CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202008Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202008
Agency receipt date: October 7, 2011 (Resubmission)
Drug: Florbetapir F 18 (18F AV-45)
Applicant: Avid Radiopharmaceuticals Inc.
Indication: Positron Emission Tomography (PET) imaging of β-amyloid aggregates in the brain.
Reviewing Division: Division of Medical Imaging Products

Background:
The pharm/tox reviewer and team leader previously concluded that the nonclinical data support approval of Florbetapir for the indication listed above. I agreed with the division pharm/tox conclusion.

No new nonclinical studies were submitted in the resubmission.

I have discussed the Established Pharmacologic Class with members of the Division. I suggested that radioactive diagnostic agent maybe an appropriate term and would be consistent with other similar product labeling. The division is considering this wording.

I also discussed other aspects of labeling with the pharm/tox supervisor and additional edits may be made.

Conclusions:
I concur with the pharm/tox supervisor and reviewer that this NDA can be approved from the pharm/tox perspective.

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/s/

PAUL C BROWN
03/27/2012

Supervisory Pharmacologist Memo

NDA: **202-008**
Drug: **Florbetapir F 18 (^{18}F AV-45)**
Sponsor: **Avid Radiopharmaceuticals Inc.**

This memo to file is in respect of NDA 202-008 resubmission application for Florbetapir F 18 Injection, (b) (4)

Dr. Sunny Awe conducted the Pharmacology/Toxicology primary review of the initial NDA and recommended approval from pharmacology/Toxicology perspectives. The sponsor did not conduct new nonclinical studies for the resubmission. Dr. Awe recommended approval based on his previous review.

I concur.

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/s/

ADEBAYO A LANIYONU
03/21/2012

MEMO TO FILE

Application number:	NDA 202-008
Supporting document/s:	Electronic submission
CDER stamp date:	October 7, 2011
Product:	Florbetapir F 18 (^{18}F AV-45)
Applicant:	Avid Radiopharmaceuticals Inc.
Review Division:	Medical Imaging Products
Reviewer:	Sunny Awe, Ph.D.
Supervisor/Team Leader:	Adebayo Laniyonu, Ph.D.
Division Director:	Dwaine Rieves, MD.
Project Manager:	Sharon Thomas

This memo to file is in respect of NDA 202008 resubmission application for Florbetapir F 18 product by Avid Pharmaceuticals Inc. The original NDA application (stamp date-September 14, 2010) was for this indication: "*Florbetapir F 18 Injection is a diagnostic radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β-amyloid aggregates in the brain. A negative ^{18}F-AV-45 -PET scan is clinically useful in ruling out the presence of β-amyloid, a definitive pathology of Alzheimer's disease (AD).*" The NDA application was reviewed and recommended for approval from nonclinical perspective. Nonclinical has no outstanding issues with the NDA application. The review is available in DARRTS.

In the current resubmission, the sponsor changed the indication to: (b) (4)

[redacted]

The sponsor did not conduct new nonclinical studies for the resubmission. The change in indication for this product does not require additional nonclinical study. Therefore, our previous recommendation of approval from nonclinical perspective stands.

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/s/

SUNNY O AWE
03/16/2012

ADEBAYO A LANIYONU
03/17/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202008 **Applicant:** Avid Pharmaceuticals Inc. **Stamp Date:** October 7, 2011

Drug Name: AMYVID™ **NDA/BLA Type:** Resubmission

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		The Agency granted the sponsor's request for the waiver from conducting carcinogenicity and reproductive and developmental toxicity studies based on indication and frequency of use.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?	X		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __ Yes_**_____**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

ADDITIONAL COMMENT: This is a resubmission in response to a complete response letter. The original submission was recommended for approval on the basis of nonclinical data on February 11, 2011. No new nonclinical data in the resubmission.

Sunny Awe 11-03-11

Reviewing Pharmacologist Date

Adebayo Laniyonu, Ph.D. 11-03-11

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

APPEARS THIS WAY ON ORIGINAL

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/s/

SUNNY O AWE
11/03/2011

ADEBAYO A LANIYONU
11/03/2011

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202008
Agency receipt date: Sept. 17, 2010
Drug: Florbetapir F 18 (18F AV-45)
Applicant: Avid Radiopharmaceuticals Inc.
Indication: Positron Emission Tomography (PET) imaging of β-amyloid aggregates in the brain.
Reviewing Division: Division of Medical Imaging Products

Background:
The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of Florbetapir for the indication listed above.

A number of nonclinical studies were not required for this application because of the patient population (subjects with potential Alzheimer's disease) and the acute use of the agent.

Genetic toxicity:
An increase in the number of revertant colonies with a non-radioactive version of Florbetapir (AV-45) was observed in *Salmonella* strainsTA98 and TA100 with or without metabolic activation. AV-45 did not cause structural chromosomal aberrations in human peripheral lymphocytes during a 3-hour treatment with or without metabolic activation, but it did induce a significant increase in structural aberrations during a 22-hour treatment without metabolic activation. A cumulative daily dose of 372 µg AV-45/kg/day for 3 consecutive days did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in either male or female Hsd:SD rats.

The applicant has proposed labeling that states that (b) (4) However, based on all the genetic toxicity information, it appears that AV-45 has some genotoxic potential. This is not likely to present a significant safety concern because the positive in vitro findings are mitigated by the lack of a positive in vivo finding, the short-term use of the product and the presence of a radioactive isotope on the active drug substance.

Conclusions:
The studies that were conducted are adequately summarized in the primary review and there are no outstanding nonclinical safety issues. I agree with the division pharm/tox conclusion that this application can be approved from a pharm/tox perspective.

Labeling will be reviewed at a later time.

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/s/

PAUL C BROWN
02/18/2011

Supervisory Pharmacologist Memo

NDA: **202-008**
Drug: **Florbetapir F 18 (^{18}F AV-45)**
Sponsor: **Avid Radiopharmaceuticals Inc.**

Florbetapir F 18 Injection is a diagnostic radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β-amyloid aggregates in the brain. A negative 18F-AV-45 -PET scan is clinically useful in ruling out the presence of β-amyloid, a definitive pathology of Alzheimer's disease (AD).

Dr. Sunny Awe conducted the Pharmacology/Toxicology primary review of the NDA and recommended approval from pharmacology/Toxicology perspectives. He proposed changes in the label.

I concur with Dr. Awe's recommendations.

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/s/

ADEBAYO A LANIYONU
02/11/2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 202-008
Supporting document/s:	Electronic submission
Applicant's letter date:	September 12, 2010
DER stamp date:	September 14, 2010
Product:	Florbetapir F 18 (^{18}F AV-45)
Indication:	Florbetapir F 18 Injection is a diagnostic radiopharmaceutical indicated for Positron Emission Tomography (PET) imaging of β-amyloid aggregates in the brain. A negative ^{18}F-AV-45 -PET scan is clinically useful in ruling out the presence of β-amyloid, a definitive pathology of Alzheimer's disease (AD).
Applicant:	Avid Radiopharmaceuticals Inc.
Review Division:	Medical Imaging Products
Reviewer:	Sunny Awe, Ph.D.
Supervisor/Team Leader:	Adebayo Laniyonu, Ph.D.
Division Director:	Dwaine Rieves, MD.
Project Manager:	Sharon Thomas

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202-008 are owned by Avid Radiopharmaceuticals Inc. or are data for which Avid Radiopharmaceuticals Inc. has obtained a written right of reference. Any information or data necessary for approval of 202-008 that Avid Radiopharmaceuticals Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of 202-008.

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1 Executive Summary

1.1. Introduction

^{18}F-AV-45 (Amyvid®Florbetapir; (E)-4-(2-(6-(2-(2-(2-[^{18}F]fluoroethoxy) ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylbenzenamine is proposed as a diagnostic radiopharmaceutical for Positron Emission Tomography (PET) imaging of β-amyloid aggregates in the brain. As proposed, a negative florbetapir-PET scan is clinically useful in ruling out the presence of pathologically significant levels of β-amyloid. Amyvid selectively binds to β-amyloid plaques with high affinity. However, ^{18}F-AV-45 demonstrates low affinity for NS and cardiovascular receptors and monoamine transporters. ^{18}F-AV-45 is intended for intravenous administration as a single bolus dose of 10 mCi and total volume of not more than 10 mL.

1.2 Brief Discussion of Nonclinical Findings

1) Proof of concept Studies: *In vitro* and *ex vivo* studies were conducted to demonstrate the affinity and selectivity of ^{18}F-AV-45 binding to β-amyloid plaque. The ability of ^{18}F-AV-45 and other compounds to bind to amyloid plaque was evaluated in a study involving the inhibition of ^{125}I- 6-iodo-2-(4'-dimethylamino-)phenyl-imidazol[1,2-α]pyridine (IMPY) binding to β-amyloid plaque in human AD brain homogenate. ^{18}F-AV-45 competitively inhibited ^{125}I-IMPY binding in the assay with K_i=5.5±0.7 nM a value that compares favorably well with those of other potential amyloid imaging agents already tested in humans.

^{18}F-AV-45 rapidly dissociates off the amyloid plaques after binding with a K_d value of 3.1±0.7 nM indicating that ^{18}F-AV-45 binding to the amyloid plaques is reversible. ^{18}F-AV-45 demonstrated high specificity in binding to its target and low binding affinity to central nervous system (NS) and ot her receptor binding sites.

Autoradiography data obtained from frozen human brain sections demonstrates ^{18}F-AV-45 labeling of β-amyloid plaques in the post-mortem brain sections of AD patients but no ^{18}F-AV-45 labeling was found in the brain sections of control human subjects. The data also showed that ^{18}F-AV-45 selectively binds to the grey matters of brain homogenates of AD patients and poorly binds to the white matters of AD where amyloid β is usually low and no binding in the brain tissues of control subjects due to absence of amyloid as shown in figure 1.

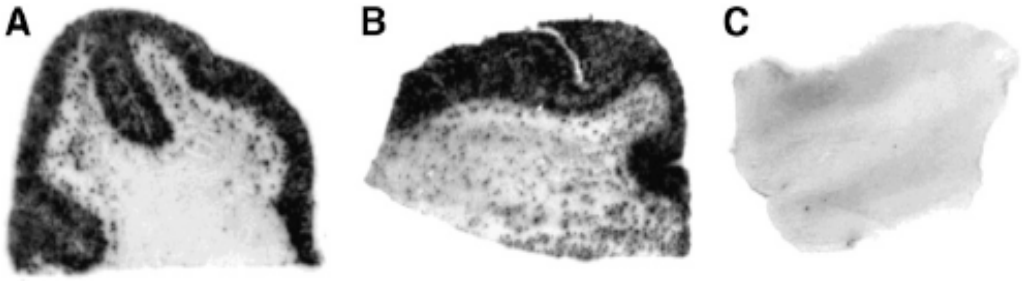


Figure 1: *In vitro* autoradiograms of frozen human brain sections labeled with ^{18}F-AV-45. (A and B) Highly intensive labeling of Aβ plaques on brain sections from AD patients. (C) Control subject exhibits no labeling by this tracer.

Transgenic mice (B6.Cg-Tg [APPswe- PSEN1]; that over express Aβ and generate Aβ plaques in the brain was employed as an animal model of AD to further evaluate the binding and specificity of Amyvid binding to β amyloid. There was a significant ^{18}F-AV-45 labeling of the Aβ plaques as shown in figure 2.

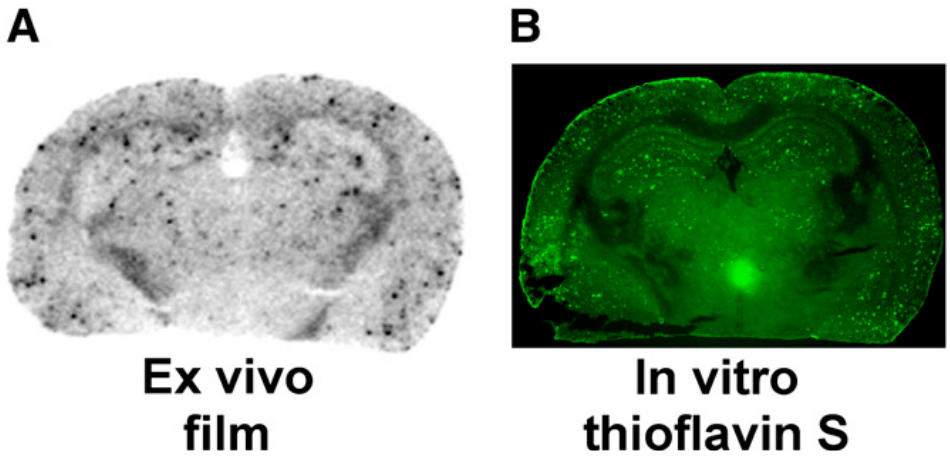


Figure 2: *Ex vivo* autoradiography of ^{18}F-AV-45 in 25-mo-old Tg (APPswe/PSEN1) mice. (A) *Ex vivo* autoradiogram of brain section. (B) Fluorescent image of comparable brain section after thioflavin S staining.

This data corroborates the binding and specificity of ^{18}F-AV-45 to Aβ plaques in brain homogenates from AD patients and in animal model of AD.

In addition, autoradiography, silver staining, thioflavin S flouresence scoring and amyloid beta specific immunohistochemistry studies on brain homogenates obtained from 48 human brain tissues were conducted. Correlation analysis performed on Amyvid binding and the data obtained from the studies indicates:

a) Correlation between Amyvid binding and neuritic plaque scores.

b) Correlation between Amyvid binding and β-amyloid plaque deposition measured by silver stain, anti-Aβ immunohistochemistry (using two different antibodies), and thioflavin S staining as shown in figure 3

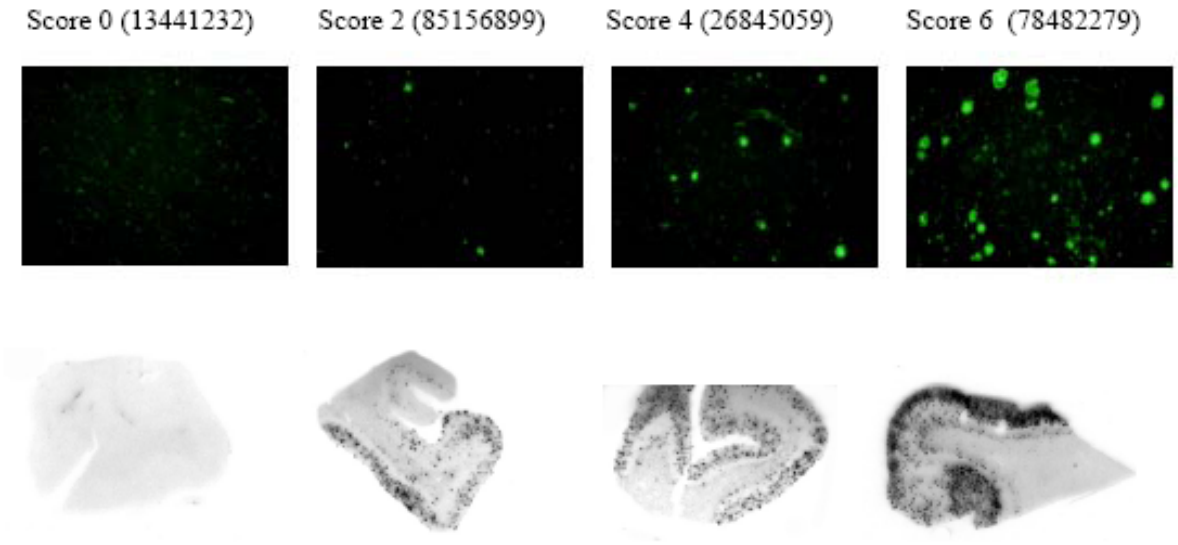


Figure **3**: Thioflavin S staining and scoring. The figure shows representative sections. Scores used were 0:none, 2:spare, 4:moderate and 6:frequent thioflavin S- positive aggregates (numbers in brackets correspond to subjects numbers in the Table below). The autoradiographs in the second row show the corresponding ^{18}F-AV-45 binding.

and

c) Correlation between Amyvid binding and immunohistochemistry quantification obtained using specific Aβ antibodies.

2) Pharmacokinetics, Distribution and Excretion: The autoradiographic and biodistribution data obtained from mice and Rhesus monkey indicate that following an intravenous injection, ^{18}F-AV-45 penetrates the brain readily and is rapidly cleared from the brain. The rapid clearance reduces non-specific binding to the brain tissue that could complicate imaging. The dosimetry data showed an estimated human effective dose of 97 mrem/mCi, which is within acceptable radiation dose limit.

^{18}F-AV-45 metabolism was studied using human and rat liver microsomes in the presence of an NADPH-generating system. The data showed that ^{18}F-AV-45 is demethylated to AV-160 and subsequently acetylated to AV-267. The binding characteristics of these metabolites to beta amyloid plaques were evaluated using autoradiography. Both metabolites demonstrated low affinity to the beta amyloid plaques. Thus, the metabolites will probably not interfere with Amyvid binding. ^{18}F-AV-45 and the metabolites are excreted from the body via the urinary route.

3) Safety Pharmacology Studies: The CNS safety of ^{18}F-AV-45 was evaluated as part of single/repeat-dose toxicity study in Sprague Dawley rats. No CNS adverse effects were reported in single, or 28-day repeated dose toxicity studies at up to 21.8X MHD

dose levels. Potential cardiovascular effect of ^{18}F-AV-45 was assessed using both *in vitro* and *in vivo* methods. ^{18}F-AV-45 inhibited hERG potassium current by 16.7±0.9% (n=4) at 12.4 µM; the only employed dose due to solubility problem, versus 0.2±0.1% (n=3) in control while the positive control, terfenadine (60nM), induced up to 83.8% inhibition. Adverse cardiovascular or respiratory effects were not observed following ^{18}F-AV-45 treatment of up to a dose of 84X MHD in a cardiovascular safety pharmacology and respiratory function study in beagle dogs.

4) Toxicity Studies: Single- and repeat-dose toxicity studies were conducted in rats and Beagle dogs. No treatment-related mortality or any serious adverse effects was reported in any of these studies. NOAELs of 87.2X- and 21.8X- MHD was obtained in a single- and 28-day repeat-dose toxicity study respectively in the rats. No cardiovascular or ocular effects were reported during 14-day repeat-dose toxicity study with a 2-week recovery period conducted in Beagle dogs and a NOAEL of 4.5X MHD was obtained. A NOAEL of 21X MHD was obtained during 28-day repeat-dose toxicity with 14-day recovery period conducted in Beagle dogs.

5) Reproductive Toxicity Studies: No reproductive toxicity study was conducted on ^{18}F-AV-45. The sponsor's request for a waiver for reproductive and developmental toxicity studies was granted by the agency. Pregnancy category C is recommended for label.

6) Genotoxicity Studies: Genotoxicity tests conducted on AV-46 includes two *in vitro* assays covering the bacterial reverse mutation assay (Ames test) and chromosomal effects (cultured human peripheral lymphocytes cells). ^{18}F-AV-45 tested positive in the *in vitro* assays and negative *in vivo* mouse micronucleus assay. This data would be reflected in the label.

7) Carcinogenicity Studies: Not required.

8) Impurities: Impurities were quantified and found to be within acceptable limits.

9) Nonclinical safety issues relevant to clinical use: The available nonclinical findings do not show any significant nonclinical safety issues that could adversely affect the clinical use of ^{18}F-AV-45 in the context of its proposed indication in this NDA.
.

10) Conclusions: Based on the review of the preclinical data, there seems to be no significant safety concerns with ^{18}F-AV-45 and the proposed indication.

1.3 Recommendations

1.3.1 Approvability

Approval is recommended from pharmacology/toxicology perspectives contingent to proposed changes in the label.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following label recommendations would more appropriately reflect findings from nonclinical studies:

(b) (4)

Label proposed by sponsor:

(b) (4)

Recommended label:

(b) (4)

(b) (4)

8.1. Pregnancy

Label proposed by sponsor:

Pregnancy Category C: (b) (4) Amyvid should be administered to a pregnant woman only if (b) (4) clearly needed.

Recommended label:

Pregnancy Category C: (b) (4)

(b) (4)

Amyvid should be administered to a pregnant woman only if clearly needed.

8.3. Nursing Mothers

Label proposed by sponsor:

(b) (4)

Recommended label:

(b) (4)

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

Label proposed by sponsor:

In an *in vitro* bacterial reverse mutation assay (Ames test), increases in the number of revertant colonies were observed in 2 of the 5 strains exposed to (b) (4) ^{19}F-AV-45, the non-radioactive form of florbetapir F 18). In a chromosomal aberration *in vitro* study with cultured human peripheral lymphocytes cells, (b) (4) did not increase the percent of cells with structural aberrations with 3-hour exposure with or without activation; however, 22 hour exposure produced a statistically significant increase in structural aberrations at all tested concentrations. Potential *in vivo* genotoxicity of (b) (4) was evaluated in a mouse micronucleus study. In this assay, (b) (4) did not increase the number of micronucleated polychromatic erythrocytes at the highest achievable dose level, 372 µg/kg/day, when given twice daily for 3 consecutive days.

(b) (4)

(b) (4)

Recommended label:

In an *in vitro* bacterial reverse mutation assay (Ames test), increases in the number of revertant colonies were observed in 2 of the 5 strains exposed to (b) (4) ^{19}F-AV-45, the non-radioactive form of florbetapir F 18). In a chromosomal aberration *in vitro* study with cultured human peripheral lymphocytes cells, (b) (4) did not increase the percent of cells with structural aberrations with 3-hour exposure with or without activation; however, 22 hour exposure produced a statistically significant increase in

structural aberrations at all tested concentrations. Potential *in vivo* genotoxicity of (b) (4) was evaluated in a mouse micronucleus study. In this assay, (b) (4) did not increase the number of micronucleated polychromatic erythrocytes at the highest achievable dose level, 372 µg/kg/day, when given twice daily for 3 consecutive days.

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

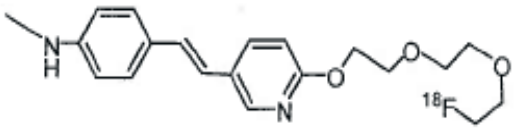
Generic Name: (E)-4-(2-(6-(2-(2-(2-[^{18}F]fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylbenzenamine

Code Name: ^{18}F-AV-45, Florbetapir F 18, Amyvid®

Chemical Name: (E)-4-(2-(6-(2-(2-(2-[^{18}F]fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylbenzenamine

Molecular Formula/Molecular Weight: $C_{20}H_{25}[^{18}F]N_2O_3$/359.4

Structure or Biochemical Description:



^{18}F-AV-45

Pharmacologic Class: Diagnostic Radiopharmaceutical Product (PET tracer)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 76862, 79511

2.3 Drug Formulation

This is a sterile product containing not less than 37 MBq/mL florbetapir F 18, apyrogenic solution and comprising 10% (v/v) Ethanol, 0.45% (w/v) Sodium Ascorbate, and 0.81% (w/v) Sodium Chloride in water.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

All the impurities and degradants were quantified and found to be within acceptable limits.

2.6 Proposed Clinical Population and Dosing Regimen

Subjects suspected to have Alzheimer's disease (AD). 370 MBq (10 mCi) of Amyvid is administered as a single intravenous bolus dose in a total volume not exceeding 10 mL.

2.7 Regulatory Background

The sponsor commenced the drug development process of ^{18}F-AV-45 with an exploratory IND (#76862) submitted to the agency in 2006. The exploratory IND was subsequently closed and replaced with a full IND (#79511).

3 Studies Submitted

3.1 Studies Reviewed

1) AV-45 binding to Alzheimer's disease tissue homogenate.
2) Binding Selectivity (binding to β-amyloid in homogenates.
3) *Ex Vivo* Autoradiography of Transgenic Mouse Brain.
4) *In Vivo* Biodistribution in Mice.
5) *In Vivo* Metabolism in Mice.
6) TR-AV-45-020: Postmortem correlation of ^{18}F-AV-45 binding to β-amyloid plaque burden in postmortem human brain tissue.
7) TR-AV-45-004 ((b) (4)-1083089): CNS and cardiovascular receptor binding of AV-45.
8) TR-AV-45-081- Potential drug-drug interactions at the ^{18}F-AV-45 binding site.
9) 1665-07667: Effects of AV-45 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cell.
10) 1665-07887: AV-45: A cardiovascular safety pharmacology and respiratory function study in beagle dogs.
11) TR-AV-011: Biodistribution of ^{18}F-AV-45 for Injection in normal mice.
12) Primary PET Imaging Study with ^{18}F-AV-45 (Rhesus Monkey).
13) TR-AV-45-012: Human Radiation dosimetry estimates based on biodistribution of ^{18}F-AV-45 for Injection in normal mice.
14) TR-AV-45-007: *In vitro* metabolism of ^{18}F-AV-19 and ^{18}F-AV-45 with human and rat liver microsomes.
15) TR-AV-45-010: *In vivo* metabolism of ^{18}F-AV-45 in normal mice and characterization of its metabolites.
16) Study 1665-07623: A Single and 28-Day Repeated Dose Intravenous Study in Sprague-Dawley Rats with a Functional Observational Battery.
17) Study 1665-07738: A 14-Day Repeat-Dose Intravenous Toxicity Study in Beagle Dogs with a 2-Week Recovery.
18) Study 08-3354: AV45: 28-Day Intravenous Toxicity Study in Dogs with a 14-day Recovery Period.
19) Study (b) (4)07-379: AV-45 Salmonella-*E-coli*/Mammalian microsome reverse mutation assay.

20) Study (b) (4) 07-380: AV-45 *In vitro* Chromosome Aberration Study with Human Peripheral Lymphocytes (HPL).
21) Study AC00HR.125.BT: Rat Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Administration of AV-45.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Reviews on INDs 76862 and 79511

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action: ^{18}F-AV-45 demonstrates high specificity in binding to amyloid plaque in AD brain homogenates. Several studies and publications were submitted to address the proof of concept and potential mechanism of action of AV-45. A brief synopsis of these is provided below. Please note that the subtitles for these sections were as provided by the sponsor.

AV-45 binding to Alzheimer's disease tissue homogenate.
Binding studies were conducted to determine binding saturation, affinity and dissociation constants. . The postmortem brain tissue employed for this study was confirmed to be from AD patient in accordance with NIA-Reagan Institute Consensus Group criteria. The brain tissues were dissected and homogenates were prepared from the gray matters. The homogenates were pooled in phosphate buffered saline, aliquoted into 1-ml portions (100 mg wet tissue/ml) and stored at -70° C. The binding affinity of AV-45 was evaluated by incubating the brain homogenates with ^{125}I-IMPY (0.04-0.06 nM diluted in PBS). AV-45 (10^{-5}-10^{-10}M) was diluted in PBS containing 0.1% BSA). The bound and free radioactivity was separated by vacuum filtration followed by washing twice with 3 ml PBS. The filters containing bound ^{125}I ligands were assayed using a gamma counter to determine the K_i values. The result indicates that AV-45 inhibited ^{125}I-IMPY binding with a K_i of 5.5 ± 0.7 nM (n=3).

