (^{18}F), there is a very low potential for substantial competitive binding between ^{19}F and ^{18}F forms of florbetapir to be expected in patients with AD pathology.
There is no evidence for any influence of clinical symptoms on the target's binding properties.

The test-retest reproducibility of florbetapir (^{18}F) PET imaging was investigated in two imaging sessions which were less than 4 weeks apart. SUVRs were highly repeatable between test and retest image results for AD and HC subjects indicating a high degree of test-retest reproducibility.

Due to rapid brain uptake of florbetapir (^{18}F) and rapid clearance of the compound from circulation and the microdose applied, influence of hepatic or renal impairment on the florbetapir F^{18} safety is not suspected and the omission of pharmacokinetic studies in special populations with hepatic or renal impairment is justified. However the respective standard text from the Radiopharmaceuticals Core SmPC is included in the SmPC for safety reasons.

A comparison of florbetapir (^{18}F) PET with alternative techniques in the field as CSF sampling for determination of β-amyloid and tau, volumetric MRI of the medial temporal lobe/hippocampus and fludeoxyglucose (^{18}F) PET shows that florbetapir (^{18}F) is the only direct measure of a defining pathological feature of AD pathology *in situ*, the presence of amyloid accumulation in the brain whereas the other modalities provide rather indirect measurements (fludeoxyglucose (^{18}F)), are invasive (CSF sampling) or are not unequivocal in determining AD (Volumetric MRI).

Interaction studies

Potential pharmacodynamic drug-drug interactions with a number of drugs belonging to classes that may be frequently used by elderly patients have been evaluated in vitro using tissue binding assays and in vitro film autoradiography (see section "Non clinical aspects"). There were no drug-drug interaction effects on florbetapir (^{18}F) cortical brain binding found in AD subjects taking acetylcholinesterase inhibitors or the NMDA receptor antagonist memantine which represent the current available therapeutical options for AD.

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

Mechanism of action

Amyloid ß (Aß) neuritic plaques are a defining neuropathology of AD. Florbetapir (^{18}F) binds to Aß neuritic plaques, and the ^{18}F isotope produces a positron signal that is detected by a PET scanner.

The correlation of florbetapir (^{18}F) binding to β-amyloid deposition was investigated in three *in vitro* studies in brain tissue from 64 subjects with a pathological diagnosis of AD, other neurological diseases, or healthy control subjects. It was confirmed that florbetapir (^{18}F) binds to and labels β-amyloid in human brain tissue. These *in vitro* studies were part of the pre-clinical studies, and are described in that section of the assessment report. There was also a correlation between florbetapir (^{18}F) binding measured by autoradiography and the density of β-amyloid measured by immunohistochemical assays.

In the pivotal study A16 in 59 end-of-life patients whose cognitive impairment status was difficult to determine, correlation (ρ=0.76; 95%CI:0.62 to 0.85) was obtained between the *in vivo* florbetapir (^{18}F) uptake in cortical grey matter (the mean of the 5-point semiquantitative visual PET rating measured by three independent readers) and the total β-amyloid burden averaged from six particular cortical regions (anterior cingulate and frontal cortex, temporal cortex, posterior cingulate, precuneus and lateral parietal cortex) using 4G8 anti-amyloid antibody that stains β-amyloid found in both neuritic and diffuse plaques.

The binding of florbetapir (^{18}F) to other β-amyloid structures or to other brain structures or receptors has not been assessed *in vivo*.

Primary and Secondary pharmacology

In a dose comparison study (**study A03** detailed in section 2.5.1), the brain uptake and distribution of florbetapir (^{18}F) was investigated in 2 different dose groups (111 MBq and 370 MBq). Based on the improvement in the visual image quality ratings and acceptable radiation dose observed, a dose of 370 MBq was chosen as the standard dose for clinical application, and this was the dose level selected for use in all subsequent clinical studies.

The time course of florbetapir (^{18}F) brain uptake was investigated in **study A01** in which the standardized uptake value ratio of target cortical brain regions versus cerebellum (SUVR) was greater in the 16 recruited subjects with AD compared with the 16 recruited cognitively healthy control subjects (HC). The target regions were precuneus, frontal, anterior cingulate, posterior cingulate, parietal and temporal. The average cortical to cerebellar SUVR values showed a continual substantial increase from time zero through 30 minutes post-administration, with only small changes thereafter, reaching asymptote by 50 minutes. There were no clinically significant differences in SUVR for images acquired at time points between 30 and 90 minutes post-injection for AD and HC subjects, and this is therefore the supposed optimal time window for image acquisition.

Study A04 was designed to evaluate test-retest reliability of florbetapir (18F) PET imaging in two imaging sessions. Fifteen AD subjects and 10 healthy control subjects were enrolled in this study. At each of two imaging sessions, less than 4 weeks apart, subjects were injected with a single IV bolus of 370 MBq of florbetapir (18F). Approximately 50 minutes after the injection of florbetapir (18F), the subject received a 20 minute continuous dynamic PET scan. SUVRs were highly repeatable between test and retest image results for AD and HC subjects indicating a high degree of test-retest reproducibility.

No clinical data on secondary pharmacology are available.

It was demonstrated that there is β-amyloid deposition in the frontal lobe and a comparison of a florbetapir (^{18}F) PET scan with a Pittsburgh compound B scan clearly shows the same distribution pattern in the frontal lobe with both compounds.

The observed residual activity found in white matter is considered to be linked to the regional cerebral blood flow in white matter, which is likely to contribute to slower radiotracer washout from the white matter relative to the grey matter. Even in healthy control subjects a characteristic white matter pattern of the PET image is observed. However, the grey matter retention is at least threefold higher than the white matter retention on the PET images of subjects with AD pathology, and this is the rationale for the visual binary read methodology.

White matter retention of florbetapir (^{18}F) was adressed by a wording included in the SmPC:

"Healthy controls show relatively low levels of florbetapir (^{18}F) retention in cortex and cerebellum. Regional analyses show slightly higher levels of retention in the caudate, putamen and hippocampus. The highest level of uptake is in regions mainly composed of white matter (pons and centrum semiovale). In AD subjects, cortical regions and putamen show significantly greater uptake compared to controls. In AD subjects, as in controls, there is low retention in cerebellum and hippocampus and high retention in pons and centrum semiovale.

The biophysical basis of the white matter retention of florbetapir (^{18}F) in the living human brain cannot be definitively explained. It is hypothesized that slower clearance of the radiopharmaceutical may contribute to white matter retention since regional cerebral blood flow in white matter is less than half of that of cortex. Uptake has also been identified in some cases in extracerebral structures such as scalp, salivary glands, muscles and cranial bone. The reason for this uptake is unknown, but may be due to accumulation of florbetapir (^{18}F) or to any of its radioactive metabolites or to blood radioactivity."

^{18}F *in vivo* uptake (radioactivity accumulation) was found in some extracerebral structures in the head (scalp, salivary glands, muscles and cranial bone) in some cases of the electronic training programme (case 103, 108, 112, 113 and others). The reason for that extracerebral uptake (if due to accumulation of florbetapir (^{18}F) or to any of its radioactive metabolites or to blood radioactivity) is unknown but the company has hypothetised that residual ^{18}F blood activity at the time of the scan may contribute to the signal. Uptake in these structures might be located in the same transverse slice in which cortical uptake should be interpreted in florbetapir (^{18}F) PET images, and then might interfere with image interpretation. Examination of sagittal images and co-registered CT or MR images could help to distinguish occipital bone from occipital grey matter. A wording was proposed to adress this proceeding in case of extracerebral uptake in the SmPC.

As cerebral amyloid angiopathy primarily shows up in the occipital lobe, which is one of the lowest neocortical sites of Aβ radiotracer retention in subjects with AD pathology, interference with cerebral amyloid angiopathy is not suspected.

Given that the maximum concentration of cold florbetapir (^{19}F) in the average human brain is about 100 fold less than the target concentration of labeled florbetapir (^{18}F), there is a very low potential for substantial competitive binding between ^{19}F and ^{18}F forms of florbetapir to be expected in patients with AD pathology.
There is no evidence for any influence of clinical symptoms on the target's binding properties.

2.4.4. Discussion on clinical pharmacology

The Applicant has provided little data on PD in this submission, however, the CHMP considered that the data provided are sufficient for a radiopharmaceutical which has (as opposed to conventional medicinal products) no pharmacological activity due to the nanodoses applied.

A dose finding study and an image time-finding study were performed before phase III trials (see section "Dose-response study(ies)").

Blood clearance, brain uptake and specific distribution in the brain and the whole body as well as elimination of florbetapir (^{18}F) have been investigated in healthy volunteers in the pharmacokinetic studies. Radiation dosimetry is similar to other (^{18}F) containing approved PET drugs. The omission of pharmacokinetic studies in special populations with hepatic or renal impairment is fully justified.

It was confirmed *in vitro* that florbetapir (^{18}F) binds to β-amyloid aggregates in human brain tissue. The correlation of *in vivo* florbetapir (^{18}F) uptake and cortical ß-amyloid deposition at pathology in the pivotal studies refers to β-amyloid plaque combining both neuritic and diffuse plaques. The binding of florbetapir (^{18}F) to other β-amyloid structures (such as cored, vascular or soluble) or to other brain structures (neurofibrillary tangles, other amyloid structures, etc.) has not been assessed in vivo. Moreover, some extracerebral structures in the head (scalp, salivary glands, muscles and cranial bone) showed high uptake (radioactivity accumulation) *in vivo* in some cases of the training programme unknowing if due to accumulation of florbetapir (^{18}F) or to any of its radioactive metabolites or to blood radioactivity. The observed residual activity found in white matter is thought to be linked to the regional cerebral blood flow in white matter.

The optimal time window for image acquisition was determined and even the test-retest reproducibility documented.

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

2.4.5. Conclusions on clinical pharmacology

Although clinical pharmacology in radiopharmaceuticals is different to clinical pharmacology in chemicals, the florbetapir (^{18}F) development programme included relevant studies on the main clinical pharmacological aspects for a MAA. The company has elucidated that *in vivo* florbetapir (^{18}F) correlates with β-amyloid plaque deposition (combining both neuritic and diffuse plaques) at autopsy. The uptake of florbetapir (^{18}F) in other ß-amyloid structures or brain structures has not been assessed. Some extracerebral structures in some cases showed uptake, and the reason is unknown.

2.5. Clinical efficacy

2.5.1. Dose response study

Dose response study A03

This study compared two different doses (**111 MBq** [3mCi] and **370 MBq** [10 mCi]) of florbetapir (^{18}F) to determine the appropriate dose range for future studies. Nine subjects (5 with clinical diagnosis of mild/moderate AD, 4 YHC) were enrolled in the 111 MBq (3 mCi) dose group and 11 subjects (4 with clinical diagnosis of mild/moderate AD, 7 YHC) in the 370 MBq (10 mCi) dose group.

Images were evaluated quantitatively by SUV and SUVR of cortex areas versus either cerebellum or semiovale centrum, and qualitatively by visual evaluation.

Concerning the quantitative evaluation of SUVR of cortex areas versus cerebellum, results for the 111 MBq and 370 MBq dose groups were about the same in the HC group, whereas there were slight quantitative differences in clinical AD subjects in favour of the 111 MBq group.
Visual assessments of the PET imaging quality for the 370 MBq dose were overall slightly better than the 111 MBq dose group. As the visual binary read of the PET images is the intended clinical practice it was concluded that the use of the 370 MBq activity is justified.
Both tested activities (111 and 370 MBq) allowed subjective visual differentiation of amyloid burden (Aβ+ or Aβ-) with acceptable image quality between 9 subjects with clinical diagnosis of mild/moderate AD and 11 cognitively healthy subjects aged less than 55 years.
For dose finding the image fractionation approach was applied. The rationale for chosing the higher 370 MBq dose, particularly to minimise movement artefacts through usage of shorter image acquisition time is considered to be acceptable particularly as the radiation exposure to the patient is comparable to the one of the widely used PET radiopharmacetical (^{18}F) fludeoxyglucose.

2.5.2. Main studies

The pivotal efficacy studies, **Study A07** and its extension **Study A16**, enrolled 152 subjects with a life expectancy of six months or less. Enrolled subjects were followed and those who came to autopsy within 1 year following the PET imaging procedure were included in study A07. Study A16 was an extension to enlarge the population at study A07 with patients autopsied within 12 months after the closure of study A07. These studies are described together below.

Methods

Pivotal study A07

This study tested the relationship between uptake in florbetapir (^{18}F) PET imaging and true levels of β-amyloid determined by histopathological analysis at autopsy.

Pivotal Study A16 (Extension of Study A07)

The **A16** trial was an expansion of the **A07** study to include all additional subjects who consented to autopsy and died within 12 months after the closure of study A07.

Study Participants

Two groups were enrolled in study A07: an autopsy cohort of end-of life subjects, and a specificity cohort of young (<40 years) subjects, cognitively and neurologically healthy. Study A16 participants were all end-of–life ones autopsied in study A07 enlarged with those patients autopsied within 12 months after the closure of study A07.

To study the relationship between PET image and histopathology results in a reasonable timeframe, an end-of-life population was chosen to verify the correlation between PET and histopathology as histopathology was more likely to be obtained in these persons.

Treatments

In study A07, all participants received 370 MBq (10 mCi) Florbetapir (18F) as one time intravenous (IV) bolus.
No radiopharmaceutical was administered in the A16 study.

Objectives

Study **A07** was designed to: (1) the test the correlation between measurements of brain uptake in florbetapir (^{18}F) PET imaging and the levels of β-amyloid measured post-mortem (Autopsy Cohort) by histopathology, and (2) confirm the specificity of florbetapir (^{18}F) PET in a cohort of young, cognitively-normal individuals with a very low likelihood of brain amyloid plaque (Specificity Cohort).

In study **A16**, by expanding the number of subjects available for image to histopathology comparisons (from the A07 trial) the following specific aims were explored:
• Determine the diagnostic performance measured as sensitivity and specificity of an independent blinded visual read assessment of the florbetapir-PET scan versus the final blinded neuropathological *modified* CERAD diagnosis made at autopsy as reference standard;
• Reassess (using a larger number of subjects) the correlation between the semiquantitative visual rating (0 to 4 scale) on an independent blinded read of the florbetapir-PET scan with true levels of cortical amyloid burden at autopsy as measured by immunohistochemistry (IHC).

Outcomes/endpoints

Subjects that came to autopsy within 1 year following the PET imaging procedure (n=35) in study A07, enlarged with those patients autopsied within 12 months after the closure of study A07 (n=24) in study A16 were evaluable for efficacy.

Endpoints tested in the efficacy population included:

- In **study A07**, 1) correlation between imaging and amyloid levels (measured by comparing the uptake of the florbetapir-PET image with the underlying amyloid levels determined by post-mortem histopathology) in the autopsy cohort, and 2) specificity of florbetapir-PET in a cohort of young, cognitively-normal individuals with a very low likelihood of brain amyloid plaque (Specificity Cohort).

In **study A16**:

•1) Determine the diagnostic performance measured as sensitivity and specificity of an independent blinded visual read assessment of the florbetapir-PET scan (majority rating among 5 blinded readers used the binary read methodology: positive or negative) versus the final blinded neuropathological diagnosis made at autopsy as reference standard (*modified* CERAD neuropathological diagnosis of probable/definite AD = positive, no/possible AD = negative) which is based on the density of neuritic plaques on sections of frontal, temporal or parietal cortex with maximum involvement in autopsy specimens (see table below). For the florbetapir studies, a *modified* CERAD neuropathological diagnosis of No, Possible, Probable and Definite AD, was assigned to each autopsy case.

Neuritic plaque level (plaque count)	***Modified* CERAD**	**Reference standard**
None (<1)	No AD	AB negative
Sparse (1-5)	Possible AD	
Moderate (6-19)	Probable AD	AB positive
Frequent (20+)	Definite AD	

The number of readers in Study A16 was defined beforehand by using statistical modeling to assess the impact of the number of readers on the Fleiss kappa statistic: 5 readers was optimal because the range of the 95% confidence interval on Fleiss kappa improved the most with 5 readers. All physicians interpreting images for the diagnostic performance endpoints of sensitivity and specificity were blinded to the neuropathological results and other clinical information.
• 2) Reassess (using a larger number of subjects) the correlation between the semiquantitative visual rating (0 to 4 scale) on an independent blinded read of the florbetapir-PET scan with true levels of cortical amyloid burden at autopsy as measured by immunohistochemistry (IHC) expressed as average area % of the slides examined for a given region of the brain (which includes both neuritic and diffuse plaques).

The cognitive status of the recruited patients was difficult to determine, as their clinical diagnosis of dementia subtype is likely inaccurate as settled only on the basis of previous clinical history and a brief cognitive battery at the screening visit without mandatorily performing either MRI or other laboratory tests to exclude the presence of either significant white matter disease or other non-neurodegenerative dementias.

In the studies, standardisation took place in several aspects of the florbetapir (^{18}F) PET scan: dosing, subject preparation, positioning and duration of imaging.

A binary visual reading methodology of florbetapir (^{18}F) PET scan as positive or negative was used in clinical study A16 and is identical to the read method proposed for clinical use in the SmPC.

A semi-quantitative (on a 5-point scale from 0 =no amyloid to 4 =high levels of amyloid) and quantitative PET reading methodology were also implemented in the pivotal studies. The company selected six particular cortical regions (frontal cortex, temporal cortex, precuneus, parietal cortex, anterior cingulate, and posterior cingulate) for the quantitative and semi quantitative PET reading methods as being showed, early in the development programme, that had consistently shown to have high florbetapir (^{18}F) uptake in patients with clinically probable AD and tracked well with the visual impression. The qualitative PET reading method, however, does not limit image assessment to specific cortical regions but to the whole cortex.

Histopathology was performed on autopsy samples by the immunohistochemistry and the Bielchowsky staining method. Immunohistochemistry (IHC), which measures both neuritic and diffuse plaques combined, was used as a histopathology standard of truth for the correlation endpoint. Standardised brain sectioning and tissue embedding were performed for each brain. The chosen brain regions and slides to read were according to published standard techniques. The six brain regions analysed were the

same already selected in the quantitative PET reading methodology, and included those 3 regions whose assessment is required for the confirmatory diagnosis of AD according to the CERAD criteria. Each region might have a probability of significant β–amyloid deposition. The modified CERAD neuropathology diagnosis method was the reference standard for the diagnostic performance endpoints (sensitivity and specificity). This method used a cut-off between none-sparse (negative) and moderate-frequent (positive) neuritic plaque density measured by Bielchowsky staining method on sections of frontal, temporal or parietal cortex with maximum involvement in autopsy specimens.

