18F-AV-1451-A16 Protocol Amendment 2

A Clinico-Pathological Study of the Correspondence Between 18F-AV-1451 PET Imaging and Post-Mortem Assessment of Tau Pathology

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A Clinico-Pathological Study of the Correspondence Between ^{18}F-AV-1451 PET Imaging and Post-Mortem Assessment of Tau Pathology

Date and Version:

18Dec2017 Amendment 2

Name of Compound:

^{18}F-AV-1451 ([F-18]T807)

Sponsor:

Avid Radiopharmaceuticals

Philadelphia, Pennsylvania USA

Approval/Signature:

[redacted]

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451	7-(6-[F-18]fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole

Title of Study: ^{18}F-AV-1451-A16

A Clinico-Pathological Study of the Correspondence Between ^{18}F-AV-1451 PET Imaging and Post-Mortem Assessment of Tau Pathology

Study Participants:

Planned number of subjects enrolled: ***Approximately 200***

Planned number of autopsies: ***Approximately 80***

Approximately 200 subjects with terminal medical conditions and projected life expectancy of ≤ 6 months will be enrolled and imaged with ^{18}F-AV-1451 in order to obtain post-mortem histological data on approximately 80 subjects. Enrollment will not be formally stratified, but an effort will be made to enroll subjects with cognitive status ranging from normal cognition to mild cognitive impairment (MCI) and dementia to achieve a full range of tau pathology. It is expected that at least 65% of the subjects enrolled will have dementia or MCI.

Front-Runners:

Up to the first 6 subjects that come to autopsy will be considered front-runners. Avid will not be blinded to the front-runner imaging and pathology results and may use these to evaluate the study methods and refine the methods if needed for the subsequent blinded cases. Subjects that are part of the front-runners will not be included in the Primary Efficacy Population.

Primary Efficacy Population:

All subjects with valid autopsies that occur after the front-runner group has completed and before the sample size requirement/trial stopping criteria (below) have been met will be considered part of the Primary Efficacy Population. Some subjects may have died and had autopsy material removed at the time the sample size requirement is met. Subjects for whom autopsies are ongoing at the time the sample size requirement is met will also be included in the Primary Efficacy Population.

Name of compound: ^{18}F-AV-1451

Dose*:* 370 MBq (10 mCi)

Route of Administration: Intravenous (IV) bolus

Study Phase: III

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451	7-(6-[F-18]fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole
Study Centers: Approximately 30 centers in the United States and Australia		

Primary Objective:

This study is designed to test the relationship between ante-mortem ^{18}F-AV-1451 Positron Emission Tomography (PET) imaging and tau neurofibrillary pathology associated with Alzheimer's disease (AD), as measured at autopsy. To accomplish this goal, two primary analyses will be performed:

Primary Analysis 1

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to Neurofibrillary Tangle (NFT) Score of B3 (Hyman et al., 2012; Montine et al., 2012) at autopsy will be evaluated; and if success criteria are met,

Primary Analysis 2

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by National Institute on Aging - Alzheimer's Association (NIA-AA) criteria (Hyman et al., 2012) will be evaluated. For individuals with cognitive impairment, high levels of AD neuropathologic change are considered adequate to explain cognitive impairment or dementia symptoms.

The trial will be considered successful if both co-primary endpoints are met according to the Statistical Analysis Plan.

Secondary Objective:

Secondary Analysis 1: To assess diagnostic performance of ante-mortem ^{18}F-AV-1451 PET imaging, based on majority interpretation of 5 independent readers, for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to Neurofibrillary Tangle (NFT) Score of B3 and for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by National Institute on Aging - Alzheimer's Association (NIA-AA) criteria.

Secondary Analysis 2: To assess agreement among readers of ^{18}F-AV-1451 PET scans.

Eligibility:

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Avid Radiopharmaceuticals	^{18}F-AV-1451	7-(6-[F-18]fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole
See Section 5.3, Selection of Subjects.		

Study Design:

This study is a clinico-pathological study of the correspondence between ^{18}F-AV-1451 PET imaging and post-mortem assessment of tau pathology and associated NIA-AA pathological diagnosis.

The screening and imaging procedures have been designed to be tolerable for eligible end-of-life patients. Individuals with terminal medical conditions with projected life expectancy of ≤ 6 months and/or their legally authorized representative (LAR) will provide informed consent. The subject will then receive a PET scan with ^{18}F-AV-1451 and will be followed to death. Subjects that have a historical brain Magnetic Resonance Imaging (MRI), acquired within 12 months of the ^{18}F-AV-1451 PET imaging visit, may consent to have the scan made available to the designated imaging core lab. If death does not occur within nine months of the ^{18}F-AV-1451 PET scan, the subject may be given the opportunity to undergo a second ^{18}F-AV-1451 PET scan and continue in the protocol at the sponsor's discretion (screening assessments will not need to be repeated). If the subject subsequently comes to autopsy, the second scan (nearest to time of death) will be used in the efficacy analyses. See below for exceptions related to cognitively normal subjects.