The sponsor cross-referenced published articles, (Kung et al., 2004; Zhang et al., 2005, 2008) in support of competitive inhibition claim. In these publications, the inhibition of ^{125}I-IMPY binding to human AD brain homogenate amyloid plaques by various compounds was evaluated. The binding affinity of the compounds was compared with that of AV-45. The K_i values of ^{18}F-AV-45 compares favorably well with those of other published potential amyloid imaging agents already tested in humans. These imaging agents include [N-methyl-]2-4'-methylaminophenyl)-6-hydroxybenzothiazole (PIB), 2-(1-{6-[2- [^{18}F]fluoroethyl](methyl)amino}-2-naphthyl)ethylidene)malononitrile (FDDNP), 6-iodo-2-(4'-dimethylamino-)phenyl-imidazol[1,2-α]pyridine (IMPY) and 4-*N*-methylamino-

4'-hydroxystilbene (SIB). The value of the *Ki* of AV-45 compares well with K_i values 2.8±0.5 nM and 239 nM for PIB and FDDNP respectively as shown in Table below:

Table 1: Binding affinity of AV-45 and other amyloid plaque ligands to AD brain homogenates (Ki vs. 125I-IMPY). Comparator values from published data (Kung et al., 2004; Zhang et al., 2005, 2008)

	AV-45	AV-1	PIB	FDDNP
K_i (nM) Mean ± SD	5.5 ± 0.7	6.7 ± 0.3	2.8 ± 0.5	239

Furthermore, the saturation binding and kinetic binding of ^{18}F-AV-45 and other amyloid plaque ligands to AD brain homogenates was evaluated. Specific binding of ^{18}F-AV-45 was determined by incubating the AD human brain homogenates with various concentrations of ^{18}F-AV-45 (0.04-10 nM, specific activity 2820 Ci/mmol) in the absence and presence of Benzotriazole-1 (BTA-1) (8 µM) at 37°C for 120 min. Four different homogenates were tested and the K_d and B_{max} were determined. The samples were then rapidly filtered and the filters washed with PBS, pH 7.4. The radioactivity retained on the filter disks was determined in a gamma counter. K_d and B_{max} were calculated by Scatchard plot and Rosenthal analysis. The K_d and B_{max} were reported as 3.72±0.30 nM and 8,811±1,643 fmol/mg protein respectively (figure 3).

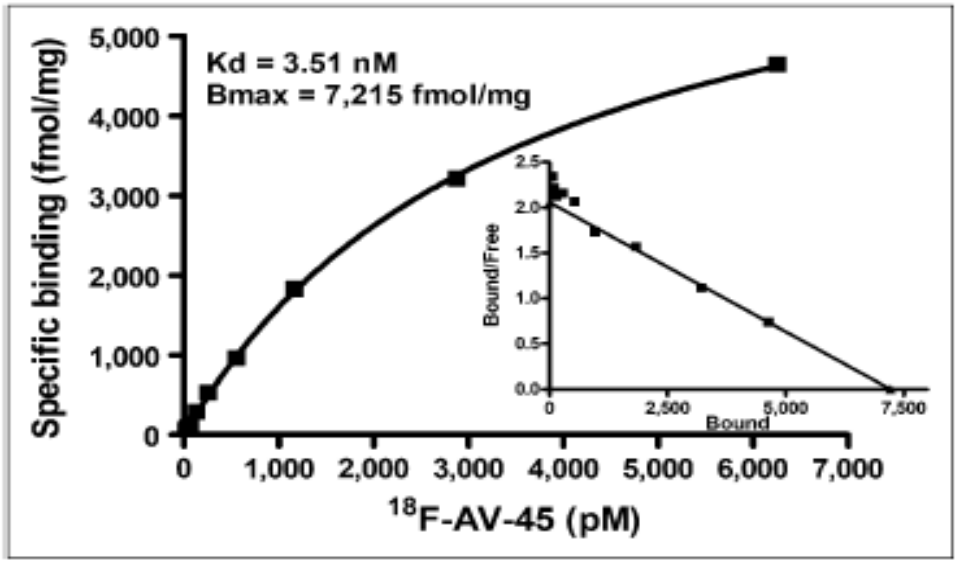


Figure 3: Representative saturation binding of ^{18}F-AV-45 to Aβ plaques in postmortem AD brain homogenates; Scatchard analysis of binding is shown. In this example, K_d was 3.51 nM and B_{max} was 7,215 fmol/mg of protein. Overall, average values for 4 AD cases were K_d = 3.72±0.30 nM and B_{max} = 8,811±1,643 fmol/mg of protein.

The dissociation constant of ^{18}F-AV-45 was also evaluated by measuring direct binding of labeled ^{18}F-AV-45 to brain homogenates. AD brain homogenates were incubated with ^{18}F-AV-45 for 2 hr at 37°C to reach the equilibrium. Benzotriazole-1 (BTA-1) (8 μM) was added to block further binding of ^{18}F-AV-45. Binding was measured at various times (0 to 120 min) to determine how rapidly ^{18}F-AV-45 dissociates off the amyloid plaques. The K_d was 3.1±0.7 nM, indicating that the compound is kinetically active in binding to its target with a high binding potential.

Table 2: Binding Affinity of AV-45 and other amyloid plaque ligands to peripheral benzodiazepine receptors and monoamine transporters.

	Peripheral Benzodiazepines		Monoamine transporters	
	% Inhibition	K_i (μM)	% Inhibition	K_i (μM)
AV-45	58	8.14	58	7.38
AV-19	61	3.63	52	3.44
AV-1	42	Not Available	13	Not Available

This data indicates that tracer dose of 10 nmol of ^{18}F-AV-45 will not cause any significant interaction with peripheral benzodiazepine or monoamine transporter sites. It is therefore very unlikely that ^{18}F-AV-45 at the planned dose would interact with any of the major receptor sites or transporters.

In a study by Choi et al (2009), another binding assay was employed to further characterize ^{18}F-AV-45. Homogenates of gray cortical brain matter from AD subjects were incubated with ^{18}F-AV-45 (0.05 nM) and competing compounds (BAY94-9172, PIB and GE-067) serially diluted to 10^{-5} to 10^{-10} M concentrations. The study involved defining nonspecific binding in the presence of 8 mM concentration of benzotriazole-1 (BTA-1), a thioflavin T analog known to bind to β-amyloid. Following incubation, the bound and free radioactivity was separated by filtration and the K_i (nM) of AV-45, BAY94-9172, PIB and GE-067 were 2.9±0.2, 0.9±0.2, 0.7±0.4 and 2.2±0.5 respectively. This indicates that ^{18}F-AV-45 demonstrated comparable binding affinity with the other tested compounds on β-amyloid.

Reviewer's Comment: Agrees with the results of this study. However, this reviewer observed that in the available data, the sponsor compared the binding affinities of ^{18}F-AV-45 with that of β-amyloid ligands already tested in AD brain homogenates. One would have preferred that the efficacy of ^{18}F-AV-45 be compared with that of approved products. However, none of these imaging agents are approved by the agency and therefore the compounds cannot be used for comparative efficacy claim.

Preclinical properties of ^{18}F-AV-45: a PET agent for Aβ plaques in the brain.
The following preclinical studies were conducted on characterization of ^{18}F-AV-45 as an Aβ plaques imaging agent:

1) Binding Selectivity (binding to β-amyloid in homogenates.

2) *Ex Vivo* Autoradiography of Transgenic Mouse Brain.
3) *In Vivo* Biodistribution in Mice.
4) *In Vivo* Metabolism in Mice.

Studies 3 and 4 are reviewed as: "Biodistribution of ^{18}F-AV-45 for Injection in normal mice (Study TR-AV-011)" and "*In vivo* metabolism of ^{18}F-AV-45 in normal mice and characterization of its metabolites" respectively under the section on pharmacokinetics below.

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Binding Selectivity (binding to β-amyloid in homogenates).

Autoradiography of postmortem brain tissue sections with ^{18}F-AV-45 was done to support selective binding of ^{18}F-AV-45 to Aβ plaques in the brain of AD patients. As shown below, there was intense labeling of Aβ plaques on brain sections from AD patients labeled with ^{18}F-AV-45. However, there was no labeling with ^{18}F-AV-45 on the images of frozen control brain sections from healthy subjects. This data shows that ^{18}F-AV-45 demonstrates specificity as a tracer in labeling the Aβ plaques present on the brain sections from AD patients and there was absence of ^{18}F-AV-45 labeling in the brain sections of healthy control subjects.

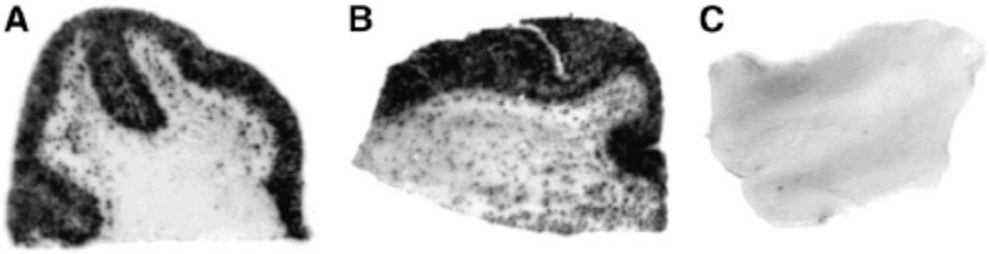


Figure 4: *In vitro* autoradiograms of frozen human brain sections labeled with ^{18}F-AV-45. (A and B) Highly intensive labeling of Aβ plaques on brain sections from AD patients. (C) Control subject exhibits no labeling by this tracer.

Reviewer's Comment: Agrees with the results of this study

Ex Vivo Autoradiography of Transgenic Mouse Brain.

This study was conducted to demonstrate the selectivity of ^{18}F-AV-45 binding to Aβ plaques in an animal model of AD. The transgenic mice (B6.Cg-Tg [APPswe-PSEN1]; engineered to over express Aβ and to generate Aβ plaques in the brain was employed as an animal model of AD. The transgenic mice were anesthetized and injected with 18,500 kBq (500 mCi) of ^{18}F-AV-45 and sacrificed 30 minutes after injection. Brain sections (20 mm) were prepared and exposed to Kodax Biomax MRI film overnight. The film was developed and the images digitized. The brain autoradiography data (figure 5), showed a dense labeling of the plaques in the cortical regions and hippocampus. This was confirmed by co-staining with thioflavin S, a dye commonly used for staining the Aβ plaques in human brain sections.

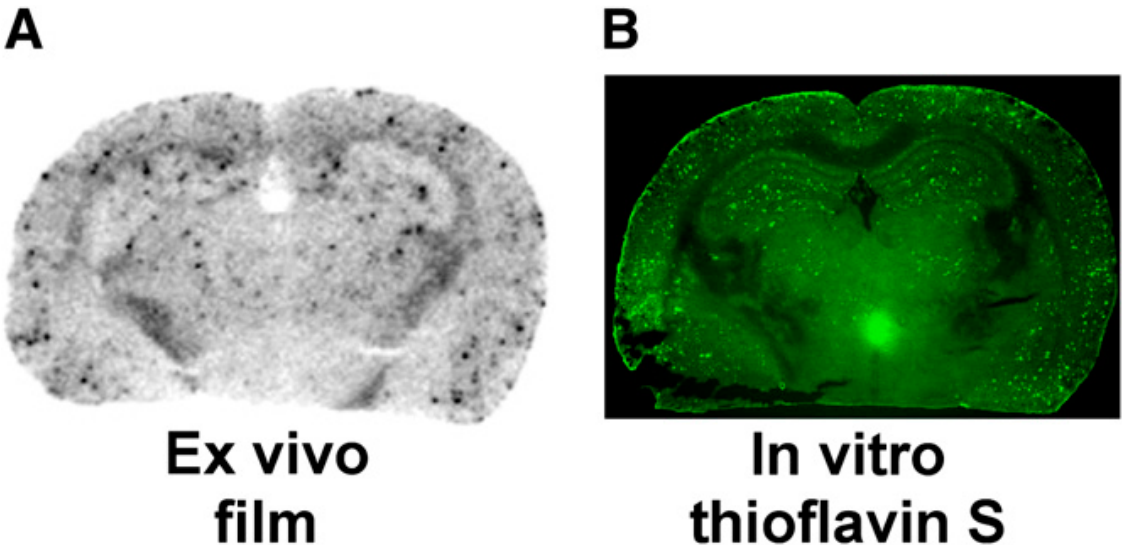


Figure 5: *Ex vivo* autoradiography of ^{18}F-AV-45 in 25-mo-old Tg (APPswe/PSEN1) mice. (A) *Ex vivo* autoradiogram of brain section. (B) Fluorescent image of comparable brain section after thioflavin S staining.

Reviewer's Comment: Efforts to develop transgenic mice to over express Aβ and to generate Aβ plaque in the brain is progressing albeit, still in its infancy. However, this reviewer is of the opinion that this *ex-vivo* study corroborates the autoradiography data obtained from post-mortem AD brain sections. Thus, the study established the selectivity of the binding of ^{18}F-AV-45 to Aβ plaque in the transgenic mice model employed.

Study: TR-AV-45-020: Postmortem correlation of ^{18}F-AV-45 binding to β-amyloid plaque burden in postmortem human brain tissue.

The studies were conducted using frozen tissue blocks from subjects that were neuropathologically defined as normal control, AD, PSP and VAD (AD: Alzheimer's disease, PSP: progressive supranuclear palsy, VAD: vascular dementia).

Histopathological evaluation of β-amyloid:

Adjacent tissue sections were identified for comparison between ^{18}F-AV-45 autoradiography, silver staining, β-amyloid immunohistochemistry (IHC) assays, and thioflavin S microfluorescence analysis. The data obtained from the histological evaluation was used for the following correlation analysis:

1. Correlation of ^{18}F-AV-45 binding to β-amyloid burden in frozen postmortem human brain tissue (Sun Health Research Institute study)
2. Correlation of ^{18}F-AV-45 binding to β-amyloid levels in sections of paraffin-embedded fixed postmortem human brain tissue (Rush University study) and
3. Correlation of ^{18}F-AV-45 binding autoradiography with β-amyloid deposit density measured by immunohistochemistry (Biospective, Inc./Avid Radiopharmaceuticals Inc. study).

1. **Correlation of ^{18}F-AV-45 binding to β-amyloid burden in frozen postmortem human brain tissue (Sun Health Research Institute study)**

The correlation study of ^{18}F-AV-45 binding to Aβ amyloid was conducted on brain tissues from 16 human brains (7 AD subjects, 4 progressive supranuclear palsy [PSP] subjects, 3 vascular dementia [VAD] subjects and 2 controls). The subjects were diagnosed post-mortem based on established neuropathological criteria for AD, PSP and VAD. ^{18}F-AV-45 binding to cortical or hippocampal tissues of these subjects was quantified by measuring the optical density of the autoradiographic signal from tissue. The maximal specific binding (B_{max}) was also determined.

The images of the film autoradiography (figure 6) showed a broad spectrum of varying signal intensities obtained over the tissue samples from the various subjects. The darkly speckled band around the edge of the positive tissue sections reflects ^{18}F-AV-45 labeling of β-amyloid plaques present in the gray matter while the light central area of the tissue reflects white matter that is not specifically labeled by ^{18}F-AV-45.

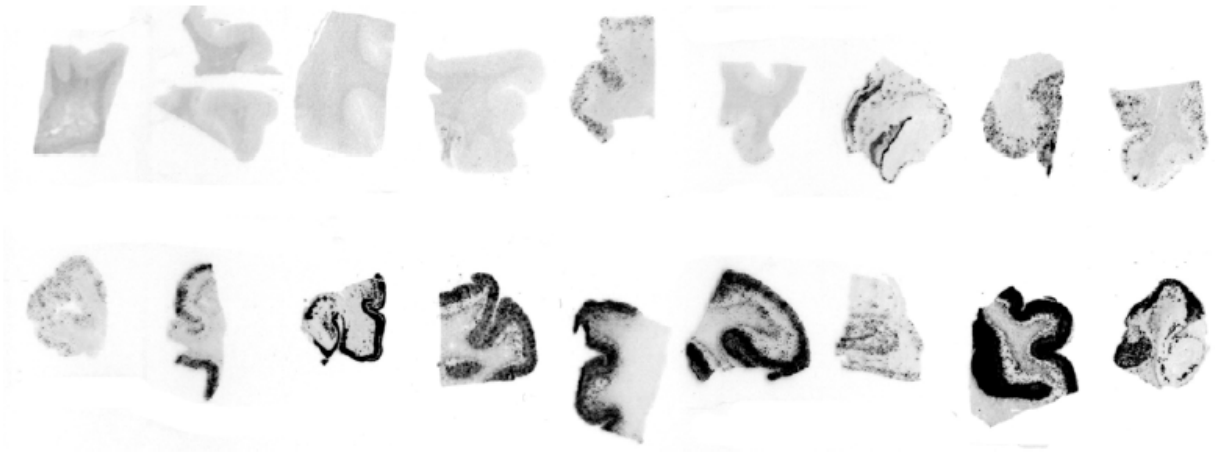


Figure 6: *In vitro* autoradiograms of frozen tissue sections with ^{18}F-AV-45 tissue sections were incubated with ^{18}F-AV-45, and binding site density was measured with film autoradiography. The figure shows representative autoradiography images used for the quantification.

The neuropathological scores of neuritic plaques or neurofibrillary tangles and ^{18}F-AV-45 binding measurements data of the tissue sections and homogenates from the 16 subjects are shown in Table 3 below:

Table 3: Neuropathological data and ^{18}F-AV-45 binding measures in tissue sections and homogenates of human brain tissue from SHR.

Case ID	Age/sex	Tangle score[1]	Plaque Score[1]	Tissue sections ARG (OD of ^{18}F-AV-45 signal; n=4-5)[2]	Tissue homogenates (Bmax, n=2-7)[3]
VAD99-20	89/F	0.0	0.0	34.2 ± 6.6	50 ± 10
VAD98-39	90/M	0.0	0.0	36.0 ± 7.0	50 ± 10
PSP02-34	82/F	0.5	0.0	27.2 ± 5.1	50 ± 10
VAD04-07	70/M	0.0	0.25	32.8 ± 8.4	398 ± 211
PSP06-01	85/M	1.5	0.75	67.6 ± 14.3	1774 ± 155
06-21	73/M	0.0	1.0	40.5 ± 4.0	833 ± 182
AD06-37 Hip	85/M	3.0	1.5	103.6 ± 17.8	3362 ± 524
06-19	84/M	0.0	1.5	84.3 ± 7.0	3213 ± 875
PSP02-30	79/M	0.5	2.0	70.6 ± 11.8	2382 ± 376
AD06-60	96/F	0.0	2.5	156.8 ± 9.4	7534 ± 424
AD03-52Hip	86/M	2.25	2.5	182.6 ± 4.7	9361 ± 432
PSP01-35	93/M	0.0	3.0	187.6 ± 13.6	8538 ± 1498
AD97-01Cx	78/F	1.5	3.0	191.4 ± 9.8	8966 ± 1067
AD06-37Cx	85/M	0.5	3.0	193.4 ± 6.7	9104 ± 2582
AD03-52Cx	86/M	0.5	3.0	224.4 ± 7.4	14099 ± 952
AD97-01 Hip	78/F	2.0	3.0	191.4 ± 15.5	5076 ± 1395

[1] Tangle and plaque scores (0 = none to 3 = very dense scale).

[2] OD values represent arbitrary absolute values.

[3] Bmax values are fmol/mg protein

The data from the correlation study (Table 4) indicates a statistically significant correlation ($r \geq 0.88$) between measurements of ^{18}F-AV-45 binding (B_{max} in tissue homogenates, and optical density of autoradiography signal in tissue sections) and amyloid plaque scores, measured using the CERAD method for scoring neuritic plaque

density. However, there were no significant correlations between the two measures of ^{18}F-AV-45 binding and the neurofibrillary tangle scores. The two measures (autoradiography [ARG] and B_{max}) of ^{18}F-AV-45 binding demonstrated a very strong (r =0.95), significant correlation between one another in this study.

Table 4: Correlation coefficients and P values for correlations between measures of ^{18}F-AV-45 binding and scores of neuritic plaques or neurofibrillary tangles.

	r	P
Plaque score vs 18F-AV-45 binding by ARG	0.95	< 0.0001
Tangle score vs ^{18}F-AV-45 binding by ARG	0.33	0.21
Plaque score vs. Bmax for ^{18}F-AV-45 binding	0.88	< 0.0001
Tangle score vs. Bmax for ^{18}F-AV-45 binding	0.20	0.45
^{18}F-AV-45 binding by ARG vs Bmax for ^{18}F-AV-45 binding	0.95	< 0.0001

A graph illustrating the correlations between neuritic plaque scores and ^{18}F-AV-45 binding measured by autoradiography in sections and homogenate assays is shown below.

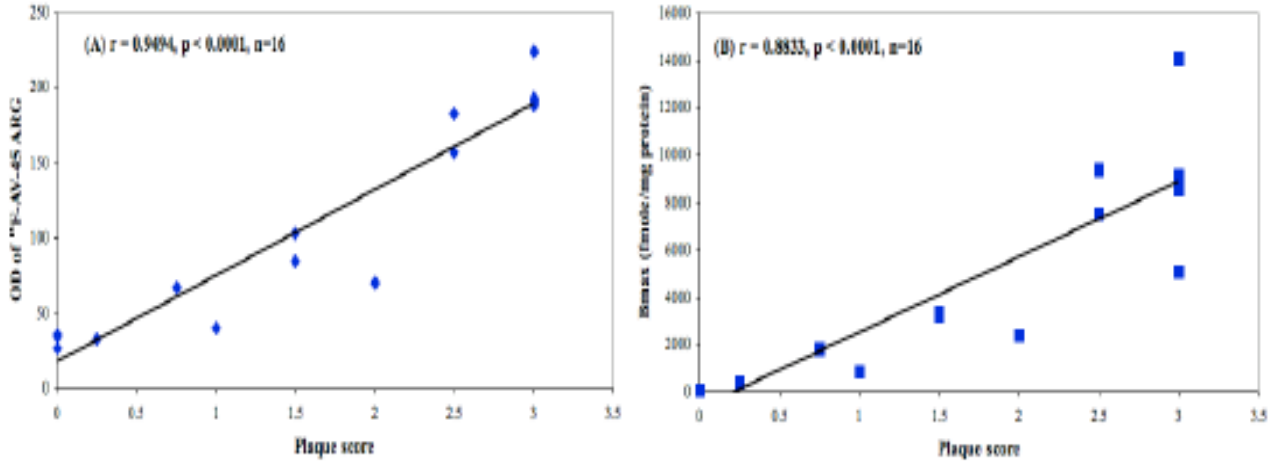


Figure 7: Correlations of plaque score with ^{18}F-AV-45 binding measured by optical density (OD) in autoradiography (panel A) and B_{max} determined in homogenate assays (panel B).

As shown in Figure 7, the binding site density data on ^{18}F-AV-45, obtained using autoradiography and B_{max} values, correlated with the density of neuritic plaques but not neurofibrillary tangles indicating selectivity of the ^{18}F-AV-45 signal for the β-amyloid plaques as well as a quantitative relationship between ^{18}F-AV-45 binding site density and β-amyloid plaque deposition.

Reviewer's Comment: The data showed that ^{18}F-AV-45 selectively binds to β-amyloid plaques in brain tissue and no binding to neurofibrillary tangles. Therefore, the data supports the proof of concept for this product.

2. **Correlation of ^{18}F-AV-45 binding to β-amyloid levels in sections of paraffin-embedded fixed postmortem human brain tissue (Rush University study).**

The study was conducted using postmortem cortical tissue sections from a total of 48 human brains. The subjects had varying degrees of β-amyloid pathology as determined by silver staining and CERAD scoring and immunohistochemistry with two specific anti-Aβ antibodies (6F/3D and 10D5). In addition, plaque burden was measured using thioflavin S fluorescence staining and semi-quantitative fluorescence microscopy. The binding of ^{18}F-AV-45 to cortical tissue sections was quantified by measuring the optical density of the autoradiographic signal. The autography images of one representative section obtained from each subject indicate signal intensity in the cortical areas as shown in the figure 8.

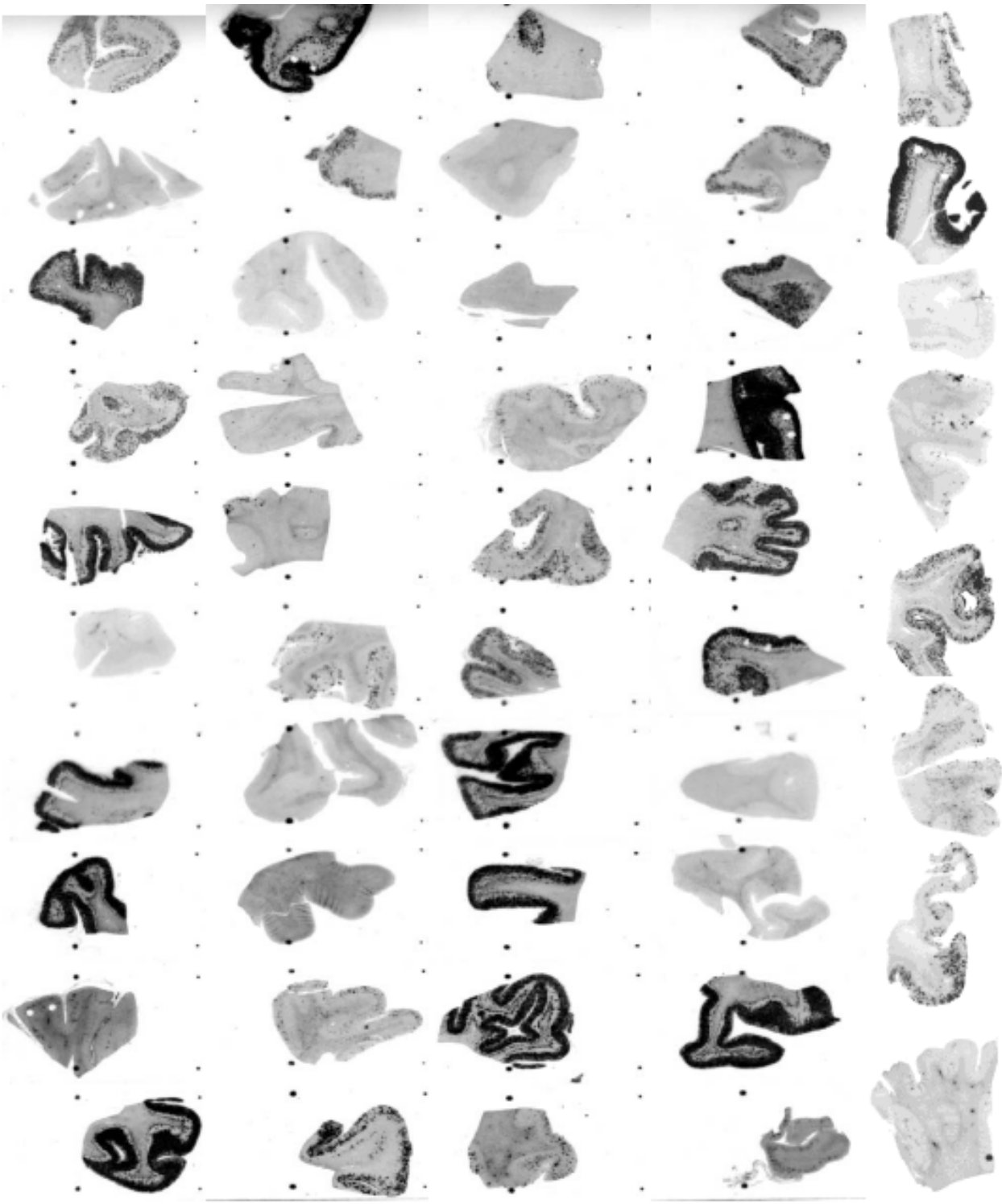


Figure 8: ^{18}F-AV-45 autoradiograms of fixed paraffin-embedded tissue sections from Rush University.