To study the relationship between image and histopathology results, FDA requested an autopsy population to verify the correlation between PET and histopathology: the end-of-life population was the one in whom histopathology was more likely to be obtained in a reasonable timeframe.

Sample size

Out of 152 subjects end of life population who consented to autopsy, all subjects who were autopsied within 12 months or their PET imaging procedure (study A07), enlarged with those patients autopsied within 12 months after the closure of study A07 (study A16).

Randomisation

Three independent imaging physicians evaluated the florbetapir-PET scans from the autopsy cohort in randomized blinded fashion in study A07 (five different readers in A16). The presumed negative florbetapir-PET images from the specificity cohort were mixed in random order with 40 images from the autopsy cohort.

Blinding (masking)

In study A07, separate groups of three readers were used for the autopsy and specificity cohort read. All PET scan readers were blinded to any clinical or histopathology data on the subject scans being evaluated. Images were read by three different readers than those used for the correlation analysis conducted on the autopsy cohort.

In study A16, there were five blinded and independent image readers for primary analyses (sensitivity and specificity calculations). Additionally, a semi-quantitative visual evaluation was done by by three blinded and independent image readers.

The possible impact of unblinded results of Study A07 on the protocol and analysis plan of study A16 was discussed. A considerable bias would have been expected for the binary read against dichotomized neuropathology results introduced in the Study A16 if results of Study A07 were known. However, it was shown that the protocol for Study A16 and Statistical Analysis Plan for Study A07 were finalized before data and results of Study A07 were available.

Readers in Study A16 were different from those in study A07. Results for sensitivity and specificity are only available for the 5 readers in Study A16.

Statistical methods

All correlation analyses were one-sided while all other statistical tests were two-sided with a significance level of $\alpha=0.05$ and were performed using statistical analysis system (SAS) version 9.0 or higher. Data were summarized using descriptive statistics.
For the primary efficacy correlation analysis and the secondary efficacy analysis, Spearman's rank order correlation was determined as well as the asymptotic standard error (ASE) and 95% CI using Fisher z-

transformation. For the primary efficacy specificity analysis, the number and percent of Aβ- and the 95% CI was determined using the florbetapir F 18 PET scan. Exploratory efficacy analyses were also conducted.
All adverse event summaries were prepared using the set of treatment-emergent adverse events only. Treatment-emergent adverse events were summarized by cohort (autopsy, non-autopsy specificity) as well as all subjects (both cohorts). The change from baseline in clinical laboratory values and vital sign measurements were analyzed within treatment group.

Results

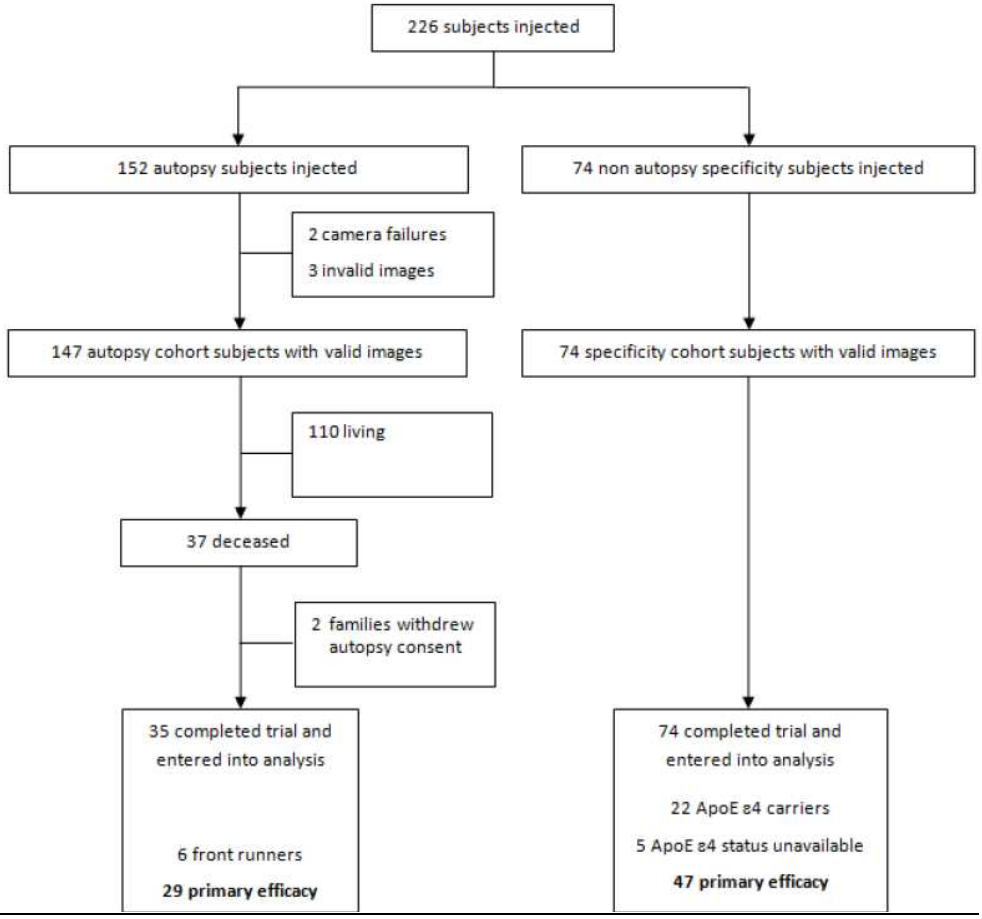
Participant flow

Autopsy Cohort
A total of 226 subjects were enrolled in the study. For the autopsy cohort, 152 subjects were enrolled from various end-of-life (e.g., hospice/hospital/nursing home) and late-life (longitudinal studies of aging) populations to yield 35 autopsies within 1 year following the PET imaging procedure. The first 6 subjects to come to autopsy were considered front runners, and an interim analysis was completed on data from these subjects in order to refine the study methods (PET and autopsy). No significant changes in the clinical study protocol, the PET image Independent Review Charter, or the Neuropathology Analysis Protocol were made following the front runner review. The front-runner analysis confirmed that an autopsy study population of 29 was sufficient to test the primary correlation hypothesis in the main phase of the trial.

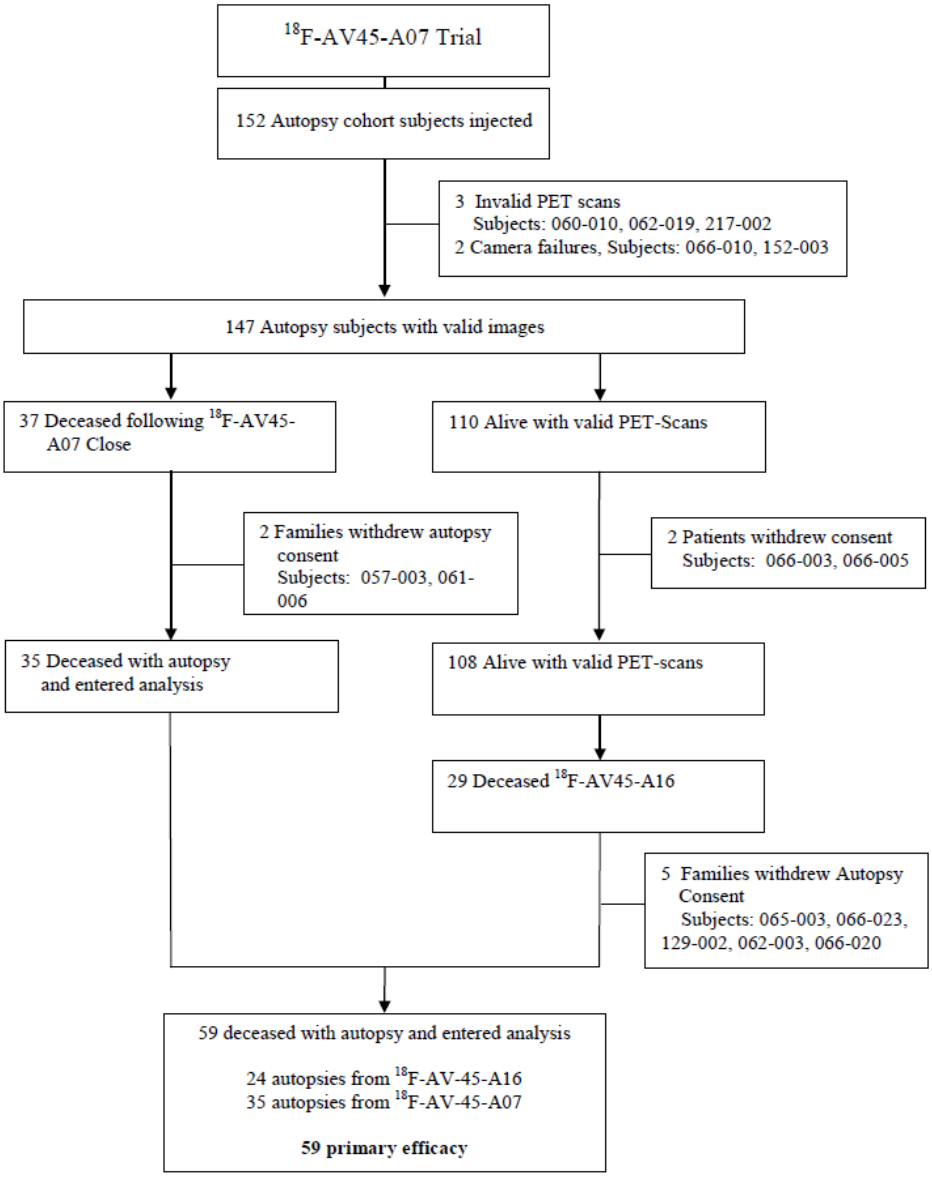
Specificity Cohort

An additional cohort of young (age < 40), cognitively and neurologically healthy individuals was enrolled for specificity analysis of florbetapir (^{18}F) PET. ApoE ε4 carriers or persons with unknown ApoE ε4 status were excluded (n=27) as age and ApoE ε4 polymorphism are the strongest risk factors for AD. Remaining subjects which were expected with high confidence to be devoid of brain amyloid were further analysed (n=47).

Study A07



Study A16



Recruitment

Study A07 was running between 24th February 2009 (first subject included), and 19th March 2010 (last subject completed). Study A16 ended 18th March 2011 (last subject completed).Out of 226 subjects recruited in the autopsy cohort, 59 autopsies were obtained in the 2 years.

Conduct of the study

Study A07 was conducted at 34 study centres in the United States. Study A16 was conducted at 22 study centres in the United States.

The main protocol amendment was the use of first 6 patients with autopsy results as test cases to refine methods. These results were not included in the primary analysis, but sensitivity analyses including these patients are in line with the primary results.

Baseline data

Characteristic	Secondary Analysis Population N=46	Primary Analysis Population N=59
Age (years)		
Mean ± SD	79.0 ± 12.38	79.4 ± 12.64
Median	82.5	83.0
Range	47 to 103	47 to 103
Gender		
Male	25 (54.3%)	29 (49.2%)
Female	21 (45.7%)	30 (50.8%)
Race		
Caucasian	42 (91.3%)	55 (93.2%)
Black or African-American	3 (6.5%)	3 (5.1%)
Other	1 (2.2%)	1 (1.7%)
Asian	0	0
Native American / Alaskan Native	0	0
Ethnicity		
Non-Hispanic or Latino	42 (91.3%)	54 (91.5%)
Hispanic or Latino	4 (8.7%)	5 (8.5%)

Of the 59 subjects composing the primary analysis population, 29 subjects had a clinical diagnosis of AD, 13 had another type of clinically-diagnosed dementing disorder, 12 had no history of cognitive impairment or dementia, and 5 had a clinical diagnosis of mild cognitive impairment (MCI). It has to be noted that the clinical diagnosis of dementia subtype is likely to be inaccurate, as attributed only on the basis of previous clinical history and a brief cognitive battery at the screening visit without mandatorily performing either MRI or other laboratory tests to exclude the presence of either significant white matter disease or other non-neurodegenerative dementias.

Numbers analysed

Fifty-nine subjects who came to autopsy within 12 months of their florbetapir-PET scan (study A07) plus those patients autopsied within 12 months after the closure of study A07 (study A16) comprised the primary analysis population. Of these, 46 had valid images and came to autopsy within 12 months of the florbetapir-PET scan and comprised the secondary analysis population. The other 13 subjects had an average interval of 16 months between image and autopsy.

Outcomes and estimation

Study A07

• Primary Endpoint -Correlation Analysis (Autopsy Cohort)

A statistically significant Spearman's ρ of 0.78 ($p<0.0001$, 95% CI: 0.58 - 0.89) was observed between the median of the independent reader semi-quantitative visual ratings of the florbetapir-PET image and the true cortical amyloid level as assessed by quantitative IHC (average percent cortical grey matter area

of β-amyloid on the IHC slides, staining both neuritic and diffuse plaques) in 29 patients of the autopsy cohort. Thus, the study met its primary hypothesis #1 as a significant correlation (ρ>0) was obtained.

• Primary Endpoint - Specificity Analysis (Specificity cohort)
In the specificity cohort, 100% (47/47) of young healthy control subjects were rated as negative on the visual binary reading of florbetapir-PET scan. The 95% CI for the primary specificity analysis blinded read was 91% to 100%. Thus, the study met its primary hypothesis as ≥90% of the florbetapir-PET scans from subjects in the specificity cohort were rated as negative on an independent visual PET reading, using the majority view of three readers.

• Secondary Endpoint - Regional Correlation Analysis of Semi-quantitative Visual Blinded PET Read with Measurement of Cortical Amyloid Burden (IHC)
Statistically significant relationships were observed between the 6 regional semi-quantitative visual ratings of the florbetapir-PET image (frontal cortex, temporal cortex, precuneus, parietal cortex, anterior cingulate, and posterior cingulate) and the regional cortical amyloid levels as assessed by quantitative IHC. The correlation coefficients between the six analysis regions and regional measures of IHC ranged from 0.68 - 0.77 ($p<0.0001$, 95% CI: 0.42 - 0.88).

Study A16
Primary Endpoint: Diagnostic Performance--Sensitivity and Specificity of florbetapir (^{18}F) PET Scan Visual Qualitative Read Compared to the Neuropathologist's modified CERAD Diagnosis

The primary analysis was performed on the 59 subjects who came to autopsy within **12 months** of the original florbetapir-PET scan or those autopsied within 12 months after closuring study A07 (efficacy population). The diagnostic agreement of the visual binary qualitative rating of the florbetapir-PET scan imaging (positive, negative) with the neuropathologist's modified CERAD diagnosis was determined. Of the 59 subjects of the efficacy population 30 (51%) subjects had a neuropathologic diagnosis of definite AD, 9 (15%) had a classification of probable AD, 5 (8%) had a classification of possible AD, and 15 (25%) had a classification of no AD. In this analysis, the visual qualitative PET rating of amyloid burden was the majority rating among five blinded readers' assessments. The sensitivity and specificity and accuracy for detection of the neuropathologist's modified CERAD diagnosis of probable/definite AD pathology were:

Sensitivity = 92% (95% CI: 78% to 98%)
Specificity = 100% (95% CI: 80% to 100%)
Accuracy = 95% (95% CI: 85% to 99%).

The NPV and PPV in the autopsy population sample were 87% (65-97%) and 100% (88-100%), respectively.

Some patients had PET-only images and others had both PET and CT images to be fused. It would appear that the fusion of PET and CT scan helps avoid interpretation errors due to technical problems in the scan itself or in brain anatomy (levels of atrophy). Therefore, the SmPC encourages the use of CT scans whenever there is uncertainty about the location of grey matter and grey/white matter border in the PET scan.

Primary Endpoint: Correlation Between Florbetapir-PET Visual Semiquantitative Rating and Cortical Amyloid Levels (IHC)

A correlation analysis between the florbetapir-PET visual semi-quantitative rating on a 5-point scale (from 0 =no amyloid to 4 =high levels of amyloid) and the cortical amyloid as determined by IHC revealed a

significant Spearman's ρ of 0.76 (95% CI: 0.62 to 0.85, p<0.0001). On the other hand, the true β–amyloid burden by IHC was very variable and overlapped for most semiquantitative PET rates.
The binary method rating method of florbetapir-PET imaging is considered to be robust in different clinical settings but the CHMP proposed to develop the quantitative method further.

Ancillary analyses

Clinical Diagnosis Comparison (study A07)
On an exploratory basis, the clinical diagnosis was compared to final autopsy diagnosis (the so-called "binary neuropathological diagnosis"). Of the 23 subjects in the autopsy cohort who had a clinical dementia diagnoses (AD or other dementias), 3 (13%) had a clinical diagnosis that did not match the final autopsy diagnosis: a single subject had a clinical diagnosis of probable AD in life, but was negative for AD at autopsy; and 2 subjects had a clinical diagnosis of other dementing disorders (one each with Parkinson's disease dementia and Lewy body dementia), but both received a final autopsy diagnosis consistent with AD. The florbetapir-PET scan blinded read result agreed with the autopsy diagnosis in all three of these cases.

Diagnostic Test Performance--Sensitivity and Specificity of Florbetapir-PET Quantitative Analysis (SUVR) versus the Neuropathologist's modified CERAD Diagnosis (study A16)
An exploratory analysis was performed on the 59 subjects who came to autopsy within 12 months of their florbetapir-PET scan or within 12 months after closuring study A07. The diagnostic agreement between the automated SUVR quantitation of the florbetapir-PET scan (Aβ+, Aβ-) using a pre-defined cut-off and the neuropathologist's modified CERAD diagnosis was determined.

The sensitivity and specificity for the neuropathologist's modified CERAD diagnosis of probable/definite AD pathology was 97% and 100% respectively. The accuracy was 98%, and the NPV and PPV were 95% and 100%, respectively. Overall, the SUVR measure was discordant with autopsy for a single subject (137-002).