Approximately 200 subjects may be enrolled and imaged with ^{18}F-AV-1451 PET. It is expected that approximately 80 individuals will undergo post-mortem autopsy evaluation (including the Primary Efficacy Cohort and up to 6 subjects in a Front-Runner Cohort). The Primary Efficacy Cohort will form the basis for the tests of diagnostic performance (subjects for whom autopsies are ongoing at the time the sample size requirement is met will also be included in the Primary Efficacy Population). The primary efficacy cohort will include all subjects with a valid PET scan that come to autopsy before the sample size requirement/trial stopping criteria are met. In order to control for the potential progressive change in tau deposition over time, cognitively impaired subjects will not have an autopsy performed or be included in the primary efficacy cohort if death does not occur within nine months of a valid imaging session. However, because cortical tau deposition is expected to occur very slowly, if at all, in cognitively normal (unimpaired) subjects, cognitively normal subjects will not be required to undergo repeat scans. Cognitively normal subjects will remain eligible for autopsy, regardless of time from first scan or second scan to autopsy, and will be included in the primary efficacy cohort if the subject comes to autopsy any time before the trial stopping criteria are met.

Up to the first 6 subjects that come to autopsy will be considered front-runners. Results from the front-runners will be analyzed on a patient-by-patient basis and may be used to refine the PET or autopsy methods (e.g. pathology staining/quantitation methods). Upon completion of the final front-runner subject analysis, the final imaging and autopsy

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methods will be specified. No additional PET image reads or autopsy analyses will be performed until the PET image read and autopsy methods have been finalized. The primary efficacy analyses will be performed on those subjects with valid imaging data that come to autopsy after the front-runners (Primary Efficacy Cohort). The front-runners will not be included in the primary efficacy analyses, but additional analyses of combined primary plus front-runner cases may be performed.

The first 30 PET scans collected will be reviewed by an independent Data Monitoring Board (DMB). The DMB will assess whether the scans can be evaluated in a manner consistent with methods developed in Phase II (^{18}F-AV-1451-A05 Exploratory Phase Cohort) as determined by criteria outlined in the Data Monitoring Board Charter. The DMB will have the ability to recommend unblinding the Sponsor to all 30 cases if a pre-specified number of scans in the autopsy population have anomalies not present in the early phase studies. Study enrollment will continue during the DMB's review. In the event the Sponsor proceeds with a DMB recommendation for unblinding, the unblinded subjects will be excluded from the Primary Efficacy Cohort (neither the scans nor any autopsies will be used in the primary analyses) or the study may be terminated. If unblinding occurs, it must be completed before the final image read and autopsy methods are specified.

Avid personnel will be blinded to the Primary Efficacy Cohort ^{18}F-AV-1451 PET images. A single Avid member will perform periodic quality assurance (QA) by reviewing a listing of the PET image header data (not to exceed 20% unless issues identified that warrant additional review). These QA reviews will serve to ensure image quality control processes at the imaging core laboratory are adequately performed. Avid staff performing the PET image data header QA assessment will not be aware of the clinical status for the subjects/images being reviewed. The independent blinded readers and DMB will be blinded to the subject clinical status and autopsy results. The pathology core lab(s) will be blinded to the subject clinical status, PET scan images, and read results. All other Avid personnel will be blinded to the Primary Efficacy Cohort ^{18}F-AV-1451 read results and pathology data.

Visits and Assessments:

Each subject will have a screening visit, up to two ^{18}F-AV-1451 PET imaging visits, and a follow-up phone call after each imaging visit. Subjects who come to autopsy during the study period will receive a post-mortem evaluation.

Specific details of assessments that will be performed at each visit are detailed in Section 7.1.

Evaluation of Imaging

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^{18}F-AV-1451 retention on PET images from subjects with valid autopsy data will be interpreted by visual examination as either

- Neocortical Uptake Not Consistent with an AD Pattern (AD-: no increased neocortical activity in any region or increased activity is isolated to the mesial temporal, anterolateral temporal or frontal region(s));
- Neocortical Uptake Consistent with an AD Pattern (AD+: increased neocortical tracer activity in the posterolateral temporal (PLT), parietal (including precuneus), or occipital region(s)); or
- Neocortical Uptake Consistent with an AD Pattern and with Likelihood of Disease Progression (AD++, which is a subset of AD+ (neocortical uptake consistent with an AD pattern) where increased tracer activity is found in the parietal (including precuneus) region(s) regardless of uptake elsewhere, or frontal region in addition to PLT, occipital, or parietal region(s)).

For the purpose of testing the primary analyses, a scan interpretation will be considered positive (hypothesized to correspond to a B3 neuropathology score in Primary Analysis 1 and an NIA-AA score of high AD pathology in Primary Analysis 2) if the scan is interpreted as at least consistent with an AD pattern (AD+ or AD++).
Details of the visual PET image review methods will be provided in the Blinded Read Manual.

Images will also be assessed quantitatively (semi-automated computerized measurement of Standard Uptake Value Ratios [SUVRs]) for exploratory analysis purposes. Details of the analysis methods will be provided in the Quantitative Methodology Document.

The ^{18}F-AV-1451 PET imaging results will not be shared with the investigator sites or subjects and their families prior to the end of the study.