The thioflavin S fluorescence scoring and ^{18}F-AV-45 autoradiography performed (as described under the respective sections) indicate a correlation between the intensities of the β amyloid detection methods and ^{18}F-AV-45 binding as shown in the figure 9.

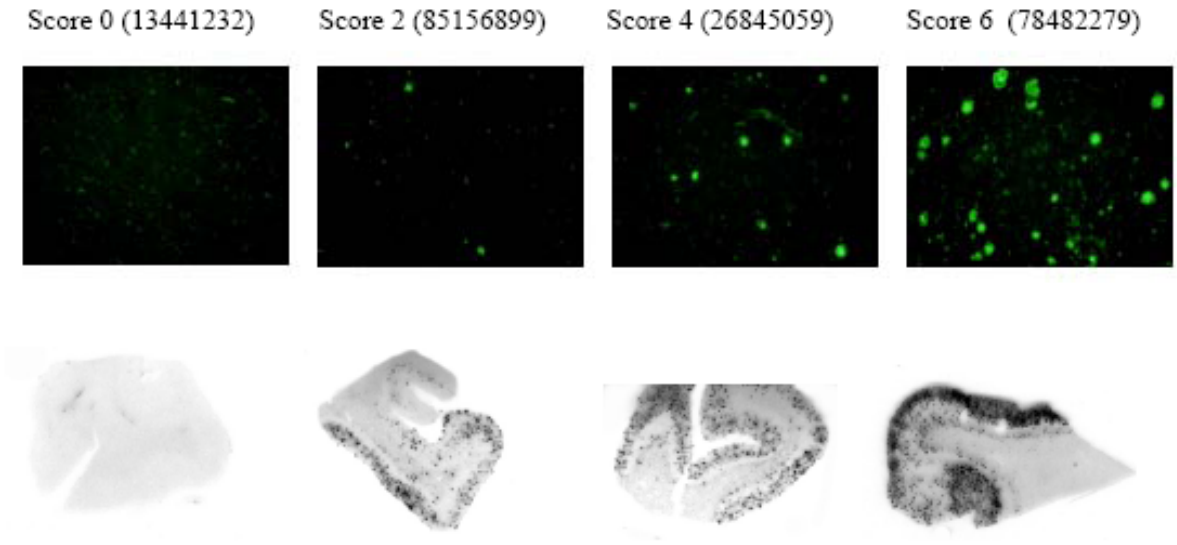


Figure 9: Thioflavin S staining and scoring. The figure shows representative sections. Scores used were 0:none, 2:spare, 4:moderate and 6:frequent thioflavin S- positive aggregates (numbers in brackets correspond to subjects numbers in the Table below). The autoradiographs in the second row show the corresponding ^{18}F-AV-45 binding.

The data showing all the 48 β-Amyloid burden evaluated by silver staining, anti-Aβ immunohistochemistry, thioflavin S fluorescence microscopy, and ^{18}F-AV-45 autoradiography signal intensity in brain sections is shown in Table 5 below.

Table 5: Table 6: β-Amyloid burden evaluated by silver staining, anti-Aβ immunohistochemistry, thioflavin S fluorescence microscopy, and ^{18}F-AV-45 autoradiography signal intensity in brain sections from Rush University.

Test	Silver staining		Anti-Aβ IHC		Thioflavin S staining	^{18}F-AV-45 ARG
ID	SP	Nft	6F/3D	10D5	SP	OD
28397614	14	0	1.41	1.75	5	104
68982187	20	0	1.35	1.85	5	95
83607395	13	0	0.73	0.98	3	57
97570354	10	0	1.13	1.47	3	78
85156899	14	0	1.07	1.34	2	72
56770304	12	0	0.91	1.16	4	84
47349836	13	0	0.46	0.75	2	54
44392663	15	0	0.81	1.33	5	62
38744268	41	0	3.21	5.68	6	197
78482279	48	11	2.82	3.68	6	139
26263589	3	0	0.00	0.00	0	17
02865527	3	0	0.37	0.79	1	47
80557472	53	8	7.10	7.11	6	204
45248304	61	1	3.94	6.05	5	217
98763727	4	0	0.00	0.00	0	21
35826797	4	0	0.00	0.00	0	7
35207786	7	0	0.63	0.80	3	30
63163524	1	0	0.24	0.38	0	0
48439135	20	0	2.19	6.91	5	191
24353928	36	0	1.72	3.23	5.5	120
06718481	24	0	1.71	4.78	4	197
14377041	24	0	1.56	4.96	4	156

34915441	12	0	0.70	0.74	3	51
47133538	7	0	0.61	0.61	1	52
47398583	41	11	1.46	6.83	6	178
79513897	61	14	2.04	5.47	6	138
22323008	18	0	0.95	1.40	5	81
26845059	15	0	0.65	1.22	4	51
55865018	23	0	0.90	2.35	4	61
93330844	12	0	1.38	3.24	3	76
33464959	0	0	0.00	0.00	1	0
35924230	0	0	0.00	0.00	0	0
29765126	19	4	2.38	2.62	6	20
95208621	24	12	2.26	2.64	6	34
71182524	45	0	0.96	2.80	5	192
02576799	56	0	0.65	4.85	5	204
70115221	0	0	0.39	2.46	2.5	34
61294868	8	0	0.82	1.63	3	35
73195385	0	0	0.00	0.00	0	0
32913467	0	0	0.00	0.00	0	0
04703286	25	0	1.66	5.08	4	172
49184971	24	0	1.34	3.81	4	200
82642208	13	0	2.25	2.50	5	93
37203674	11	0	1.74	2.40	4.5	55
46567282	0	0	0.00	0.55	0	12
13441232	0	0	0.00	0.00	0	0
76371687	7	0	0.22	0.47	0	0
63944979			0.43	1.21	0	0

Silver staining of amyloid plaques (SP) and neurofibrillary tangles (Nft) as well as thioflavin S staining were quantified using CERAD scoring systems. Anti-Aβ values represent the percent area covered by staining.

A statistically significant correlation is demonstrated between ^{18}F-AV-45 binding, indicated by the autoradiographic signal intensity, and β-amyloid plaque deposition measured by silver stain, anti-Aβ immunohistochemistry (using two different antibodies), and thioflavin S staining is shown in Table 6 and Figure 10 below.

Table 6: Correlation coefficients and P values of data shown in Table above.

	r	p
^{18}F-AV-45 ARG (OD) vs β-amyloid SP score (silver stain)	0.84	< 0.0001
^{18}F-AV-45 ARG (OD) vs β-amyloid burden measured by 6F/3D IHC	0.66	< 0.0001
^{18}F-AV-45 ARG (OD) vs β-amyloid burden measured by 10D5 IHC	0.89	< 0.0001
^{18}F-AV-45 ARG (OD) vs. β-amyloid NPs (thioflavin S)	0.73	< 0.0001
^{18}F-AV-45 ARG (OD) vs tangles score (silver stain)	0.23	0.113
6F/3D IHC vs. silver stain	0.69	< 0.0001
10D5 vs. silver stain	0.82	< 0.0001
6F/3D IHC vs 10D5 IHC	0.77	< 0.0001
Thioflavin S vs 10D5 IHC	0.75	< 0.0001

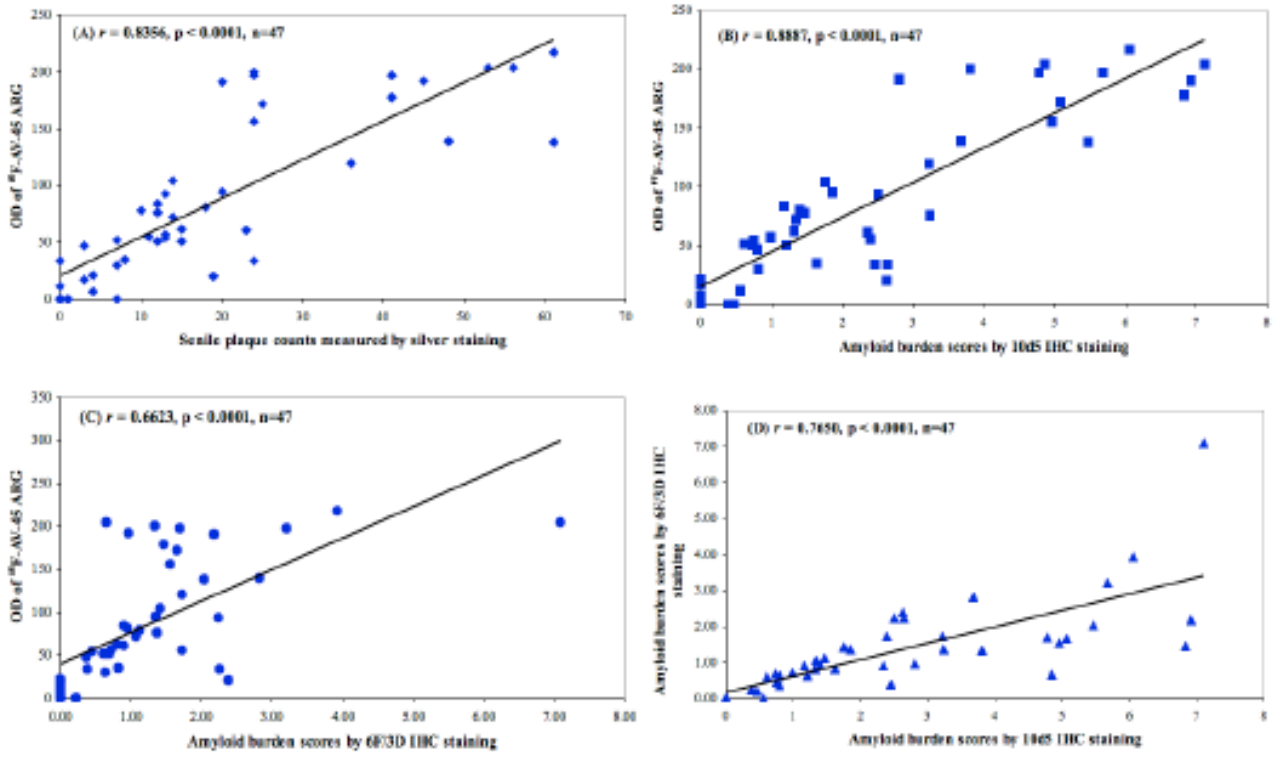


Figure 10: Correlations of ^{18}F-AV-45 ARG with β-amyloid plaque scores based on silver staining (A), immunohistochemical staining with the 10D5 anti-Aβ antibody (B), immunohistochemical staining with the 6F/3D anti-Aβ antibody (C) and immunohistochemical staining with the 10D5 and 6F/3D anti-β antibodies (D).

Reviewer's Comment: The sponsor demonstrated a quantitative relationship between ^{18}F-AV-45 binding site and β-amyloid plaque deposition in postmortem human brain tissue. A sufficiently high sample size was employed and the data indicates that the binding intensity of ^{18}F-AV-45 correlates with the Aβ plaques deposition. However, no correlation was found between ^{18}F-AV-45 binding and tangle pathology. The correlation between binding intensity and amyloid plaque deposition could be of great potential in screening and be useful as a measure of the severity of Alzheimer's disease.

3. **Correlation of ^{18}F-AV-45 binding autoradiography with β-amyloid deposit density measured by immunohistochemistry (Biospective, Inc./Avid Radiopharmaceuticals Inc. study).**

The sponsor employed human brain tissue sections for additional correlation studies with ^{18}F-AV-45 autoradiography and anti-Aβ immunohistochemistry using 4G8. The monoclonal anti-human Aβ antibody recognizes an epitope formed by amino acids 17-24 in the middle of the Aβ-peptide. This study complements the other two antibodies; 10D5 and 6F/3D that bind to the N-terminal of the Aβ peptide (Walker et al., 1994), and an epitope near the N-terminus of the Aβ peptide respectively. The sponsor compared the immunohistochemistry with the individual antibodies on sections from 9 human subjects and correlated them with ^{18}F-AV-45 autoradiography data from same brain tissue sections to assess whether the three antibodies produced equivalent results. In figure 11 below shows the 4GS anti-Aβ immunostaining and the ^{18}F-AV-45 autoradiography of adjacent brain sections.

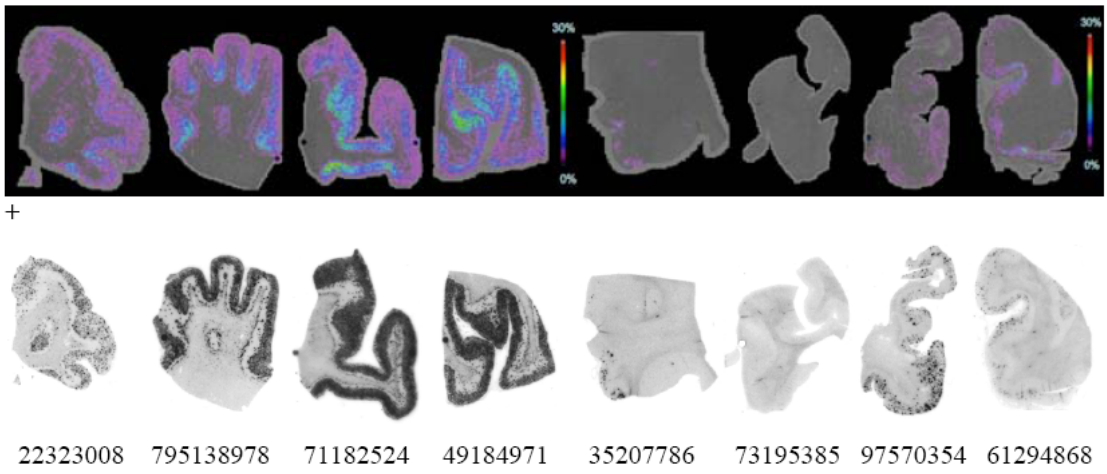


Figure 11: Anti-Aβ immunohistochemistry with antibody 4G8 and ^{18}F-AV-45 autoradiography on adjacent sections of human brain tissue (numbers correspond to subject numbers in the Rush University study). Top row: 4G8 immunohistochemistry performed at Biospective Inc.; pseudo-color images showing staining intensity. Spectral color scale shows tissue β-amyloid burden per unit area (0-30%). Bottom row: ^{18}F-AV-45 autoradiography performed at Avid Radiopharmaceuticals Inc.

The correlation analysis of the 4G8 Aβ immunohistochemistry and ^{18}F-AV-45 autoradiography of the nine brain sections (Figure 12 and Table 7 below) indicates a statistically significant correlation between the two parameters.

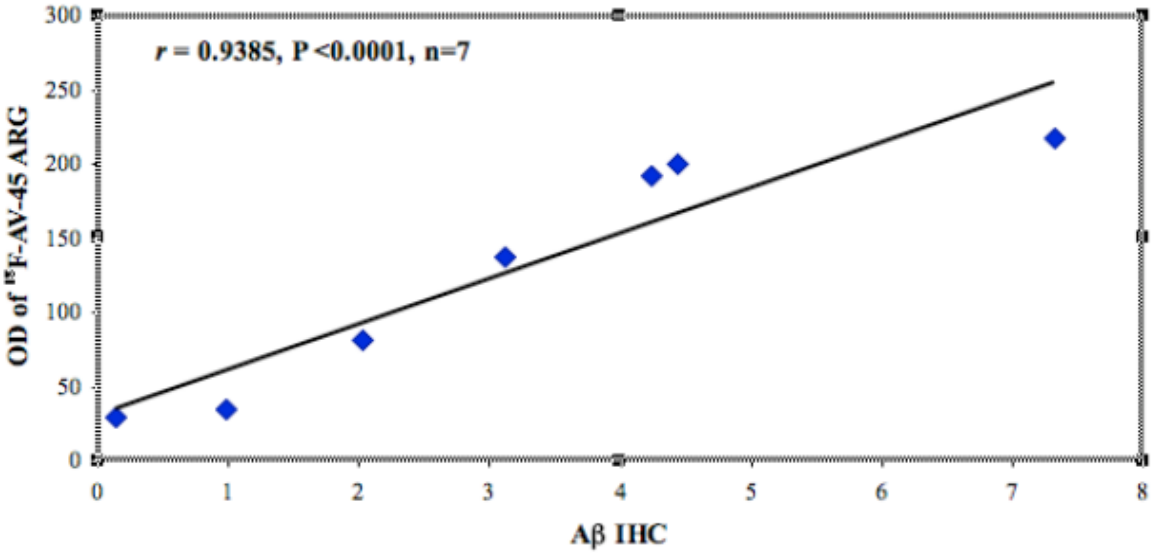


Figure 12: Correlation of ^{18}F-AV-45 autoradiography with 4G8 Aβ immunohistochemistry in sections of cortical tissue.

Table 7 below compares the anti-Aβ IHC values obtained with antibody 4G8 at Biospective Inc. with the corresponding values obtained at Rush university using antibodies 6F/3D and 10D5 in the employed brain tissues.

Table 7: Quantitation of anti-Aβ immunohistochemistry and ^{18}F-AV-45 autoradiography optical density in sections of human brain tissue.

Tissue ID	^{18}F-AV-45 (OD)	Aβ IHC 4G8	Aβ IHC 6F/3D	Aβ IHC 10D5
35207786	30	0.14	0.63	0.80
73195385	0	-	0.00	0.00
97570354	78	-	1.13	1.47
61294868	35	0.98	0.82	1.63
22323008	81	2.03	0.95	1.40
79513897	138	3.11	2.04	5.47
71182524	192	4.24	0.96	2.80
49184971	200	4.44	1.34	3.81
45248304	217	7.32	3.94	6.05

	r	p
^{18}F-AV-45 ARG (OD) vs 4G8 IHC	0.94	0.002
^{18}F-AV-45 ARG (OD) vs 6F/3D IHC	0.72	0.029
^{18}F-AV-45 ARG (OD) vs 10D5 IHC	0.86	0.003
4G8 IHC vs 6F/3D IHC	0.83	0.020
4G8 IHC vs 10D5 IHC	0.83	0.020
6F3D IHC vs 10D5 IHC	0.87	0.002

This study indicates that a statistically significant correlation was demonstrated among the immunohistochemistry quantifications obtained with the three antibodies (r>0.83).

Reviewer's Comment: This reviewer agrees with the study result. A good correlation (r values between 0.83-0.87) between the immunohistochemistry quantifications and each of the different antibodies, binding to a different epitope of the Aβ peptide employed was demonstrated.

Selectivity (binding to other receptors)

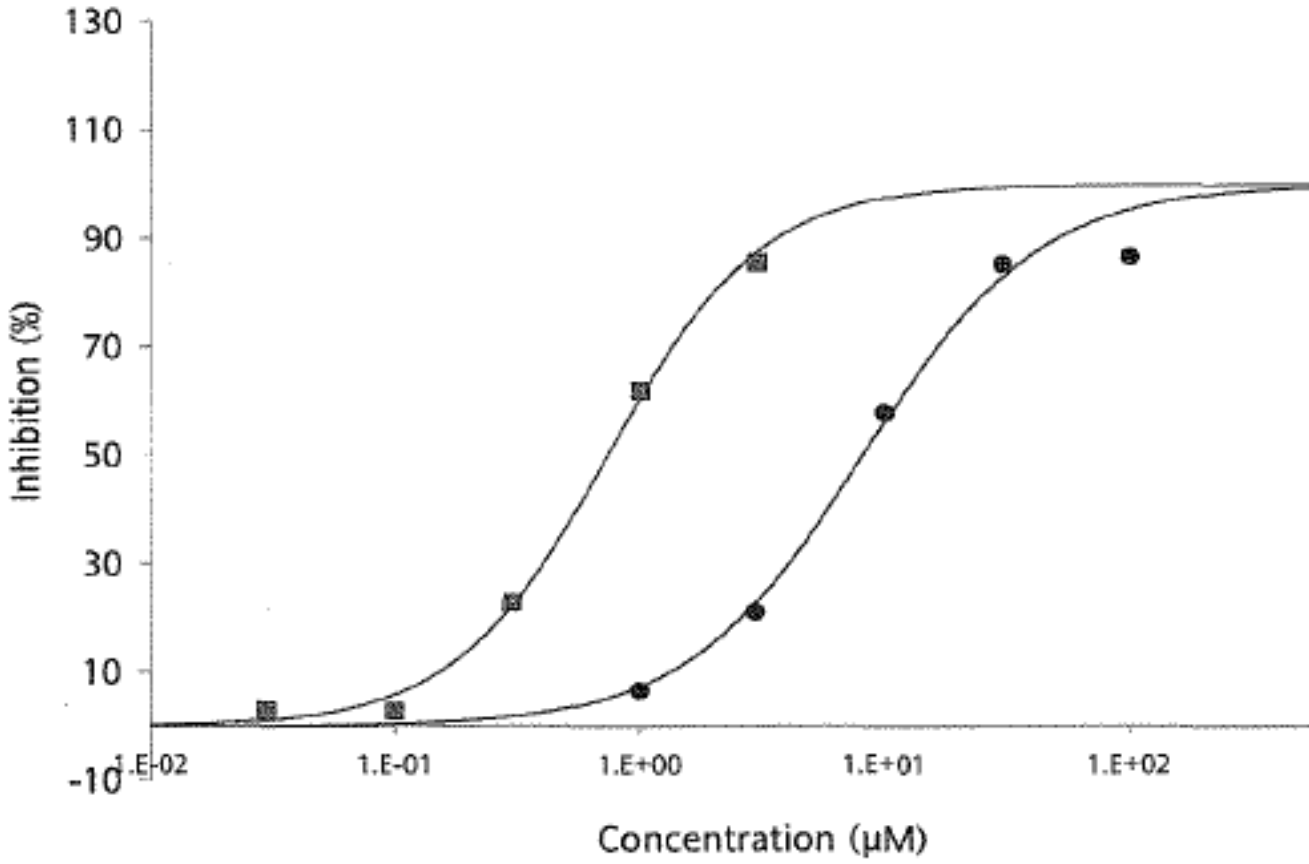
The sponsor evaluated possible binding of AV-45 to other target receptors using radioligand binding assay.

Study title: CNS and cardiovascular receptor binding of AV-45.

Study no.:	TR-AV-45-004 ([redacted]-1083089)
Study report location:	2.6.3.1: Page 1 – 24
Conducting laboratory and location:	[redacted]
Date of study initiation:	November 15, 2006
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	NA

The activity of AV-45 was evaluated using radioligand binding assay. The specific criteria for both radioligand assays of peripheral benzodiazepine and monoamine transporter receptors were ≥ 50% of maximum stimulation or inhibition.

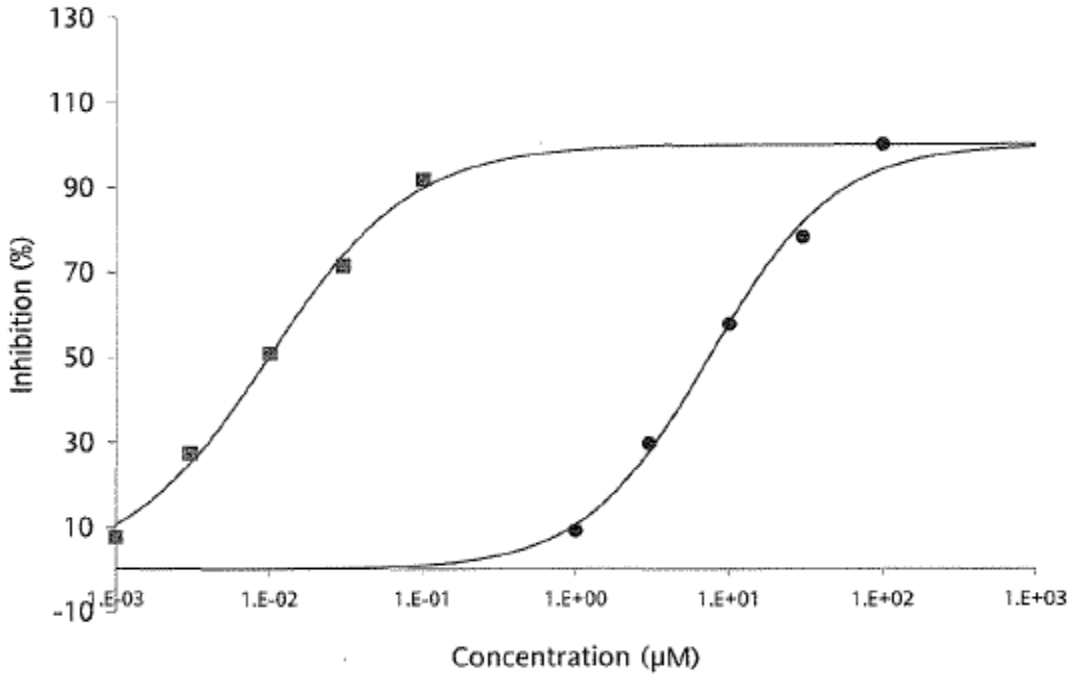
ASSAY: 226700 - 1 Benzodiazepine, Peripheral



Compound	IC_{50}	K_i	n_H
● AV-45 (1083089)	8.15 µM	7.21 µM	1.21
■ Diazepam	0.733 µM	0.649 µM	1.39

Figure 13: Radioligand binding assays of peripheral benzodiazepine.