Secondary analysis in study A16 (subgroup of subjects with autopsies within 12 months)

Inter-Reader Reliability
A total of 295 image evaluations of florbetapir-PET scans were performed and compared with autopsy results.
The pooled sensitivity was 87% (95% CI: 82% to 91%), pooled specificity was 95% (95% CI: 88% to 98%), and the accuracy was 90% (95% CI: 86% to 93%). The Fleiss' kappa statistic was 0.75 for the inter-reader agreement analysis using qualitative (binary) reads. In only 6% of all image reads was an individual reader's result different from the majority reader outcome. The inter-reader agreement (kappa) was ≥0.82 (p<0.0001) for all reader comparisons *except those for reader 5*. Such reader presented sensitivity and negative predictive values as low as 69% while for the other four readers were closed to the majority analysis (87-95%). There is not really an explanation for the different behaviour of reader 5 in comparison to the other readers. It was however hypothesised that due to the fact that advanced AD subjects being prone to movement, or with atrophy and a thinned cortical ribbon, the images from these subjects are more difficult to interpret. A wording was proposed for inclusion in the SmPC, section 4.4:
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.

The interreader agreement for the correlation analysis between semiquantitative PET rating and histopathology was only moderate (kappa=0.47) between reader 1 and 2 while mildly substantial (kappa values of 0.64 and 0.62) for the other paired readers.

Clinical Pathologic Comparison

On an exploratory basis, clinical diagnosis was compared to final autopsy diagnosis (the so-called "binary neuropathological diagnosis"). Of the 42 subjects in the autopsy cohort who had clinical dementia diagnoses (AD or other dementias), one subject had a clinical diagnosis of probable AD in life, but was negative for AD at autopsy; and 8 subjects did not have a clinical diagnosis of AD in life, but did have a neuropathologic diagnosis of AD at autopsy. In addition, one of the 12 subjects in the autopsy cohort with a clinical diagnosis of normal cognition at enrolment had an autopsy diagnosis of definite AD. The florbetapir-PET scan blinded read result agreed with the autopsy diagnosis in all of the cases where there was discordance between clinical and autopsy diagnosis.

Range and Distribution of β-amyloid Pathology for Study Subjects

The β-amyloid histology observed in this autopsy study population ranged from none to frequent neuritic plaques and from 0% to 14% β-amyloid by IHC staining. The distribution of the modified CERAD diagnosis revealed that 30 (51%) subjects had a neuropathologic diagnosis of definite AD, 9 (15%) subjects had probable AD, 5 (8%) had possible AD, and 15 (25%) were classified as no AD.

When PET images were quantitatively read, correlation of PET uptake (averaging 6 prespecified cortical regions) versus the measurement of β-amyloid deposition by IHC was $\rho=0.75$ ($p<0.0001$).
Among the nine cases that had sparse/no plaques at autopsy, all were correctly rated as "negative" using the binary PET read method. Among the four cases that had moderate/frequent plaques at autopsy, two were correctly read as "positive" and two were read as "negative" (i.e. false negative scan results, which in one case is explained by a possible change between image acquisition and autopsy) at PET images.

Summary of main studies

The following table summarises the efficacy for pivotal trial A07 and its extension Pivotal Study A16

Study A07: A Phase III study of the correlation between florbetapir (^{18}F) positron emission tomography imaging and amyloid pathology **Study A16**: Autopsy follow-up of subjects previously imaged with Florbetapir (^{18}F) PET in trial A07	
Study identifier	A07 /A16
Design	Study **A07** was designed to: (1) the test the correlation between uptake at florbetapir-PET imaging and the levels of β-amyloid measured post-mortem (**Autopsy Cohort**) by histopathology, and (2) confirm the specificity of florbetapir-PET in a cohort of young, cognitively-normal individuals with a very low likelihood of brain amyloid plaque (**Specificity Cohort**). Study **A16** was designed to test the relationship between uptake at florbetapir (^{18}F) PET imaging and true levels of amyloid burden determined at autopsy, and to evaluate the diagnostic performance of florbetapir (sensitivity and specificity) for detecting the modified CERAD neuropathological diagnosis of probable/definitive AD based on histopathology

<table>
<tr><td></td><td colspan="3"></td></tr>
<tr><td>Hypotheses</td><td colspan="3">Study A07 Primary hypothesis #1: <u>Correlation analysis</u>
There is a statistically significant correlation (ρ>0) between the semi-quantitative visual rating of the florbetapir-PET scan and the cortical amyloid burden (combining both neuritic and diffuse plaques) at autopsy as assessed by quantitative immunohistochemistry (IHC). Spearman's Rank Order Correlation, one-sided, p < 0.05, ρ>0, is used to assess a significant correlation
Study A07 Primary hypothesis #2: <u>Specificity analysis</u>
The observed specificity of florbetapir-PET imaging is ≥90% in young healthy controls (i.e., ≥90% of the florbetapir-PET scans from subjects in the specificity cohort would be rated as negative, which yields 95% CI bounds of 80% to 98% for n=40).
Study A16 Primary hypothesis A: Observed sensitivity of florbetapir-PET scan is ≥ 80%.
Study A16 Primary hypothesis B: Observed specificity of florbetapir-PET scan is ≥ 80%.</td></tr>
<tr><td rowspan="3">Treatments groups</td><td rowspan="2">Out of 152 subjects end of life population who consented to autopsy</td><td>Study A07
N=35 in autopsy cohort</td><td rowspan="3">All participants received
370 MBq (10 mCi) Florbetapir (^{18}F) as one time intravenous (IV) bolus</td></tr>
<tr><td>Study A16
additional N=24 subjects in autopsy cohort
i.e. total N=59 subjects in autopsy cohort</td></tr>
<tr><td colspan="2">Study A07
Out of 74 YHC N=47 in Specificity Cohort</td></tr>
<tr><td rowspan="3">Endpoints and definitions</td><td colspan="3">Study A07 Primary Endpoint - Correlation Analysis (Autopsy Cohort)</td></tr>
<tr><td colspan="3">Study A07 Primary Endpoint - Specificity Analysis (Specificity cohort)</td></tr>
<tr><td colspan="3">Study A16 Primary Endpoint: Diagnostic Performance--Sensitivity and Specificity of Florbetapir-PET Scan visual binary Read Compared to the Neuropathologist's modified CERAD Diagnosis
Study A16 Primary Endpoint: <u>Correlation Between Florbetapir-PET Visual Semiquantitative Rating and Cortical Amyloid Levels (IHC)</u></td></tr>
<tr><td>Database lock</td><td colspan="3">Study A07: After N=35 in autopsy cohort
Study A16 after additional N=24 subjects in autopsy cohort</td></tr>
<tr><td colspan="4"><u>Results and Analysis</u></td></tr>
<tr><td>Analysis description</td><td colspan="3">Primary Analyses</td></tr>
<tr><td>Analysis population</td><td colspan="3">See treatment groups above</td></tr>
</table>

Descriptive statistics	**Study A07** Statistics All correlation analyses were one-sided while all other statistical tests were two-sided with a significance level of α=0.05 and were performed using statistical analysis system (SAS) version 9.0 or higher. Data were summarized using descriptive statistics. For the primary efficacy correlation analysis and the secondary efficacy analysis, Spearman's rank order correlation was determined as well as the asymptotic standard error (ASE) and 95% CI using Fisher z-transformation. For the primary efficacy specificity analysis, the number and percent of Aβ- and the 95% CI was determined using the florbetapir (^{18}F) PET scan.
	Study A16 Statistics All correlation analyses were tested with a one-sided significance level of α=0.05. The diagnostic statistical measurements (e.g., sensitivity, specificity) were provided with two-sided 95% CIs. Analyses were performed using Statistical Analysis System (SAS) version 9.0 or higher. Data were summarized using descriptive statistics. Statistical analyses evaluated the performance of the qualitative PET image read (using neuropathology as the reference standard). Additional analyses evaluated the Spearman's rank correlation between global and regional PET image scores and neuropathology measurements.
	Study A07 Participant flow A total of 226 subjects were enrolled in the study, 152 in the autopsy cohort and 74 (all cognitively normal) in the specificity cohort. At the end of the study, 110 subjects in the autopsy cohort were alive and had valid images, and 37 subjects had died. Of the 37 subjects who had died, consent to perform the autopsy for 2 subjects was withdrawn by their families. Thus, there were 35 subjects in the autopsy cohort who completed the trial and had data available for the correlation efficacy analyses. The first six subjects to come to autopsy were used in the front-runner analysis, and the remaining 29 subjects comprised the primary efficacy population for the autopsy cohort. Of the 74 subjects in the specificity cohort, 47 were identified as non-ApoE ε4 carriers and were included in the primary specificity efficacy analyses. All 226 subjects injected with florbetapir (^{18}F) were included in the safety analyses.
	At the close of the **A07** study, 35 subjects were part of the autopsy efficacy population and 108 subjects with valid imaging data were alive and enrolled in the **A16** extension study. At the end of the **A16** study, 79 subjects were alive and an additional 29 subjects had died. Five subjects withdrew consent for autopsy resulting in 24 subjects added to the efficacy population. Thus, a total of 59 subjects had completed brain autopsy neuropathology procedures. All 59 had valid images and had come to autopsy within 24 months of their florbetapir-PET scan. Twenty-nine subjects had a clinical diagnosis of AD, 13 had another type of clinically-diagnosed dementing disorder, 12 had no history of cognitive impairment or dementia, and 5 had a clinical diagnosis of MCI. These 59 subjects comprised the primary analysis population.

Effect estimate per comparison	Primary Endpoint Correlation Analysis (Autopsy Cohort) Study A07	A Spearman's ρ of 0.78 (p<0.0001, 95% CI: 0.58 - 0.89) was observed between the median of the independent reader semi-quantitative visual ratings of the florbetapir-PET image and the true cortical amyloid level as assessed by quantitative IHC (average percent cortical grey matter area of β-amyloid on the IHC slides including both neuritic and diffuse plaques).
	Primary Endpoint Specificity Analysis (Specificity cohort) study A07	In the specificity cohort, 100% (47/47) of young healthy control subjects were rated as negative on the florbetapir-PET scan. The 95% CI for the primary specificity analysis blinded binary visual PET read was 91% to 100%. Thus, the study met its primary endpoint as ≥90% of the florbetapir-PET scans from subjects in the specificity cohort were rated as negative on an independent read, using the majority view of three readers.
	Primary Endpoint: Diagnostic Performance--Sensitivity and Specificity of Florbetapir-PET Scan visual binary Read Compared to the Neuropatho-logist's modified CERAD Diagnosis Study A16	The diagnostic agreement of the visual binary rating of the florbetapir-PET scan majority rating (positive, negative) with the neuropathologist's modified CERAD diagnosis was determined. The sensitivity and specificity and accuracy for detection of the neuropathologist's modified CERAD diagnosis of probable/definite AD pathology were: Sensitivity = 92% (95% CI: 78% to 98%) Specificity = 100% (95% CI: 80% to 100%) Accuracy = 95% (95% CI: 85% to 99%). The NPV and PPV in the autopsy population sample were 87% and 100%, respectively.
	Primary Endpoint: Reassess Correlation Analysis between florbetapir-PET visual semiquantitative rating and cortical amyloid levels (IHC) Study A16	A correlation analysis between the florbetapir-PET visual semi-quantitative rating and the cortical amyloid as determined by IHC revealed a Spearman's ρ of 0.76 (95% CI: 0.62 to 0.85, p<0.0001)
Analysis description	For Secondary analyses see clinical assessment	

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

An analysis of subpopulation factors known to influence β-amyloid deposition (clinical presentation, age and ApoE status) was performed by pooling data across the following studies: A01, A03, A04, A05 and A07. Furthermore an analysis according to gender, race and concomitant use of Alzheimer's disease medication was undertaken. In order to do a common analysis on the maximum number of subjects, the quantitative SUVR evaluation of florbetapir-PET scans was chosen as the primary imaging parameter for the integrated analysis. A quantitative cut-point was applied so that SUVR values > 1.10 were considered amyloid positive and values less than 1.10 were considered amyloid negative.
No meta-analysis was done.

Relationship between clinical presentation and PET Amyloid Levels (SUVR)
Mean florbetapir-PET signal was highest in subjects with clinical diagnosis of AD, lowest in HC.
MCI subjects showed concentrations of both high and low SUVR values consistent with the expected heterogeneity of this group. Approximately 85% of clinical AD subjects, 40 % of MCI subjects, and 15% of cognitively normal subjects were rated positive using any florbetapir-PET measure, which is similar with findings from autopsy literature

The applicant states that the cognitive scales collected from subjects of the Autopsy Cohort of Study A07 were excluded since they were not considered to be reliable in this terminally ill population.

Age

The integrated analysis demonstrated a positive association between age and uptake on florbetapir-PET, particularly in the clinically normal HC group and the MCI group.

ApoE Status

Genotyping data were collected in Studies A04, A05, and A07. In the integrated analysis of these data, ApoE ε4 was highly associated with increased β-amyloid florbetapir-PET scans across multiple presentation groups. The pooled analysis indicates that the florbetapir-PET signal is consistent with the increased risk of amyloid pathology of ApoE ε4 and seems to be also sensitive to detect the reduced risk associated with ApoE ε2 allele. In the clinical setting sporadic Alzheimer cases without obvious association to genetics are of interest.

Gender and Race

There is no evidence for differences in efficacy based on gender and race.

Concomitant use of Alzheimer's disease medication

The integrated database was used to investigate whether there is an interaction of florbetapir (^{18}F) with AD medications. The use of AD medication remained in the model as a possible explanatory factor ($0.05 < p < 0.15$) for only memantine, but was not statistically significant. However, memantine is considered to be a symptomatic treatment and not a disease modifying treatment. Therefore interaction is unlikely.

Clinical studies in special populations

There were no studies in renal or hepatic impaired patients. With respect to subpopulation factors see analysis performed across trials below. The use of florbetapir (^{18}F) in children cannot be recommended, and it is not expected.

Supportive study(ies)

Data to the clinical efficacy of florbetapir-PET was provided from a Phase 2 study **(A05)** and its clinical follow-up extension study **(A11)**, primarily aimed to differentiate clinical probable AD from normal cognition by both a quantitative and a binary visual interpretation of florbetapir (^{18}F) PET images, and secondarily to differentiate them from MCI. 151 subjects who received florbetapir-PET scans in Study **A05** agreed to participate in Study **A11**, a 36-month longitudinal study to evaluate cognitive outcomes. Interim results from the 18-month follow-up visit of Study A11 and only very limited results from the 36-month follow-up were provided.

The potential of florbetapir-PET to impact diagnostic thinking was investigated in **Study A13** by simulating a diagnostic situation. In this study, experts in the clinical diagnosis and management of AD reviewed case histories prepared for 44 subjects enrolled in Study A05 while blinded to the subject's florbetapir-PET results. The experts were asked to assign a diagnosis, a confidence value associated with the diagnosis, and a proposed clinical management plan. The subject's florbetapir-PET results were then revealed, and the experts were again asked to provide the diagnosis, confidence value, and a new clinical management plan, if applicable.

Supportive studies were also those assessing the training methods in the sense of diagnostic performance and inter/intra-reader reproducibility (**Study A08, A09 and PT01**) and the test-retest reproducibility (**Study A04**).

Study A05

This study was an open label, parallel group, multicenter study, evaluating the safety and imaging characteristics of 18F-AV-45 in healthy volunteers, patients with MCI and patients with clinical diagnosis of probable Alzheimer's disease. The study was primarily aimed to differentiate clinical AD from normal cognition by both a quantitative and a binary visual interpretation of florbetapir (^{18}F) PET images, and secondarily to differentiate them from MCI.

A total of 184 subjects (45 subjects with clinical diagnosis of probable AD and mild/moderate dementia, 60 subjects with MCI as cognitive impairment of non-obvious cases lasting less than 12 months, and 79 cognitively normal subjects) were enrolled in the study.

Results

- Florbetapir ^{18}F PET image assessments: The semi-quantitative median PET rating (0-4) was highly correlated with the quantitative mean cortical SUVR (r=0.808, *P*<0.0001), and there was excellent agreement between the qualitative binary (Aβ+ and Aβ-) and semi-quantitative visual reads (100% agreement) and between mean cortical SUVR and qualitative visual reads (91% agreement).

- Concerning the technical performance for binary reads (positive or negative) there were differences observed between the different readers. A very good agreement was observed for 2 of the three readers (Kappa= 0.86), while Reader 2 had unacceptable agreement with the two other readers (Kappa=0.46 and Kappa=0.48, respectively). These results are in line with observations of in the A16 study, indicating that results for individual readers could vary substantially, and further supporting the importance of adequate training.

- Clinical diagnostic group: The 18F-AV-45 PET cortical brain signal was highest in subjects with a clinical diagnosis of AD, lowest in cognitively normal subjects, and intermediate in subjects with MCI. Results show 75.6% of subjects with AD, 38.3% of subjects with MCI, and 14.1% of cognitively normal subjects were rated as positive in PET images. Subjects with clinically diagnosed probable AD were rated as negative by qualitative rating of the 18F-AV-45 PET scan in 24.4% of cases.

- Age: In cognitively normal subjects, rates of PET positivity increased with age: 5.3%, 10.5%, 15.0%, and 25.0% of cognitively normal subjects aged 50 to 59, 60 to 69, 70 to 79, and 80 years or more, respectively, were rated as PET positive.

- ApoE genotype: Subjects in the ApoE2 group had statistically significantly lower mean cortical SUVRs than subjects in the ApoE4 group, regardless of diagnostic category, and no subjects in the ApoE2 group were rated as PET positive, regardless of diagnostic category.

- Cognitive testing: For subjects with clinical AD, there were statistically significant negative correlations between median PET uptake and scores on the Digit-Symbol Substitution (P=0.0162) and on the GDS (P=0.0310). Subjects with clinically diagnosed AD and low PET uptake, tended to score higher on the depression scale. The applicant concludes that these data suggest clinical depression as an alternative cause for clinical dementia in these subjects. This is not totally endorsed since patients with AD also have depressive symptoms. For subjects with MCI, there was a statistically significant negative correlation between median PET uptake and scores on the Digit-Symbol Substitution (P=0.0239), with high PET uptake correlating with poorer performance. Overall there is only a weak correlation between generally used cognitive tests such as ADAS-cog and ADCS-ADL and PET uptake in the MCI and AD group.