Neuropathology Evaluation

All neuropathological measurements on brain tissue in this trial will be evaluated in a standardized and blinded (with respect to PET image results and subject identifying/clinical information) fashion in a qualified laboratory. A Technical Autopsy Manual and Neuropathology Analysis Plan will be developed for this study containing all methods and measurement procedures. Neuropathological assessment will be derived from the NIA-AA guidelines (Hyman et al., 2012; Montine et al., 2012). Sections from brain regions recommended for pathological assessment of AD, as well as additional, pre-specified neocortical regions will be sampled. Staining will follow the procedures recommended in the NIA-AA practical approach guidelines. NFT extent will be assessed using standard procedures and NFT scores (B0-B3) will be recorded (Hyman et al., 2012; Montine et al., 2012). The Truth Standard (TS) for Primary Analysis 1 will be constructed from NFT scores as shown in Table 1.

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Table 1: Autopsy NFT Score Truth Standard (Primary Analysis 1)

Braak Stage	NFT Score[a]	Truth Standard (TS)
0 (no NFTs)	B0	Negative
I-II	B1	
III-IV	B2	
V-VI	B3	Positive

[a] adapted from Hyman et al., 2012

Amyloid pathology will be evaluated using Thal and CERAD rating systems. Distribution of amyloid (Thal plaque Score A0-A3; Montine et al., 2012) will be recorded. The frequency of neuritic amyloid plaques will be evaluated semi-quantitatively (CERAD score: none, sparse, moderate or frequent). Using Thal plaque score, CERAD score and NFT score, level of AD neuropathologic change will be recorded as none, low, intermediate or high per NIA-AA guidelines (Hyman et al. 2012). The TS for Primary Analysis 2 will be constructed from levels of AD neuropathologic change as shown in Table 2.

Table 2: NIA-AA Autopsy Diagnosis Level of AD Neuropathologic Change Truth Standard (Primary Analysis 2)

Level of AD Neuropathologic Change Present[a]	Truth Standard (TS)
Not	Negative
Low	
Intermediate	
High	Positive

[a] adapted from Table 2 in Hyman et. al, 2012

Statistical Methods:

This study is designed to test the relationship between ante-mortem ^{18}F-AV-1451 PET imaging and tau neurofibrillary pathology associated with AD, as measured at autopsy. To accomplish this goal, two primary analyses will be performed:

Primary Analysis 1

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The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to NFT Score of B3 (Hyman et al., 2012; Montine et al., 2012) at autopsy will be evaluated; and if success criteria are met,

Primary Analysis 2

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by NIA-AA criteria (Hyman et al., 2012) will be evaluated. For individuals with cognitive impairment, high levels of AD neuropathologic change are considered adequate to explain cognitive impairment or dementia symptoms.

The trial will be considered successful if both co-primary endpoints are met according to the Statistical Analysis Plan.

For Primary Analysis 1, the hypothesis to be tested is that for the same 3 out of 5 independent readers the lower bound of the two-sided 95% confidence intervals (CI) for both sensitivity and the specificity of ^{18}F-AV-1451 PET reading results will be ≥ 50%. As detailed in Table 1 above, NFT scores for autopsy cases will be categorized as either B0/B1/B2 or B3 level and will serve as the NFT Score TS for this analysis. ^{18}F-AV-1451 PET imaging will be classified as either AD- or AD+/AD++ by each reader. Sensitivity and specificity will be calculated for each reader as the proportions of True Positive (TP) and True Negative (TN) cases correctly identified as such, according to Table 3 below:

Table 3: Primary Analysis 1: Diagnostic Performance Calculations for AV-1451 PET Scan Reader Interpretation vs. Autopsy NFT Score TS

	Autopsy NFT Score TS	
Physician Reader Interpretation	**NFT Score B3 [Truth Positive]**	**NFT Score B0–B2 [Truth Negative]**
AV-1451 Neocortical uptake; AD pattern (AD+ or AD++)	True Positive (TP)	False Positive (FP)
Neocortical uptake not consistent with AD (AD-)	False Negative (FN)	True Negative (TN)

Two-sided 95% CIs for sensitivity and specificity will be calculated using the Wilson score method. The first primary endpoint is considered to be met if for the same 3 out of 5

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readers, the lower bound of the 95% CIs for both sensitivity and specificity are ≥ 50% (i.e., statistically significant at two-sided significance level of 0.05).

Like Primary Analysis 1, the hypothesis to be tested for Primary Analysis 2 is that for the same 3 out of 5 readers, the lower bound of the 95% CIs for both sensitivity and the specificity will be ≥ 50%. For Primary Analysis 2, autopsy cases will be categorized as either [not, low or intermediate] vs. [high] level of AD neuropathologic change according to NIA-AA criteria, considering Braak stage, Thal plaque score, and CERAD amyloid neuritic plaque score, and this level of AD neuropathologic change will be used as the NIA-AA Autopsy Diagnosis TS (Table 2, above).

^{18}F-AV-1451 PET images will be interpreted by visual examination as either AD- or AD+/AD++ by each reader. Sensitivity and specificity will then be calculated for each reader as the proportions of True Positive (TP) and True Negative (TN) cases correctly identified as such, according to Table 4 below:

Table 4: Primary Analysis 2: Diagnostic Performance Calculations for AV-1451 PET Scan Reader Interpretation vs. NIA-AA Autopsy Diagnosis TS

	NIA-AA Autopsy Diagnosis TS	
Physician Reader Interpretation	**High AD Neuropathologic Change [Truth Positive]**	**Not, Low or Intermediate AD Neuropathologic Change [Truth Negative]**
AV-1451 Neocortical uptake; AD pattern (AD+, AD++)	True Positive (TP)	False Positive (FP)
AV-1451 Neocortical uptake not consistent with AD pattern (AD-)	False Negative (FN)	True Negative (TN)

Two-sided 95% CIs will be calculated using the Wilson score method. The second primary endpoint is met if for the same 3 out of 5 readers the lower bounds of the 95% CIs for both sensitivity and specificity are ≥ 50%.