ASSAY: 252010 - 1 Transporter, Monoamine



Compound	IC_{50}	K_i	n_H
AV-45 (1083089)	7.38 µM	6.13 µM	1.07
Ketanserin	9.81 nM	8.15 nM	0.937

Figure 14: Radioligand binding assays of monoamine transporter.

The data indicates that AV-45 demonstrates specificity in binding. As shown in Figures 13 and 14 above, IC_{50} values of 8.15 µM and 7.38 µM for the peripheral benzodiazepine (K_i values of 7.21 µM) and monoamine transporter (K_i values of 6.13 µM) respectively were reported. These K_i values indicate that the receptors and transporters were significantly inhibited. However, the K_i values of AV-45 for amyloid β were about 1000X lower than the affinity for these transport sites. Thus, it seems unlikely that a 10 nmol of ^{18}F-AV-45 will cause any significant interaction with peripheral benzodiazepine or monoamine transporter sites. The significance of this study is that it is highly unlikely that AV-45 would induce any pharmacology effect at these receptor sites or affect the transporter mechanism at the intended human dose.

Reviewer's Comment: Agrees with the result of this study.

Study Title: TR-AV-45-081- Potential drug-drug interactions at the ^{18}F-AV-45 binding site.

Key Findings: The possible effect of a total of 23 commonly used drugs and drug candidates on ^{18}F-AV-45 binding to β-amyloid was investigated using *in vitro* tissue binding assay and *in vitro* film autoradiography. The tested approved drugs were NSAIDs ibuprofen, naproxen, and celecoxib; the acetylcholinesterase inhibitors tacrine, physostigmine, galantamine and donepezil; the cholesterol-lowering drug simvastatin; the anti-diabetic drug troglitazone; the anti-psychotic drug haloperidol; the anxiolytic diazepam; and the antidepressants citalopram, fluoxetine, paroxetine, nisoxetine, one anti-Aβ-antibody currently in clinical studies and four γ-secretase inhibitors (L-685458, S1288, Compound W, and DAPT). No risk for drug-drug interactions at the ^{18}F-AV-45 binding sites were found in this study. This indicates that the commonly used drugs for Alzheimer's disease did not interfere with ^{18}F-AV-45 binding.

Study Design:

The list of the compounds tested in this study is shown in Table 8.

Table 8: Compounds tested for binding to the ^{18}F-AV-45 binding site.

Compound	Pharmacological action	Brand name	Manufacture
AV-45	Probe for β-amyloid	Florbetapir	Avid RP
Ibuprofen	Non-selective cyclooxygenase (COX-1 and COX-2) inhibitor	Advil, Motrin	(b) (4)
Naproxen	Non-selective cyclooxygenase (COX-1 and COX-2) inhibitor.	Aleve, Naprosyn, Naprelan	
Troglitazone	Anti-diabetic thiazolidinedione (TZD)	Rezulin	
Tacrine	Acetylcholinesterase inhibitor	Cognex	
L-685458	γ-secretase inhibitor		
Physostigmine (Eserine)	Acetylcholinesterase inhibitor	Antilirium	
Galantamine	Acetylcholinesterase inhibitor	Razadyne, Reminyl	
Haloperidol	antipsychotic; D2, D3, and D4 dopamine receptor antagonist.	Haldol	
Celecoxib	cyclooxygenase-2 (COX-2) selective inhibitor.	Celebrex	
Memantine	Low-affinity NMDA glutamate receptor antagonist	Namenda	
Simvastatin	Inhibitor of HMG-CoA reductase	Zocor	
Donepezil	acetylcholinesterase inhibitor	Aricept	
Diazepam	Benzodiazepine anxiolytic	Valium	
BTA-1	Probe for β-amyloid		
S1288	γ-secretase inhibitor		
Compound W	γ-secretase inhibitor		
DAPT	γ-secretase inhibitor		
Citalopram	Antidepressant: Selective serotonin reuptake inhibitor (SSRI)	Celexa, Cipramil	
Floxetine	Antidepressant:SSRI	Prozac, Sarafem	
Paroxetine	Antidepressant:SSRI	Paxil, Seroxat	
Nisoxetine	Antidepressant: selective norepinephrine uptake inhibitor		
Anti-Aβ antibody	Anti-Aβ antibody		undisclosed

The drugs were evaluated for competitive binding assay using five dilutions at concentrations ranging from 0.2-97 μM. The K_i values for compounds that demonstrated over 50% inhibition at any of the selected concentrations employed for the binding assays were determined.

Results: A table showing the % inhibitions induced by the various employed concentrations of the drugs on ^{18}F-AV-45 binding to the β-amyloid in AD brain tissue sections is shown in table 9.

Table 9: The Percentage inhibitions of the drugs on ^{18}F-AV-45 binding to the β-amyloid in AD brain tissue sections.

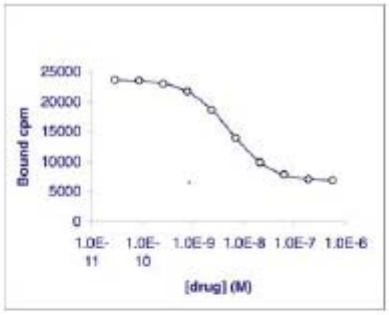
Drug	Drug conc. (μM)	% inhibition	Drug	Drug conc. (μM)	% inhibition
AV-45	56.0	**83.6**	**Haloperidol**	49.0	8.8
	14.0	**85.3**		12.3	3.8
	3.5	**75.6**		3.1	5.4
	0.9	**73.3**		0.8	8.7
	0.2	**70.2**		0.2	20.4
Ibuprofen	97.0	12.2	**Celecoxib**	52.0	**71.1**
	24.3	6.9		13.0	27.2
	6.1	9.5		3.3	15.2
	1.5	18.5		0.8	11.1
	0.4	15.4		0.2	20.9
Naproxen	79.0	11.8	**Memantine**	93.0	26.3
	19.8	11.6		23.3	16.5
	4.9	13.6		5.8	11.4
	1.2	25.6		1.5	14.4
	0.3	15.7		0.4	12.2
Troglitazone	45.0	**82.4**	**Simvastatin**	48.0	2.3
	11.3	21.8		12.0	17.1
	2.8	19.2		3.0	12.6
	0.7	21.4		0.8	13.0
	0.2	11.9		0.2	12.9
Tacrine	78.0	19.7	**Donepezil**	53.0	2.5
	19.5	7.5		13.3	0.2
	4.9	9.5		3.3	-0.6
	1.2	14.2		0.8	12.2
	0.3	14.2		0.2	2.7
L-685458	60.0	47.4	**Diazepam**	70.0	12.3
	15.0	-140.5		17.5	3.0
	3.8	-210.6		4.4	-8.0
	0.9	-37.1		1.1	-3.0

	0.2	4.6		0.3	-1.0
Physostigmine	58.0	-0.1	**BTA-1**	83.0	**78.2**
	14.5	1.1		20.8	**70.3**
	3.6	15.3		5.2	**65.6**
	0.9	9.4		1.3	**61.4**
	0.2	8.0		0.3	**57.6**
Galantamine	54.0	-0.3	**S1288**	56.7	12.6
	13.5	4.8		14.2	-0.5
	3.4	20.6		3.5	-2.1
	0.8	16.4		0.9	-1.6
	0.2	17.2		0.2	-2.9
Compound W	50.5	8.1	**DAPT**	46.2	3.2
	12.6	11.3		11.6	-4.5
	3.2	4.0		2.9	-6.2
	0.8	-0.1		0.7	4.0
	0.2	1.1		0.2	-10.1
Citalopram	48.3	2.5	**Fluoxetine**	58.0	1.4
	12.1	-1.3		14.5	1.2
	3.0	1.6		3.6	1.3
	0.8	-5.1		0.9	-2.5
	0.2	-10.7		0.2	3.7
Paroxetine	60.7	14.8	**Nisoxetine**	65.1	14.7
	15.2	8.6		16.3	4.8
	3.8	5.5		4.1	8.2
	0.9	-4.3		1.0	5.9
	0.2	6.9		0.3	2.2

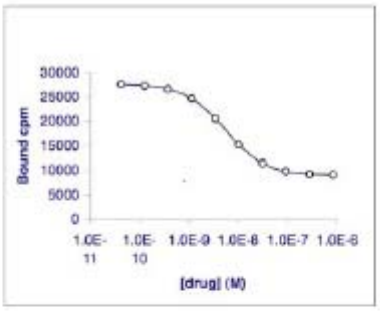
The K_i values of the compounds showing >50% inhibition at any concentration in the primary analysis in the table above is shown in table 10 and figure 15 below:

Table 10: K_i values of compounds showing >50% inhibition at any concentration in the primary analysis.

Compound	Ki
AV-45	5.22 nM
BTA-1	4.94 nM
Celecoxib	>10 µM
Troglitazone	>10 µM



BTA-1: Ki = 5.22nM



AV-45: Ki = 4.94nM

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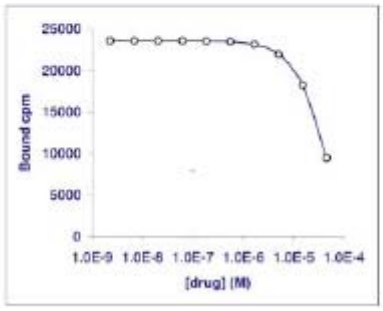
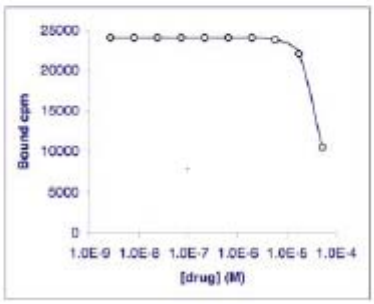


Celecoxib: Ki > 10μM



Troglitazone: Ki > 10μM

Figure 15: Competitive binding curves of the four compounds listed in the Table above. (Vertical axis: cpm)

The data showed that high concentrations of L-685458 increased ^{18}F-AV-45 binding to the β-amyloid in AD brain. The sponsor investigated whether or not this effect was seen with or without human brain tissue homogenates in the test solution. It was found that L-685458 does not inhibit ^{18}F-AV-45 binding to β-amyloid (Table 11). Thus, it is very unlikely L-685458 will interact with ^{18}F-AV-45 binding to β-amyloid.

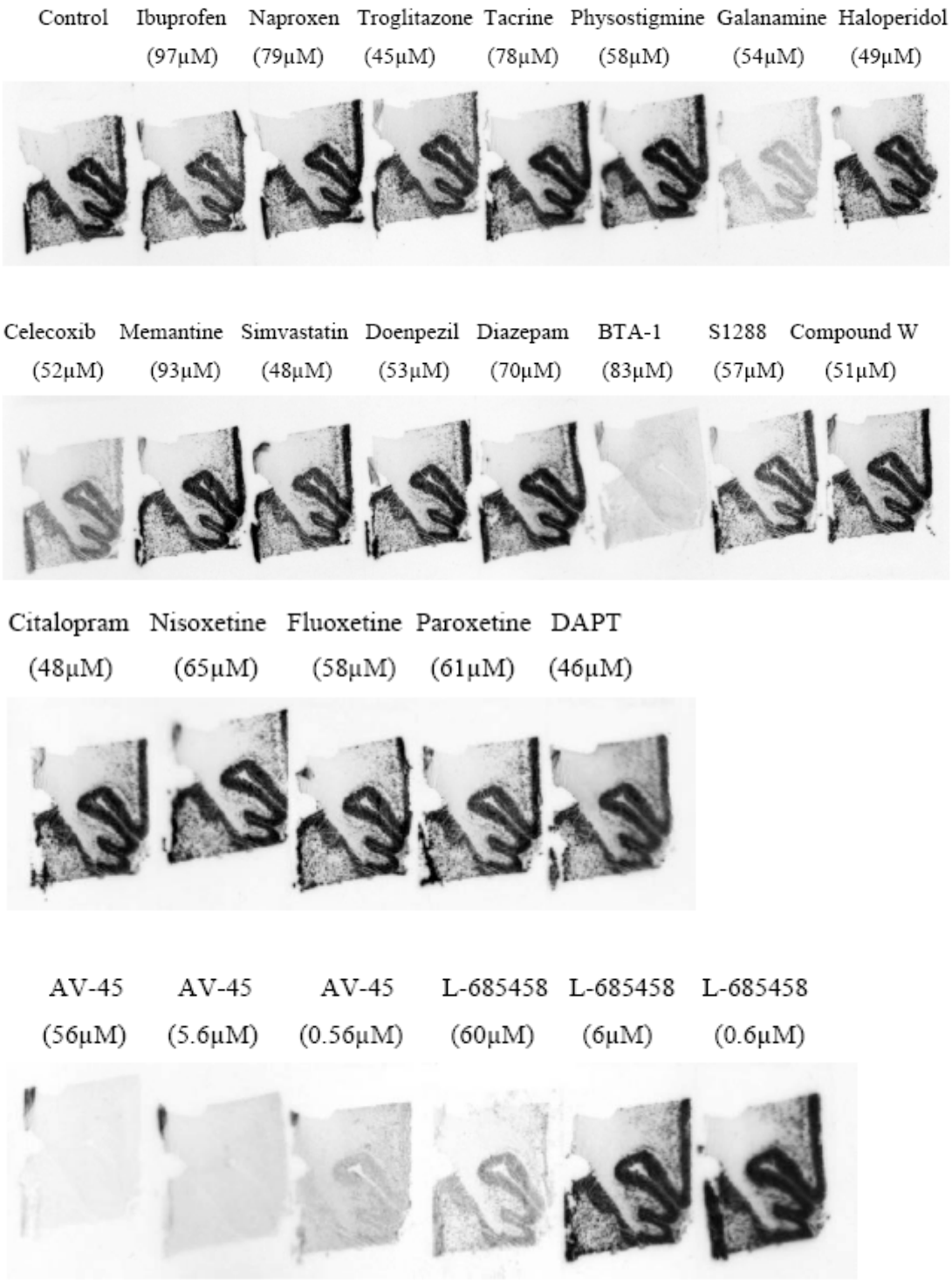
Table 11: L-685458 at concentrations higher than 1μM increases the precipitation of ^{18}F-AV-45 from the test solution in absence of tissue homogenate.

L-685458 concentration (M)	With AD brain homogenates (cpm)	Without AD brain homogenates (cpm)
3.05E-09	23450	7488
9.14E-09	22893	7290
2.74E-08	22198	6488
8.23E-08	23122	7196
2.47E-07	22649	7291
7.41E-07	22050	7411
2.22E-06	33155	20431
6.67E-06	62432	60667
2.00E-05	89184	90368
6.00E-05	94978	98582
Total binding	22713	6708
Nonspecific binding	4311	5766

The table shows cpm values of the retentate on the filter in the binding assay, in presence or absence of AD tissue homogenates.

In vitro ^{18}F-AV-45 autoradiography with section of human brain tissue- The method employed is described above under the section on Binding Specificity (binding to β-amyloid in homogenates). During the *in vitro* autoradiography, the potential interference of the compounds at concentrations ranging from 46 μM to 97 μM on ^{18}F-AV-45 binding to β-amyloid in AD brain tissue sections was assessed. AV-45, L-685458, galantamine and celecoxib were also tested at lower concentrations.

Results: The autoradiography data showed that none of the test compound reduced the autoradiographic signal intensity of ^{18}F-AV-45 at the highest concentration tested, except for AV-45, BTA-1, galantamine, L685458 and celecoxib. The data from the binding studies indicates that AV-45 reduced the signal intensity at concentrations in the μM range. BTA-1 reduced the signal intensity, L-685458 reduced the signal intensity at the highest concentration only (60 μM) while no reductions were reported at lower concentrations of 6 μM and 0.6 μM. Galantamine and celecoxib slightly decreased ^{18}F-AV-45 binding at the highest concentration (54 μM and 52 μM, respectively), but there were no effect at 5 μM and 0.5 μM concentrations.


Control
Ibuprofen (97μM)
Naproxen (79μM)
Troglitazone (45μM)
Tacrine (78μM)
Physostigmine (58μM)
Galanamine (54μM)
Haloperidol (49μM)
Celecoxib (52μM)
Memantine (93μM)
Simvastatin (48μM)
Doenpezil (53μM)
Diazepam (70μM)
BTA-1 (83μM)
S1288 (57μM)
Compound W (51μM)
Citalopram (48μM)
Nisoxetine (65μM)
Fluoxetine (58μM)
Paroxetine (61μM)
DAPT (46μM)
AV-45 (56μM)
AV-45 (5.6μM)
AV-45 (0.56μM)
L-685458 (60μM)
L-685458 (6μM)
L-685458 (0.6μM)

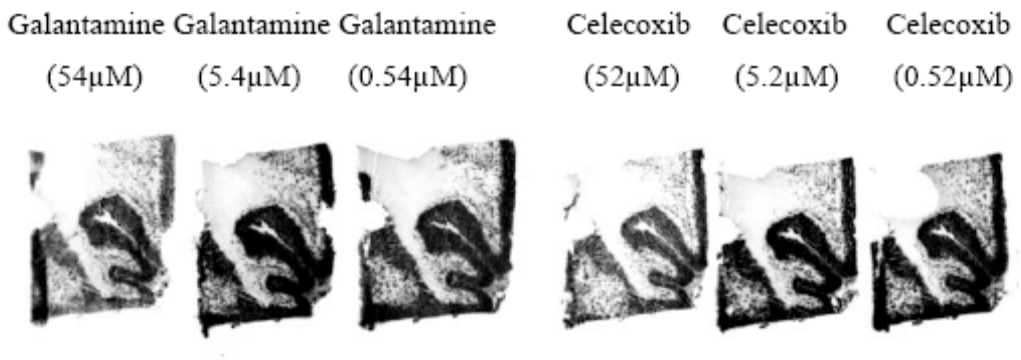


Figure 16: ^{18}F-AV-45 autoradiography to AD brain tissue section and inhibition by test compounds (control: ^{18}F-AV-45 only; concentrations indicate test compound concentration in the incubation solution).

No potential risk for drug-drug interactions at the ^{18}F-AV-45 binding site was identified in this study. This indicates that the commonly used drugs for Alzheimer's disease do not interfere with ^{18}F-AV-45 binding to β-amyloid in the brain.

Reviewer's Comment: *In vitro* tissue binding assay and *in vitro* autoradiography studies were conducted to assess possible interaction between common medications used by AD patients and ^{18}F-AV-45 binding to β amyloid. The study showed that none of the commonly used drugs and drug candidates studied interfered with ^{18}F-AV-45 binding to β amyloid. This reviewer believes that the sponsor has adequately demonstrated that it is very unlikely that administration of commonly used Alzheimer's medications would complicate imaging with ^{18}F-AV-45.

4.2 Secondary Pharmacology

Not Available

4.3 Safety Pharmacology

Neurological effects: The sponsor conducted CNS safety evaluation of AV-45 during single/repeat-dose toxicity study in Sprague Dawley rats. No CNS adverse effects were observed in any of the groups exposed to single dose or 28-day daily dosing of up to 21.8X MHD.

Cardiovascular effects: The sponsor conducted both *in vitro* and *in vivo* studies to evaluate potential cardiovascular effects of AV-45. The full study reports of the *in vitro* studies using hERG potassium channel and the GLP *in vivo* studies in dogs were provided.

In vitro studies: The *in vitro* effect of AV-45 on the hERG potassium channels expressed in human embryonic kidney cells was examined. 17% inhibition was observed at 12.4 μM. This indicates that it is highly unlikely there would be any cardiovascular adverse effect due to AV-45 interaction with hERG/Ik_r currents at the intended dose levels of 50 μM in humans.

Study title: Effects of AV-45 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cell

Study no.:	1665-07667
Study report location:	2.6.3.1- page 1 – 68
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 7, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ1-07-207 and purity is stated to be 97.6%

Key study findings: The potential of AV-45 to inhibit hERG tail current was evaluated in human ether-a-go-go-related gene (hERG)-encoded channel tail current recorded from HEK293 cells stably transfected with hERG cDNA. AV-45 significantly ($P<0.05$) inhibited hERG tail current by 16.7±0.9% at 12.4 µM (n=4) as against 0.2±0.1% control while the positive control (60 nM terfenadine) inhibited the hERG potassium current by 83.8±0.2%. Thus, indicating the sensitivity of the test system to hERG inhibition. However, the IC_{50} for the inhibitory effect of AV-45 on hERG potassium current was not determined because the maximum inhibition observed was less than 50%. However, the sponsor estimated the IC_{50} to be > 12.4 µM.

Study Design:

Doses: 12.4 µM. This dose was selected following an initial testing during which 12.4 µM gave 16.7 % inhibition and limited solubility of AV-45 in the recording solution was reported at higher concentrations.

Vehicle: Dimethyl sulfoxide (DMSO); 0.3%

Reference substance: E-4031 (500 nM)

Positive control: Terfenadine (60 nM)

Control article: HEPES-buffered physiological saline (HB-PS) solution (composition in mM): NaCl, 137; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use), supplemented with 0.3% DMSO.

Main Study: Groups of cells were treated with vehicle, reference substance, or AV-45 as below:

a) AV-45 (12.4 µM); n = 4 cells per concentration
b) Vehicle (0.3% DMSO); n = 3 cells and
c) Reference substance (Terfenadine 60 nM); n = 2 cells (vehicle treated).

Cells stably expressing hERG were held at -80 mV. Onset and steady state block of hERG potassium current due to AV-45 were measured using a pulse pattern with fixed amplitudes (conditioning pre pulse: +20 mV for 1 sec; repolarizing test ramp to –80 mV (-0.5 V/s) repeated at 5 s intervals. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. AV-45 at a target concentration of 12.4 µM was applied to four cells (n = 4). An inhibitory effect on hERG potassium current amplitude of 16.7% was observed. However, additional concentrations selected based on this result could not be tested because of limited solubility of AV-45.

Results: The sponsor provided a typical hERG potassium current records acquired during control, after equilibration with AV-45 at 12.4 µM and after equilibration with the reference substance (0.5 µM E-4031) are superimposed as shown in the figures below;
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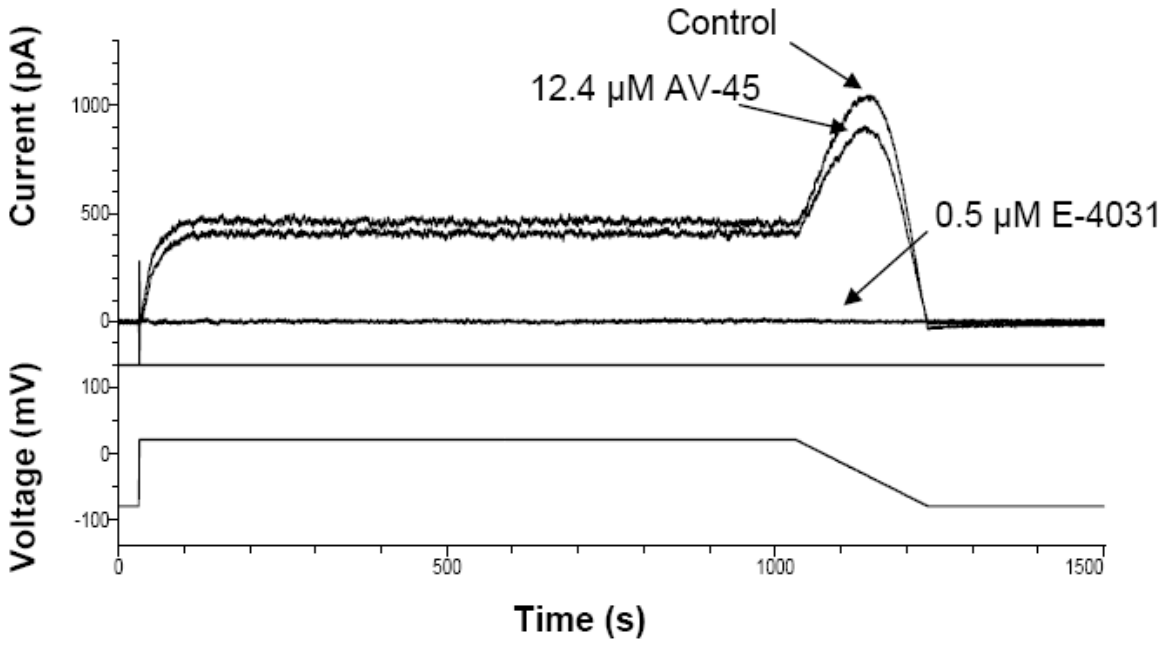

Figure 17: Typical hERG potassium current traces. Upper panel [Current (pA); Time (s)] shows superimposed, records of hERG potassium currents obtained in a single cell during application of control, test article and reference substance. HERG potassium currents were evoked by the voltage protocol shown in the lower panel [Voltage (mV)].

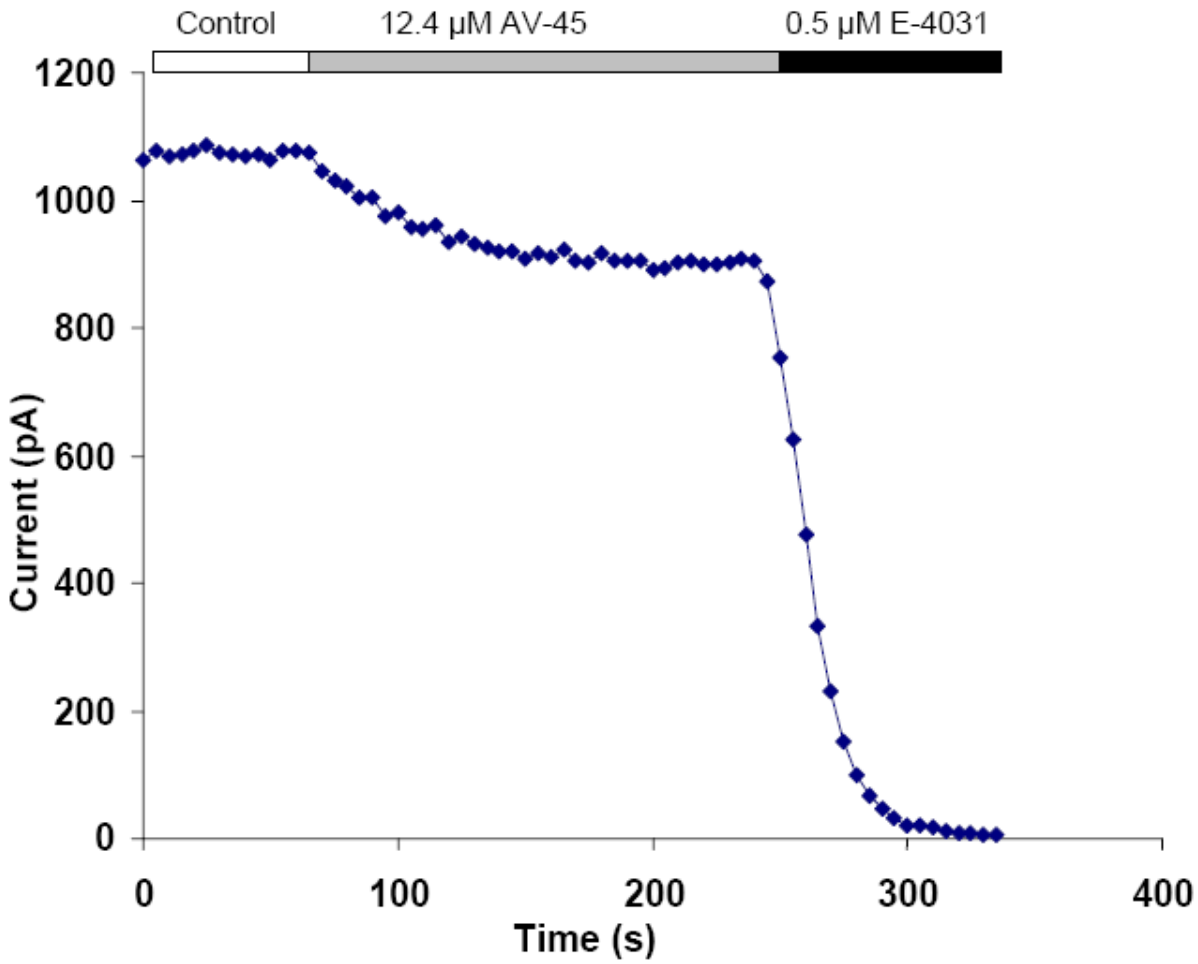

Figure 18: Typical time course of the effect of AV-45 on the hERG current Peak current amplitude during application of vehicle (control), test article, and reference substance. The horizontal bars indicate the control, test article concentration and E-4031.