The CHMP considered that enrolled subjects may not encompass the anticipated population in which florbetapir (18F) will be used. Not all tests standardized for management of patients suspected of AD (e.g. concurrent MRI, blood tests, …) were utilised to confirm the absence of systemic disorders or other brain diseases that could account for the progressive cognitive impairment.

Overall there was only a weak correlation between generally used cognitive tests such as ADAS-cog and ADCS-ADL and PET uptake in the MCI and AD group.
Study A05 is therefore not considered a study of confirmatory nature but supportive.

Statistical methods for exploratory analyses performed were considered appropriate.

Study A11- Diagnostic utility

This was an extension of study A05, a longitudinal study of long-term (36 month) cognitive outcomes in healthy volunteers, patients with MCI and patients with AD who have previously had PET imaging with Florbetapir (^{18}F). A total of 151 subjects (31 subjects with clinical diagnosis of probable AD and mild/moderate dementia, 51 subjects with MCI as cognitive impairment of non-obvious cases lasting less than 12 months, and 69 CN-cognitively normal- subjects) who participated in the previous phase II study (A05) were enrolled in this study. A total of 140 subjects completed the study as of Month 18 (27 subjects with AD, 46 subjects with MCI, and 67 CN subjects), and 142 as of Month 36 (28 with clinical AD, 47 MCI and 67 CN).
The primary analysis focused on an analysis of the risk of cognitive deterioration in MCI in a 36-month follow-up in subjects with PET positive and negative, and also depending on a pre-defined value of SUV.

There was a planned interim analysis at 18 of 36 months, a report of which was included in the MAA. The study concluded during the dossier evaluation procedure, and a limited summary of the final results at month 36 were provided by the applicant (the final study report was not available at the time of opinion).

Primary Outcomes (in MCI Subjects)

● **Clinical conversion (improvement or worsening) in MCI subjects.** Most of the patients had an unchanged MCI status after 18 or 36 months irrespective of the PET result (see table below).

Table: Change from baseline clinical diagnosis for the MCI clinical diagnostic group – efficacy set

		MCI to CN	MCI Unchanged	MCI to AD	Number of subjects
Month 18	Subjects with PET positive, No, (%)	1 (5.9%)	11 (64.7%)	5 (29.4%)	17
	Subjects with PET negative, No, (%)	6 (20.7%)	21 (70.0%)	3 (10.0%)	30
Month 36	Subjects with PET positive, No, (%)	1 (5.9%)	10 (58.8%)	6 (35.3%)	17
	Subjects with PET negative, No, (%)	5 (16.7%)	22 (73.3%)	3 (10.0%)	30

Using the clinical diagnosis as reference standard, diagnostic performance values of florbetapir (18F) PET are tabulated below

	For diagnosis of MCI N=51	*For diagnosis of clinical AD* N=31
Sensitivity	37.3% (95% CI: 24.1-51.9%)	67.7% (95% CI: 48.6-83.3%)
Specificity	69.0% (95% CI: 59.0-78.9%)	75.8% (95% CI: 67.2-83.2%)
Positive likelihood ratio	1.20 (95% CI: 0.76-1.91)	2.80 (95% CI: 1.88-4.18)

At the 36-month follow-up, considering those 47 patients with a baseline diagnosis of MCI that completed the follow-up:

- 9 out of the 47 (19%) converted from MCI at baseline to AD 36 months later
- Out of the total of 17 MCI subjects who had a positive PET scan, only 6 (35%) were classified clinically as converted to clinical AD after 36 months.
- Sensitivity of Amyvid scan to show the MCI conversion rate to AD in 9 converters was 66.7% (95% CI: 35-88%), specificity in 38 non-converters was 71.0% (95% CI: 55-83%) and positive likelihood ratio was 2.31 (95% CI: 1.2-4.5%). The design of this study does not allow estimating the risk of MCI progression to clinical AD.

• Progressive cognitive impairment in MCI subjects:

- At the 18-month follow-up visit, 41.2% out of the MCI subjects at baseline who had a positive florbetapir-PET scan experienced a clinically significant deterioration in ADAS-Cog score of ≥4 while such deterioration was observed in 13.3% of MCI subjects with PET images rated as negative (p<0.0001).
- For subjects with MCI at baseline, there was a significant relationship between PET scan positivity and decreases in cognitive performance, as measured on a number of the functional and psychometric assessments. Within this clinical diagnostic group, there were statistically significant relationships between the majority of the functional and psychometric assessments and PET uptake, as measured by the binary visual image assessment (positive or negative) or the quantitative image assessment (SUVR), with positive scans associated with worse cognitive performance. As a group, the MCI subjects with a positive PET scan showed a greater mean worsening of functional and psychometric test scores over time than MCI subjects with a negative PET scan. Of the 3 clinical diagnostic groups, differences in the changes from baseline functional and psychometric test scores at Month 18 between subjects with PET images rated as positive and those with images rated as negative were most pronounced in subjects with MCI.

Secondary Outcome

• Stability of clinical diagnosis of AD

• One subject who had a clinical diagnosis of AD at baseline received a different clinical diagnosis at the 18-month follow-up visit. This subject had a PET image rated negative and was diagnosed with non-AD dementia at the 18-month follow-up visit.

Additional Results

• Progressive cognitive impairment in CN subjects

• CN subjects showed relatively minor changes in most functional and psychometric test scores over the 18-month interval; however, both ADAS-Cog (p=0.0112) and the CDR global score (p=0.0151) showed statistically significant declines in subjects whose PET images were classified as positive.

• From the CN subjects at baseline with a positive florbetapir-PET scan, 40.0% experienced a clinically significant change (≥4 points) on the ADAS-Cog. It happened in 5.3% of CN subjects at baseline with a negative florbetapir-PET scan, respectively) (p<0.0001).

Most individual functional and psychometric test scores showed only minor changes which were not statistically significant over the 18-month follow-up interval.

• Clinical conversion in CN subjects

• 20% of CN subjects with a positive baseline florbetapir-PET scan and 3.5% of CN subjects with a negative baseline florbetapir-PET scan converted to a clinical diagnosis of MCI or AD at the 18-month follow-up. This difference was not statistically significant (p=0.1029). Percentage comparisons are based on very low absolute numbers. Only 2 patients in the CN group with PET scans rated as positive converted to MCI or clinical AD after 18 months. Of note the absolute number was identical in the CN group with negative PET scan.

• Baseline PET results in all three groups

It should be pointed out that of the 31 patients initially diagnosed with cllinical AD 10 had a negative PET scan, while the remaining 21 had a positive scan. There were 19 MCI patients at baseline with a positive PET scan, while 32 MCI patients had a negative scan. In cognitively normal subjects at baseline, there were 10 with a positive PET while 59 had a negative scan. Agreement of PET scan results with the baseline diagnosis of MCI or clinical AD were as follows:

	Agreement with baseline diagnosis of MCI N=51	*Agreement with baseline diagnosis of clinical AD* N=31
Sensitivity	19/51 = **37.3%** (95% CI: 24.1-51.9%)	21/31 = **67.7%** (95% CI: 51.3-84.2%)
Specificity	*Using non-MCI cases (cognitively normal & clinical AD)* 69/100 = **69.0%** (95% CI: 59.9-78.1%)	*Using non-AD cases (cognitively normal & MCI)* 91/120 = **75.8%** (95% CI: 68.2-83.5%)
Positive likelihood ratio	**1.20** (95% CI: 0.76-1.91)	**2.80** (95% CI: 1.88-4.18)

Conclusions: Sensitivity for diagnosis of AD by PET was 70 % of patients using the clinical diagnosis of probable AD as standard, and it was only 37% for the diagnosis of MCI.

After the 36-month follow-up, 9 out of 47 (19%) MCI subjects had converted from MCI at baseline to AD, independently if they had a positive or a negative florbetapir (^{18}F) PET scan. Out of the total of 17 MCI

subjects at baseline who had a positive PET scan, only 6 (35%) were classified clinically as converted to AD after 36 months. 10.0 % (n=3) of patients with MCI rated PET negative progressed to clinical AD.

With respect to clinical diagnosis of AD sensitivity of florbetapir PET imaging is not as in the autopsy population. The data indicate that Florbetapir-PET scan results are more closely related to the histopathological changes than to the clinical symptoms of AD and that they may somehow complement but not replace the complex clinical diagnosis of AD.

Although the primary aim of the study is clinically very relevant, the power of the study is considered to be rather low. With full enrollment and the assumptions the applicant presents (60 MCI patients with one half with positive PET and one half with negative PET, population MCI to AD conversion rate 15% for 18-month period) the study would have a power of 75% for a conversion ratio of 8/1 for positive PET and negative PET subjects, respectively. For a conversion ratio of 7/2 for positive PET and negative PET subjects, respectively, the power would be only 41%. Actually the A11 protocol enrolled only 51 MCI patients and this further diminishes the power.

The statistical methods are considered appropriate and methods for additional analyses are acceptable.

Study A13 ***(Impact on Diagnostic Thinking)***

The primary objective of this study was to determine the impact of qualitative visual read of florbetapir (18F) PET (as performed in study A05) on diagnostic thinking by 3 independent expert clinicians.
The following exploratory endpoints were determined: If a florbetapir-PET scan could:

a) change expert clinicians' initial clinical diagnoses to be consistent with the florbetapir-PET scans
b) change expert clinicians' diagnostic certainty
c) change expert clinicians' patient management plans

Clinical data and florbetapir imaging results from 44 subjects who participated in a previous phase II study (**A05**) were included. New subjects were not evaluated in this study. Twenty-two subjects with enrolling clinical diagnosis of AD and 22 subjects with enrolling clinical diagnosis of MCI were included in this study. Half the subjects in each group had a scan previously (in **A05**) read as PET negative; the other half had scans previously read as PET positive (based on majority read).

It is a retrospective study and there were no longitudinal observations. An expert clinician review panel (ECRP) issued a pretest and posttest diagnosis among many clinical diagnostic categories (cognitively normal, MCI -indeterminate etiology, due to AD or unlikely due to AD- or dementia -indeterminate etiology, due to AD and unlikely due to AD-).

Study Results:

Primary Endpoint:

After reviewing the clinical case report (blinded to the initial clinical diagnosis made by the enrolling physician in A05), and prior to receiving information about the patient's florbetapir-PET amyloid imaging results, the expert's diagnosis indicated either cognitive impairment of **indeterminate origin** or suggested an origin **inconsistent** with PET scan results (e.g., dementia due to AD in a patient with an amyloid negative PET scan) in 59% of the 44 cases (range 50-66%). When presented with the florbetapir-PET scan results, these experts changed their diagnosis in 85% (range 66-100%) of the cases with indeterminate origin or inconsistent with PET scan results. This represented a significant shift in diagnostic thinking ($p < 0.001$, 95% CI 80-100%).

Exploratory Endpoints:

Exploratory #1: Consistency of Diagnosis with florbetapir-PET:

Overall, the ECRP provided a diagnosis that was consistent with the florbetapir-PET-scan results 87% of the time. This is less than the pre-specified outcome of consistency of diagnosis with florbetapir scan of

at least 90% and is explained by the fact that only two of three expert clinicians had diagnoses consistent with florbetapir-PET 95% and 98 % of the time.

Exploratory #2: Clinician diagnostic certainty:
For cases where the clinician's initial and final diagnoses were the same, regardless of agreement with the PET scan, confidence in their final diagnoses improved after unblinding of scan results, as indicated by a shift from 76 to 92 on a 100 point scale, where higher numbers indicated increased confidence ($p < 0.0001$).

Exploratory #3: Clinician management plan:
For the entire group of 44 cases, the three expert clinicians altered specific components of their management plan in 80% of the cases they reviewed (range 75-84%, $p < 0.0001$) as a consequence of the information provided by the florbetapir-PET scan.

Conclusions: This study attempted to demonstrate that information from a florbetapir-PET scan could have a significant effect on a physician's clinical diagnosis, diagnostic confidence, and patient management. However, it could not be concluded whether this observed change in diagnostic thinking leads to a clinically relevant change in patient management.

Study A06 (Time window imaging Study)
This study is a comparison of PET images acquired at 30 min and 50 min post injection of 18F-AV-45 in Healthy Volunteers and AD Patients. It serves as the primary source for characterisation of the time course of florbetapir (^{18}F) PET imaging in this MAA. It is about the results of an independent, randomized blinded read of images collected in two previous studies, **A01** and **A03**, at 5 imaging centers. The objectives of this trial were:

- To test whether a qualitative read of a PET scan collected at 30-40 minutes post-injection (the 30-minute image) provides equivalent results to a qualitative read of a PET scan collected at 50-60 minutes post-injection (the 50-minute image).
- To compare the results of a semi-quantitative read of the 30-minute image to a semiquantitative read of the 50-minute image.
- To measure and summarize inter-reader reliability (intra-class correlation coefficients) for qualitative and semi-quantitative assessments of the 30-minute images and 50-minute images.
- To measure and summarize SUVRs obtained from the 30-minute images and 50-minute images.

Results:
Using the majority qualitative read of the 3 readers, there was 100% agreement between the 30-minute image evaluation and the 50-minute image evaluation (100% agreement within each clinical diagnostic subgroup). Similarly, agreement measured using the kappa statistic was 100% for each diagnostic subgroup. Overall inter-reader reliability (i.e. intraclass correlation coefficient) using the qualitative reads was observed to be 0.816 at both 30-minute and 50-minute post-injection time points. Using the median semi-quantitative read of the 3 readers, agreement between the 30-minute images and 50-minute images measured using the Cohen-Fleisser (quadratic) kappa statistic was 0.946 for the overall efficacy population. Correlation between the 30-minutes images and 50-minutes images semi-quantitative median reads was observed to be 0.948 which is highly statistically significant (1-sided p-value <0.0001). Overall inter-reader reliability (ICC) using the semi-quantitative reads was observed to be 0.812 at both the 30-minute and 50-minute post-injection time points. The average cortical to cerebellar SUVR values in AD patients, but not the HV subjects, show continual substantial increases from time zero through 30 minutes post-administration, with only small changes thereafter up to 90 minutes post-injection. The time activity curve for YHV was similar to that for HV. The ratio of AD to HV cortical average SUVRs appeared relatively constant between 30 and 90 minutes, and the effect size using Cohen's d were comparable at 30 and 50 minutes (3.25 and 2.84, respectively). Similar results were obtained comparing

AD subjects to YHV at 30 and 50 minutes. The correlation between the two time points, across all subjects analyzed, was 0.988 and was highly statistically significant (1-sided p-value <0.0001).

Conclusions: The results of this study demonstrated that there is no significant difference between florbetapir-PET scans acquired at 30 and 50 minutes post-injection with respect to the blinded reader visual assessment or the SUVR quantitative analysis.

Study A09

This was an evaluation of physician training for interpretation of florbetapir-PET scans, in particular evaluation of inter-reader reliability using images from subjects with a clinical presentation of AD or MCI. This study was to evaluate an "in person" reader training program developed to educate physicians in the binary visual interpretation of florbetapir-PET images and using images previously collected in Study **A05**. The training program was evaluated by measuring the consistency (inter-reader agreement) of image assessment among readers trained in this program.

Results:

Inter-reader agreement was high for 6 of the 7 readers. One reader (reader 3) had a strong bias to rate scans as positive, and thus had low agreement (kappa=0.25) with the other readers. With the exception of this reader, median kappa values for individual reader compared to every other reader ranged from 0.68 to 0.83 and each inter-reader comparison produced significantly higher than chance agreement, with p values <0.0001. The Fleiss' kappa across all inter-reader comparisons was 0.61 (p<0.0001), and with reader 3 excluded, the overall kappa was 0.76 (p<0.0001) indicating excellent agreement across readers for the binary read in the population of interest. This study also demonstrated the consistency of the reader results as compared to the majority read; kappa statistics comparing individual reader results to the majority read were generally near perfect (values ranging from 0.75 to 0.95, excluding reader 3) and agreement between individual readers and the median read ranged from 88% to 98%. Overall (including reader 3), 89% (248/280) of individual reads agreed with the majority read. Excluding reader 3, 93% (223/240) of individual reads agreed with the majority read.

Conclusions: The results of study A09 indicate that the proposed reader training methodology, using a prospectively-defined binary visual rating scale, produces consistent image interpretation of florbetapir-PET scans from patients with recently diagnosed MCI or clinical diagnosis of probable AD with mild/moderate dementia, patients not unlike those included in the population of intended florbetapir use.

Supportive studies concerning Diagnostic performance

Study A08 ("in-person" training program)

The objective of this study was to evaluate an "in-person" training programme developed to educate physicians in the binary visual interpretation of florbetapir-PET images on images from all 35 subjects who received florbetapir-PET scans and subsequently came to autopsy in study A07.

Results:

The median sensitivity, specificity, and accuracy across the 9 individual reviewers relative to the pathology reference standard (to estimate the neuritic plaque density) was 100%, 93.8% and 94.3% respectively. In addition, 8 of the 9 readers achieved greater than 90% accuracy relative to the reference standard. Overall, 96.2% of individual reads agreed with the majority read. The overall Fleiss' kappa was 0.85 (P < 0.0001) comparing multiple individual readers, indicating excellent reader-to-reader agreement.

Conclusions: The results of study A08 indicate that the proposed reader training methodology is effective in teaching readers how to accurately interpret florbetapir-PET scans using a prospectively-defined binary visual rating scale. In addition, the reader training methodology resulted in excellent interreader reproducibility using the binary assessment.

Study PT01 (web-based self-study reader training programme)

The primary objective of this study was to validate a web-based self-study reader training programme that would be used to educate Nuclear Medicine and Radiology physicians in the methods of interpreting florbetapir PET scans in a standard clinical setting. The secondary objective was to demonstrate the sensitivity and specificity of reader assessments to estimate the neuritic plaque density following web-based training using the florbetapir scans from subjects who came to autopsy in five individual readers.

Results:

The primary aim was to examine the inter-reader reliability in florbetapir PET scan interpretation. The Fleiss' kappa statistic of 0.81 (95% CI: 0.75 to 0.87) for the inter scans in the initially defined primary data set, exceeded the target value of 0.64 (95% LCI 0.58). The percent of image reads that were different from the majority of readers was 5.5% (95% CI: 4% to 7.7%). The kappa values for each individual reader relative to every other reader also exceeded the pre-specified target value of 0.64.