The secondary efficacy analyses will assess the performance of the blinded ^{18}F-AV-1451 PET scan readers in the following two ways:

Secondary Analysis 1: Assess diagnostic performance of ante-mortem ^{18}F-AV-1451 PET imaging based on majority interpretation of 5 independent readers: Diagnostic performance will be assessed relative to the Autopsy NFT score TS as per Primary Analysis 1 and again relative to the NIA-AA Autopsy Diagnosis TS as per Primary Analysis 2. In each case (image), a majority reading result will be derived using each

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individual reader's scan interpretation results. Sensitivity and specificity, and two-sided 95% CI thereof, will be calculated for the majority interpretation results relative to the appropriate TS. The hypotheses to be tested are:

1a: the majority reading result will have the lower bounds of two-sided 95% CI ≥ 55% for both sensitivity and specificity for detection of tau neurofibrillary pathology that corresponds to NFT Scores of B3, and

1b: the majority reading result will have the lower bounds of two-sided 95% CI ≥ 55% for both sensitivity and specificity for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by NIA-AA criteria.

Secondary Analysis 2: Assess agreement among readers of ^{18}F-AV-1451 PET scans:
Fleiss' Kappa statistics will be used to assess inter-reader agreement for the diagnostic decisions associated with Primary Analyses 1 and 2. For evaluation of agreement in the context of Primary Analysis 1, kappa will be calculated according to the reader interpretation classification in Table 3. If Primary Analysis 1 is successful, a second kappa will be performed using the reader interpretation classification in Table 4 . The hypothesis to test is the observed kappa values are ≥ 0.64 and the lower bound of the two-sided 95% CIs for this assessment are ≥ 0.55.

Safety data will be tabulated.

Sample Size Calculation and Trial Stopping Criteria

Assuming sensitivity/specificity of approximately 80%, a minimum of 14 autopsy cases in each of the truth standard groups (pathological positive or negative) is required for the lower bound of the two-sided 95% CI to be > 50% for each individual reader. The sponsor is blinded to the pathology results (truth standard) until after the study is completed and the database is locked. Therefore, in order to ensure that an adequate number of subjects are recruited and come to autopsy for each truth standard group, the sponsor will assume that subjects diagnosed on clinical grounds as having dementia due to AD will be truth standard positive, subjects diagnosed as having no cognitive impairment (cognitively normal) will be truth standard negative. Based on these assumptions, the study sample size requirement and trial stopping criteria will be deemed to have been met when

- the number of subjects diagnosed as having impairment due to AD that have come to autopsy, is greater than or equal to 14, AND
- the number of subjects diagnosed as having no cognitive impairment that have come to autopsy is greater than or equal to 14

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The sponsor recognizes that there may be some errors in clinical diagnosis such that some patients diagnosed clinically as having impairment due to AD may have a nonAD pathology, or limited amounts of AD tau pathology, and thus be truth standard negative, and, less often, subjects considered clinically normal may have AD pathology and be truth standard positive. However, given the excess frequency of cases with AD diagnoses relative to clinically normal subjects already recruited in this trial population, any diagnostic errors will likely help ensure a minimum of 14 truth standard negative subjects without jeopardizing the criterion for truth standard positive subjects.

In addition to subjects diagnosed as clinically normal or AD patients, it is expected that approximately 10-15 subjects with nonAD impairments will be enrolled and come to autopsy in this trial. However, the proportion of these that will have truth standard positive/negative AD tau pathology is very difficult to predict and will depend on the precise cases enrolled. Thus, it is not expected that these cases will be useful in estimating when a sufficient number of cases have occurred in each truth standard group and are not employed in the stopping criteria.

No new autopsies will begin once the sample size requirement/trial stopping criteria are met. However, some subjects may have died and had autopsy material removed at the time the sample size requirement is met. Histology analysis will be completed in subjects for whom autopsies are ongoing at the time the sample size requirement is met and these subjects will also be included in the Primary Efficacy Population.

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ABBREVIATIONS AND DEFINITIONS

Aβ	Beta amyloid
AD	Alzheimer's disease
Adverse Event (AE)	Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.
electronic Case Report Form (eCRF)	An electronic form for recording study participants' data during a clinical study, as required by the protocol.
CNS	Central Nervous System
CRO	Contract Research Organization: A person or organization (commercial, academic, or other) contracted by the sponsor to perform one or more of the sponsor's trial-related duties and functions.
CT	Computed Tomography
DMB	Data Monitoring Board
Efficacy	Efficacy is the ability of a treatment to achieve a beneficial intended result.
FDA	US Food and Drug Administration
FDG	^{18}F – Fluorodeoxyglucose
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
Institutional Review Board /Independent Ethics Committee	A board or committee (institutional, regional, or national) composed of medical and nonmedical members whose responsibility is to verify that the safety, welfare and human rights of the subjects participating in a clinical study are protected.
Investigator	A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
IV	Intravenous