The IC_{50} for the inhibitory effect of AV-45 on hERG potassium current was not determined because the maximum inhibition observed was less than 50% but was estimated to be >12.4 µM. Higher concentrations could not be tested since visual precipitation was observed. The reference control substance, Terfenadine (60 nM) inhibited hERG tail current by 83.8±0.2%.

Reviewer's Comments: The reviewer agrees with the result of this study indicating that AV-45 inhibited hERG potassium current by 16.7±0.9% at the only concentration of AV-45 tested,12.4 µM (n=4), verses 0.2±0.1% (n=3) in control while the reference positive control, terfenadine (60 nM), induced up to 83.8% inhibition on the hERG cells. The sponsor estimated the IC_{50} for the inhibitory effect of AV-45 on hERG potassium to be >12.4 µM. It is noted that 12.4 µM concentration is several thousands higher than the

possible achievable concentration of AV-45 in the heart following the administration of the planned single dose of AV-45.

In vivo Studies: A study titled- "AV-45: A cardiovascular safety pharmacology and respiratory function study in beagle dogs" was conducted by the sponsor.

Study title: AV-45: A cardiovascular safety pharmacology and respiratory function study in beagle dogs.

Study no.:	1665-07887
Study report location:	Volume 1, page 1 – 139
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 24, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ1-07-207 and purity is stated to be 98.7%

Key Study Findings

AV-45 at doses 0, 21X, 42X or 84X MHD based on human body surface area (0, 32, 64 or 128 µg/kg) did not affect the cardiovascular, respiratory and other parameters assessed in this study. AV-45 did not alter the blood pressure, heart rate, RR, PR, QT and QTc intervals as well as QRS duration values.

Methods

Doses:	0, 21X, 42X or 84 X MHD (0, 32, 64 or 128 µg/kg)
Frequency of dosing:	Cardiovascular Evaluation- Vehicle on Days 1 and 29, and AV-45 on Days 9, 15, 22, and 30 at 0, 21X, 42X or 84 X MHD levels. Respiratory Evaluation- Vehicle on Day 29 and AV-45 on Day 30 only.
Route of administration:	Intravenous bolus injection via cephalic vein.
Dose volume:	0.5 mL/kg
Formulation/Vehicle:	AV-45/10% 2-hydroxypropyl-β-cyclodextrin/0.5% sodium ascorbate in 10% ethanol in saline
Species/Strain:	Dogs, Beagle strain
Number/Sex/Group:	4 dogs/sex/dose
Age:	6 months (M) 6 months (F)
Weight:	7.9 - 9.9 kg (M); 7.2 - 7.4 kg (F)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	1) The dogs were not exercised following telemetry implant surgery to prevent dislodging or damaging the telemetry units. 2) The test article formulation were prepared up to 3 days prior to dosing day and stored at 2-8°C contrary to 6 hours stated in study protocol to allow for dose concentration confirmation .analysis.

The heart rate, body temperature, systolic and diastolic blood pressure, mean arterial pressure and ECG wave were recorded with DSI Telemetry System DataQuest. The respiration rate was measured manually and the saturated blood oxygen (SpO_2) and end-tidal O_2 were measured using capnography.

Observations and Results

Cardiovascular Assessments:

Heart Rate: No AV-45-mediated changes in heart rate.

Mean systolic, diastolic, and mean arterial blood pressures: The data on mean systolic, diastolic, and mean arterial blood pressures of these animals showed no change attributable to AV-45 treatment.

Respiratory evaluations: The treatment with AV-45 had no effect on respiration rate, SpO_2, or end-tidal O_2. These respiratory parameters were within normal physiological ranges for beagle dogs following administration of the vehicle or AV-45.

ECG

Treatment with AV-45 up to the employed maximum dose of 28 µg/kg did not produce any adverse effect on the electrocardiographic parameters. All the electrocardiograms were within normal limits and there were non-treatment related slight sinus bradycardias in few animals.

Reviewer's Comment: This reviewer agrees with the conclusion of the sponsor that no potential cardiovascular or respiratory effect was demonstrated by AV-45 treatment up to a dose of 84X MHD in this study..

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

^{18}F-AV-45 penetrates the brain readily and is rapidly cleared from the brain. Non-clinical data on the biodistribution, pharmacokinetics and metabolism of ^{18}F-AV-45 in the rodents, primate and human was provided. The data showed that rat and human liver microsomes rapidly metabolized AV-45.

Distribution: The distribution of ^{18}F-AV-45 was evaluated in mice, non-rodent primate and human as described below:

Mice:
Study TR-AV-011: Biodistribution of ^{18}F-AV-45 for Injection in normal mice
An injection of ^{18}F-AV-45 (up to 20 µCi) was given directly into the tail vein of male and female IRC mice (25-30 g) under isoflurane anesthesia. The mice (n=3 for each time point/sex) were sacrificed, the organs of interest were removed, weighed, and assayed with an automatic gamma counter. The percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activity in blood was calculated under the assumption that they were 7% of the total body weight. The % dose/g of samples was calculated by comparing the sample counts with the count of the diluted initial dose.

Results: Following the injection into the tail vein, ^{18}F-AV-45 was distributed primarily to the liver, kidneys and the small intestine. An uptake into the brain was also demonstrated. Within 2 min post-injection, there was 6.23% injected dose per gram (% id/g) in the brain of female mice and 7.33% id/g in male mice. However, the activity cleared rapidly from the brain dropping to 1.88% id/g in males and 1.84% id/g in females as shown in tables 12-14 below. The data on ratios of brain and blood uptake and clearance in the treated mice showed an initial uptake in the brain and blood of both male and female mice followed by a rapid clearance from the brain.

Table 12: Percent injected dose of ^{18}F-AV-45 per organ in normal male mice.

%dose/organ				
Organ	2 min	60 min	120 min	180 min
Blood	3.74 ± 0.46	3.60 ± 0.51	2.96 ± 0.45	2.08 ± 0.09
Heart	0.70 ± 0.15	0.21 ± 0.02	0.18 ± 0.02	0.13 ± 0.02
Muscle	29.83 ± 1.49	11.49 ± 0.40	8.83 ± 0.93	6.73 ± 0.44
Lung	1.08 ± 0.10	0.44 ± 0.08	0.31 ± 0.03	0.23 ± 0.03
Kidney	4.22 ± 1.00	1.92 ± 0.28	1.24 ± 0.27	0.67 ± 0.04
Spleen	0.27 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.09 ± 0.01
Liver	18.47 ± 4.74	13.53 ± 0.91	9.26 ± 0.47	5.51 ± 0.15
Skin	3.44 ± 0.21	5.21 ± 0.26	4.16 ± 0.58	2.98 ± 0.23
Brain	3.77 ± 0.86	0.87 ± 0.06	0.82 ± 0.07	0.70 ± 0.03
Bone	3.97 ± 0.72	11.12 ± 0.67	19.57 ± 1.74	23.60 ± 3.53
Thyroid	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
Pancreas	0.63 ± 0.21	0.31 ± 0.08	0.23 ± 0.05	0.13 ± 0.01
Stomach	0.99 ± 0.31	2.21 ± 0.37	1.77 ± 0.71	0.87 ± 0.10
Intestine	10.34 ± 1.54	28.23 ± 1.43	28.10 ± 1.04	28.12 ± 1.16
Urogenital system	0.33 ± 0.06	2.90 ± 1.06	2.37 ± 0.60	1.51 ± 1.09
Testes	0.28 ± 0.08	0.22 ± 0.02	0.19 ± 0.03	0.12 ± 0.01
Tail	13.38 ± 7.01	1.35 ± 0.09	1.34 ± 0.20	1.29 ± 0.15
Fat	0.25 ± 0.08	0.18 ± 0.01	0.18 ± 0.04	0.07 ± 0.01
Carcass	38.74 ± 2.69	22.86 ± 0.92	23.50 ± 1.86	19.60 ± 0.59

Table 13: Percent injected dose of ^{18}F-AV-45 per organ in normal female mice.

%dose/organ Organ	2 min	60 min	120 min	180 min
Blood	3.23 ± 0.19	4.23 ± 0.57	2.95 ± 0.18	2.47 ± 0.50
Heart	0.56 ± 0.03	0.23 ± 0.04	0.17 ± 0.03	0.13 ± 0.02
Muscle	31.07 ± 4.16	12.35 ± 0.03	8.33 ± 1.24	6.36 ± 0.51
Lung	0.87 ± 0.03	0.47 ± 0.09	0.32 ± 0.05	0.27 ± 0.03
Kidney	3.22 ± 0.30	1.36 ± 0.22	0.80 ± 0.09	0.52 ± 0.09
Spleen	0.25 ± 0.02	0.17 ± 0.01	0.10 ± 0.00	0.09 ± 0.02
Liver	17.10 ± 5.37	13.09 ± 0.66	8.40 ± 1.11	5.61 ± 0.40
Skin	2.42 ± 0.41	6.16 ± 0.72	4.21 ± 0.73	3.24 ± 0.63
Brain	2.96 ± 0.67	0.89 ± 0.09	0.75 ± 0.08	0.65 ± 0.11
Bone	3.95 ± 0.50	10.63 ± 0.66	18.94 ± 1.22	21.62 ± 2.54
Thyroid	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00
Pancreas	0.73 ± 0.04	0.34 ± 0.12	0.27 ± 0.05	0.19 ± 0.02
Stomach	1.05 ± 0.42	2.38 ± 0.04	1.63 ± 0.29	1.01 ± 0.20
Intestine	9.57 ± 1.33	27.71 ± 1.34	27.43 ± 1.50	28.58 ± 1.50
Urogenital system	0.20 ± 0.04	0.51 ± 0.18	0.30 ± 0.17	0.11 ± 0.03
Ovary & Uterus	0.43 ± 0.05	0.57 ± 0.25	0.41 ± 0.13	0.16 ± 0.07
Tail	14.19 ± 2.32	1.34 ± 0.02	1.24 ± 0.11	1.43 ± 0.33
Fat	0.11 ± 0.03	0.32 ± 0.11	0.19 ± 0.11	0.06 ± 0.03
Carcass	40.77 ± 4.65	24.32 ± 1.26	21.88 ± 2.35	20.04 ± 1.51

Table 14: Ratios of brain and blood uptake and clearance in normal mice with ^{18}F-AV-45.

	Brain 2 min :60 min	Blood 2 min :60 min	Brain:Blood 2 min	Brain:Blood 60 min
Male	3.9	1.1	2.9	0.74
Female	3.4	0.76	3.2	0.73

Reviewer's Comment: Agrees with the result of this study showing that ^{18}F-AV-45 readily penetrates and is rapidly cleared from the brain of normal mice. The results indicating an early uptake and washout is a favorable characteristic of ^{18}F-AV-45 as a brain imaging agent in that the rapid clearance reduces non-specific binding to the brain tissue which could complicate imaging.

Primate:

Study: Primary PET Imaging Study with ^{18}F-AV-45 (Rhesus Monkey): During the study, the monkey was anesthetized with isoflurane and intubated. A venous catheter was inserted into one hind limb of the animal which was positioned on the bed of the

Concorde microPET P4 gantry and injected with ^{18}F-AV-45 (173.9 MBq [5 mCi] in 3 mL of 95% isotonic saline and 5% ethanol) as a bolus over 1 min. Emission data were collected for 90 min, corrected for attenuation and scatter, and reconstructed using the 3-dimensional maximum a priori method. The regions of interest (ROIs) were drawn manually on multiple planes to obtain volumetric ROIs for the striatum, thalamus, cortex, and white matter, using a summed image of the last 3 frames (60- to 90-min data). The regional tissue time-radioactivity data was obtained from the volumetric ROIs.

Results: The primate study showed that ^{18}F-AV-45 readily enters the primate brain and is rapidly washed out within 90 min, as shown in sponsor's Figure 19, thus, supporting the mice data.

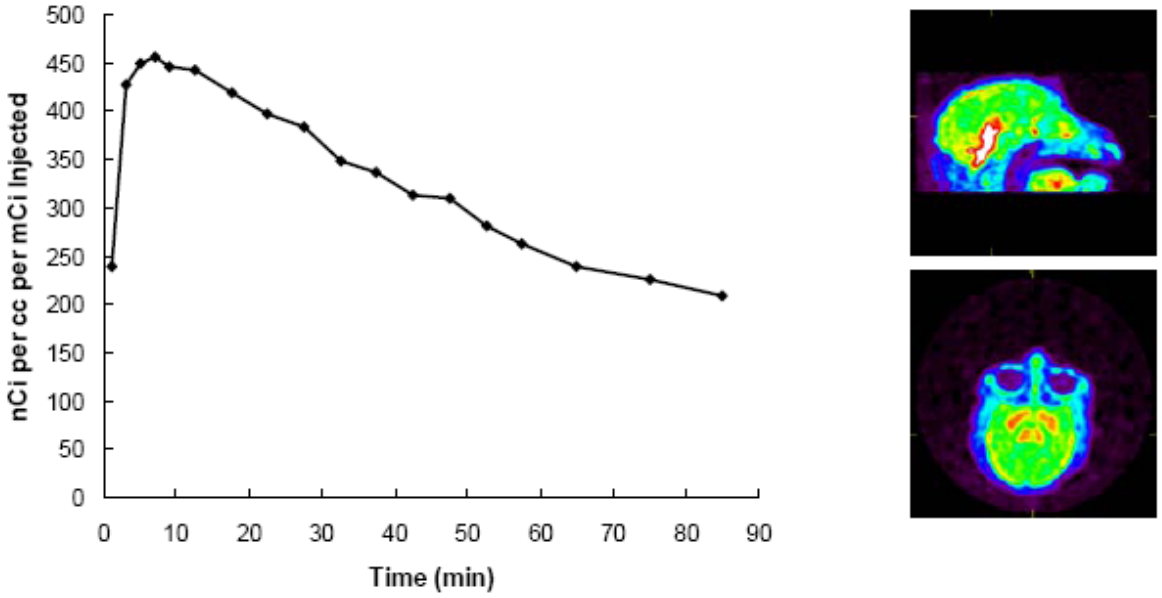

Figure 19: PET Study of Distribution and Washout of ^{18}F-AV-45 in Rhesus Monkey Brain.

As shown in figure 20, following the intravenous injection, ^{18}F-AV-45 penetrated brain and cortex activity peaked at 7 min. There was an initial uptake in the white matter region; that was followed by a rapid washed out. The sponsor stated that at 20 min post-injection, the cortex and white matter regions demonstrated similar radioactivity without providing the data to support the statement. It was also stated that at the peak, there was about 4.4% of the injected dose localized in the brain. However, there was no specific binding or retention in to the monkey brain.

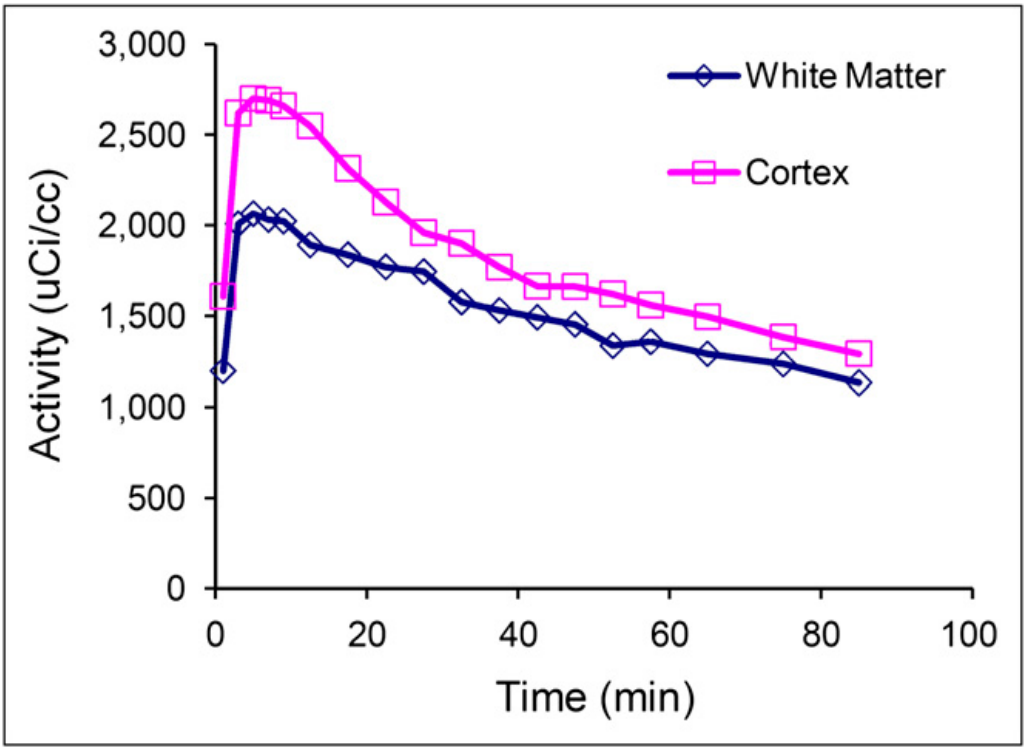

Figure 20: Kinetics of brain uptake and washout in rhesus monkey after intravenous injection of ^{18}F-AV-45 (173.9 MBq [4.7 mCi]) are presented. It is evident that uptake in cortex peaked at 7 min, and activity was washed out quickly thereafter. White matter area also displayed good initial uptake, and washout rate was also rapid. It is estimated that brain uptake at peak was about 4.4% of injected dose, which suggested that ^{18}F-AV-45 penetrated normal blood–brain barrier efficiently.

Reviewer's comment: The absence of Aβ plaques in the healthy monkey employed in this study was responsible for the lack of display of specific binding or retention of the injected ^{18}F-AV-45 in the brain.

Human: The sponsor provided whole body scan series within 6 hours after administration of ^{18}F-AV-45 as shown in figure 21. This scan series showed that ^{18}F-AV-45 was rapidly distributed and cleared from the body. This data corroborates the animal data on the distribution of ^{18}F-AV-45 and so positively supports its potential use as a radiodiagnostic tracer.

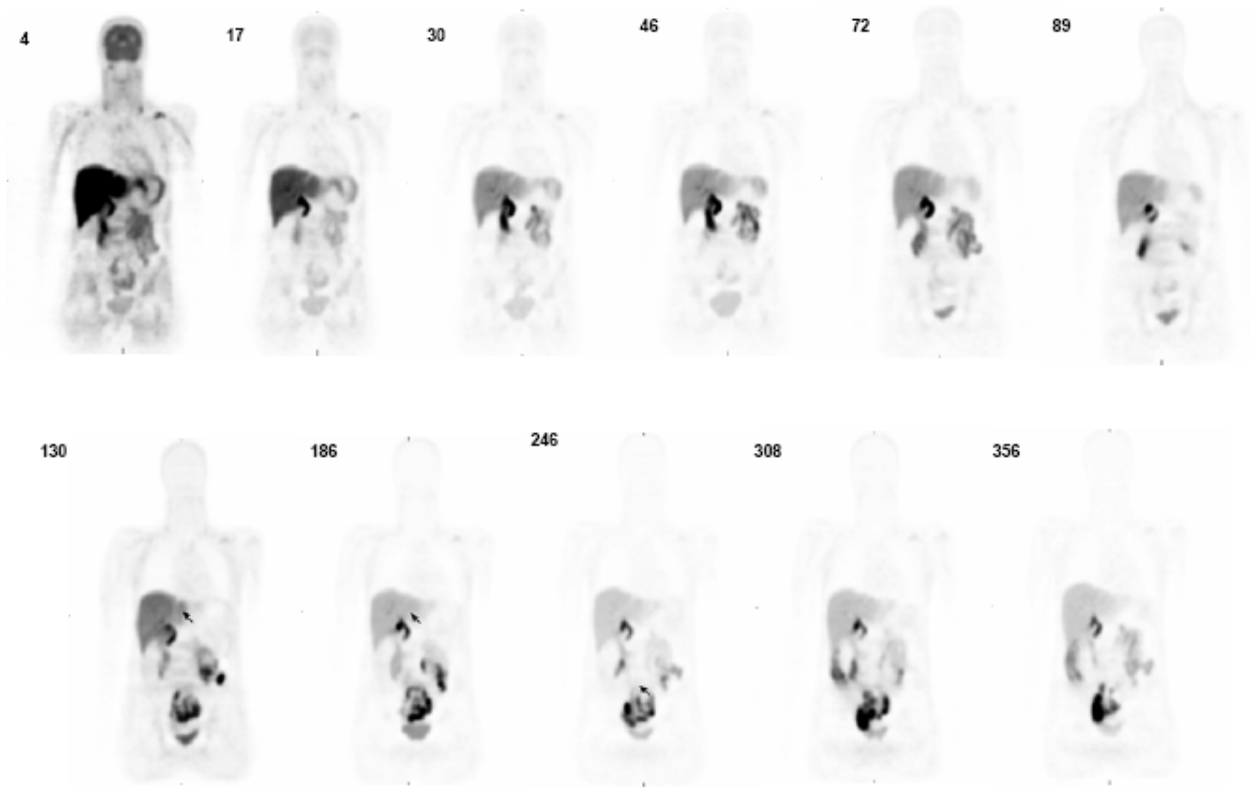

Figure 21: Whole body scan series taken from 4 to 356 minutes post administration of ^{18}F-AV-45 in a single representative patient.

Human Dosimetry:

Study Title: TR-AV-45-012: Human Radiation dosimetry estimates based on biodistribution of ^{18}F-AV-45 for Injection in normal mice.

The distribution of the radioactivity concentrations were measured in various organs of male and female mice at 0.033, 1, 2 and 3 hours after injection of ^{18}F-AV-45. The radiation dose estimates were calculated for human organs, based on an extrapolation of the animal data to humans. The percent in the human organs is derived using organ masses taken from a standard model of the human body for adult males and the data were fit using the SAAM II software. Time integrals of activity were calculated and converted to residence times (Loevinger et al. 1988). The organ residence times were entered into the OLINDA/EXM software (Stabin et al. 2005), using the adult male model. The sponsor also evaluated how the doses might change with an irregular bladder voiding intervals (voiding at 90, 140 and 205 minutes post-injection) and then at regular bladder voiding interval of 3.5 hour.