The secondary aim was to evaluate the performance of each of the five readers in florbetapir (^{18}F) PET scan interpretation by calculating the sensitivity and specificity among those scans in patients with autopsy data to estimate the neuritic plaque density. In the All-Autopsy Population (comprised of scans from all 59 subjects that came to autopsy,) all 5 of the 5 readers achieved the protocol specified success criterion of lower bound of 95% confidence interval for both sensitivity and specificity > 0.50, relative to the autopsy reference standard. The median sensitivity and specificity across the 5 individual reviewers relative to the pathology reference standard were 82% and 95%, respectively. The lowest sensitivity was seen in Reader #3 with 69% (95% CI: 53.6% - 81.4%) and the lowest specificity was seen in Readers #1 and 2 with 90% (95% CI: 69.9% - 97.2%).

In the Autopsy Analysis Population (comprised of scans from those 46 subjects that came to autopsy within 12 months of having a PET scan) all 5 of the 5 readers again achieved the protocol specified success criterion of lower bound of 95% confidence interval for both sensitivity and specificity > 0.50, relative to the autopsy reference standard. The median sensitivity and specificity across the 5 individual reviewers relative to the pathology reference standard were 89% and 94%, respectively indicating that the performance for each reader was slightly higher in this population. In the All Autopsy population, which includes all autopsies even when they occurred greater than 12 months after PET scan (n=59), all five readers exceeded the pre-specified success criteria of lower bounds of a 95% CI for both sensitivity and specificity above 50%. The mean (pooled, GEE) sensitivity and specificity were 82% and 93% respectively and the median sensitivity and specificity for the five readers were 82% and 95%.
The lowest sensitivity was seen in Reader #3 with 69% (95% CI: 53.6% - 81.4%) and the lowest specificity was seen in Readers #1 and 2 with 90% (95% CI: 69.9% - 97.2%).

The difference in sensitivity between the a.m. two populations was attributable to two patients, 054-001 and 137-002 that were borderline positive for amyloid by neuropathology (highest neuritic plaque score in diagnostic regions 6 and 7 respectively, with a score of 6 required for a classification of moderate plaques) at autopsy 22 and 14 months, respectively, post scan. All 5 PET readers classified these cases as negative and post-hoc review of these cases confirms that they did not meet criteria for a positive scan.

Conclusion: in this study all pre-defined success criteria with respect to inter-reader reproducibility, intra-reader reproducibility and sensitivity and specificity were met.

Study A04 (Test-retest reproducibility)

This study was done to evaluate test-retest reliability of florbetapir (^{18}F) PET imaging. In fifteen clinical AD subjects and 10 healthy control subjects which were imaged twice less than 4 weeks apart.

There was a high degree of test-retest reproducibility. SUVRs were highly repeatable between test and retest image results for AD and control subjects whether comparing the 10-minute (50 to 60 minutes post-injection) or 20-minute (50 to 70 minutes post-injection) scans. SUVRs for the cortical average relative to cerebellum of test versus retest 20-minute scans were 1.42 ± 0.25 versus 1.41 ± 0.27 for AD subjects and 1.00 ± 0.06 versus 1.01 ± 0.06 for control subjects.

Conclusion: Results showed that: 1) test-retest reproducibility of florbetapir (^{18}F) PET was good both in AD and control subjects, with intra-subject variance of <3% observed between the two scans, and 2) a 10-minute florbetapir (^{18}F) PET image provided equivalent results to a 20-minute image. The rate of dose administration (slow versus fast injection) did not substantially alter the results for the 4 AD subjects.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

For the validation of florbetapir (18F) 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 florbetapir (18F) in the relevant areas.

b. Further development 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.

Those two stages were attempted in the pivotal study A07 and its follow-up extension study A16 using histopathology as the standard of truth. The approach using autopsy data as standard of truth is acceptable to demonstrate that the results obtained with the investigational diagnostic agent are valid for estimation of the Aβ deposition (quantity and topography). Such standard of truth, without considering the dementia status and the patient age, is however not acceptable for establishing the probable/definitive diagnosis of AD.

The choice of endpoints and populations of the pivotal studies was discussed by the CHMP SAWP which considered how the choice of the "end-of-life" population and healthy subjects for the primary analyses has an impact on the external validity of the pivotal study, and the diagnostic utility in the intended clinical population has to be established.

The overall study design of pivotal study A07 was acceptable, including the primary correlation analysis. The co-primary analysis of specificity in healthy controls is adequate to rule out false positive scans, but conclusions on external validity are limited to the enrolled population for this analysis, which may not reflect real life population.

The statistical methods used in the pivotal study A07 are considered appropriate. The main change in study conduct was the use of first 6 patients with autopsy results as test cases to refine methods which is acceptable. Their results were not included in the primary analysis. Changes to the statistical analysis plan were considered acceptable and had no impact on the interpretation of the study results.

The overall study design of pivotal study A16, which was implemented as an extension study of protocol A07 to include further patients from the study 07 autopsy cohort, was also acceptable. Statistical methods for analysis and calculation of confidence intervals are considered appropriate. The number of readers, however, was changed from 3 to 5 in the Statistical Analysis Plan. The motivation for this change was not described and the change in readers was not implemented by amendment in the study protocol. Several changes to the study protocol were implemented via amendment, including change of a co-primary aim of the study. This reflects the change in focus towards a sensitivity/ specificity analysis. An influence of the protocol amendments on the conduct of the study is, however, unlikely.

The pivotal study A07 and its extension study A16 have however the following limitations:

1. For practical reasons, the participants recruited in the autopsy cohort had a prerequisite of an anticipated life expectancy ≤6 months (resulting in patients with very different pathologies: cancer, heart failure, as well as pre-defined dementia and other non-dementia medical conditions). Even if they were age-matched population to the AD population, their end-of-life status could impact on their brains in unanticipated ways, reflecting neither the actual range/distribution of brain β–amyloid deposits nor the cognitive status expected in the intended population for routine clinical use of florbetapir (^{18}F). This limitation was also discussed in the Scientific Advice. There is the possibility that their clinical diagnosis of dementia subtype (if any) is inaccurate, as settled only on the basis of previous clinical history and a brief cognitive battery at the screening visit without mandatorily performing either MRI or other laboratory tests to exclude the presence of either significant white matter disease or other non-neurodegenerative dementias.

2. In 93 out of 152 recruited patients, brain CT images were not available. However, the remaining 59 patients had PET images acquired or fused (manually or automatically) with CT images, and then structural information (cortical atrophy) from CT images was available at PET reading and may have influenced the PET's reader decision. This was addressed by the CHMP request to couple a co-registered CT/MRI scan if needed to correctly assign the position of the grey matter.

3. Study A16 was designed as an extension of pivotal study A07 in order to reach a more significant sample size. However, the main objective of study A16 did not respect the original aim of analyzing the correlation between PET imaging and pathology and was expanded to include also diagnostic performance. Moreover, the first 6 subjects to come to autopsy in study A07 were included in the efficacy population in study A16 whenever they had been recruited exclusively to refine the study methods and then excluded from the efficacy population in study A07. The number of raters for qualitative evaluation of PET images in study A16 increased from study A07 (5 vs 3), which might result in greater indices of sensitivity and specificity because the final rating used to establish sensitivity and specificity was that expressed by the majority of readers.

4. Three different methods of PET image interpretation were implemented for different statistical analyses: visual qualitative binary, visual semi-quantitative and quantitative. The qualitative method (the one to be adopted) changed between the pivotal study and its extension (different color scale, different reference area, and different interpretation criteria). Histopathological techniques used (IHC and the Bielschowsky silver staining method) are well-accepted for the assessment of β-amyloid deposition in the brain postmortem and, as stated by the company, performed according to previously published standardized methods. However,

- The six brain regions to read were chosen to match with those regions involved in the quantitative PET reading method. Each region might have a probability of significant β–amyloid deposition. Only 3 of those 6 regions are accepted for the confirmatory diagnosis of AD at autopsy.

- The Bielschowsky method requires that a neuropathologist usually counts the number of stained neuritic plaques in an autopsy brain specimen, and then inter-reader agreement becomes crucial but was not confirmed.

The company should continue to develop and validate a quantitative PET reading methodology based on their product.

The pivotal studies did not address the impact on diagnostic thinking or on patient management of florbetapir (^{18}F) PET, and this should be further explored.

Therefore, the choice of primary endpoints in pivotal studies A07 and A16 allows, by comparison to the histopathology, only conclusions on correlation on the brain β–amyloid deposition (combining both

neuritic and diffuse plaques) and diagnostic performance (as implemented by additional exploratory analyses in supportive studies A08 and PT01) related to the brain β–amyloid neuritic plaque density.

Efficacy data and additional analyses

Diagnostic Performance

In two pivotal **studies A07** and its extension **A16** diagnostic performance was evaluated using histopathology as the standard of truth. 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, but not for diagnosis of a particular disease.

In **pivotal study A07** the diagnostic performance of florbetapir (^{18}F) PET imaging was evaluated in a population with a life expectancy of six months or less by a primary analysis of the comparability of florbetapir (^{18}F) PET with histopathology. Semi-quantitative florbetapir measurements of the amyloid burden were compared with histopathological levels of amyloid burden determined at autopsy.
152 subjects were enrolled from various end-of-life (e.g., hospice/hospital/nursing home) and late-life (longitudinal studies of aging) populations to yield 35 autopsies within 1 year following the PET imaging procedure. At the end of the study 29 of 37 subjects who had died were the primary analysis population (withdrawal in 2 cases and 6 subjects were used in a front-runner analysis).

The correlation (Spearman's ρ of 0.78, p<0.0001, 95% CI: 0.58 - 0.89) was assessed between florbetapir-PET (semiquantitative PET reading) and histopathologic measurements of β-amyloid by IHC (combining neuritic and diffuse plaques). However, of the 19 (of 35) subjects which met neuropathologic modified CERAD criteria for AD only 18 were florbetapir-PET scan positive, whereas all 19 were considered positive by quantitative analysis (i.e. in one neuropathologic positive case the visual PET interpretation was negative).

Considering the clinical dementia diagnoses of 23 end-of-life patients, in 3 cases the clinical diagnosis did not match the final autopsy diagnosis: one subject with a clinical diagnosis of probable AD was negative for AD at autopsy; two other subjects with a clinical diagnosis of other dementing disorders than AD (one each with Parkinson's disease dementia and Lewy body dementia) were positive for AD at autopsy. The clinical diagnoses in this end-of-life population are likely to be inaccurate, as discussed above. This outcome shows, however, the necessity of an additional validated test supporting the clinical decision making. The true histopathologic status of the subjects remains unclear (until post mortem), as it is possible to have β-amyloid deposition in the brain without any cognitive symptoms.

The specificity of florbetapir-PET to identify the absence of amyloid plaque deposition was additionally evaluated in a cohort of 47 young healthy controls (HC). In this population the qualitative visual PET rating was 100% negative, thereby establishing that clinically defined negatives can be confirmed negative by florbetapir-PET in healthy subjects. The true histopathologic status of these subjects is however unclear as it is possible to have β-amyloid deposition in the brain without any symptoms regarding cognition.

Limitations of study 07 were the use of end-of life patients and YHC instead of the population of intended use, that the performed correlation statistic (between florbetapir and β-amyloid) is not directly translatable into diagnostic performance statistics, that the semi-quantitative visual PET read training did not define criteria for readers to classify images as positive versus negative which is needed for measuring diagnostic performance and that the size of the autopsy population was too small to set tight confidence intervals around sensitivity and specificity measurements.

The pivotal study A16 included additional subjects from the **A07** study end of life population who consented to autopsy and died within 24 months of the florbetapir-PET scan. It was designed to overcome the fact that the size of the autopsy population was too small to set tight confidence intervals around sensitivity and specificity measurements. 24 more subjects were included and a pooled data a set of 59 subjects was analysed which had both valid florbetapir-PET images and autopsy data. Primary objectives of study A16 were 1.) to determine the correlation between the visual semi-quantitative read of florbetapir (^{18}F) PET scan and quantitative assessement of amyloid burden at autopsy by IHC (staining and counting both neuritic and diffuse plaques) and also 2.) to assess sensitivity and specificity of a binary visual read (positive or negative) of the florbetapir-PET scan in relation to the histopathology diagnosis (the estimation of the neuritic plaque density) as standard of truth.

This study aimed at demonstrating correlation between uptake by the whole cortex on florbetapir (18F) PET images (evaluated by three readers on a 5-point semiquantitative visual scale) and the quantitative measurement by IHC at pathology as an appropriate gold standard. Achieving success required a statistical significant ($p \leq 0.05$) clinically irrelevant correlation of only Spearman's $\rho > 0$. The actual correlation was 0.76 (95% CI: 0.62 to 0.85, $p < 0.0001$), similar to the correlation versus the neuritic plaque density measured at autopsy by the Bielschowksy method ($\rho = 0.71$) on an exploratory analysis but inferior to that obtained in vitro ($\rho = 0.88$, $p < 0.0001$, $n = 47$).

On the other hand, the true β–amyloid burden by IHC was very variable and overlapped for most semiquantitative PET rates. Almost no subjects were considered as PET rating 2. Moreover, by using the median of the semiquantitative PET rating measured by 3 independent readers, a biased greater correlation might have been obtained because the attenuation produced by the lack of reproducibility across readers has been diminished. Indeed, the interreader agreement was only moderate (kappa=0.47) between reader 1 and 2 while mildly substantial (kappa values of 0.64 and 0.62) for the other paired readers. This underlines the importance of appropriate reader training.

When PET images were quantitatively read, correlation of uptake by PET (averaging 6 prespecified cortical regions) versus the measurement by IHC or Bielschowsky was $\rho = 0.75$ ($p < 0.0001$).

In this study, the prespecified hypotheses with respect to sensitivity and specificity were confirmed and a sensitivity of 92% (78%-98%) and specificity of 100% (80%-100%) was shown (data with 95% CI) to estimate the neuritic plaque density. Concerning subjects with positive modified CERAD neuropathology diagnosis, 36 subjects were correctly identified as PET positives while 3 subjects were falsely rated as PET negative. Concerning subjects with negative modified CERAD neuropathology diagnosis a diagnostic agreement was found in 20 of 20 subjects rated as PET negative with no subjects declared as false positive. Diagnostic performance in this population translates into a Negative Predictive Value (95% CI) of 87% (65%-97%) for patients with negative results at PET, and a Positive Predictive Value (95% CI) of 100% (88%-100%) for PET positive patients. These results provide strong support that florbetapir-PET images estimate the neuritic plaque density in the brain with high sensitivity (92%) and specifity (100%).

Although the expected sensitivity and specificity (>80%) of the qualitative PET reading was met, it might be biased due to the already mentioned five study limitations and the following ones:

1. By using the majority of the qualitative PET rating of 5 independent readers for the analysis of sensitivity and specificity, the inter-reader variability was obscured. On an exploratory analysis in study A16 it was seen that the inter-reader agreement was almost perfect ($kappa \geq 0.82$, $p < 0.0001$) for all reader comparisons except those for reader 5. Sensitivity and negative predictive value of this reader was as low as 69% while for the other four readers were closed to the majority analysis (87-95%).

2. Sensitivity and specificity in study A16 was calculated versus an endpoint called "final pathologic diagnosis of AD" or also called "binary neuropathology diagnosis" made at autopsy, which is neither the

gold standard nor an acceptable reference standard. It is a non-validated subjective interpretation of the standardized widely used post-mortem CERAD criteria for the definitive diagnosis of AD. The company modified these CERAD criteria to convert the semiquantitative assessment of neuritic plaques of 3 particular neocortical areas directly into a neuropathologic diagnosis of AD, notwithstanding patient age or clinical information regarding the presence or absence of dementia. This way, the "final pathologic diagnosis of AD" just refers to the presence of moderate/frequent or none/sparse neuritic plaque density and not to the definitive diagnosis of AD. The clinical relevance of this endpoint is unknown (when and in what clinical circumstances the detection of beta-amyloid deposition is useful).

Quantitative measurement of brain florbetapir (^{18}F) retention performed in 6 pre-specific cortical regions, and averaged for the whole brain, yielded better results of sensitivity and specificity: 97% (CI 85-100%) and 100% (CI 80-100%), respectively.

Additional data show a significant positive correlation between the florbetapir-PET visual semi-quantitative rating on a 5-point scale (from 0 =no amyloid to 4 =high levels of amyloid) and the neuropathology diagnoses. Data from the pivotal studies A07 and A16 were further assessed in the supportive studies A08 and PT01, in which different sets of readers interpreted images from the A07/A16 study autopsy subjects using similar criteria of PET positivity/negativity of than in study A16 to calculate diagnostic performance of the visual binary PET reading. However, readers trained ("in-person" and "web-based", respectively) were different than the in-person training in study A16 (see table below). For limitations of the training methods implemented during florbetapir (^{18}F) development clinical program, see discussion hereinafter.

Table: Visual Read Training and Scoring Techniques across Florbetapir Studies

	A05, A06	A07 (Autopsy Cohort)	A08, A09	A16	PT01
# of readers	3, 3	3	9, 7	5	5
Scoring method	0-4 semi-quantitative with explicit binary Aβ+/Aβ-	0-4 semi-quantitative	binary Aβ+/Aβ-	binary Aβ+/Aβ-	binary Aβ+/Aβ-
Palate	Colour and Black and white	Colour and Black and white	Black and white	Black and white	Black and white
Required Visualisation	No required approach	No required approach	Axial, coronal, sagittal planes	Axial required, sagittal and coronal optional	Axial required, sagittal and coronal optional
In person vs. Web-based	In-person	In-person	In-person	In-person	Web-based
# of demonstration Cases	5	5	5	5	5
# of Practice cases	21	42	7	7	7
# of self-assessment cases	None	25	None	None	20

In **Study PT01** an additional 5 nuclear medicine physicians, with no previous experience in amyloid imaging, completed an interactive web-based reader training program before conducting a blinded binary image interpretation of 151 subject scans. These scans included the 59 autopsy case images from Study A16 randomized together with 92 subject scans from AD, MCI, and HC subjects of Study A05. In Study PT01 results from the autopsy cases were less convincing about diagnostic performance when compared to Study A16 with 5 false negative cases with negative PET results and 1 false positive case with positive PET scan. This translates into a NPV of 79% (58%-93%) for subjects with negative PET results and a PPV 97% (85%-100%) for subjects with positive PET results in the population under study (data with exact binomial 95% CIs). Overall Kappa for all interrater comparisons was high (0.81 (0.75-0.87))

In **Study A08**, nine independent Nuclear Medicine readers, with no previous experience in amyloid imaging, provided binary interpretations of the florbetapir-PET scans from the original 35 cases that came to autopsy in Study A07. Readers underwent an "in-person" training slightly modified for Study A16 (see table above). Sensitivity and specificity in study A08 were calculated for each reader by comparing a binary read (positive or negative) of the florbetapir-PET scan in relation to the histopathology diagnosis as standard of truth. The median sensitivity and specificity across the 9 individual reviewers relative to the estimation of the neuritic plaque density was 100% and 93.8% respectively and 8 of the 9 readers achieved greater than 90% accuracy relative to the reference standard. Two readers (#5 and #6) had

lower results for specificity (#5) or sensitivity (#6). Overall Kappa for all interrater comparisons was high (0.85) and the results of study A08 are more convincing than the inter-reader comparisons from study A16. Outcome of this study is that a reader training for interpretation of florbetapir-PET scans can lead to high sensitivity, specificity and accuracy in the binary interpretation of flobetapir-PET scan images.