K_d	Dissociation Constant
LAR	Legally Authorized Representative
MBq	Megabecquerel
mCi	Millicurie
MCI	Mild Cognitive Impairment
MHD	Maximum Human Dose
MRI	Magnetic Resonance Imaging
NIA-AA	National Institute on Aging – Alzheimer's Association
NFT	Neurofibrillary Tangle
NOAEL	No Observable Adverse Effect Level
OCN	Older Cognitively Normal
PET	Positron Emission Tomography
QA	Quality Assurance
SUVRs	Standard Uptake Value Ratios
TN	True Negative
TP	True Positive
TS	Truth Standard
YCN	Young Cognitively Normal

1. INTRODUCTION

Molecular imaging biomarkers have the potential to aid in the diagnosis of patients with cognitive impairment (Dubois et al., 2010; McKhann et al., 2011). PET ligands such as florbetapir F 18 (Wong et al., 2010) provide a minimally invasive estimate of cortical beta amyloid (Aβ) neuritic plaque deposition, which is a hallmark pathology of Alzheimer's disease (AD) and a required element for the neuropathologic diagnosis of AD (Hyman et al., 2012). More recently, imaging biomarkers for tau protein, the other required pathology for AD, have become available (Chien et al., 2103; Xia et al., 2013).

Amyloid is believed to accumulate very early in the AD disease process (Jack et al., 2010) and may be present in other diseases or as an incidental finding in patients with other dementias (Siderowf et al., 2014; Serrano et al., 2014). While amyloid deposition is required for the diagnosis of AD, its density or distribution is not associated with disease duration or severity (Nelson et al., 2012).

In contrast to Aβ neuritic plaques, the density and distribution of phosphorylated tau, aggregated in neurofibrillary tangles, increases with AD-related cognitive impairment and correlates with neurodegeneration (Dickson et al., 1997; Duyckaerts et al., 1987; Nelson et al., 2012). Thus, a PET imaging agent that binds to phosphorylated tau has potential application as a biomarker for disease severity and may be useful for selecting patients for therapy, monitoring disease progression and assessing response to putative disease modify treatments.

^{18}F-AV-1451 (originally named [F-18]T807 by Siemens Molecular Imaging Biomarker Research group) has been developed as a positron emitting radiopharmaceutical for in vivo imaging of tau protein aggregates (Xia et al., 2013; Chien et al. 2013). Autoradiography results using tissue sections from human brains showed a strong signal in the grey matter of cortical slices from tau positive brains but weak or no binding in tau negative, Aβ positive, or tau and Aβ negative tissue. Scatchard analysis based on this heterogeneous autoradiography assay yielded an estimated Kd of 15nM. A saturation binding experiment using purified Paired Helical Filament (PHF) Tau isolated brains of AD patients yielded a Kd value of 0.54 nM.

AV-1451 was assessed in competitive binding assays against a panel of 72 of the most common central nervous system (CNS) targets and no clinically relevant inhibition was seen. AV-1451 was positive in the in vitro hERG assay; however, in vivo cardiovascular safety pharmacology assessments in dogs showed no evidence of QT prolongation at doses up to 50x the intended maximum human dose (MHD). Nonetheless, until sufficient human cardiovascular safety data are available, clinical studies will exclude subjects with a history of risk factors for Torsades de Pointes and subjects taking drugs known to prolong the QT interval.

In vivo safety pharmacology studies were also conducted in rats to determine potential effects on the CNS and respiratory systems. In these studies no clinically relevant effects were reported at doses exceeding 100x the intended MHD. Additionally, non-radioactive AV-1451 has been tested in single and repeat-dose toxicology studies in rat and dog. In each of these studies the no observable adverse effect levels (NOAELs) were the highest doses tested (150x MHD for single, 50x MHD for repeat).