Results: The estimated human radiation doses estimates are as shown below:

Males:

Table 15: Estimated human radiation doses (males)

	Estimated Radiation Dose				
	Regular Bladder Voids		Irregular Bladder Voids		Ratio
Target Organ	mSv/MBq	rem/mCi	mSv/MBq	rem/mCi	
Adrenals	7.58E-03	2.81E-02	7.50E-03	2.77E-02	1.01E+00
Brain	5.09E-03	1.88E-02	5.09E-03	1.88E-02	1.00E+00
Breasts	3.63E-03	1.34E-02	3.62E-03	1.34E-02	1.00E+00
Gallbladder Wall	1.64E-02	6.08E-02	1.62E-02	6.00E-02	1.01E+00
LLI Wall	4.39E-02	1.62E-01	4.11E-02	1.52E-01	1.07E+00
Small Intestine	1.03E-01	3.81E-01	1.02E-01	3.77E-01	1.01E+00
Stomach Wall	9.27E-03	3.43E-02	9.14E-03	3.38E-02	1.01E+00
ULI Wall	1.16E-01	4.29E-01	1.15E-01	4.26E-01	1.01E+00
Heart Wall	6.46E-03	2.39E-02	6.43E-03	2.38E-02	1.00E+00
Kidneys	1.44E-02	5.33E-02	1.42E-02	5.27E-02	1.01E+00
Liver	2.10E-02	7.77E-02	2.09E-02	7.74E-02	1.00E+00
Lungs	6.31E-03	2.33E-02	6.29E-03	2.33E-02	1.00E+00
Muscle	7.41E-03	2.74E-02	6.73E-03	2.49E-02	1.10E+00
Ovaries	2.52E-02	9.31E-02	2.27E-02	8.38E-02	1.11E+00
Pancreas	9.31E-03	3.44E-02	9.20E-03	3.40E-02	1.01E+00
Red Marrow	1.19E-02	4.39E-02	1.14E-02	4.22E-02	1.04E+00
Osteogenic Cells	1.99E-02	7.34E-02	1.96E-02	7.25E-02	1.02E+00
Skin	4.28E-03	1.58E-02	4.04E-03	1.49E-02	1.06E+00
Spleen	6.67E-03	2.47E-02	6.58E-03	2.43E-02	1.01E+00
Testes	7.99E-03	2.96E-02	6.17E-03	2.28E-02	1.29E+00
Thymus	4.29E-03	1.59E-02	4.27E-03	1.58E-02	1.00E+00
Thyroid	4.88E-03	1.81E-02	4.88E-03	1.81E-02	1.00E+00
Urinary Bladder Wall	1.88E-01	6.97E-01	9.38E-02	3.47E-01	2.00E+00
Uterus	2.71E-02	1.00E-01	2.12E-02	7.86E-02	1.28E+00
Total Body	9.50E-03	3.52E-02	8.88E-03	3.29E-02	1.07E+00
Effective Dose Equivalent	3.84E-02	1.42E-01	3.15E-02	1.16E-01	1.22E+00
Effective Dose	2.62E-02	9.70E-02	2.24E-02	8.29E-02	1.17E+00

Females:

Table 16: Estimated human radiation doses (females)

	Estimated Radiation Dose				
	Regular Bladder Voids		Irregular Bladder Voids		Ratio
Target Organ	mSv/MBq	rem/mCi	mSv/MBq	rem/mCi	
Adrenals	7.25E-03	2.68E-02	7.16E-03	2.65E-02	1.01E+00
Brain	5.09E-03	1.88E-02	5.09E-03	1.88E-02	1.00E+00
Breasts	3.51E-03	1.30E-02	3.50E-03	1.29E-02	1.00E+00
Gallbladder Wall	1.60E-02	5.93E-02	1.58E-02	5.84E-02	1.01E+00
LLI Wall	4.39E-02	1.62E-01	4.10E-02	1.52E-01	1.07E+00
Small Intestine	1.03E-01	3.80E-01	1.02E-01	3.76E-01	1.01E+00
Stomach Wall	9.10E-03	3.37E-02	8.95E-03	3.31E-02	1.02E+00
ULI Wall	1.16E-01	4.28E-01	1.15E-01	4.25E-01	1.01E+00
Heart Wall	6.46E-03	2.39E-02	6.44E-03	2.38E-02	1.00E+00
Kidneys	1.29E-02	4.77E-02	1.27E-02	4.71E-02	1.02E+00
Liver	1.92E-02	7.11E-02	1.91E-02	7.08E-02	1.01E+00
Lungs	6.24E-03	2.31E-02	6.23E-03	2.30E-02	1.00E+00
Muscle	7.30E-03	2.70E-02	6.56E-03	2.43E-02	1.11E+00
Ovaries	2.52E-02	9.32E-02	2.25E-02	8.32E-02	1.12E+00
Pancreas	9.07E-03	3.36E-02	8.96E-03	3.31E-02	1.01E+00
Red Marrow	1.14E-02	4.21E-02	1.09E-02	4.03E-02	1.05E+00
Osteogenic Cells	1.86E-02	6.86E-02	1.83E-02	6.76E-02	1.02E+00
Skin	4.18E-03	1.55E-02	3.92E-03	1.45E-02	1.07E+00
Spleen	6.64E-03	2.46E-02	6.54E-03	2.42E-02	1.02E+00
Testes	7.86E-03	2.91E-02	5.89E-03	2.18E-02	1.33E+00
Thymus	4.15E-03	1.54E-02	4.14E-03	1.53E-02	1.00E+00
Thyroid	5.56E-03	2.06E-02	5.55E-03	2.05E-02	1.00E+00
Urinary Bladder Wall	1.95E-01	7.21E-01	9.27E-02	3.43E-01	2.10E+00
Uterus	2.78E-02	1.03E-01	2.14E-02	7.92E-02	1.30E+00
Total Body	9.30E-03	3.44E-02	8.63E-03	3.19E-02	1.08E+00
Effective Dose Equivalent	3.88E-02	1.43E-01	3.12E-02	1.15E-01	1.24E+00
Effective Dose	2.64E-02	9.77E-02	2.21E-02	8.18E-02	1.19E+00

This data showed an agreement between the dosimetry data for males and females within a range of 15% in all cases.

The sponsor presented the average estimated radiation dose for the regular bladder and irregular bladder as shown below:

Table 17: Average radiation dose estimates for regular and irregular bladders.

	Estimated Radiation Dose				
	Regular Bladder Voids		Irregular Bladder Voids		Ratio
Target Organ	mSv/MBq	rem/mCi	mSv/MBq	rem/mCi	
Adrenals	7.42E-03	2.75E-02	7.33E-03	2.71E-02	1.01E+00
Brain	5.09E-03	1.88E-02	5.09E-03	1.88E-02	1.00E+00
Breasts	3.57E-03	1.32E-02	3.56E-03	1.32E-02	1.00E+00
Gallbladder Wall	1.62E-02	6.01E-02	1.60E-02	5.92E-02	1.01E+00
LLI Wall	4.39E-02	1.62E-01	4.11E-02	1.52E-01	1.07E+00
Small Intestine	1.03E-01	3.81E-01	1.02E-01	3.77E-01	1.01E+00
Stomach Wall	9.19E-03	3.40E-02	9.05E-03	3.35E-02	1.02E+00
ULI Wall	1.16E-01	4.29E-01	1.15E-01	4.26E-01	1.01E+00
Heart Wall	6.46E-03	2.39E-02	6.44E-03	2.38E-02	1.00E+00
Kidneys	1.37E-02	5.05E-02	1.35E-02	4.99E-02	1.01E+00
Liver	2.01E-02	7.44E-02	2.00E-02	7.41E-02	1.01E+00
Lungs	6.28E-03	2.32E-02	6.26E-03	2.32E-02	1.00E+00
Muscle	7.36E-03	2.72E-02	6.65E-03	2.46E-02	1.11E+00
Ovaries	2.52E-02	9.32E-02	2.26E-02	8.35E-02	1.12E+00
Pancreas	9.19E-03	3.40E-02	9.08E-03	3.36E-02	1.01E+00
Red Marrow	1.17E-02	4.30E-02	1.12E-02	4.13E-02	1.04E+00
Osteogenic Cells	1.93E-02	7.10E-02	1.90E-02	7.01E-02	1.02E+00
Skin	4.23E-03	1.57E-02	3.98E-03	1.47E-02	1.06E+00
Spleen	6.66E-03	2.47E-02	6.56E-03	2.43E-02	1.01E+00
Testes	7.93E-03	2.94E-02	6.03E-03	2.23E-02	1.31E+00
Thymus	4.22E-03	1.57E-02	4.21E-03	1.56E-02	1.00E+00
Thyroid	5.22E-03	1.94E-02	5.22E-03	1.93E-02	1.00E+00
Urinary Bladder Wall	1.92E-01	7.09E-01	9.33E-02	3.45E-01	2.05E+00
Uterus	2.75E-02	1.02E-01	2.13E-02	7.89E-02	1.29E+00
Total Body	9.40E-03	3.48E-02	8.76E-03	3.24E-02	1.07E+00
Effective Dose Equivalent	3.86E-02	1.43E-01	3.14E-02	1.16E-01	1.23E+00
Effective Dose	2.63E-02	9.74E-02	2.23E-02	8.24E-02	1.18E+00

Reviewer's Comment: The dosimetry data showed that the upper large intestine and urinary bladder received the highest doses of 0.1-0.2 mSv/MBq respectively while other organs received much less doses of about 0.003-0.009 mSv/MBq. A change in the bladder voiding interval from regular 3.5 hour voids to the irregular voiding interval increased the radiation dose of the urinary bladder wall by up to 50% and the dose to ovaries and testes by 10-30%. The level of this estimated human effective dose equivalent (97 mrem/mCi) is below the range of other approved brain imaging agents

such as ^{18}F-FDG which is 900 mrem/mCi. Radiation related safety concern on the use of ^{18}F-AV-45 minimal

Metabolism: The following studies were conducted to investigate the *in vitro* and *in vivo* metabolism of ^{18}F-AV-45 in normal mice, rat and human.

Study Title: TR-AV-45-007: *In vitro* metabolism of ^{18}F-AV-19 and ^{18}F-AV-45 with human and rat liver microsomes.

Key Findings: The metabolism of ^{18}F-AV-19 and ^{18}F-AV-45 was studied using human and rat liver microsomes in the presence of an NADPH-generating system at 37°C for 2, 5, 10, 30, 60 and 120 min. The radioactive metabolites and the parent compounds were extracted and assayed using HPLC and the metabolites were characterized using reverse-phase (RP)-HPLC. The study shows that both ^{18}F-AV-19 and ^{18}F-AV-45 were rapidly metabolized by human and rat liver microsomes. ^{18}F-AV-19 was metabolized to ^{18}F-AV-45,which was further N-demethylated to form ^{18}F-AV-160. After 60 min incubation with the microsomes, only 4% of the parent ^{18}F-AV-45 was detected and the dominant metabolite was ^{18}F-AV-160 accounting for 71%. The biological half life of ^{18}F-AV-45 was estimated to be <5 min in this study. Thus, the final metabolic product of both ^{18}F-AV-19 and ^{18}F-AV-45 in human and rat liver microsomes was ^{18}F-AV-160.

Study Design:
Microsomal Incubations using NADPH-generating system- The NADPH-generating system was composed of 5 mL of phosphate buffer solution (pH 7.4) added to 30 mg of $MgCl_2.6H_2O$ (30 mM), 39 mg of glucose-6-phosphate sodium salt, 30 units of glucose-6-phosphate dehydrogenase and 23.2 mg of NADPH (6 mM). The NADPH-generating system in a vial was placed on ice. 100 µL of the NADPH-generating system, 50 µL of ^{18}F-AV-19 or ^{18}F-AV-45 in phosphate buffer solution, and 5 µL of human or rat liver microsomes were employed in the study. The control vials were prepared by adding 100 µL of the NADPH-generating system to 50 µL of ^{18}F-AV-19 or ^{18}F-AV-45 solution. The capped vials were gently shaken and placed in an incubator at 37°C for 2, 5, 10, 30, 60 and 120 min. The mixtures were quenched with 200 µL of acetonitrile, shaken vigorously for 10 sec and centrifuged for 10 sec at 14, 000 rpm. The acetonitrile layer was removed shortly before HPLC analysis. The RP-HPLC was performed using Agilent 1100 Series HPLC equipped with an Agilent XDB C8 column and the radiometric peaks were detected using a Bioscan flow detector.

Results:
^{18}F-AV-19 metabolism- ^{18}F-AV-19 was rapidly metabolized to ^{18}F-AV-45 followed by further N-demethylation of ^{18}F-AV-45 to form ^{18}F-AV-160. Thus, two metabolites (^{18}F-AV-45 and ^{18}F-AV-160) were detected by RP-HPLC following the incubation of ^{18}F-AV-19 in rat/human liver microsomes for 2 min. The final metabolic product of ^{18}F-AV-19 and ^{18}F-AV-45 in human and rat liver microsomes was the des-N-methylated derivative, ^{18}F-AV-160, within 30 min as shown in Figure below. However, no metabolism of the parent ^{18}F-AV-19 was found in the absence of rat or human microsomes.

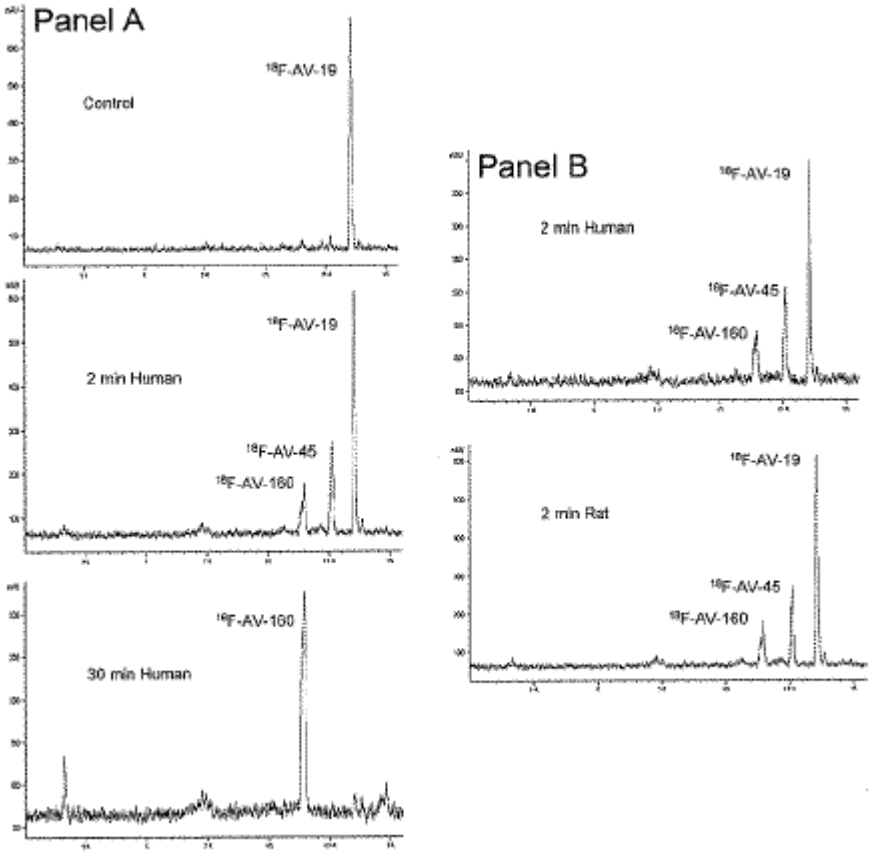

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Figure 22: Radiometric HPL chromatograms of Panel A: ^{18}F-AV-19 in control (top); ^{18}F-AV-19 after treatment with human microsomes for 2 min (middle) and ^{18}F-AV-19 after treatment with human microsomes at 30 min (bottom). Panel B: ^{18}F-AV-19 after treatment with human microsomes for 2 min (top) and with rat liver microsomes at 2 min.

^{18}F-AV-45 metabolism: The chromatograms (figure 23) shows that in rat and human microsomes, ^{18}F-AV-45 is rapidly metabolized to des-N-methylated ^{18}F-AV-160 at 2 min while a longer incubation of 30 min yielded also ^{18}F-AV-160. The rate of metabolism of ^{18}F-AV-19 and ^{18}F-AV-45 was analyzed by plotting the amount of the parent and each of the metabolites against incubation time as shown in figure 23. The data indicates that ^{18}F-AV-19 was rapidly metabolized to ^{18}F-AV-45 and ^{18}F-AV-160. At about 3 min, the amount of ^{18}F-AV-19 and ^{18}F-AV-45 present are equal and thereafter they follow a similar rate of metabolism. The graph shows that the initial metabolism of ^{18}F-AV-19 to ^{18}F-AV-45 was at a faster rate than the metabolism of ^{18}F-AV-45 to ^{18}F-AV-160. This leads to accumulation of ^{18}F-AV-45 in quantities equivalent to ^{18}F-AV-19. Thus, with the exhaustion of ^{18}F-AV-19, ^{18}F-AV-45 is completely metabolized to ^{18}F-AV-160.

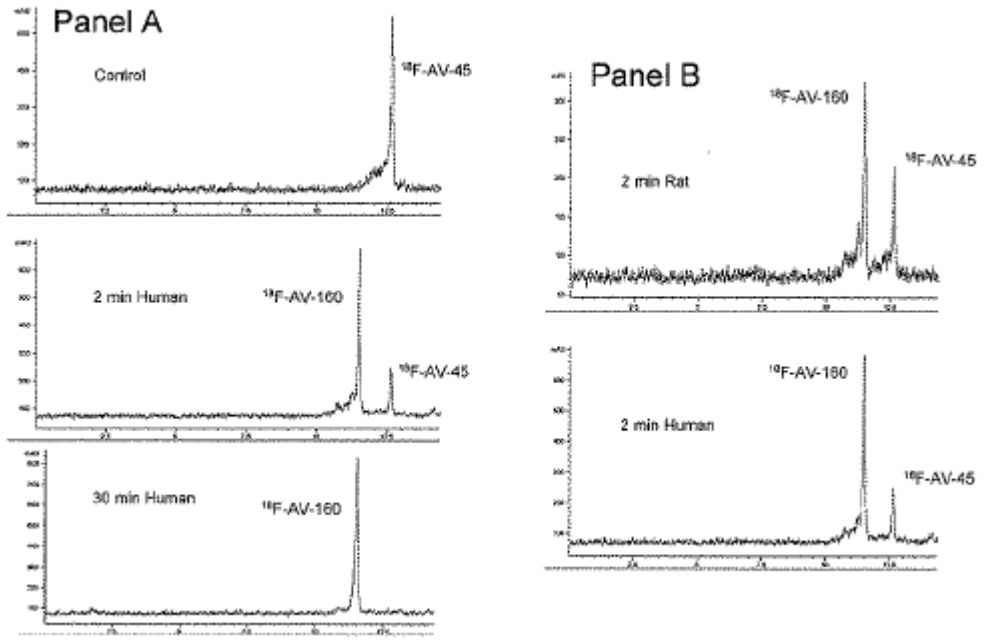

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Figure 23: Radiometric HPL chromatograms of Panel A: ^{18}F-AV-45 in control (top); ^{18}F-AV-45 after treatment with human microsomes for 2 min (middle) and ^{18}F-AV-45 after treatment with human microsomes at 30 min (bottom). Panel B: ^{18}F-AV-45 after treatment with human microsomes for 2 min (top) and with rat liver microsomes at 2 min.

As shown in figure 24, ^{18}F-AV-45 is rapidly metabolized to form ^{18}F-AV-160 as the dominant long-lived metabolite (up to 71%). The biological half life of ^{18}F-AV-45 was estimated to be <5 min in this study.

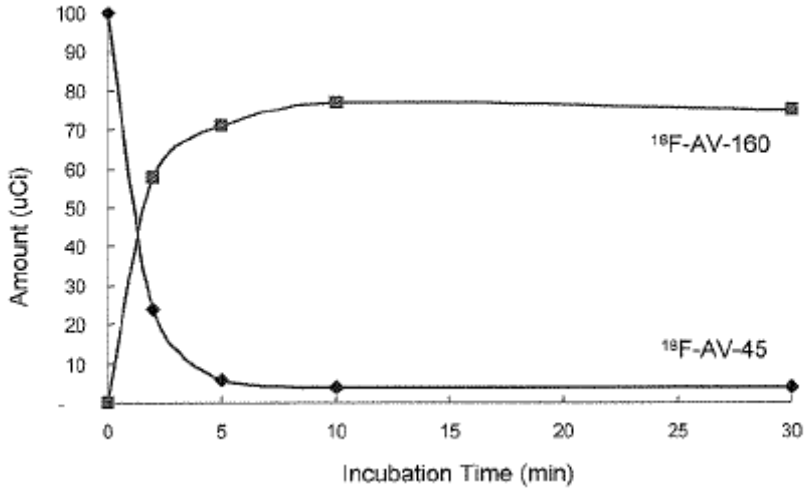

Figure 24: Plot of ^{18}F-AV-19 metabolism with human liver microsomes showing the rates of formation of ^{18}F-AV-160.

Based on this *in vitro* metabolism study, the sponsor proposed a metabolic pathway indicating that ^{18}F-AV-19 and ^{18}F-AV-45 produced a dominant metabolite, ^{18}F-AV-160, as shown figure 25 below:

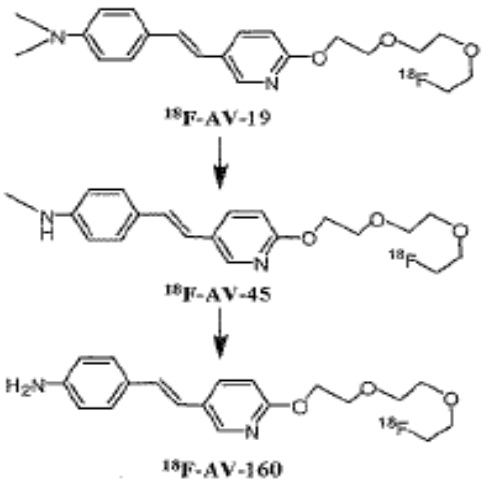

Figure 25: Proposed metabolic pathway of ^{18}F-AV-19 and ^{18}F-AV-45 to produce the dominant long-lived metabolite, ^{18}F-AV-160.

Reviewer's Comment: Agrees with the result of this *in vitro* study.

Study Title: TR-AV-45-010: *In vivo* metabolism of ^{18}F-AV-45 in normal mice and characterization of its metabolites.

Key Findings: This study showed that ^{18}F-AV-45 was metabolized rapidly *in vivo* in normal mice. Within 30 min after injection, only 30% of the parent ^{18}F-AV-45 was present in plasma. The profiling and identification of the metabolites was performed by HPLC with radioactive detection and liquid chromatography/mass spectroscopy (LC/MS) analysis. The dominant metabolite was ^{18}F-AV-160, the ^{18}F-labeled N-desmethylated primary amine derivative (48% at 30 min post injection). The other radioactive metabolite was identified as ^{18}F-AV-267, the N-acetylated AV-160. These two metabolites demonstrated some brain uptake but rapid washout from normal mice brain. However, no significant binding to amyloid plaques was observed with either metabolite using AD brain section autoradiography; the inhibition constants of AV-160 (K_i = 150 nM) and AV-267 (K_i = 48 nM) indicate at least 10-fold less affinity to amyloid plaques than that for AV-45 (K_i = 5.5 nM). This study indicates that it is unlikely that the metabolites of ^{18}F-AV-45 will affect its binding to the amyloid plaques.

Study Design:

Metabolite Analysis- ^{18}F-AV-45 (10-15 mCi) was injected into the tail vein of mice and the animals were sacrificed by cardiac exsanguination at 2, 10, 30 or 60 min post-injection. The blood samples were centrifuged at 3000 rpm for 2 min to separate plasma and the plasma samples were mixed with equal volumes of acetonitrile followed by centrifugation at 5000 rpm for 5 min to remove the denatured proteins. The supernatant was then analyzed directly by HPLC. The metabolism of ^{18}F-AV-45 and its metabolites was determined in liver and brain homogenates.

Biodistribution after injection of ^{18}F-AV-160 or ^{18}F-AV-267 (10-15 µCi), radioactive metabolites of ^{18}F-AV-45 in ND-1 mice (25-30g; n=3 for each time point) was evaluated.

Biding Studies- The method employed for the binding study is as described above under the section on *Binding Selectivity (binding to β-amyloid in homogenates)*.

In vitro autoradiography of AD brain section with ^{18}F-AV-45 and its metabolites- The method employed has been described above under the section on Binding Specificity (binding to β-amyloid in homogenates).

Results: The sponsor provided a prototype of the HPLC spectra of ^{18}F-AV-45 and the metabolites in plasma, liver and brain tissue of mice as shown below:

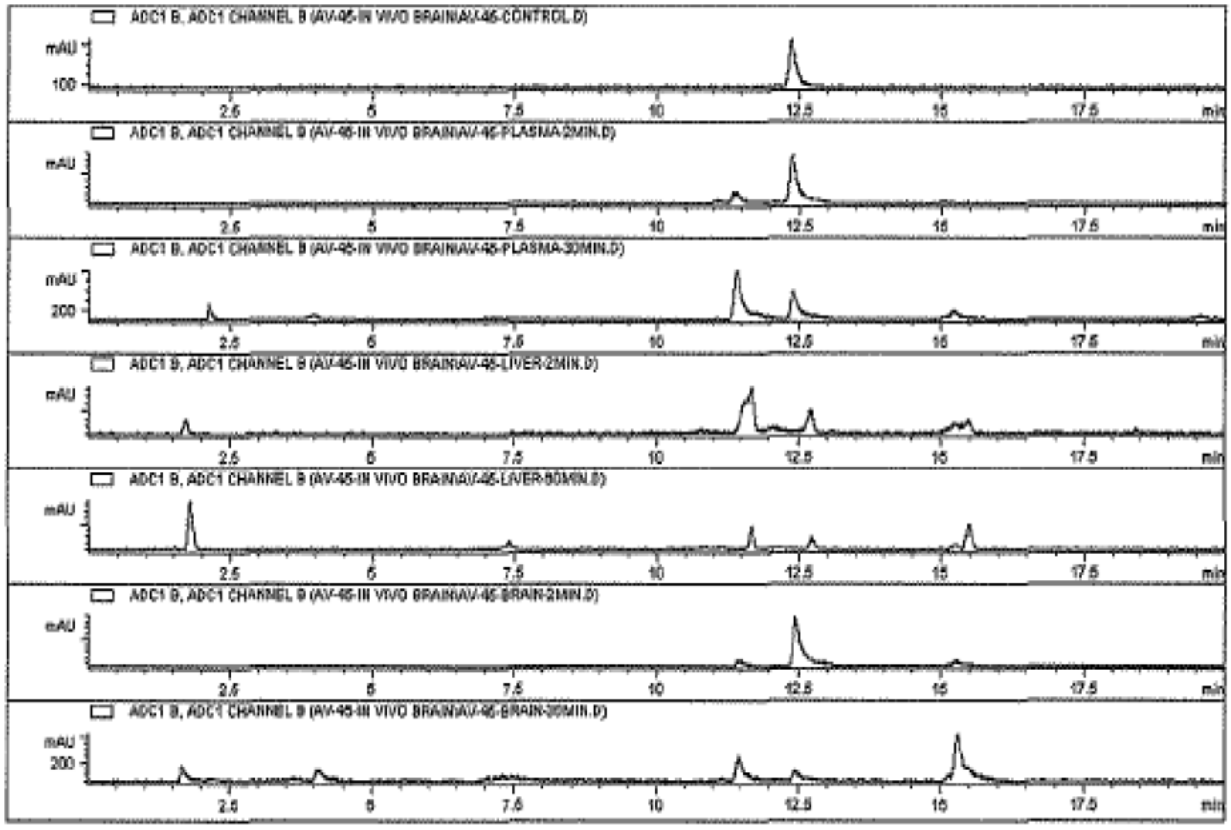

Figure 26: HPLC analyses of plasma, liver and brain after intravenous injection of ^{18}F-AV-45.

The HPLC spectra and the data on percentage radioactivity of ^{18}F-AV-45 and its ^{18}F-labeled metabolites in plasma, brain and liver summarized in the Tables below indicate that ^{18}F-AV-45 was rapidly metabolized in normal mice *in vivo*. The initial metabolic route of ^{18}F-AV-45 was N-demethylation. This metabolite was further identified by injecting the cold reference standard for AV-160, into the RP-HPLC. The RP-HPLC shows that the ^{18}F-labeled metabolite co-eluted with the des-N-methylated derivative. Mass spectroscopy confirmed the identity of the AV-160 metabolite. Similarly, AV-267 was identified as N-acetylated AV-160 by mass spectrometry and co-elution of the cold reference standard synthesized.

Table 18: ^{18}F-AV-45 and its metabolites in plasma of normal mice (% of total activity).

Time (min)	Retention time on HPLC (min)					
	2.2	4.1	7.3	11.5	12.4	15.3
	Polar metabolite #1	Polar metabolite #2	Polar metabolite #3	AV-160	AV-45	AV-267
2	0.0	0.0	0.0	15.5	84.5	0.0
10	2.3	3.6	0.0	33.2	49.8	11.1
30	6.2	4.8	0.0	48.3	30.3	10.4
60	12.4	2.9	9.7	19.5	30.3	25.3

Table 19: ^{18}F-AV-45 and its metabolites in brain of normal mice (% of total activity).