In total, diagnostic performance (sensitivity and specificity) to estimate the neuritic plaque density has been investigated for a total of 19 readers (9 in A08, 5 in A16 and 5 in PT01) for the 35 autopsy cases from Study A07, including 10 readers (5 in A16 and 5 in PT01) for all 59 autopsy cases from Study A16. Results show that readings of single individual readers can deviate from the majority read and this finding emphasizes the importance of reader training.

Clinical usefulness

The ability of a positive florbetapir-PET scan to differentiate patients with clinical diagnosis of AD, of MCI and HC was the objective of Phase 2 study **A05** and its clinical follow-up extension -the longitudinal study **A11-** which actually aimed to evaluate the risk of cognitive deterioration or progression to AD**.** It has to be taken into account that in these studies no histopathological confirmation of imaging results could be obtained. The reference standard in this setting is the clinical evaluation only (since nor other biomarkers have been measured), which is not an appropriate reference to support claims for neither imaging AD pathology nor AD diagnosis, and the link to populations of known brain amyloid status (autopsy study, YHC) has to be justified. Moreover, enrolled subjects do not encompass the overall anticipated population in which florbetapir (^{18}F) will be used. Indeed, not all tests standardized for management of patients suspected of AD (e.g. concurrent MRI, 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. For all these reasons, neither study A05 nor study A11 directly support the proposed indication for use.

Study A05 represents a clinical scenario where amyloid imaging (both quantitative and binary qualitative read) is used to confirm the clinical diagnosis. 75.6% of subjects with clinical diagnosis of probable AD with mild/moderate dementia, 38.3 % of patients with cognitive impairment of non-obvious cause lasting less than 12 months and even 14.1% of cognitively normal subjects were Aβ positive. The numbers for AD are slightly below the expected prevalence of amyloid-negative individuals in a clinically diagnosed AD population, based on literature reports of the false positive rate for the clinical diagnosis of AD versus autopsy. Lim et al. (1999) reported that 20% of clinically-diagnosed AD subjects did not have AD at autopsy and lacked amyloid pathology and Pearl et al. (1997) reported that 23% of clinically diagnosed AD subjects did not have AD at autopsy and lacked amyloid pathology.
The observation that 38.3% of MCI subjects were positive by florbetapir-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). Finally, the observation that 14.1% of HC were rated as positive on visual read of the florbetapir-PET scan is consistent with literature reports that 13% to 45% of apparently cognitively healthy subjects have significant β-amyloid pathology at autopsy (Hulette et al 1998, Davis et al 1999, Price et al 1999, Schmitt et al 2000, Knopman et al 2003, Aizenstein et al 2008) and also consistent with findings from other PET amyloid tracers (Mintun, 2006; Jagust, 2010).
Amyloid imaging detects brain pathophysiology but in itself does not make a clinical diagnosis. Overall there was only a weak correlation between generally used cognitive tests such as ADAS-cog and ADCS-ADL and PET uptake in the MCI and AD group in this study.

Study A11, was developed to help to understand the clinical relevance of detecting a pathologically significant density of Aβ neuritic plaques and specifically to determine whether florbetapir (^{18}F) is predictive of cognitive decline by following subjects from study A05 over a 36-month period. However, the design of this study does not allow estimating the risk of MCI progression to clinical AD.

Study A11 is an extension of study A05 and subjects in A11 were previously enrolled in Study A05. The primary analysis focuses on a longitudinal analysis of conversion from MCI at baseline to AD or CN in subjects with PET positive and PET negative. The planned analysis is an interim analysis at 18 of 36 months, but the study concluded during this application and some preliminary final results at the 36-month follow-up were also presented.

Although the primary aim of the study is clinically very relevant, the power of the study is considered to be rather low. With full enrolment and the assumptions the applicant presents (60 MCI patients with one half with positive PET scan and one half with negative PET scan, population MCI to AD conversion rate 15% for 18-month period) the study would have a power of 75% for a conversion ratio of 8/1 for PET positive and PET negative subjects, respectively. For a conversion ratio of 7/2 for PET positive and PET negative subjects, respectively, the power would be only 41%. Actually the A11 protocol enrolled only 51 MCI patients and this further diminishes the power. Of the originally 31 subjects with AD, 51 subjects with MCI, and 69 CN subjects that were enrolled, 27 subjects with AD, 46 subjects with MCI, and 67 CN subjects completed the study as of month 18. Overall these numbers are considered to be very low.

At 36-month follow-up, of the patients who had been clinically diagnosed with MCI at study entry, 9 (19%) converted to clinical AD. This value is not particularly remarkable since studies have shown that MCI progresses to dementia at a rate of about 10% to 15% per year (Petersen 2007; Plassman et al 2008). Those who have amnestic mild cognitive impairment (prodromal AD) or prodromal vascular dementia (those who have had at least 1 stroke) make the conversion to dementia at an even more rapid rate of 17% to 20% per year.

Of 17 MCI patients at baseline who had a positive PET scan, 6 (35%) were diagnosed with clinical probable AD 36 months later compared to 3 (10%) of 30 who had a negative scan. Sensitivity of Amyvid scan to show the MCI conversion rate to AD in 9 converters was 66.7% (95% CI: 35-88%), specificity in 38 non-converters was 71.0% (95% CI: 55-83%) and positive likelihood ratio was 2.31 (95% CI: 1.2-4.5%). But it has to be taken into account that most of the patients had an unchanged MCI status after 36 months irrespective of amyloid status (58.8% with positive PET and 73.3 % with negative PET). Moreover, 16.7 % (n=5) patients converted to a cognitive normal status at the 36-month follow-up.

Predictive values ratios provide information on the probability that a subject may in fact have the disease when the test is positive. Alternatively, likelihood ratios, when used in the context of diagnostic assessment, provides an estimate of how much a test result will change the odds of having (or not having) the disease. Both parameters are considered to be appropriate endpoints to evaluate the diagnostic performance of a diagnostic agent (CPMP/EWP/1119/98/Rev1.). The PPV in the MCI population is low and the +LR is at the lower threshold of modest incremental improvement. Positive likelihood ratios between 2 and 5 are considered to provide modest incremental improvements to the ultimate diagnosis while likelihood ratios of greater than 5 provide a significant improvement (EMEA/H/SAB/005/1/FU/2/QA/2011).

Of the 51 patients initially diagnosed with MCI only 19 had a positive PET scan (37.3%). The positive lilkelihood ratio of florbetapir (18F) for agreement with baseline diagnosis of MCI was 1.20.

Furthermore, of the 31 patients initially diagnosed with AD 10 had a negative PET scan. Taken out the one patient who after 18 months had non-AD dementia this means that only 70% (21/30) of clinically AD patients had a positive PET scan which is below the expected rate of histopathologically confirmed AD based on literature data (Lim et al 1999, Pearl et al 1997). An alternative explanation for the lower sensitivity in the AD group of study A11 may be attributed to a bias for selective dropout of subjects with positive scans. 10 of the 31 study participants with diagnosis of AD dementia might indeed have had another etiologic diagnosis. Apart from that recent publications report that the binding patterns of tracers

may be affected by atypical amyloidβ assembly structure or plaque organization which might also increase the potential for false-negative scans (*Ringman JM et al. The exception makes the rule. Not all Aβ plaques are created equal; Neurology 2012; 79:206-207; Schöll M et al. Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers: Neurology 2012: 79:229-236*).

Overall, the available data after 36 months do not support that amyloid detection alone allows accuracy for diagnosis of AD or other cognitive diseases nor prognostic accuracy. Respective wording was introduced in sections 4.1 and 4.4 of the SmPC. Unfortunately, no correlation with other potential biomarkers (CSF β42amyloid, tau, hippocampal volume) was provided. On the other hand, a prognosis indication is not the requested intended use for florbetapir (^{18}F) at the moment.

Impact on diagnostic thinking

The potential of florbetapir-PET to impact diagnostic thinking was investigated in **study A13**. After reviewing the clinical case report (blinded to the initial clinical diagnosis made by the enrolling physician in A05), and prior to receiving information about the patient´s florbetapir-PET amyloid imaging results, the expert´s diagnosis indicated either cognitive impairment of indeterminate origin or suggested an origin inconsistent with PET scan results (e.g., dementia due to AD in a patient with an amyloid negative PET scan) in 59% of the 44 cases (range 50-66%). When presented with the florbetapir-PET scan results, these experts changed their diagnosis in 85% (range 66-100%) of the cases with indeterminate origin or inconsistent with PET scan results. This represented a significant shift in diagnostic thinking ($p < 0.001$, 95% CI 80-100%).

When evaluating these data it has to be taken into account that only two of the three physicians changed diagnosis in nearly every case where the algorithm classified the scan result as inconsistent with pre-scan diagnosis. The third clinician differed from the other two primarily in the evaluation of cases given a pre-scan diagnosis MCI of indeterminate aetiology or dementia of indeterminate aetiology. When the florbetapir-PET was positive, this clinician changed the diagnosis to indicate an impairment likely due to AD, but when the scan was negative this clinician maintained a diagnosis of indeterminate aetiology (rather than unlikely due to AD). So in clinical practice a negative scan may not change diagnostic thinking.

The subgroup of 44 subjects with cognitive impairment included, from those previously recruited in study A05, are likely not representative of the population in whom this radiopharmaceutical has demonstrated diagnostic performance (who are still pending to be confirmed), not even of the intended population. Neither the impact was assessed in the particular diagnostic purpose in which the test has been validated (which is none yet) to judge whether changes in patient management are likely to be based on correct test results or in scan interpretation errors. Nor was assessed for the intended diagnostic purpose (i.e. to change a pretest diagnosis of "suspected but not confirmed diagnosis of AD" to a posttest one of either "confirmed diagnosis of AD pathology" or "excluded diagnosis of AD").

Because of its retrospective design and of the absence of longitudinal observations, this study might show the potential impact, but it would NOT report the actual impact florbetapir (^{18}F) PET imaging has on diagnostic thinking. Indeed, the experts did not decide to request the test, as happens in clinical practice, and just reviewed all participants in which the test was mandatorily done as recruited for a clinical trial. Nor did they have option to interview the patient or to ask for any particular additional specific test, especially considering that this radiopharmaceutical is intended to be used in combination to other diagnostic evaluations (not defined yet).

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, or the benefit/risk balance is unclear…..**".** This applies to florbetapir (^{18}F) although this has not been demonstrated for this diagnostic agent.

As therapeutic consequences of the diagnosis of labelling brain β-amyloid are not obvious, the company is recommended to perform a study to assess the impact on diagnostic thinking and patient management.

These findings have an impact on the initially proposed indication: the wording of the indication refers to "AD pathology" (AD-P), expression referring to a pathology which is a still non-validated entity, and it is different from clinical AD (AD-C) (Jack et al 2011) and implies furthermore that the detection of amyloid plaques with PET scan can be regarded as a reliable biomarker for AD pathology. The wording of the indication continues that a negative scan on the other hand makes the diagnosis of AD unlikely. Here two distinct domains although pathopysiologically connected are mixed. Recently the National Institute on Aging and the Alzheimer´s Association workgroups published a consensus for a revision of diagnostic and research criteria for AD, which are still non validated. Whereas the original NINCDS-ADRDA criteria (Mc Khann e al. 1984) assumed that AD is a clinical-pathological entity the new criteria take into account that AD dementia is part of a continuum of clinical and biological phenomena (McKhann et al 2011). Accordingly, in the revised NIA-Alzheimer 'Association criteria, a semantic and conceptual distinction is made between AD pathophysiological process (AD-P) and the clinical manifestation (AD-C) (Jack et al 2011, Dubois et al. 2010).

The amyloid cascade hypothesis suggests that accumulation of Aβ is the key pathological step in the pathogenesis of AD (Karran et al 2011). However, imperfect correlation between cognitive status and Aβ deposits in brain have been described (Golde at al 2011) as amyloid deposition can also occur in normal aging as well (Davis et al 1999, Price et al 1999, Knopman et al 2003, Aizenstein et al 2008) and amyloid pathology has been observed in autopsy brains of older persons without dementia (Bennett et al 2006). β-amyloid plaques may also be present in patients with MCI, with other dementias (dementia of Lewy Body, Parkinson disease dementia), Niemann-Pick disease type C and severe brain injury. This has led to the view that Aβ is only one of the factors that causes AD and that other non Aβ factors also contribute to AD (Pimplikar et al 2009). Indeed, 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).

Aβ-accumulation may reach a plateau early in the course of the disease and does not change much with disease progression. This makes amyloid an useful marker for early diagnosis of AD pathology, but not for determining further prognosis (van Rossum et al 2012). Genetic, other pathological and environmental factors could modulate progression, disease course and manifestation of illness. Factors that enhance neuroplasticity may make an individual more resistant to and delay clinical manifestations of the illness (Golde et al 2011). Protective factors also exist which may modify a relationship between Aβ pathology and clinical expression of cognitive impairment (Quigley et al 2011).

The last statement of the indication is crucial since it may trigger decisions that patients with clinical features of AD may not be treated on the basis of these findings. Although florbetapir (18F) imaging is not proposed as a stand-alone diagnostic tool for the definitive diagnosis of AD its role in the clinical setting has to be more clearly defined. Amyloid imaging only captures one aspect of Alzheimer pathology and does not assess other potentially important changes in CSF tau and other markers. No correlation with other biomarkers has been shown in the intended population and it remains unclear how to grade conflicting results. Taking all this into account the following revised indication is proposed:

This medicinal product is for diagnostic use only.

Amyvid 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. Amyvid 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.

It is clearly indicated that it has to be prescribed by physicians skilled in the clinical management neurodegenerative disorders.

The SmPC recommends a dose of 370 MBq of florbetapir (18F) with a total volume not exceeding 10 mL given as an IV bolus administration, and to acquire a 10-minute PET image starting approximately 30 to 50 minutes after injection. The only dose finding trial was the study A03. Although both tested activities (111 and 370 MBq) allowed to subjective visual differentiation of uptake (positive or negative) between mild/moderate clinical AD and cognitively healthy subjects with acceptable image quality, the highest dose was chosen for the phase III and subsequent trials. However, the lowest dose would have been enough for visual PET interpretation avoiding exposition to an excessive dose of radioactive.

Quantitative measurements of PET images showed consistent uptake levels in the time period between 30-90 minutes postinjection, with clear separation between activity in cortical target areas and cerebellum in subjects with clinical diagnosis of AD beginning around 15 minutes after dosing but not in the control subjects. The florbetapir (18F) uptake at 50-60 minutes postadministration in clinical AD subjects and controls should be justified on the basis of concordance with histopathology, particularly if the target(s) are amyloid plaques which occupy cortical gray matter exclusively. This pattern consisted on the highest SUV values appearing in the centre semiovale white matter similarly in both AD and controls independently of the dose, a high SUV in the neocortex and putamen in AD patients, and in normal cognitively controls a high SUV in both basal ganglia, pons and hippocampus.

The primary source for characterisation of the time window for image acquisition was the study A06 in AD patients and cognitively normal subjects. There was agreement/correlation between the 30-40 min and 50-60 min time points for the qualitative, semi-quantitative and quantitative PET interpretation. The applicant presented data in study A04 showing that there is demonstrated test-retest reproducibility of PET findings AD and controls in a time interval of 4 weeks. Results from image acquisition of 10-minutes showed to be similar to a 20 -minute period.

Despite all this above, the selected activity of florbetapir (18F) to inject and the time window for image acquisition could not be useful for the full spectrum of intended population but only to the subjects with mild/moderate AD by clinical criteria with mild/moderate dementia. Neither patients with any type of cognitive impairment without dementia nor patients with probable AD severely demented were recruited. The reason for selection of the six brain regions to consider on the quantitative PET interpretation methods (either as target or reference areas) was provided, and the 3 regions to be assessed for the confirmatory diagnosis of AD were included amongst them.

Concerning interpretation of PET images, three distinct methods have been used during the development programme: a binary qualitative visual, a semi-quantitative visual, and a quantitative one. The PET reading method to be considered for principal analysis, the scoring technique, the interpretation criteria and the readers' training changed during the clinical development. In the most recent studies (A08, A09, A16 and PT01) and in the SmPC, the Applicant has chosen the binary qualitative method as principal for image PET reading, readers were trained in this methodology and inter-reader concordance addressed in different populations using different reference/truth standards.

For the qualitative reading in pivotal study A16, which is the visual inspection method recommended in the SmPC, the visual global determination of whether the scan was positive or negative was used. An

influence of choice of the regions on the visual global determination of amyloids status by florbetapir ^{18}F PET scan is not expected.

The chosen qualitative (binary) reading criteria of florbetapir (^{18}F) PET scans as positive or negative to be used in clinical practice 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 interpretation criteria in the binary method is looking at loss of reduction of contrast 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 the two can be challenging. Moreover, 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), it may become challenging to accurately interpret florbetapir (^{18}F) PET scans based on visual assessment.

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 analyzed. 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 interpretate 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 and has collaborated to the development of quantitative methodology software. For all this, the CHMP highly recommends that the company should continue to develop and validate a quantitative reading PET methodology based on their product.