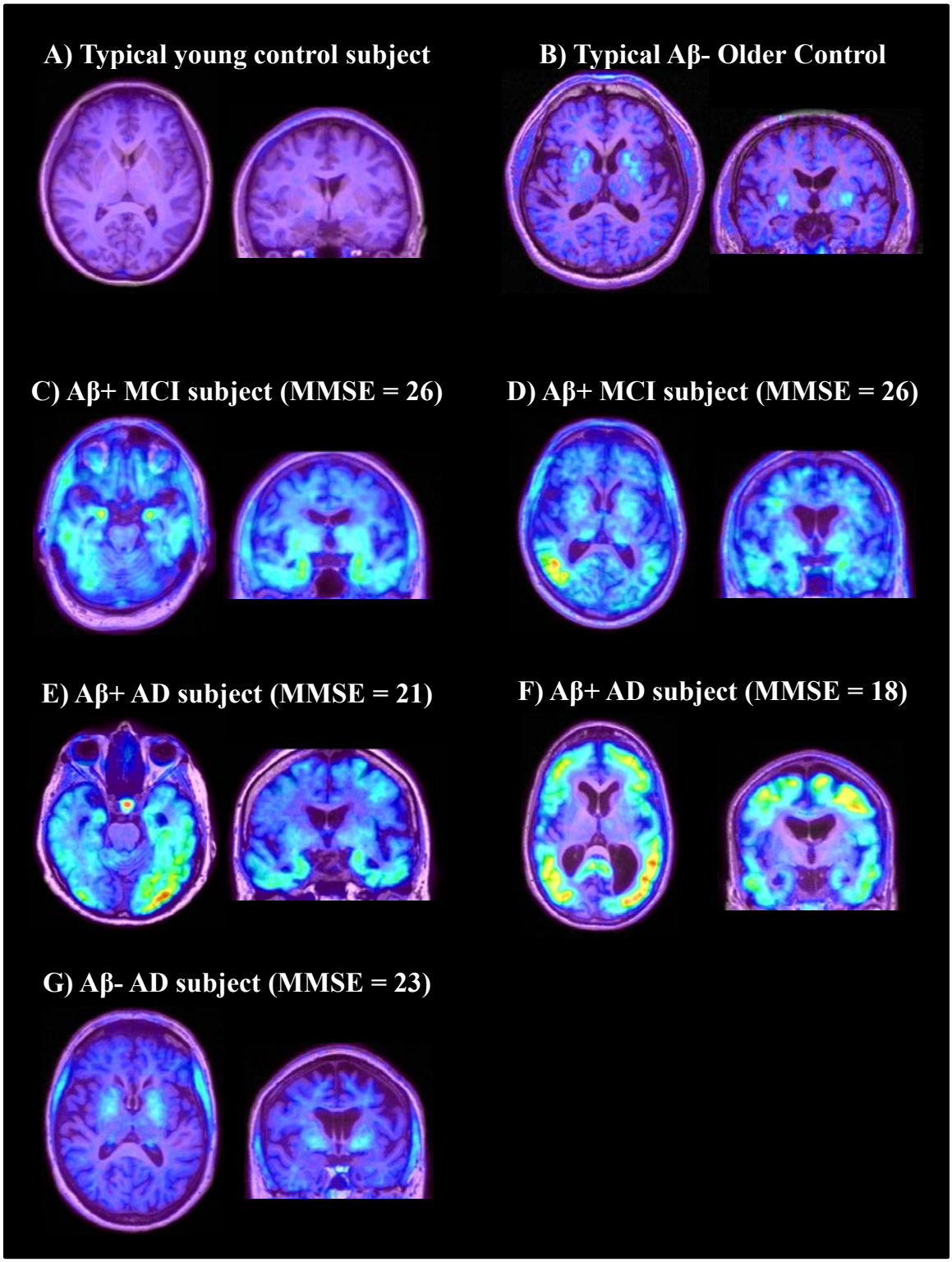
Potential genotoxicity of non-radioactive AV-1451 was tested in both *in vitro* and *in vivo* assays. In the *in vitro* assays, AV-1451 tested positive for potential genotoxicity. However, in the *in vivo* rat micronucleus assay at doses up to 750x MHD (scaled allometrically), AV-1451 showed no evidence of genotoxicity. The different results in the in vitro genotoxicity assays and the *in vivo* micronucleus study are likely related to differences in the exposure conditions encountered by the target cells in the different test systems. In vivo, AV-1451 is cleared rapidly; however, the i*n vitro* experiments employ static, prolonged exposure of cells to high concentrations of the test article. While the *in vitro* data show the potential for genotoxicity, the *in vivo* data provide assurance that genotoxicity is unlikely to occur at clinically-relevant doses for human diagnostic studies.

Three human clinical studies have been completed with ^{18}F-AV-1451. A total of 59 subjects have been exposed to ^{18}F-AV-1451 in these studies. The following side effects have been reported in clinical studies: diarrhea, headache, and altered taste. All reported events were mild in intensity and all subjects recovered from these events.

Human dosimetry has been obtained in nine subjects. Generally, the radiotracer distribution was consistent among the subjects and showed rapid hepatobiliary clearance. There were three organs that received estimated doses higher than 0.05 mSv/MBq. The organ that received the largest estimated dose was the upper large intestinal wall (0.0962 ± 0. 0134 mSv/MBq), followed by the small intestine and the liver. The Effective Dose was 0.0241 ± 0.0016 mSv/MBq. This results in an estimated Effective Dose of 8.70 mSv for an anticipated 370 MBq (10 mCi) injection and is comparable to the effective dose of approved 18F-labeled compounds such as fluorodeoxyglucose (FDG) and florbetapir F 18 injection.

Evaluation of the PET images from completed studies demonstrates little focal cortical retention of ^{18}F-AV-1451 in either young cognitively normal (YCN) or older cognitively normal (OCN) subjects known to be amyloid negative (florbetapir PET SUVR <1.10; Figure 1 Panels A and B). However, OCN subjects frequently demonstrated retention in the mesial temporal lobes and some OCN subjects also demonstrated retention in the brainstem or striatum (Figure 1 Panel B). In MCI and AD subjects, retention appeared to spread from mesial temporal lobes to isocortical areas (Figure 1 Panels C-F). The pattern of ^{18}F-AV-1451 distribution seen on PET imaging, across subjects with various levels of impairment, paralleled the pattern reported in the classic autopsy series of older persons and subsequently used to define the Braak stage (Braak and Braak, 1991). Two clinically diagnosed AD subjects that were found to be amyloid negative on a florbetapir PET scan were the exception to this pattern, as their scans were similar to clinically normal elder controls and showed no cortical ^{18}F-AV-1451 retention (Figure 1 Panel G).