Time (min)	Retention time on HPLC (min)					
	2.2	4.1	7.3	11.5	12.4	15.3
	Polar metabolite #1	Polar metabolite #2	Polar metabolite #3	AV-160	AV-45	AV-267
2	0.0	0.0	0.0	7.4	82.8	9.8
10	4.1	0.0	0.0	22.8	40.8	32.3
30	13.3	8.7	8.8	18.8	8.4	42.0

Table 20: ^{18}F-AV-45 and its metabolites in liver of normal mice (% of total activity).

Time (min)	Retention time on HPLC (min)					
	2.2	4.1	7.3	11.5	12.4	15.3
	Polar metabolite #1	Polar metabolite #2	Polar metabolite #3	AV-160	AV-45	AV-267
2	6.1	0.0	0.0	48.3	24.5	21.1
60	29.9	0.0	7.8	28.0	8.8	25.5

The analysis of the rate of metabolism of ^{18}F-AV-45 involved plotting the percent amount of the parent ^{18}F-AV-45 and each of the metabolites versus time after the injection as shown in figure 27 indicates that ^{18}F-AV-45 was rapidly metabolized to ^{18}F-AV-160 and an unidentified polar species. The $t_{1/2}$ of ^{18}F-AV-45 was estimated to be <30 min in normal mice.

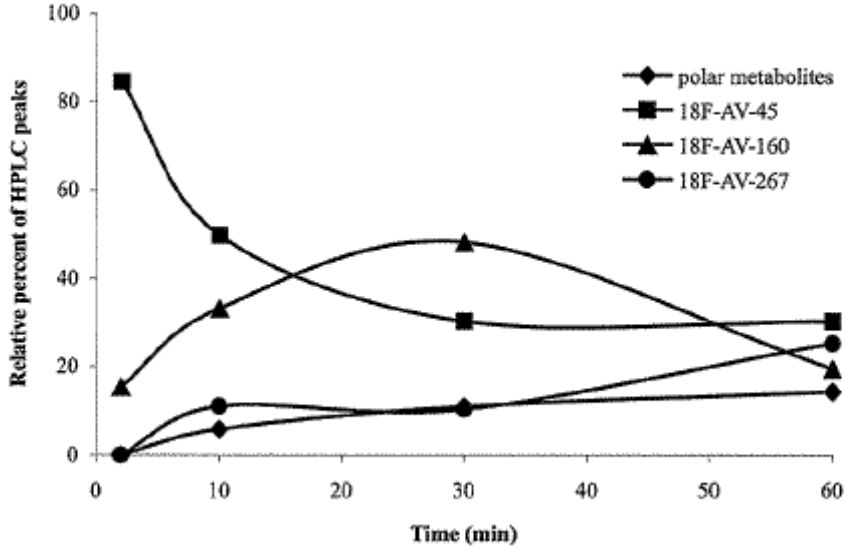

Figure 27: Plot of ^{18}F-AV-45 metabolism showing the rates of metabolism of ^{18}F-AV-45 and formation of ^{18}F-AV-160, ^{18}F-AV-267 and polar metabolites in plasma of normal mice.

Based on the above analysis of the metabolites, the sponsor proposed the metabolic pathway of ^{18}F-AV-45 as shown below:

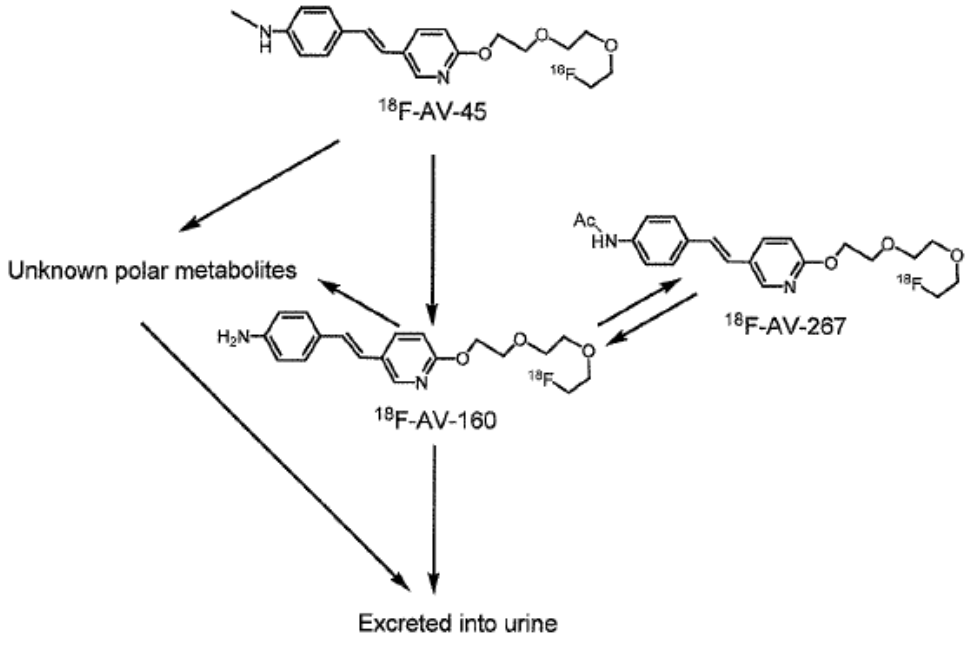

Figure 28: Proposed metabolic pathway of ^{18}F-AV-45.

The brain uptakes and wash out of ^{18}F-AV-45 and its metabolites following their injection to normal mice as shown in the Table below indicates that the parent ^{18}F-AV-45 and its metabolites demonstrate good brain uptake and fast wash out from the brain. The brain uptake at 2 min post injection for ^{18}F-AV-45, ^{18}F-AV-160 and ^{18}F-AV-267 of 7.3%, 4.5% and 3.1% injected dose/gram tissue (ID/g) respectively all decreased to 1.8% ID/g at 60 min post injection.

Table 21: Brain uptake and wash out in normal mice after injection of ^{18}F-AV-45 and its metabolites.

Ligand	2 min	60 min	120 min	180 min
AV-45	7.33 ± 1.54	1.88 ± 0.14	1.80 ± 0.07	1.48 ± 0.15
AV-160	4.49 ± 0.31	1.81 ± 0.10	1.61 ± 0.06	1.39 ± 0.01
AV-267	3.08 ± 0.21	1.82 ± 0.04	1.35 ± 0.05	1.^{18} ± 0.06

The specificity of the amyloid plaque binding to ^{18}F-AV-45 and its metabolites was characterized using a direct *in vitro* binding assay with AD and control brain tissue homogenates. ^{18}F-AV-45 demonstrates specific binding in the gray matter homogenates of AD, as shown in figure 30 below. This suggests selective binding of ^{18}F-AV-45 to the amyloid plaques. There was very low or complete absence of binding signal for the metabolites and brain homogenates of control brain as shown below.

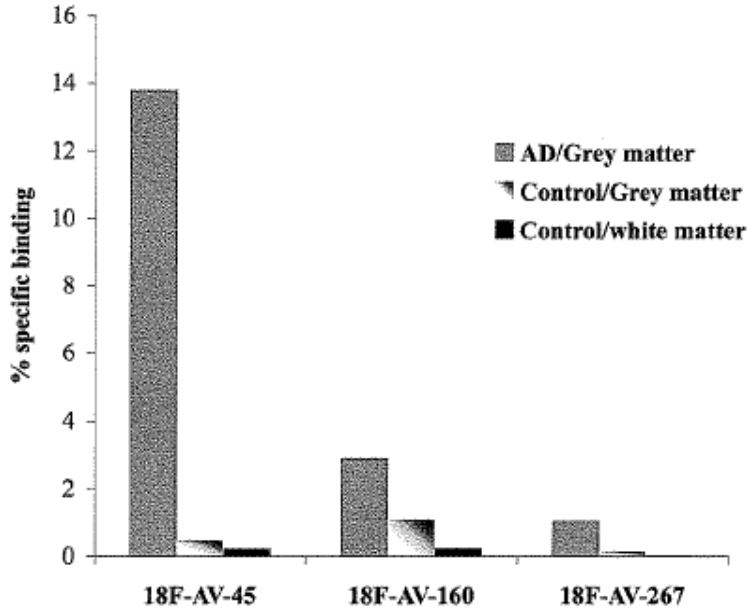

Figure 29: Specific binding of ^{18}F-AV-45 and its major two metabolites to tissue homogenates prepared from AD patient and control human brain.

The autoradiography studies involving incubation of the metabolites with frozen sections from AD brain did not show specific localization to areas of amyloid plaques as shown in figure 30. This indicates that it is very unlikely the metabolites would interfere with ^{18}F-AV-45 binding to the amyloid plaques.

Figure 30: Autoradiography showing excellent labeling of amyloid plaques by ^{18}F-AV-45 in post-mortem brain sections from a patient with AD (left) but very weak binding by ^{18}F-AV-160 and no labeling by ^{I8}F-AV-267 (right).

Furthermore, the sponsor determined the inhibition constant of ^{18}F-AV-45 and its metabolites using cold AV-160 and AV-267 by using ^{125}I-IMPY as the competitor.

Table 22: Binding affinity of AV-45 and its metabolites in AD brain homogenates (Ki vs. ^{125}I-IMPY).

	AV-45	AV-160	AV-267	Polar metabolite
Ki (nM)	5.5 ± 0.7	150	48.0 ± 12.6	NA

As shown in table 22, the inhibition constants of AV-160 (K_i = 150 nM) and AV-267 (K_i = 48 nM) were at least l0-fold less affinity to amyloid plaques than that of AV-45 (K_i = 5.5 nM). Thus, it is very unlikely that the metabolites of ^{18}F-AV-45 will affect its binding to amyloid plaques.

Reviewer's Comment: This reviewer agrees with the results of this study which indicate that the identified metabolites of ^{I8}F-AV-45 demonstrate poor binding affinity for the amyloid plaques as shown by their inhibition constant. The autoradiography data also confirmed that it is highly unlikely these metabolites would complicate imaging of the amyloid plaques with ^{I8}F-AV-45. This reviewer observed that the sponsor has not identified the polar metabolite. However, there seems to be no concern with the unidentified metabolite because it would not cross the blood brain barrier and so it is not expected to interfere with the ^{I8}F-AV-45 binding to the amyloid plaques in the brain. The imaging properties of ^{18}F-AV-45 are stable despite decreasing ratio of parent to metabolite over the later time points of between 60 and 90 minutes (as shown in the data on brain uptake and wash out of parent ^{18}F-AV-45 and metabolites). Therefore,

^{18}F-AV-45 is more likely the primary contributor to the imaging and the metabolites do not contribute to the activity detected in the brain.

Excretion: The human data showed that ^{18}F-AV-45 was eliminated following metabolism in the liver through the gall bladder into the gastrointestinal tract. Some renal clearance with urinary accumulation was also observed. The urine samples were collected from two subjects in Study ^{18}F-AV-45 -A01 at 75, 90, 120 and 200 minutes post injection. The radioactivity was measured and analyzed to determine the radioactive species using HPLC as shown in figure 32. The data showed that the radioactivity cleared rapidly from the body via urinary system over 200 minutes after injection with an estimated total dose of 6.2 MBq. This is mostly attributable to ^{18}F-polar metabolite while the parent and other metabolites were in minor quantities of less than 5% of the total activity. The data showed that ^{18}F-polar species metabolite is likely rapidly cleared via the urinary system within a short time it is generated.

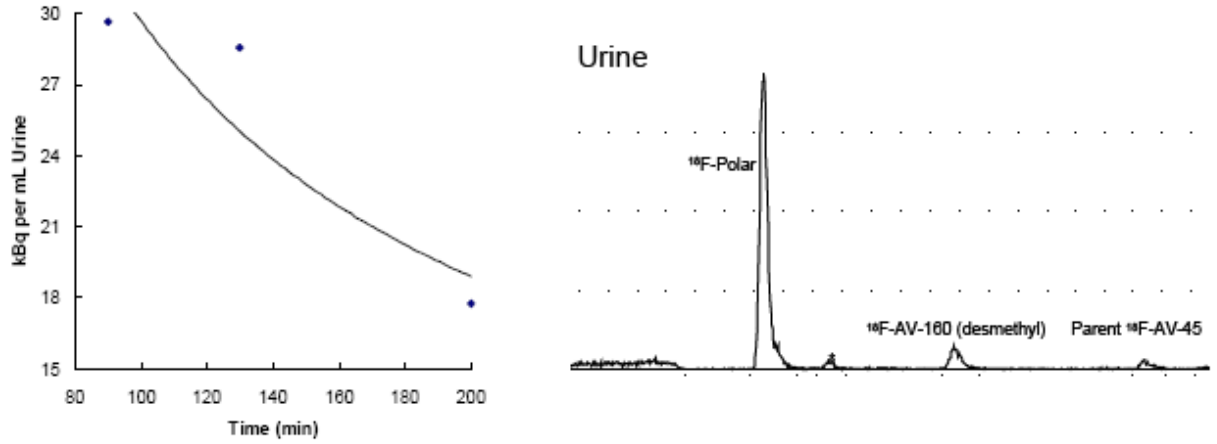

Figure 31: Left- Clearance of radioactivity through urine over time. Right- HPLC separation profile with radiometric detection of urine sample 75 minutes after an injection of ^{18}F-AV-45.

Pharmacokinetic drug interactions: Not available

Reviewer's Comment: Available data showed that ^{18}F-AV-45 selectively binds to the grey matters of the isolated brain homogenates of AD patients and poorly binds to the white matters of AD patients and grey and white matters of normal elderly where amyloid β is usually low. ^{18}F-AV-45 demonstrates very high specificity and selectivity in binding and very low dose is required for use as a potential amyloid β imaging agent. Based on the pharmacokinetics data provided, it is not envisaged that the pharmacokinetics of AV-45 would hamper its potential use as an amyloid β imaging agent.

6 General Toxicology

6.1 Single-Dose Toxicity

The sponsor conducted a combined single- and repeat-dose toxicity studies in Sprague Dawley rats as reviewed under section 6.2 below.

6.2 Repeat-Dose Toxicity

Study title: A Single and 28-Day Repeated Dose Intravenous Study in Sprague-Dawley Rats with a Functional Observational Battery

Study no.:	1665-07623
Study report location:	2.2.6.1 Pages 1-454
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	October 22, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ1-07-207 and purity is stated to be 98.4%

Key Study Findings

No mortality or changes in clinical observations, body weights, food consumption, functional findings, coagulation, urinalysis, gross necropsy findings, organ weight data, and histopathological findings were induced by a single or repeated dose intravenous administration of up to 87.2X MHD and 21.8X MHD dose of AV-45 respectively. Clinical chemistry evaluation did not show any serious adverse findings. However, in animals treated with 87.2X and 21.8X MHD of AV-45 during single- or repeat-dose study respectively, there were mild clinical signs varying from slight increased globulins, total white blood cell count, absolute neutrophils and absolute lymphocytes.

Study Design:

The study was designed to determine the potential toxicity including neurotoxicity of AV-45 in Sprague Dawley rats following intravenous administration for 28 days to four groups of 5 male and 5 female rats. Three additional groups of rats were assessed for toxicity and reversibility following administration of a single intravenous dose. The main phase animals were sacrificed on Day 3 while the recovery phase animals were sacrificed on Day 15 after dosing. The repeat dose study also evaluated delayed onset, or reversibility of effects during a 2 week non-treatment recovery period. The main phase and recovery phase animals were sacrificed on Days 29 and 43 post-dosing respectively.

Methods –Single Acute Dose Toxicity Study in Rats

Doses:	0, 224 or 448 µg/kg (0, 43.6X or 87.2X MHD)
Frequency of dosing:	Once
Route of administration:	Intravenous Injection
Dose volume:	4 mL/kg
Formulation/Vehicle:	AV-45/10% 2-hydroxypropyl-β-cyclodextrin/0.5% sodium ascorbate in 10% ethanol in saline
Species/Strain:	Sprague Dawley/Rats
Number/Sex/Group:	5/sex/group
Age:	8-9 Weeks (M) 8-9 Weeks (F)
Weight:	265.3-305.6g (M) 210.0-259.1g (F)
Satellite groups:	Recovery group
Unique study design:	None
Deviation from study protocol:	None

Methods -28-day Repeated Dose Toxicity Study in Sprague-Dawley Rats

Doses:	0, 24, 56 or 112 µg/kg (0, 4.7X 10.9X, 21.8X MHD)
Frequency of dosing:	Days 1-28
Route of administration:	Intravenous Injection
Dose volume:	4 mL/kg
Formulation/Vehicle:	AV-45/10% 2-hydroxypropyl-β-cyclodextrin/0.5% sodium ascorbate in 10% ethanol in saline
Species/Strain:	Sprague Dawley/Rats
Number/Sex/Group:	5/sex/group
Age:	8-9 Weeks (M) 8-9 Weeks (F)
Weight:	265.3-305.6g (M) 210.0-259.1g (F)
Satellite groups:	Recovery group
Unique study design:	Functional Observations on Day 28
Deviation from study protocol:	FOB for most animals were performed 5-7 minutes post-dose instead of 10 minutes post-dose stated in the protocol. Some tissues were not microscopically examined or only one of the paired organs was examined.

Observations and Results

Mortality

No mortality was reported in any of the animals.

Clinical Signs

No treatment-related clinical signs were observed.

Body Weights

No effect on weight gains in treated animals.

Feed Consumption

No effect on food intake in the animals throughout the study.

Ophthalmoscopy

No ophthalmic lesion was reported in AV-45 treated animals.

Hematology

There was a significant decrease in fibrinogen on study day 29 in the 21.8X MHD AV-45 treated males. There were also reports of mildly increased globulins, total white blood count, absolute neutrophils, and absolute lymphocytes in the treated groups at various time points.

Clinical Chemistry

None of biological or toxicological significance.

Urinalysis

The urine composition was not affected.

Gross Pathology

No treatment-related macroscopic findings were reported. However, in the single dose toxicity study, there were reports of red thymic discoloration which correlated histologically with minimal multifocal hemorrhage in two males rats administered 87.2X MHD. Uterine distension was also reported in a female rat administered 87.2X MHD AV-45. In the repeat-dose study, there were right epididymal mass and correlated sperm granuloma in a male rat administered AV-45 (21.8X MHD); red thymic discoloration in a control male. There were also reports of uterine distension in a female administered 21.8X MHD AV-45 and alopecia of the forelimbs in another female at this dose.

Organ Weights

No treatment-related organ weight change was reported.

Histopathology

Adequate Battery –Yes

Peer Review –Yes

Histological Findings

Study	Repeat Dose Study			
Species	Rat			

Adrenals	x *			
Aorta	x			
Bone Marrow smear	x			
Bone (femur)	x			
Brain	x *			
Cecum	x			
Cervix	x			
Colon	x			
Duodenum	x			
Epididymis	x			
Esophagus	x			
Eye	x			
Fallopian tube				
Gall bladder				
Gross lesions	x			
Harderian gland	x			
Heart	x *			
Ileum	x			
Injection site	x			
Jejunum	x			
Kidneys	x *			
Lachrymal gland	x			
Larynx				
Liver	x *			
Lungs	x			
Lymph nodes, cervical				
Lymph nodes mandibular	x			
Lymph nodes, mesenteric	x			
Mammary gland	x			
Nasal cavity				
Optic nerves	x			
Ovaries	x *			
Pancreas	x			
Parathyroid	x			
Peripheral nerve				
Pharynx				
Pituitary	x *			
Prostate	x			
Rectum	X			
Salivary gland	x			

Sciatic nerve	x			
Seminal vesicles	x			
Skeletal muscle	x			
Skin	x			
Spinal cord	x			
Spleen	x *			
Sternum				
Stomach	x			
Testes	x *			
Thymus	x *			
Thyroid	x			
Tongue	x			
Trachea	x			
Urinary bladder	x			
Uterus	x *			
Vagina	x			
Zymbal gland				

x, histopathology performed
* organ weight obtained

Reviewer's Comment: This reviewer agrees with the result of this study. In the single and repeated dose study, AV-45 there was no mortality or treatment-related adverse effects on clinical or cage side observations, body weights, body weight change, organ weight data, food consumption, FOB results, ophthalmic findings, urinalysis, hematology parameters, clinical chemistry (repeated dose) gross necropsy findings or histopathology findings. AV-45 caused mild increased in total white blood cell count, absolute neutrophils, and absolute lymphocytes in the treated animals. This observation could probably be caused by a mild immune response following an exposure to AV-45. The NOAEL for AV-45 for the single- and 28-day repeat-dose study in the rat were 87.2X- and 21.8X- MHD respectively. Thus, this safety margin is adequate for the intended single dose administration of ^{18}F-AV-45.

Study title: A 14-Day Repeat-Dose Intravenous Toxicity Study in Beagle Dogs with a 2-Week Recovery

Study no.:	1665-07738
Study report location:	2.2.6.1 pages 1-271
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 3, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ11-07-27 and purity is stated to be 98.4%

Key study findings: No changes in clinical observations, body weights, body weight changes, food consumption, functional findings, coagulation, urinalysis, gross necropsy findings, organ weight data, or histopathology findings was induced by up to 4.5X MHD of AV-45. No mortality, serious adverse findings, cardiovascular or ocular effects were reported.

Methods

Doses: 0, 4.5X, 10.3X or 20.6X MHD (0, 7, 16 or 32 µg/kg respectively)
Species/strain: Dogs, Beagle strain
Number/sex/group or time point (main study): 3 dogs/sex/dose
Route, formulation, volume, and infusion rate: Intravenous bolus administration via cephalic vein at a dose volume of 0.5 mL/kg.
The test and control articles are shown in the table below:

Table 23: Purity and descriptions of the Test and Control articles.

Name	Lot No.	Supplier	Purity[a]	Description
AV-45-009 (AV-45)	WZI-07-207	Avid Radiopharmaceuticals, Inc. Philadelphia, PA	98.4%	Light brown powder (Light yellow powder[a])
Ethyl Alcohol Absolute	B0513572 B0513969	(b) (4)	99.9%	Clear, colorless liquid
0.9% Sodium Chloride, USP	38-203-JT		97.2%	Clear, colorless liquid
Sodium Ascorbate	WA1487		100.1%	White powder

a – Information presented as provided by Certificates of Analysis.

Satellite groups used for toxicokinetics or recovery: Yes
Age: 4-6 months
Weight: 5.7 – 7.3 kg (male) and 4.4 – 6.2 kg (female).
Unique study design or methodology (if any): None.
The experimental design is shown below:

Table 24: The study information.

Group	Treatment	Test Article Dosage (µg/kg/day)	Animal Numbers			
			Main Phase		Recovery Phase	
			Males	Females	Males	Females
1	Control[a]	0	15819–15821	15825–15827	15822–15824	15828–15830
2	AV-45 Low (5x)	7	15831–15833	15837–15839	15834–15836	15840–15842
3	AV-45 Mid (10x)	16	15843–15845	15849–15851	15846–15848	15852–15854
4	AV-45 High (25x)	32	15855–15857	15861–15863	15858–15860	15864–15866

[a]0.5% Sodium Ascorbate/10% Ethanol in 0.9% Saline

Study Design:
The study was designed to determine the potential toxicity of AV-45 in beagle dogs following intravenous administration for 14 consecutive days and to assess any potential late onset, or extent of reversibility, during a 14-day no-treatment recovery period. The dosage and treatment groups employed during the repeated dose toxicity studies are shown in table 24.

Results:
Clinical signs : No treatment-related clinical signs were observed in males and females dogs treated with AV-45 up to 20.6X MHD during this study. No mortality was reported and all the animals survived until the scheduled termination. However, there were reports of diarrhea, discolored feces, mucoid or soft feces, and salivation. The sponsor regarded this observation as incidental and this reviewer agrees with the sponsor since the sporadic observations occurred in both control and AV-45 treated dogs. There was also lack of dose relationship.

Body weights: No effect on weight gains in treated animals.

Food consumption: No effect on food intake in the animals.

Ophthalmoscopy: No ophthalmic lesion was reported in AV-45 treated animals. There were no ocular lesions in any animal.

Electrocardiogram: No atrioventricular conduction defects or premature atrial or ventricular complexes were observed in any animal. The electrocardiograms were within normal limits.

Hematology: There was no treatment-related significant change in the red blood cells, hematocrit, absolute basophils and reticulocytes in the animals. There was however a slight and dose unrelated increase in white blood cells, neutrophils, lymphocytes, monocytes and platelets in treated males and females on Day 15. There was also an increase in white blood cells, neutrophils, and monocytes in dosed females on Day 29. However, these changes could not be attributable to AV-45 compound. The prothrombin time in the groups 3 and 4 females was statistically significantly higher than controls on Day 29. Although, these values were outside the historical control reference ranges, the sponsor stated that this finding has no biological or toxicology relevance.

Clinical chemistry : No biological or toxicological changes of significant important.

Urinalysis: The sponsor reported a statistically significantly higher specific gravity in 10.3X MHD-treated females than the controls.

Gross pathology: No treatment related histological findings were reported. However, there were inflammatory and fibrotic lesions around the veins of the injection sites in many animals. The lesions subsided in recovery-sacrificed animals indicating that the lesions were reversible.

Organ weights: The sponsor provided a detailed histopathology table and no treatment related organ weight change was reported. However, adrenal gland weights and relative liver-to-body weight ratios were significantly increased in 4.5X MHD males at Day 15 necropsy. This observation was considered sporadic and unrelated to treatment.

Histopathology: specify groups examined, special stains, etc
Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Histopathology inventory (optional)

Study	14 Day Repeat-Dose Study			
Species	Dog			
Adrenals	X *			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X *			
Cecum	X			
Cervix	X			
Colon	X			
Duodenum	X			
Epididymis	X*			
Esophagus	X			
Eye	X			
Fallopian tube				
Gall bladder				
Gross lesions	X			
Harderian gland	X			
Heart	X *			
Ileum	X			
Injection site	X			
Jejunum	X			
Kidneys	X *			
Lachrymal gland	X			
Larynx				
Liver	X *			
Lungs	X			
Lymph nodes,				

cervical				
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X			
Mammary gland	X			
Nasal cavity				
Optic nerves	X			
Ovaries	X *			
Pancreas	X			
Parathyroid	X			
Peripheral nerve				
Pharynx				
Pituitary	X *			
Prostate	X			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	X *			
Sternum				
Stomach	X			
Testes	X *			
Thymus	X *			
Thyroid	X			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X *			
Vagina	X			
Zymbal gland				

x, histopathology performed
*, organ weight obtained

Reviewer's Comment: This reviewer agrees with the result of this study as presented by the sponsor. AV-45 demonstrated no adverse effects on mortality, clinical or cage side observations, body weights, body weight change, organ weight data, food consumption, ophthalmic findings, urinalysis, hematology parameters, clinical chemistry (repeat-dose) gross necropsy findings or histopathology findings. There were several findings which are of no biological relevance to AV-45 treatment. However, the available evidence does not support the sponsor's claim that the elevated prothrombin time in females administered AV-45 at 10.3X- and 20.6X-MHD on Day 29 is of no biological or

toxicological relevance. Consequently, this reviewer disagrees with the NOAEL value of 16 µg/kg (10.3X MHD) as given by the sponsor. The NOAEL of AV-45 in this study is 4.5X MHD (7 µg/kg).