The company acknowledges that technical problems in the scan itself or in brain anatomy (levels of atrophy) can affect the anatomical location of the gray matter/white matter border and is important to consider in the interpretation of a florbetapir (^{18}F) PET scan. They also suggest that CT scans may also be helpful for discerning anatomy in cases in which atrophy or a low quality scan complicate the PET-only image interpretation. Therefore, availability of a co-registered recent CT scan or MR image is highly recommended for the qualitative (binary) interpretation of florbetapir (^{18}F) PET scans. This has been included in the SmPC.

Due to the difficulties for visual qualitative interpretation of florbetapir (^{18}F) PET scans, it is mandatory to complete an appropriate reader training prior to routine clinical image interpretation. The company says to be committed to providing high quality training in all countries where florbetapir (^{18}F) is available, and that the training materials will be available in both in-person and electronic programmes in clinical practice. The company assures that both training programmes will contain identical material to that used in 3 studies (A08, A16 and PT01), which were handicapped by lack of training on fused PET-CT images, a low number of cases and lack of qualification of readers after reading.

Only information from the electronic training programme, but not for the in-person training, has been recently supplied by the company by either digital means or via a log-in to www.amyvidtraining.com

(currently for clinical training use by US physicians) to be reviewed. The CHMP expressed concerns about the lack of readers's validation after training and lack of training on PET-CT images. Some training cases presented with drawbacks which might falsely influence the image interpretation: incorrect image orientation, images very noisy or very smooth, images of very low quality or serious misregistration PET-CT. These drawbacks should be addressed in the training programme.

As the training programme on the visual qualitative reading method should be changed to be adapted to the European market (different indication wording than in the US, different languages), it should be improved as follows:

- Information on amyloid pathology in Alzheimer Disease; relevant information on Amyvid as an β-amyloid PET tracer, including the approved indication according to the SmPC, limitations of Amyvid use, interpretation errors, safety information and the results of clinical trials informing on the diagnostic use of Amyvid
- Review of the PET reading criteria, including method of image review, criteria for interpretation, and images demonstrating the binary read methodology
- The material should include Amyvid PET demonstration cases with correct PET scan interpretation by an experienced reader; Amyvid-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 both electronic and in-person training should be ensured.

This improvement of the training programme should be considered in parallel of the study which the company will conduct in the USA to assess the impact of different reader training methods on the reliability of Amyvid scan interpretations as they are performed in clinical practice and to help determine the performance of the reader training processes as compared to the experts at the central reading facility.

No efficacy subanalysis has been presented for the use of florbetapir (^{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.

No dedicated clinical studies have been presented to evaluate efficacy of florbetapir (^{18}F) in patients with impaired renal function and impaired hepatic function.

2.5.4. Conclusions on the clinical efficacy

The primary evidence of efficacy of florbetapir-PET is derived from the diagnostic performance (i.e., sensitivity and specificity) reported in pivotal Study A16 using the binary read method (positive or negative) conducted by 5 independent academic nuclear medicine physicians. This is the read method proposed in the SPC for clinical use. The florbetapir-PET scan was compared to autopsy as standard of truth for detection of pathologically significant density of Aβ neuritic plaques (i.e. moderate to frequent neuritic plaque density).

In this study, a sensitivity of 92% (78%-98%) and specificity of 100% (80%-100%) was shown for detection of pathologically significant density of Aβ neuritic plaques (data with 95% CI). 36 subjects with positive modified CERAD neuropathology diagnosis were correctly identified as PET positive while 3

subjects were falsely rated as PET negative. A diagnostic agreement was found in 20 of 20 subjects with negative modified CERAD neuropathology diagnosis rated as PET negative with no subjects declared as false positive. Due to the limited number of subjects, confidence intervals have considerable width.

Another evidence of efficacy is the correlation of florbetapir-PET read results with total amyloid (both neuritic and diffuse plaques) by histopathology at autopsy initially evaluated in the first 35 autopsy subjects in pivotal Study A07 and later expanded in Study A16 to include all 59 autopsy cases. A statistically significant and relevant correlation between the semi-quantitative image assessment and the quantitative amyloid levels measured at autopsy was found. Additionally, in YHC subjects in the pivotal Study A07 a specificity (with 95% CI) of 100% (91-100%) was observed. 47 of the 47 subjects in the specificity cohort were correctly rated as PET negative by the majority read of three readers.

The selection of an "end-of-life" population and healthy subjects for the primary analyses in the pivotal studies implies that extrapolation of the efficacy results to the intended target population for florbetapir application would be necessary.

However, the two pivotal studies in end-of-life patients have limitations which might have biased their main results.

The sensitivity and specificity of florbetapir-PET for detection of moderate to frequent neuritic plaque density was further confirmed in two supportive studies A08 and PT01, in which different sets of readers interpreted images from the A07/A16 study autopsy subjects. In total, diagnostic performance (sensitivity and specificity) has been investigated for a total of 19 readers (9 in A08, 5 in A16 and 5 in PT01) for the 35 autopsy cases from Study A07, including 10 readers (5 in A16 and 5 in PT01) for all 59 autopsy cases from Study A16. Results show that readings of single individual readers can deviate from the majority read and this finding emphasizes the importance of appropriate reader training.

Overall, the proposed florbetapir (^{18}F) PET reading methodology to be used in clinical practice has not been shown to be the one with the highest technical performance. 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 achievable nowadays in clinical practice. Therefore it is strongly recommended that the company should continue to develop and validate a quantitative reading methodology.

For visual qualitative interpretation of florbetapir (^{18}F) PET scans, a co-registered recent CT or MR imaging should be available in cases of uncertainty about the location of grey matter and of the grey/white matter border in the PET scan. Moreover, it is mandatory to complete an appropriate reader training prior to routine clinical image interpretation. The proposed programme should be changed to be adapted to the European market (different indication wording than in the US, different languages, information about study A16 and not A07), and improved as discussed above.

It is clearly indicated that Amyvid PET examinations have to be prescribed by physicians skilled in the clinical management of neurodegenerative disorders.

The clinical usefulness was investigated in Study A11 with a focus on conversion from MCI at baseline to AD or CN in relation to the PET scan result (positive or negative). Study A11 is an extension of study A05 and subjects in A11 were previously enrolled in Study A05 were amyloid imaging was related to clinical diagnosis and more or less matched autopsy literature data. The available data after 18 and 36 months however, do not support that in the clinical setting amyloid detection alone allows prognostic accuracy in patients with cognitive impairment being evaluated for suspected AD. No correlation with other potential biomarkers (CSF β42amyloid, tau, hippocampal volume) was provided.

Overall, florbetapir-PET imaging, with its high sensitivity and specifity in the autopsy population, has the potential of being a valuable additional diagnostic tool in the clinical evaluation of patients with cognitive impairment who are being evaluated for AD and other causes of cognitive decline.

The indication has been reworded to clearly indicate that a positive scan alone is not synonymous with the diagnosis of AD or other cognitive diseases and emphasizes that a negative scan is considered inconsistent with a diagnosis of AD. Moreover, the low diagnostic performance of florbetapir (^{18}F) PET in subjects with MCI at baseline, and particularly their conversion rate at 36-month follow-up when the PET was positive, are a reason of concern and consequently provided in the SmPC.

The limitations of use of florbetapir (^{18}F) are described in section 4.4 of the SmPC.

The actual impact of florbetapir (18F) PET scan on diagnostic thinking and patient management has not been assessed, and the company is highly encouraged to further investigate these aspects.

The CHMP considers the following measures necessary to address issues related to efficacy:

1. The company should continue to develop and validate a quantitative PET reading methodology based on their product.

2. The company is encouraged to perform a study to assess the impact on diagnostic thinking and patient management since the therapeutic consequences of the diagnosis of labelling brain β-amyloid are not obvious. For the design, parallel HTA/scientific advice is recommended.

2.6. Clinical safety

Patient exposure

In the database at submission, the integrated safety database for florbetapir (18F) contained safety data from 496 subjects who received one dose or, in the case of 25 individuals, two doses in one study of florbetapir (18F). At day 150, the company also included the safety data from 3 company's sponsored studies which were ongoing at submission but had already been completed. These 3 studies involved 59 new patients who were intravenously injected florbetapir (18F) – 370 MBq-. One of those 3 studies, study A11, did not involve additional patients receiving the radiopharmaceuticals but 86 cases in which a second dose of florbetapir (18F) was administered 18-24 months after the first one.

Overall, the sample exposed to a single administration of florbetapir (18F) in the safety database was 286 subjects with cognitive impairment and 269 cognitively normal controls. Additionally, 110 out of them received two doses (24 patients within 4-weeks in study A04, and 86 cases delayed at 18-24 months after the first injection in study A11). All them received 370 MBq except 9 cases who received 110 MBq.

The company also updated the profile of severe AEs with the data of the 6 still ongoing company's sponsored trials (amounted a total of 1,124 exposed subjects) and with data from other ongoing trials with florbetapir (18F) sponsored by other companies or investigator-sponsored (with 1,455 subjects) as to date 15 April 2012.

Adverse events

In the database at submission, the overall rate of adverse events (AEs) was low, with 47 of 496 (9.5%) subjects experiencing a total of 63 Treatment Emergent AEs nearly all of which were assessed as mild or moderate in severity (62 of 63 AEs), and the majority was assessed by the clinical investigators as not

related (43 of 62 AEs) to study drug. No subject was discontinued from a study because of an AE. The most frequently reported TEAEs (>0.5%; in descending order of frequency) were headache, musculoskeletal pain, fatigue, and nausea. AEs are summarized in the tables below.

Table: Overview on Adverse events in Completed Florbetapir (^{18}F) Studies

Study ID	**Study Phase**	**Exposed Population (N)**	**Study Drug Doses**	**All Adverse Events**	**Not related Adverse Events** (of these serious)	**related Adverse Events** (of these serious)
A01	1	32	370 MBq single dose	1	0 (0)	1 (0)
A02	1	9	370 MBq single dose	10	10 (0)	0 (0)
A03	1	20	111 MBq and 370 MBq single dose	3	2 (0)	1 (0)
A04	1	25	370 MBq two doses within four weeks	3	2 (0)	1 (0)
A05	2	184	370 MBq single dose	20	16 (1)	4 (0)
A07	3	226	370 MBq single dose	25	13 (1)	12 (0)
Total		496		62	43 (2)	19 (0)

Table: Single Adverse events in Completed Florbetapir (^{18}F) Studies

Study	**Related**	**Unrelated**
A01	1 Headache	
		1 Claustrophobia
A02		3 Musculoskeletal pain
		2 Nausea
		1 Anxiety
		1 Back pain
		1 Chest pain
		1 Palpitations
A03	1 Injection site irritation	
		1 Diarrhea
		1 Vomiting
A04	1 Dysgeusia (metallic taste)	
		1 Supraventricular extrasystoles
		1 Ventricular extrasystoles

Study	Related		Unrelated		
A05	1	Feeling cold			
	1	Hematuria			
	1	Generalized pruritus			
	1	Flushing			
			1	Back pain	
			1	Musculoskeletal pain	
			1	Musculoskeletal stiffness	
			1	Neck pain	
			1	Fatigue	
			1	Infusion site rash	
			1	Injection site haemorrhage	
			1	Vessel puncture site haematoma	
			1	Abdominal distension	
			1	Flatulence	
			1	Blood pressure increased	
			1	White blood cell count increased	
			2	Headache	
			1	Upper limb fracture	SAE
			1	Claustrophobia	

A07	3	Headache			
	1	Insomnia			
	1	Chills			
	1	Constipation			
	1	Hypertension			
	1	Neck pain			
	1	Nausea			
	1	Pain in extremity			
	1	Parosmia	1		
	1	Sinus headache			
			2	Headache	
			2	Fatigue	
			1	Insomnia	
			1	Blood pressure increased	
			1	Infusion site extravasation	
			1	Edema peripheral	
			1	Respiratory failure	fatal SAE
			1	Urine color abnormal	
			1	Urticaria	
			1	Anxiety	
			1	Dizziness	

Total	**19**	**related**	**43**	**unrelated**

Table: Adverse Events in Descending Order of Frequency

MedDRA Preferred Term (PT)	No. (%)[a)] Overall (N = 496)
Number of Subjects With at Least One Adverse Event	**47 (9.5)**
Headache	8 (1.6)
Musculoskeletal pain	4 (0.8)
Fatigue	3 (0.6)
Nausea	3 (0.6)
Anxiety	2 (0.4)
Back pain	2 (0.4)
Claustrophobia	2 (0.4)
Hypertension	2 (0.4)
Insomnia	2 (0.4)
Neck pain	2 (0.4)
Abdominal distension	1 (0.2)
Blood pressure increased	1 (0.2)
Chest pain	1 (0.2)
Chills	1 (0.2)
Constipation	1 (0.2)
Diarrhoea	1 (0.2)
Dizziness	1 (0.2)
Dysgeusia[b]	1 (0.2)
Feeling cold	1 (0.2)
Flatulence	1 (0.2)
Flushing	1 (0.2)
Haematuria	1 (0.2)
Infusion site extravasation	1 (0.2)
Infusion site rash	1 (0.2)
Injection site haemorrhage	1 (0.2)
Injection site irritation	1 (0.2)
Musculoskeletal stiffness	1 (0.2)
Oedema peripheral	1 (0.2)
Pain	1 (0.2)
Palpitations	1 (0.2)
Parosmia	1 (0.2)
Pruritus generalized	1 (0.2)
Respiratory failure	1 (0.2)
Sinus headache	1 (0.2)
Supraventricular extrasystoles	1 (0.2)
Upper limb fracture	1 (0.2)
Urine color abnormal	1 (0.2)
Urticaria	1 (0.2)
Ventricular extrasystoles	1 (0.2)
Vessel puncture site haematoma	1 (0.2)
Vomiting	1 (0.2)
White blood cell count increased	1 (0.2)
Total AES	62

Only 5 additional related AEs were identified in the updated database: 2 reports of headache and 1 report of injection site pain, one report of dizziness, and one case of insomnia. This makes the profile of related AE to change from 15 cases (3.0%) in the original database to 20 cases (3.6%) in the updated database. The percentages are almost similar for cognitively impaired and cognitively normal subjects. The most commonly reported related AE are headache (n=6 (1.1%)) and nausea (n=2 (0.4%)).

The causality of adverse events which were considered to be related to the study drug by the investigator was evaluated, taking into consideration the biological plausibility, frequency, severity and the PET procedure itself. The rationale for exclusion of adverse events as not related to florbetapir (18F)

administration was elucidated by the applicant: exclusion of adverse events due to reasons such as lack of temporal relationship, lack of biological plausibility or the presence of clear confounding factors was considered acceptable. The following adverse reactions are proposed to be added in SmPC section 4.8:

- Common: headache
- Uncommon: nausea, dysgeusia.

Serious adverse event/deaths/other significant events

A total of 7 SAEs, including 3 deaths have been reported worldwide across florbetapir (^{18}F) studies involving a total of 2,578 subjects exposed. None of these events was considered to be related to florbetapir (^{18}F) by the investigators, and none of the deaths was inconsistent or unusual for the population and individuals in which the event occurred. The currently ascertained profile of serious drug related related AEs is 0%.

Laboratory findings

Clinical laboratory investigations, vital signs and ECG were evaluated as a function of subject age, cognitive status, gender, race, comorbid cardiac rhythm disturbance (by baseline ECG), and presence of AD medications or medications that might prolong QTc.

There were no clinically meaningful pre-dose to post-dose changes in the mean values associated with any laboratory value when considering the entire safety population or when evaluating changes by cognitive status. There were no changes in vital signs thought to be due to drug administration. However, statistically significant increases in blood pressure were seen between screening and baseline measurements (i.e., prior to administration of drug) as well between baseline and both t = 0 and t = 75 minutes post dose after the completion of the PET scanning session. Changes in blood pressure were not related to the mass dose of compound administered and no other notable changes in vital signs were observed in the integrated analysis. In the 344 subjects with pre- and post-treatment ECG measurements, the only statistically significant finding was a small (3 msec) mean increase in QTcF at the 75 minutes post-dose time point shortly after completion of imaging. This change may be a consequence of the algorithm used to correct for heart rate decrease rather than a true physiological change, as the algorithm tends to under-correct when heart rate is low and produce spurious high QTc values. This is supported by the observation that the mean QTcB did not change significantly from baseline at any post-dose time point. No individuals had increases in QTcF or QTcB more than 60 msec from baseline, and no absolute QTc values exceeded 500 msec. Combined with the absence of hERG channel binding and the lack of effects on cardiovascular function in preclinical studies, these results suggest florbetapir (^{18}F) has no significant effect on cardiac electrophysiology.

Overall there were no significant differences in clinical laboratory investigations, vital signs and ECG across any of the examined populations.

Safety in special populations

Subpopulation analyses were conducted to look for any differential safety effects related to gender, age, race, concomitant use of AD medications, history of cardiac rhythm disturbances, and concomitant use of medications that could prolong QT. No consistent changes in safety parameters were observed in any of these subpopulations. In particular, there seems to be no selective vulnerability in the likely target population of older individuals.

An analysis of subjects with altered renal function (i.e. elevated serum creatinine) or hepatic function (i.e. elevated serum liver enzymes) from Study A07 did not show any discernible differences in AEs or lab values versus those with normal-range renal or hepatic parameters.

Amyvid is not expected to be used in women of child-bearing potential.

Immunological events

Although the applicant states that no immunological events have been reported, the fact that chills, rash, and urticaria have been observed might indicate a potential for development of at least slight hypersensitivity reactions, although only chills were considered by the investigators to be related to the product. Based on the available safety data it is currently not possible to draw definite conclusions but hypersensitivity reactions can not be ruled out.

Safety related to drug-drug interactions and other interactions

AD medications included the cholinesterase inhibiting drugs donepezil, rivastigmine, galantamine, and memantine. Overall, there were no significant differences in the incidence of AEs in subjects taking (7.0%) and those not taking (10.2%) AD medications. There are no known drug-drug interactions. Patients taking or not taking AD medications tolerated florbetapir-PET similarly well. Thus, the currently available data do no indicate safety concerns related to drug-drug interactions.

Discontinuation due to adverse events

No discontinuation due to AEs has been reported from any of the florbetapir (18F) trials.

Dosimetry and radiation protection

The human radiation dosimetry of florbetapir (18F) has been studied in three different clinical studies, two conducted by the sponsor (study A01 and A02) and one investigator-sponsored study of Lin et al. 2010. The mean human effective dose of 0.013 mSv/MBq in Study A01, 0.019 mSv/MBq in Study A02, and 0.019 mSv/MBq in the Lin study.