Figure 1: Representative ^{18}F-AV-1451 PET Scans



Comparison of tracer binding to aggregated protein with autopsy material is one means of validating a novel PET agent. Multiple studies have been completed comparing amyloid PET scans to histopathologic assessment of amyloid burden subjects for whom biopsy samples were available or who came to autopsy after receiving a PET amyloid scan. These studies support the relationship between PET amyloid imaging results and cortical neuritic plaque density (Clark et al., 2011, 2012; Leinonen et al., 2008; Sojkova et al., 2011; Kantarci et al., 2011; Burack et al., 2010). The larger autopsy studies (Clark et al., 2012; Curtis et al., 2015; Sabri et al., 2015) demonstrated a high sensitivity and specificity for amyloid PET to discriminate subjects with subsequent autopsy findings of no or sparse neuritic plaques (amyloid negative) from those with moderate to frequent plaques (amyloid positive).

By contrast, systematic studies correlating imaging data from tau ligands with post-mortem verification of AD pathology have not been completed. The goal of this protocol is to generate evidence to support the relationship between PET imaging with ^{18}F-AV-1451 PET imaging and AD neuropathology assessed at autopsy.

2. TRIAL OBJECTIVES

2.1. Primary Objective:

This study is designed to test the relationship between ante-mortem ^{18}F-AV-1451 PET imaging and tau neurofibrillary pathology associated with AD, as measured at autopsy. To accomplish this goal, two primary analyses will be performed:

Primary Analysis 1

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to NFT Score of B3 (Hyman et al., 2012; Montine et al., 2012) at autopsy will be evaluated; and if success criteria are met,

Primary Analysis 2

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by NIA-AA criteria (Hyman et al., 2012) will be evaluated. For individuals with cognitive impairment, high levels of AD neuropathologic change are considered adequate to explain cognitive impairment or dementia symptoms.

The trial will be considered successful if both co-primary endpoints are met according to the Statistical Analysis Plan.

2.2. Secondary Objective:

To assess performance of independent, blinded readers of ^{18}F-AV-1451 PET scans:

Secondary Analysis 1: To assess diagnostic performance of ante-mortem ^{18}F-AV-1451 PET imaging, based on majority interpretation of 5 independent readers, for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to Neurofibrillary Tangle (NFT) Score of B3 and for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by National Institute on Aging - Alzheimer's Association (NIA-AA) criteria.

Secondary Analysis 2: To assess agreement among readers of ^{18}F-AV-1451 PET scans.

3. SPONSOR, INVESTIGATOR(S) AND OTHER PARTICIPANTS

The trial is sponsored by:

Avid Radiopharmaceuticals

[redacted]

The medical contact is:

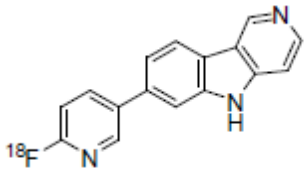
[redacted]

Approximately 30 centers in the United States and Australia will participate.

4. TEST DRUG AND CONTROL AGENTS

4.1. Descriptive Name: ^{18}F-AV-1451

7-(6-[F-18]fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole



MW = 262.27 amu

4.2. Radioactive Labeling

The compound is labeled with [^{18}F] fluorine that decays by positron (β^+) emission and has a half-life of 109.77 min. The principal photons useful for diagnostic imaging are the 511 keV gamma photons, resulting from the interaction of the emitted positron with an electron.

4.3. Decay Characteristics

The time course of radioactive decay for Fluorine [^{18}F] is shown below

Min.	Fraction Remaining
0	1.000
30	0.827
60	0.685
90	0.567
120	0.469
150	0.388
180	0.321
210	0.266
240	0.220

Physical decay chart for Fluorine [^{18}F]. Half-life = 109.77 min.

4.4. Formulation and Dose ^{18}F-AV-1451 Injection

^{18}F-AV-1451 Injection is a sterile, apyrogenic clear solution for intravenous bolus administration. ^{18}F-AV-1451 Injection contains ^{18}F-AV-1451 (drug substance) formulated in 10% (v/v) ethanol, USP in 0.9% sodium chloride injection, USP.

The shelf-life of ^{18}F-AV-1451 Injection is dependent on the strength or specific activity calculated at End-of-Synthesis (EOS) but is not more than 10 hours post EOS. The ^{18}F-AV-1451 Injection expiration time and date will be provided on the label on the secondary packaging of each vial or syringe.

4.5. Packaging ^{18}F-AV-1451 Injection

^{18}F-AV-1451 Injection is contained in a sterile, non-pyrogenic septum sealed Type I glass vial or a sterile apyrogenic syringe. Vials and syringes containing ^{18}F-AV-1451 Injection are stored in an opaque shield (secondary packaging) which protects personnel from

radiation exposure. The shield may be packaged in a Type A shipping container for shipment to the imaging facility.

4.6. Storage and Handling ^{18}F-AV-1451 Injection

^{18}F-AV-1451 Injection is stored at room temperature. ^{18}F-AV-1451 Injection should be stored within the original container or equivalent radiation shielding.