Study title: AV45: 28-Day Intravenous Toxicity Study in Dogs with a 14-day Recovery Period.

Study no.:	08-3354
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 19, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ11-00-108 and purity is stated to be 99.5%

Key Study Findings

The potential toxicity of AV-45 in beagle dogs following intravenous administration for 28 consecutive days was evaluated. No serious clinical observations, body weights, body weight changes, food consumption, functional findings, coagulation, urinalysis, gross necropsy findings, organ weight data, or histopathological findings was induced by up to 20.6X MHD (32 µg/kg dose) of AV-45 in this study. No mortality was reported and the clinical chemistry analysis did not show any serious adverse findings and no cardiovascular or ocular effects were reported. A NOAEL of 20.6X MHD was obtained.

Methods

Doses:	0, 7.4X or 20.6X MHD (0, 11.2 or 32 µg/kg/day respectively)
Frequency of dosing:	28 consecutive days
Route of administration:	Intravenous injection
Dose volume:	0.5 mL/kg
Formulation/Vehicle:	AV-45 and 10% [v/v] ethanol, 10% [w/v] (2-hydroxypropyl)-β-cyclodextrin and 0.5% [w/v] sodium ascorbate in 0.9% saline respectively.
Species/Strain:	Dogs, Beagle strain
Number/Sex/Group:	3 dogs/sex/dose
Age:	12 months
Weight:	7.9 – 10.9 kg (male) and 6.1 – 9.3 kg (female)
Satellite groups:	Recovery group- 3 dogs/sex/dose
Unique study design:	None

Observations and Results

Mortality

No mortality was reported

Clinical Signs

No treatment-related clinical signs were observed in males and females dogs treated with AV-45 up to 20.6X MHD dose during this study. However, there were reports of diarrhea, mucoid or soft feces, and salivation in the animals. The sponsor regarded this observation as incidental since the observations were sporadic and occurred in both control and AV-45 treated dogs. It is pertinent to note that similar findings were reported in the 14 day repeat dose toxicity study in dogs. Therefore, whether or not the diarrhea, mucoid or soft feces, and salivation reported in the dogs are AV-45 induced cannot be ruled out completely.

Body Weights

No effect on weight gains in treated animals.

Feed Consumption

No effect on food intake in the animals throughout the study.

Ophthalmoscopy

No ophthalmic lesion was reported in AV-45 treated animals. There were no ocular lesions in any animals.

Hematology

There was a statistically significant increase in monocytes relative to pre-test (up to 1.78X) and to controls (up to 1.60X) in males administered 20.6X MHD dose at weeks 2 and 4. However, at the end of the recovery period, the monocytes levels in males administered 20.6X MHD dose were comparable with the controls. This indicates that

the enhanced monocyte levels subsided. No other treatment-related significant changes were observed in the evaluated hematology parameters.

There were reports of statistically significant but minor increases in fibrinogen in the 20.6X MHD-treated males at weeks 2 and 4 and in the 20.6X MHD-treated females at week 2. However, the histopathology data showed no evidence of inflammatory processes.

Clinical Chemistry

There were statistically significant increase in globulin and subsequently albumin/globulin (A/G) ratios in males at 20.6X MHD at weeks 2 and 4. However, the total protein levels were not affected. In addition, at the end of the treatment period, there were statistically significant increases in glucose at ≥7.4X MHD (at weeks 2 and 4) and increased triglycerides at 20.6X MHD.

Urinalysis

No effect.

Gross Pathology

No treatment related microscopic or macroscopic histological findings were reported in these animals.

Organ Weights

The sponsor provided a detailed histopathology table showing an increased kidney weight in the animals administered 21X MHD. However, the increase was not statistically significant. At the end of the dosing period, there was also a significantly decrease in relative (vs. body weight) thymus weight in males at ≥7.4X MHD and increased absolute adrenal weight in the MHD females. This effect was reversible and at the end of the recovery period, the affected organ weights were comparable with the controls.

Histopathology

Adequate Battery- Yes
Peer Review - Yes
Histological Findings

Study	28 Day Repeat-Dose Study			
Species	Dog			

Adrenals	x *			
Aorta	x			
Bone Marrow smear	x			
Bone (femur)	x			
Brain	x *			
Cecum	x			
Cervix	x*			
Colon	x			
Duodenum	x			
Epididymis	x*			
Esophagus	x			
Eye	x			
Fallopian tube				
Gall bladder				
Gross lesions	x			
Harderian gland	x			
Heart	x *			
Ileum	x			
Injection site	x			
Jejunum	x			
Kidneys	x *			
Lachrymal gland	x			
Larynx				
Liver	x *			
Lungs	x			
Lymph nodes, cervical				
Lymph nodes mandibular	x			
Lymph nodes, mesenteric	x			
Mammary gland	x			
Nasal cavity				
Optic nerves	x			
Ovaries	x *			
Pancreas	x			
Parathyroid	x*			
Peripheral nerve				
Pharynx				
Pituitary	x *			
Prostate	x			
Rectum	x			
Salivary gland	x			

Sciatic nerve	x			
Seminal vesicles	x			
Skeletal muscle	x			
Skin	x			
Spinal cord	x			
Spleen	x *			
Sternum				
Stomach	x			
Testes	x *			
Thymus	x *			
Thyroid	x*			
Tongue	x			
Trachea	x			
Urinary bladder	x			
Uterus	x *			
Vagina	x			
Zymbal gland				

x, histopathology performed
* organ weight obtained

Reviewer's Comment: This reviewer agrees with the study results. However, the sponsor did not provide adequate evidence to show that diarrhea, mucoid or soft feces, and salivation reported in dogs administered up to 21X MHD is not AV-45 –treatment related.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: AV-45 Salmonella-*E-coli*/Mammalian microsome reverse mutation assay.

Study no.: (b) (4) 07-379
Study report location: 4.2.3.3. Pages 597-619
Conducting laboratory and location: (b) (4)
Date of study initiation: November 2, 2007
GLP compliance: YES
QA statement: YES
Drug, lot #, and % purity: AV-45, lot # WZ1-07-207 and purity is stated to be 98.4%

Key Study Findings: AV-45 did not induce mutation in three of the five histidine-requiring strains of *Salmonella triphimurium* when tested at concentrations up to 5000 µg/plate, in the absence or presence of a rat liver metabolic activation system. There

was no increase in the number of revertant colonies in strains TA1537, TA1535 and WP2 *uvrA* while there was 2- and 3-folds increases in the number of revertant colonies for TA100 and TA98 strains.

Methods	
Strains:	Five histidine-requiring strains of *Salmonella triphimurium* –TA98, TA100, TA1535, TA1537 and *E.coli* strain WP2 *uvrA*.
Concentrations in definitive study:	25, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate
Basis of concentration selection:	Initial assay
Negative control:	Sterile dimetylsulfoxide (DMSO).
Positive control:	2-nitrofluorene for TA98, sodium azide for TA100 and TA1535, ICR-191 acridine for TA1537 and 4-nitroquinoline-N-oxide for WP2 *uvrA*. However, for testing with metabolic activation, 2-aminoanthracene was used as the positive control for all strains: TA98, TA100, TA1535, TA1537 and *E.coli* strain WP2 *uvrA*.
Formulation/Vehicle:	AV-45/sterile DMSO
Incubation & sampling time:	Not provided

This test was conducted on each of these strains: TA98, TA100, TA1535, TA1537 and *E.coli* strain WP2 *uvrA* in the presence and absence of a rat liver S-9 liver homogenate which allows detection of potentially indirect- and direct-acting mutagens.

Study Validity

For positive response the test article should induce a concentration dependent increase at least two times the vehicle control background frequency for strains with high spontaneous levels (TA100) and three times for those with low spontaneous levels (TA98, TA1535, TA1537 and *E.coli* strain WP2 *uvrA*). The test article is therefore considered to be negative for inducing mutagenicity if it did not induce a response which fulfils the criteria for a positive response.

Study outcome: The sponsor conducted an initial assay and reported presence of precipitates at ≥100 µg/plate in strain TA100 without metabolic activation, at ≥50 µg/plate in strain TA98 without metabolic activation and at ≥25 µg/plate in strains TA98 and TA100 with metabolic activation. However, strains TA1535, TA1537 and *E.coli* strain WP2 *uvrA* with and without metabolic activation met criteria for negative response. The sponsor tested 100, 250, 500, 1000, 2500 and 5000 µg/plate during the confirmatory assay. As in the initial assay, precipitates were observed at ≥1000 µg/plate in all strains both with and without metabolic activation. The criteria for positive

response were met at ≥100 µg/plate in strains TA98 and TA100 both with and without metabolic activation while strains TA1535, TA1537 and *E.coli* strain WP2 *uvrA* with and without metabolic activation met criteria for negative response.

Reviewer's Comment: This reviewer agrees with the result of this study which indicates increases in the number of revertant colonies with AV-45 in *Salmonella* strains TA98 and TA100 with or without metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: AV-45 *In vitro* Chromosome Aberration Study with Human Peripheral Lymphocytes (HPL)

Study no.:	(b) (4) 07-380
Study report location:	4.2.3.3. Pages 1-83
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 7, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ1-07-207 and purity is stated to be 98.4%

Key Study Findings

AV-45 did not induce any structural and numerical chromosomal aberrations in human peripheral lymphocytes in the 3-hour treatment conditions with and without metabolic activation, but was positive in the 22-hour treatment without metabolic activation at all tested concentrations. Thus, AV-45 showed intrinsic ability to induce chromosome structural damage or change the number of chromosomes in the continuous treatment without metabolic activation.

Methods

Cell line:	Cultured human peripheral blood lymphocytes obtained from non-smoker healthy donors (ages 21-48).
Concentrations in definitive study:	14. 45, 28.91, 57.81, 115.6, 231.3, 462.5, 925.0, 1850 and 3700 µg/mL.
Basis of concentration selection:	Initial assay
Negative control:	Treatments with DMSO
Positive control:	Mitomycin C (MMC) and cyclophosphamide (CP).
Formulation/Vehicle:	AV-45 test articles prepared as a solution in 370 mg/mL DMSO.
Incubation & sampling time:	3-hour incubations with and without S9 metabolic activation and 22-hour incubations without S9 activation. The slides were randomly selected and coded for analysis by an individual not involved with the scoring process to control bias. Out of 1000 cells analyzed for mitotic index from each culture by a single investigator, a total of 200 metaphase (100 from each of the duplicate culture) or ≥30 aberrant cells from each test article or positive control concentration and from vehicle control were analyzed. The sponsor analyzed the percent polyploidy and endoreduplication by evaluating the 200 metaphases per duplicate culture.

Study Validity

The assay was considered positive if it induces a dose-dependent and statistically significant increase in the number of cells with chromosomal aberrations when compared to the relevant vehicle control at two or more test concentrations. A statistically significant increase in the percentage of cells with more than one aberration is an indication of the severity of a positive response. The test article is considered negative for inducing chromosomal aberration if no statistically significant increase is observed in the number of cells with chromosomal aberrations at any of the tested AV-45 concentrations. Another validity criteria employed in this study was the proportion of cells having structural aberration in the negative control cultures that is within the normal range of the historical data.

Results

Study outcome: A precipitate was observed at 1850 and 3700 µg/mL concentrations of AV-45 at the time of wash and at harvest in this study. Cytotoxicity was observed at 115.6 µg/mL during the 3-hour treatment condition without activation (53%) (Table 25) and at 462.5 µg/mL during 3-hour treatment with activation (55%) as shown in Table 27.

With the 22-hour treatment condition without activation, the cytotoxicity was 55% at 462.5 µg/mL as shown in table 26. However, no statistically significant increase in numerical aberrations was observed. The data from the vehicle controls were however, within the historical control ranges.

Table 25: Mitotic and Aberration Summary: 3-Hour without metabolic activation.

Treatment	Structural Mitotics Scored	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO	200	0%	0.0	0.0	0.0	0.0
MMC 0.6 µg/mL	81	44%	37.0 *	7.4 *	0.0	0.0
AV-45 14.45 µg/mL	200	21%	1.0	0.0	0.0	0.0
57.81 µg/mL	200	43%	0.5	0.0	0.0	0.0
115.6 µg/mL	200	53%	1.5	0.0	0.0	0.0

MMC - mitomycin C Endos = Endoreduplicated Cells
* Significantly greater than vehicle control, p≤0.05 (Fisher's Exact).

Table 26: Mitotic and Aberration Summary: 22-Hour without metabolic activation.

Treatment	Structural Mitotics Scored	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO	200	0%	0.5	0.0	0.0	0.0
MMC 0.3 µg/mL	68	51%	44.1 *	5.9 *	0.0	0.0
AV-45 28.91 µg/mL	200	23%	10.0 *	0.5	0.0	0.0
231.3 µg/mL	200	34%	11.0 *	0.0	0.0	0.0
462.5 µg/mL	200	55%	10.0 *	0.0	0.0	1.0

MMC - mitomycin C Endos = Endoreduplicated Cells
*Significantly greater than vehicle control, p≤0.05 (Fisher's Exact)

Table 27: Mitotic and Aberration Summary: 3-Hour with metabolic activation.

Treatment	Structural Mitotics Scored	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO	200	0%	2.5	0.0	0.0	0.0
CP 30.0 µg/mL	93	21%	32.3 *	0.0	0.0	0.0
AV-45 14.45 µg/mL	200	0%	1.5	0.0	0.0	0.0
115.6 µg/mL	200	27%	2.5	0.0	0.0	0.5
462.5 µg/mL	150	55%	3.3	0.0	0.0	0.0

CP - cyclophosphamide Endos = Endoreduplicated Cells
*Significantly greater than vehicle control, p≤0.05 (Fisher's Exact)

Reviewer's Comment:
This reviewer agrees with the result of this study which indicates that AV-45 does not cause structural aberrations in human peripheral lymphocytes during the 3-hour treatment with or without metabolic activation, but induced a significant increase in structural aberrations during 22-hour treatment without metabolic activation.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Rat Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Administration of AV-45

Study no.:	AC10HR.125.BT
Study report location:	4.2.3.3. Pages 1-85
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AV-45, lot # WZ1-07-207 and purity is stated to be 98.4%

Key Study Findings

The administration of AV-45 twice daily up to a cumulative daily dose of 372 µg/kg/day for 3 consecutive days did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in either male or female Hsd:SD rats.

Methods	
Cell line:	Hsd:SD rats
Concentrations in definitive study:	12, 38.5 or 186 µg/kg twice per day for three consecutive days.
Basis of concentration selection:	A confirmatory toxicity study was based on toxicity and solubility information of AV-45. The solubility limit of this compound was 50 µg/mL and acute intravenous doses of 224 or 448 µg/kg did not induce any mortality or severe signs in male and female rats.
Positive control:	Cyclophosphamide monohydrate (CP).
Formulation/Vehicle:	AV-45/0.5% (w/v) ascorbic acid, sodium ascorbate in 10% (v/v) ethanol in 0.9% saline solution
Incubation & sampling time:	The test and vehicle treated groups were killed 24 hours after the second dose while the CP-treated mice were killed 24 hours after the single dose. Both femurs from each animal were removed, cleaned and the slides of the bone marrow prepared. This was followed by slide scoring of the polychromatic erythrocytes (PCE), normachromatic (MCE) and micronucleated erythrocytes (M) for each animal. The PCE/NCE ratios were assessed for any reduction in the AV-45-treated group as evidence of bone marrow toxicity.

The sponsor initially projected to administer AV-45 to male and female rats at 50, 150 or 500 µg/kg twice per day to achieve cumulative daily doses of 100, 300 or 1000 µg/kg/day. However, the study evaluated AV-45 in rat bone marrow micronucleus test at three dose levels (12, 38.5 or 186 µg/kg twice per day for three consecutive days) during the definitive micronucleus study. The doses were selected based on AV-45 solubility limit of 50 µg/mL. AV-45 or vehicle (0.5% (w/v) ascorbic acid, sodium ascorbate in 10% (v/v) ethanol in 0.9% saline solution) was intravenously (10 mL/min) administered once daily on two consecutive days. The positive control, CPA (40 mg/kg) was given as a single dose at a dose volume of 10 mL/kg. The animals were observed daily before and after the administration of the dose and mortality assessed throughout the observation period.

Table 28: Treatment information.

Group	Treatment (10 mL/kg/2x day/3days)	
	Planned/Targeted Daily Doses	Achieved Daily Doses
1	**Vehicle Control** (0.0 µg/kg/2 x day/3 days)	**Vehicle Control** (0.0 µg/kg/2 x day/3 days)
2 3 4	**Test Article:** 50 µg/kg/2 x day = 100 µg/kg/day 150 µg/kg/2 x day = 300 µg/kg/day 500 µg/kg/2 x day = 1000 µg/kg/day	**Test Article:** 1.2 µg/kg/2 x day = 24 µg/kg/day x 3 days 3.85 µg/kg/2 x day = 77 µg/kg/day x 3 days 18.6 µg/kg/2 x day = 372 µg/kg/day x 3 days
5	**Positive Control:** Cyclophosphamide monohydrate (CP, 40 mg/kg/1x day)	*

*CP was administered only once; formulation was not analyzed for accuracy. The accuracy of preparation was confirmed by acceptable results that met criteria for valid test

The test and vehicle treated groups were sacrificed 24 hours after the second dose while the CPA-treated mice were sacrificed 24 hours after the single dose. The femurs from each animal were removed, cleaned and the slides of the bone marrows prepared. This was followed by slide scoring of the polychromatic erythrocytes (PCE), normachromatic (MCE) and micronucleated erythrocytes (M).

Study Validity

The acceptability of the assay was determined by 1) Comparing the group mean frequencies of micronucleated PCE in vehicle control animals with the historic vehicle control range and 2) The CP-treated group induced a statistically significant increase in the frequency of micronucleated PCE.

Results

Study outcome: In the definitive study, no mortality was reported and all the rats appeared normal following each dose administration and throughout the observation period. No reduction in group mean weights or mortality was observed following each dose administration and throughout the observation period. A cumulative daily dose of 372 µg/kg/day for 3 consecutive days did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in either male or female Hsd:SD rats.

Reviewer's Comment: This reviewer agrees with the sponsor that the result of rat micronucleus assay on AV-45 is negative.

8 Carcinogenicity

Not required.

9 Reproductive and Developmental Toxicology

The sponsor requested for waiver from conducting reproductive and developmental toxicity studies. The sponsor justified the request as follows:

1) Intended Population: The disease occurs most commonly in the elderly. Alzheimer's disease is the most common type of dementia found in 50% to 70% of dementia cases. The sponsor provided data showing that the prevalence of dementia ranges from 1.5% for patients (65-69 years old), up to 16-25% for those over 85 years old. Thus, the intended population for AV-45 would be elderly male or post-menopausal females and reproductive toxicity is of less concern relative to the potential benefit from an administration of ^{18}F-AV-45 to Alzheimer's patients.

2) Data from Toxicity Studies in Animals: No toxic effects on male or females were reported in the single- and repeat-dose toxicity studies conducted on non-radioactive AV-45 in rats and dogs. No macroscopic or microscopic histopathological effects was found in any organs including the reproductive organs of female (uterus and ovaries) and male (testes) rats administered up to 88X and 22X Multiple Human Dose (MHD) respectively. Similar findings were reported in a 28-day repeat dose toxicity study in dogs administered up to 22X MHD.
3) Pharmacokinetics and Distribution Properties of AV-45: The human pharmacokinetic data from studies ^{18}F-AV45-A02 and ^{18}F-AV45-A03 showed rapid uptake and clearance of AV-45 via hepatobiliary system. Over 90% of the radioactive dose is cleared from the circulation within 5 minutes after administration.

Dosimetry Data from 9 Healthy Volunteers: The dosimetry data obtained from 9 healthy volunteers (Study ^{18}F-AV45-A02) indicates low radiation exposure of 0.018±0.004, 0.018±0.003 and 0.007±0.001 in the ovaries, uterus and testes respectively. Thus, AV-45 is not concentrated in these organs.

4) Labeling: The sponsor is willing to accept Pregnancy Category C labeling and proposes the following:
Pregnancy Category C
(b) (4)

Reviewer's Comment: The request for a waiver from reproductive and developmental toxicology studies for ^{18}F-AV-45 was granted by the Agency.

10 Special Toxicology Studies

No special toxicity studies were conducted on ^{18}F-AV-45.

11 Integrated Summary and Safety Evaluation

The sponsor conducted several *in vitro* and *ex vivo* studies to demonstrate the affinity and selectivity of ^{18}F-AV-45 binding to β-amyloid plaque. In an *in vivo* binding study, ^{18}F-AV-45 competitively inhibited ^{125}I-IMPY binding with K*i*=10.0±3.3 nM. ^{18}F-AV-45 rapidly dissociates off the amyloid plaques after binding with a K_d value of 3.1±0.7 nM indicating that ^{18}F-AV-45 binding to the amyloid plaques is reversible. ^{18}F-AV-45 demonstrates high specificity in binding to its target and low binding affinity to central nervous system (CNS) and cardiovascular receptors.

An *ex-vivo* autoradiography study showed that ^{18}F-AV-45 selectively binds to the grey matters of the isolated brain homogenates of AD patients and poorly binds to the white matters where amyloid β is usually low. There was no binding in control brain tissues due to absence of amyloid. An animal model of AD was employed to evaluate the binding and specificity of ^{18}F-AV-45 binding to β amyloid. The data showed a significant labeling of the Aβ plaques following the injection of ^{18}F-AV-45 into B6.Cg-Tg [APPswe-PSEN1] transgenic mice.

Correlation analysis of data obtained from studies involving brain tissues obtained from up to 48 post mortem humans indicates correlations between ^{18}F-AV-45 binding and (a) neuritic plaque density but not neurofibrillary tangles (b) β amyloid plaque deposition (c) immunohistochemistry quantifications obtained using specific amyloid β antibodies.

Autoradiographic and biodistribution data from mice and Rhesus monkey indicate that ^{18}F-AV-45 penetrates the brain site readily and is rapidly cleared from the brain. The dosimetry data showed an estimated human effective dose of 97 mrem/mCi, a dose within acceptable radiation dose limit. ^{18}F-AV-45 is demethylated to AV-160 and subsequently acetylated to AV-267. The metabolites demonstrate low affinity in binding to the beta amyloid plaques indicating that the metabolites will probably not interfere with ^{18}F-AV-45 binding. ^{18}F-AV-45 and the metabolites are excreted from the body via urinary route.

There was no CNS adverse effect reported in any of the groups exposed to single dose what is the dose multiple or 28-day repeated dosing of up to 21.8X multiple human dose

(MHD) levels. The *in vitro* cardiovascular study shows that AV-45 inhibited hERG potassium current by 16.7 ± 0.9% (n=4) at 12.4 µM; the only employed dose due to solubility problem, verses 0.2 ± 0.1% (n=3) in control while the reference positive control, terfenadine (60nM), induced up to 83.8% inhibition on the hERG cells. The sponsor estimated the IC_{50} to be >12.4 µM, the only concentration employed during the study. However, no potential cardiovascular or respiratory effect was observed following AV-45 treatment of up to a dose of 84X MHD during safety pharmacology study conducted in beagle dogs.

The sponsor evaluated any possible interaction between drugs commonly used by AD patients and ^{18}F-AV-45 binding to β amyloid. None of the drugs evaluated interfered with ^{18}F-AV-45 binding to β amyloid. In a radioligand binding assay, AV-45 demonstrates high specificity in binding. AV-45 also gave IC_{50} values of 8.15 µM and 7.38 µM for the peripheral benzodiazepine (K_i values of 7.21 µM) and monoamine transporter (K_i values of 6.13 µM) respectively. Though, the receptors and transporters were significantly inhibited, however, the K_i values of AV-45 were about 1000X lower than the affinity for these transport sites. Thus, it is highly unlikely that AV-45 would interact with any of the evaluated receptors sites or affect the transporters at the intended human dose.

The sponsor conducted single- and repeat-dose toxicity studies in rats and Beagle dogs and no treatment-related mortality or any serious adverse effects was reported. During the rat studies, NOAELs of 87.2X- and 21.8X- MHD were obtained in a single- and 28-day repeat-dose toxicity study respectively as shown in the table below:

Toxicity	Species	NOAEL (mg/kg) M/F	Safety Margin
Single-Dose	Sprague Dawley rats	448 µg/ml	87.2X MHD
Repeat-Dose	Sprague Dawley rats	112 µg/kg/day	21.8X MHD
	Beagle dogs	7 µg/kg/day	4.5X MHD
	Beagle dogs	32 µg/kg/day	21X MHD

There were no reports of cardiovascular or ocular effects during 14-day repeat-dose toxicity study with a 2-week recovery conducted in Beagle dogs and NOAEL of 4.5X MHD was obtained. A NOAEL of 21X MHD was obtained during 28-day repeat-dose toxicity with 14-day recovery period conducted in Beagle dogs.

The sponsor's request for a waiver for reproductive and developmental toxicity studies was granted. Pregnancy category C is recommended for label.

Genotoxicity tests conducted include two *in vitro* assay the bacterial reverse mutation assay (Ames test) and chromosomal effects (cultured human peripheral lymphocytes cells). ^{18}F-AV-45 tested positive to *in vitro* assays and negative during *in vivo* mouse micronucleus assay.

The available nonclinical data has not demonstrated any significant nonclinical safety issues that could adversely affect the clinical use of ^{18}F-AV-45 in the context of its proposed indication in this NDA.

Overall Conclusion: The sponsor demonstrated that ^{18}F-AV-45 selectively binds to β-amyloid plaques and showed that no adverse cardiovascular or respiratory effect is envisaged if the product is administered at the proposed clinical dose. Adequate safety margins were demonstrated for AV-45 at the proposed dose during single-dose and repeat-dose toxicity studies in rats and dogs. There is no significant safety issue with the proposed indication for AV-45.

Recommendation: The approval of NDA 202008 is recommended from nonclinical perspective.

12 Appendix/Attachments

References

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/s/

SUNDAY O AWE
02/11/2011

ADEBAYO A LANIYONU
02/11/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202008 **Applicant:** Avid Pharmaceuticals Inc. **Stamp Date:**

Drug Name: AMYVID **NDA/BLA Type:** 05(b)(1)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		The Agency granted the sponsor's request for the waiver from conducting carcinogenicity and reproductive and developmental toxicity studies based on indication and frequency of use.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __ Yes**_____**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Sunny Awe	10-28-10
Reviewing Pharmacologist	Date
Adebayo Laniyonu, Ph.D.	10-28-10
Team Leader/Supervisor	Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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