As results of weight-adjusted calculations show no significant differences over the range of 50 to 80 kg body mass, no dose adjustments are recommended based on patient's weight.

The SmPC includes specifications related to radiation protection in the context of manipulation and elimination of the radiopharmaceutical by healthcare professionals, and radiation protection for the family.

Post marketing experience

Not applicable

2.6.1. Discussion on clinical safety

The overall number of patients exposed to florbetapir (^{18}F) in the completed clinical trials sponsored by the company up to September 15th, 2012 is small (n= 555), particularly considering the prevalence of AD in the general population. All of them received 370 MBq as a single dose except 5 patients with AD and 4 HC who received 110 MBq. No post-marketing data is available since no marketing authorization had been issued in the world until the start date of this application procedure.

Overall, the sample exposed to a single administration of florbetapir (^{18}F) in the updated safety database is 286 with cognitive impairment and 269 cognitively normal controls. Additionally, 110 out of them received two doses (24 patients within 4-weeks in study A04, and 86 cases delayed at 18-24 months after the first injection in study A11).

Florbetapir was generally well tolerated. The most common AE related with the study drug was headache and nausea occurring in less than 2% of subjects. Other notable AEs were, however, more likely related to the procedure of IV injection (<1% of subjects with injection site bleeding, bruising or pain) or to the PET-procedure (musculoskeletal pain in 0.8% of subjects) than the study drug itself. This view is supported by the observation that the rate of these AEs was highest in studies requiring prolonged imaging times for dosimetry measurement.

Only few serious adverse events have been reported, and none was considered related to the study drug, the administration, or the imaging procedure.

There were small but statistically significant changes in lab parameters and vital signs, but most appeared non-detrimental. No changes in safety labs or vital sign measurements suggested toxicity of the study drug.

There was no safety signal related to the cognition status of the subject (cognitively impaired subjects versus cognitively normal subjects). Even in the A07 Autopsy Cohort end-of-life population, in which several individuals had many severe concomitant illnesses, the study drug was well tolerated.

No consistent changes in safety parameters were observed in any subpopulation related to gender, age, race, concomitant use of AD medications, history of cardiac rhythm disturbances, and concomitant use of medications that could prolong QT.

The human radiation dosimetry of florbetapir (^{18}F) yields an effective dose of 0.019 mSv/MBq. The results of weight-adjusted calculations show no significant differences over the range of 50 to 80 kg body mass. Therefore no dose adjustments are needed in principle based on patient's weight.

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

No safety for repeated injections can be concluded due to the low number of patients exposed within a short time period (only 24).

The specifications of use of this radiopharmaceutical in pregnancy and lactation was drafted in line with the EMA core SmPC for radiopharmaceuticals.

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

An analysis of subjects with altered renal/hepatic function from study A07 showed no discernible differences in AEs or lab values versus those with normal-range renal or hepatic parameters. No specific safety data in patients with impaired renal function or impaired hepatic function have been provided. In these cases, 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 is reflected in the SmPC in the sense that careful consideration of the activity to be administered is required.

2.6.2. Conclusions on Clinical Safety

Amyvid has been studied in a limited number of patients (safety population of completed clinical trials n=555). Overall, there were no significant safety signals identified with florbetapir-PET imaging. In particular there were no safety signals in cognitively impaired subjects compared to cognitively normal subjects or as compared to the whole safety population. In addition, the study drug was well tolerated even in the A07 Autopsy Cohort end-of-life population which had many severe concomitant medical illnesses.

Considered together with the substantial safety margins in non-clinical studies, these safety results meet the expectations for a single-dose diagnostic agent and in particular for low, non-pharmacodynamically active amounts of a diagnostic radiopharmaceutical.

The paediatric use of florbetapir (^{18}F) is not recommended, and the use in patients with impaired renal function and impaired hepatic function should be recommended with caution.

The available nonclinical findings do not show any significant nonclinical safety issues that could adversely affect the clinical use of Amyvid in the context of the proposed indication. Non-clinical radiation safety studies showed a radiation exposure risk for florbetapir (^{18}F) which is similar to that of the approved PET imaging agent fludeoxyglucose (^{18}F).

No studies have been conducted with pregnant or nursing females or with subjects under the age of 18. In addition, patients with hepatic or renal impairment, clinically meaningful cerebrovascular disease, or subpopulations carrying known and relevant genetic polymorphism (other than the ApoE gene), patients with BMI<19 and BMI>32 were not included in clinical trials. Due to the microdose administered and the rapid clearance of Amyvid, safety in patients with renal impairment is not considered to represent important missing information. Based on the data available it is not anticipated that florbetapir binding or radiation exposure will differ in patients with ApoE polymorphisms, BMI<19 and BMI>32.

As Amyvid is not intended nor expected to be used in pregnant or nursing females, the safety in this population is not considered relevant for inclusion in the RMP as an important safety concern.

The applicant discussed risks related to incorrect diagnosis. The key risk for Amyvid leading to incorrect diagnosis is considered to be incorrect scan interpretation. As the SmPC states that diagnosis cannot be established based on Amyvid scans alone without taking into account a clinical evaluation, the risks related to incorrect diagnosis beside image interpretation errors are not considered to represent safety concerns for Amyvid.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

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

Risk Management Plan

The applicant submitted a risk management plan, which included a risk minimisation plan.

Safety Issue	Proposed Pharmacovigilance Activities	Proposed Risk-Minimisation Activities
Important Potential Risk #1: Hypersensitivity reactions	Routine Pharmacovigilance	Routine (SmPC section 4.3 Contraindications, Annex 1)
Important Potential Risk #2: Carcinogenicity and hereditary effects	Routine Pharmacovigilance	Routine (SmPC Undesirable Effects section 4.8; Patient Leaflet Side Effects section 4, Annex 1) Administration within a controlled clinical setting and by trained staff
Important Potential Risk #3: PET-imaging interpretation errors	Routine Pharmacovigilance Post-Authorisation Safety Study to assess the effectiveness of the additional risk minimization activities Post-Authorisation Safety Study to evaluate patterns of use, including off label use.	Routine SmPC section 4.2 Posology and Method of Administration; Image Interpretation; Warnings and Precautions section 4.4; Interpretation of Amyvid Images; Training of physicians to optimize interpretation of PET scan.

The below pharmacovigilance activity(ies) in addition to the use of routine pharmacovigilance are needed to investigate further some of the safety concerns:

Description	Due date
Post-authorisation safety study to assess a) the effectiveness of the reader training programme including different training methods; b) understanding of and compliance of readers with the approved indication; and c) the frequency of reading errors in the routine clinical practice following implementation of the reading training	Submission of draft protocol for review within Q1 2013 Further timelines to be agreed upon protocol review.
Post-authorisation safety study to evaluate usage patterns of Amyvid including off-label use	Submission of draft protocol for review within Q1 2013. Further timelines to be agreed upon protocol review.

The following additional risk minimisation activities were required:

Training should be provided to users of florbetapir in order to ensure accurate and reliable interpretation of the PET images. The training should include:

- Information on amyloid pathology in Alzheimer Disease; relevant information on Amyvid as an β-amyloid PET tracer, including the approved indication according to the SmPC, limitations of Amyvid use, interpretation errors, safety information and the results of clinical trials informing on the diagnostic use of Amyvid
- Review of the PET reading criteria, including method of image review, criteria for interpretation, and images demonstrating the binary read methodology
- The material should include Amyvid PET demonstration cases with correct PET scan interpretation by an experienced reader; Amyvid-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 both electronic and in-person training should be ensured.

2.8. 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.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

Accuracy of clinical diagnosis of AD may be as low as 70% when compared to the definitive diagnosis of AD (based on a pre-specified levels of age-related brain beta-amyloid neuritic plaque density at autopsy in the presence or not of clinical history of dementia). This clearly shows the high need for better diagnostic procedures for AD.

The intended use of florbetapir (^{18}F) is for diagnosis. According to the Guideline on Clinical Evaluation of Diagnostic Agents (CPMP/EWP/1119/98/Rev.1) two aspects should be considered for assessment of efficacy of a diagnostic:

- Diagnostic Performance (e.g. sensitivity and specificity against (reference) standard of truth)

- Clinical usefulness (impact on diagnostic thinking and/or patient management)

For florbetapir (^{18}F), the diagnostic performance was evaluated versus the histopathological diagnosis at autopsy of the β-amyloid neuritic plaque density. On the other hand, clinical usefulness was assessed regarding the clinical diagnosis of AD. There is a wide gap between the clinical diagnosis of AD and the histopathological diagnosis of neuritic plaque density at autopsy. The uncertainties introduced by this gap must be considered when the efficacy of florbetapir (^{18}F) is assessed for either diagnostic performance or clinical usefulness.

Diagnostic performance was the primary focus of the pivotal efficacy studies A07 (n=226, 152 autopsy cohort; 74 young healthy controls) and its extension study A16 (N= 108 from autopsy cohort of A07), 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 achieved the pre-specified study objectives. The sensitivity (92%, 95% CI: 78% to 98%), and specificity (100%, 95% CI: 80% to 100%) of the majority visual PET read score for detecting moderate-frequent or none-sparse β-amyloid neuritic plaque score at autopsy, clearly exceeded the target value of 80%.

Florbetapir (^{18}F) accurately estimates moderate-frequent or non-sparse neuritic plaque density, 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. .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 "end–of-life" patients and data from young healthy volunteers presumably without amyloid burden in the submitted pivotal studies are not representative of the intended patient population. Various extrapolations are made to conclude from the patients in those studies to the intended use. The selection of an "end-of-life" population for the primary analyses in the pivotal studies implies the extrapolation of the efficacy results to the intended target population of patients with remaining therapeutic options, preferably NOT "end-of-life" patients. In the healthy subjects (demonstration of selectivity) no histopathological confirmation of the absence of amyloid is available, leaving the (admittedly highly improbable) possibility that a significant amyloid burden may have been present in a putatively healthy volunteer. More importantly, the absence of false positive results in young healthy volunteers cannot be

considered conclusive proof of the absence of false positive results in (elderly) patients with neurological diseases other than AD. There are very little data on patients with other neurological diseases.

Amyvid is not a stand-alone diagnostic tool, and needs to be used in conjunction with a clinical evaluation.

Due to the limited number of subjects in the pivotal trials, confidence intervals for both sensitivity and specificity have considerable width, but the CHMP concluded it was reassuring that the lower limits were still at about 80%.

The impact of reader's subjectivity on the subjective interpretation of florbetapir-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 readers' training in the clinical trials may be representative for the effect of a training of users post-marketing.This is addressed by a post-authorisation measure regarding the assessment of the effectiveness of readers training.

Actual impact on physician's diagnostic thinking (diagnosis and diagnostic intervals) was not demonstrated except for exclusion of amyloid burden and it is unclear to what extent any change in diagnostic thinking will lead to changes in patient management. Further to this, it remains unclear whether a tangible benefit for the patients can be expected from a change in patient management.

In the future, the clinical usefulness of florbetapir (^{18}F) may change in line with the ongoing discussions on the importance and exact meaning of amyloid burden for the pathophysiology and clinical course of AD. 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 florbetapir (^{18}F) would not avoid other diagnostic tests (eg. MRI, CT, blood tests, etc.): these are generally performed before PET to exclude non-neurodegenerative dementias when suspicions of AD exists.

The Applicant is invited to seek HTA/scientific advice on the design of a study to explore the impact on diagnostic thinking and patient management.

Risks

Unfavourable effects

Preclinical and clinical studies did not reveal specific safety concerns. Whereas the radiation exposure appears acceptable, a further minimization might be considered. A dose of 370 MBq (10 mCi) provided slightly better visual imaging quality than a lower dose of 111 MBq in dose-finding studies; however, it is not clear whether an intermediate activity (e.g. of about 185 MBq) might be used with the same imaging quality.

Uncertainty in the knowledge about the unfavourable effects

The high specificity, and particularly the high sensitivity to estimate the neuritic plaque density are appreciated. Nevertheless, false positive findings with the possible consequence of a wrong diagnosis of AD (and its consequences) cannot be excluded. Although no immunological events have been reported, the observed chills, rash, and urticaria might indicate a potential for development of these events, of at least of slight hypersensitivity reactions. These are included in the RMP.

Benefit-risk balance

Importance of favourable and unfavourable effects

Diagnosis of AD has severe consequences for the patients. The current diagnosis of AD based on standardized clinical criteria has a limited accuracy. A final "true" diagnosis can be made only after the patients' death and includes autopsy histopathology. Florbetapir (^{18}F) accurately estimates neuritic plaque density, only achievable at autopsy nowadays, when the patient is still alive. This density is one of the key issues, but not the only one, for the definitive diagnosis of AD at autopsy. This is regarded as a significant improvement in the diagnostic procedures for patients with cognitive impairment suspected of AD.

With no relevant adverse effects, the fact remains that false positive readings of florbetapir-PET images may result in wrongly diagnosed AD with the medical, psychological, and sociological consequences that this entails.

Benefit-risk balance

Discussion on the benefit-risk balance

There is clear evidence that florbetapir-PET images reflect the presence of moderate to frequent β-amyloid neuritic plaque density in the brain, with high sensitivity and even higher specificity, in a population of end-of-life patients whose cognitive status was not accurately established. Such capability has been only achievable at autopsy up to now, not when the patient is still alive. This is regarded as a significant improvement in the diagnostic procedures for adult patients with cognitive impairment who are being evaluated for AD and other causes of cognitive impairment. Currently, for a significant percentage of patients clinically diagnosed with AD on the basis of clinical criteria, the post-mortem neurohistopathology findings fail to confirm this diagnosis.

Based on current estimates on specificity and sensitivity of the neuritic plaque density, the use of florbetapir (^{18}F) is expected, but not confirmed, to reduce number of patients with discrepancies between clinical diagnosis and histopathological findings at autopsy. Such density is one of the defining components of the criteria for definitive diagnosis of AD at autopsy. The absence of increased amyloid is not compatible with a diagnosis of AD, while there might be β-amyloid neuritic plaque density in the brain in asymptomatic elderly and other neurodegenerative demented patients.

While the safety profile of florbetapir (^{18}F) is reassuring, the risk remains of false positive readings of florbetapir-PET images that may result in wrongly diagnosed AD. Therefore every effort must be made to minimize false positive readings. This includes restricting the use of florbetapir (^{18}F) to its approved use, ensuring that florbetapir-PET scans are perceived as an additional diagnostic tool to be associated with clinical investigation, addressing inter-reader variability (e.g. by readers' training and continued monitoring of the success of the training) and possible user non-compliance.

In the submitted supportive trials in the intended population physicians frequently changed their previous diagnosis when the findings in the florbetapir-PET were made available. However, information came from a retrospective study, which could introduce bias. Therefore it is highly recommended that the company performs a well-designed prospective study to assess impact on diagnostic thinking and patient management.

The immediate consequences of an improved diagnostic procedure for AD on the clinical course of the disease are less straightforward: it has not been shown that a changed diagnosis results in an altered treatment strategy translating in a tangible clinical benefit for the patients. This is at least partly due to

the rather limited effect of all currently available treatments for AD. In the absence of treatments to stop or revert AD, it is not clear that early AD diagnosis means benefits and omitting/delaying treatment in false negative cases is not crucial.

The improvement in the diagnostic procedures of AD with a non-invasive method and with the good safety profile of florbetapir (^{18}F) is therefore considered of benefit even without strong evidence for an immediate improvement in the patient's management and patient's outcome of the intended population.

4. 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 Amyvid in the following indication

Positron Emission Tomography (PET) diagnostic 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 Amyvid 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

Pharmacovigilance system
The MAH must ensure that the system of pharmacovigilance presented in Module 1.8.1 of the Marketing Authorisation, is in place and functioning before and whilst the medicinal product is on the market.

Risk Management System
The MAH must ensure that the system of pharmacovigilance, presented in Module 1.8.1 of the marketing authorisation, is in place and functioning before and whilst the product is on the market.

The MAH shall perform the pharmacovigilance activities detailed in the Pharmacovigilance Plan and the Risk minimisation Plan, as agreed in the Risk Management Plan presented in Module 1.8.2. of the Marketing Authorisation and any subsequent updates of the RMP agreed by the Committee for Medicinal Products for Human Use (CHMP).

As per the CHMP Guideline on Risk Management Systems for medicinal products for human use, the updated RMP should be submitted at the same time as the next Periodic Safety Update Report (PSUR).

In addition, an updated RMP should be submitted

- When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan or risk minimisation activities
- Within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached
- At the request of the European Medicines Agency.

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

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 discussions and agreement with the National Competent Authorities in each Member State where Amyvid is marketed, at launch and after launch, all physicians who are expected to use Amyvid have access to a training course in order to ensure accurate and reliable interpretation of the PET images.

The physician training course should contain the following key elements:

- Information on amyloid pathology in Alzheimer Disease; relevant information on Amyvid as an β-amyloid PET tracer, including the approved indication according to the SmPC, limitations of Amyvid use, interpretation errors, safety information and the results of clinical trials informing on the diagnostic use of Amyvid
- Review of the PET reading criteria, including method of image review, criteria for interpretation, and images demonstrating the binary read methodology
- The material should include Amyvid PET demonstration cases with correct PET scan interpretation by an experienced reader; Amyvid-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 both electronic and in-person training should be ensured.

Obligation to complete post-authorisation measures

The MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
RMP Post-authorisation safety study To assess a) the effectiveness of the reader training programme including different training methods; b) understanding of and compliance of readers with the approved indication; and c) the frequency of reading errors in the routine clinical practice following implementation of the reading training; Submission of draft protocol for review by 5 months after CHMP opinion. Further timelines to be agreed upon protocol review.	1st quarter 2013
RMP Post-authorisation safety study To evaluate usage patterns of Amyvid including off-label use; Submission of draft protocol review by 5 months after CHMP opinion. Further timelines to be agreed upon protocol review.	1st quarter 2013

* Classification: Annex II (specific obligations; obligations), RMP

Proposed list of recommendations:

Description of post-authorisation measure(s)
The company should continue to develop and validate a quantitative PET reading methodology based on their product.
The company is strongly encouraged to perform a study to assess the impact on diagnostic thinking and patient management since the therapeutic consequences of the diagnosis of labelling brain β-amyloid are not obvious. For the design, parallel HTA/scientific advice is recommended.

New Active Substance Status

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