5. INVESTIGATIONAL PLAN

5.1. Overall Design and Plan of Trial

This study is a clinico-pathological study of the correspondence between ^{18}F-AV-1451 PET imaging and post-mortem assessment of tau pathology and associated NIA-AA pathological diagnosis.

The screening and imaging procedures have been designed to be tolerable for eligible end-of-life patients. Individuals with terminal medical conditions with projected life expectancy of ≤ 6 months and/or their LAR will provide informed consent. The subject will then receive a PET scan with ^{18}F-AV-1451 and will be followed to death. Subjects that have a historical brain MRI, acquired within 12 months of the ^{18}F-AV-1451 PET imaging visit, may consent to have the scan made available to the designated imaging core lab. If death does not occur within nine months of the ^{18}F-AV-1451 PET scan, the subject may be given the opportunity to undergo a second ^{18}F-AV-1451 PET scan and continue in the protocol at the sponsor's discretion (screening assessments will not need to be repeated). If the subject subsequently comes to autopsy, the second scan (nearest to time of death) will be used in the efficacy analyses.

Approximately 200 subjects may be enrolled and imaged with ^{18}F-AV-1451 PET. It is expected that approximately 80 individuals will undergo post-mortem autopsy (including the Primary Efficacy Cohort and 6 subjects in a Front-Runner Cohort). The Primary Efficacy Cohort will form the basis for the tests of diagnostic performance. The Primary Efficacy Cohort will include all subjects with a valid PET scan that come to autopsy before the sample size requirement/trial stopping criteria (Section 8.6) are met (subjects for whom autopsies are ongoing at the time the sample size requirement is met will also be included in the Primary Efficacy Population). In order to control for the potential progressive change in tau deposition over time, cognitively impaired subjects will not have an autopsy performed or be included in the Primary Efficacy Cohort if death does not occur within nine months of a valid imaging session. However, because cortical tau deposition is expected to occur very slowly, if at all, in cognitively normal (unimpaired) subjects, cognitively normal subjects will not be required to undergo repeat scans. Cognitively normal subjects will remain eligible for autopsy, regardless of time from first scan or second scan to autopsy, and will be included in the primary efficacy cohort if the subject comes to autopsy any time before the trial stopping criteria are met. No new autopsies will begin once the sample size requirement and trial stopping criteria (Section 8.6) are met. However, some subjects may have died and had autopsy material removed at the time the sample size requirement is met. Histology analysis will be completed in

subjects for whom autopsies are ongoing at the time the sample size requirement is met and these subjects will also be included in the Primary Efficacy Population.

Up to the first 6 subjects that come to autopsy will be considered front-runners. Results from the front-runners will be analyzed on a patient-by-patient basis and may be used to refine the PET or autopsy methods (e.g. pathology staining/quantitation methods). Upon completion of the final front-runner subject analysis, the final imaging and autopsy methods will be specified. No additional PET image reads or autopsy analyses will be performed until the PET image read and autopsy methods have been finalized. The primary efficacy analyses will be performed on those subjects with valid imaging data that come to autopsy after the front-runners (Primary Efficacy Cohort). The front-runners will not be included in the primary efficacy analyses, but additional analyses of combined primary plus front-runner cases may be performed.

The first 30 PET scans collected will be reviewed by an independent DMB. The DMB will assess whether the scans can be evaluated in a manner consistent with methods developed in Phase II (^{18}F-AV-1451-A05 Exploratory Phase Cohort) as determined by criteria outlined in the Data Monitoring Board Charter. The purpose of establishing these criteria for review by the DMB is to ensure that scans in the autopsy population do not appear significantly different than scans obtained in early phase studies, where subjects were not at end-of-life. The DMB will have the ability to recommend unblinding the Sponsor to all 30 cases if a pre-specified number of scans in the autopsy population have anomalies not present in the early phase studies. Study enrollment will continue during the DMB's review. In the event the Sponsor proceeds with the DMB recommendation for unblinding, the unblinded subjects will be excluded from the Primary Efficacy Cohort (neither the scans nor any autopsies will be used in the primary analyses) or the study may be terminated. If unblinding occurs, it must be completed before the final image read and autopsy methods are specified. If clinically significant incidental findings are identified on a PET scan, the DMB will communicate the findings to Avid. This process will be described in the DMB Charter.

Avid personnel will be blinded to the Primary Efficacy Cohort ^{18}F-AV-1451 PET images. A single Avid member will perform periodic quality assurance (QA) by reviewing a listing of the PET image header data (not to exceed 20% unless issues identified that warrant additional review). These QA reviews will serve to ensure quality control processes at the imaging core laboratory are adequately performed. Avid staff performing the PET image data header QA assessment will not be aware of the clinical status for the subjects/images being reviewed. The independent blinded readers and DMB will be blinded to the subject clinical status and autopsy results. The pathology core lab(s) will be blinded to the subject clinical status, PET scan images, and read results. All other Avid personnel will be blinded to the Primary Efficacy Cohort ^{18}F-AV-1451 read results and pathology data.

5.2. Planned Dosage and Duration of Treatment

5.2.1. Dosage and Administration

All subjects will receive a single IV bolus administration target dose of 370 MBq (10 mCi) of ^{18}F-AV-1451 Injection.

5.2.2. Rationale for Dosage

This trial is designed to evaluate the brain tau protein imaging properties of ^{18}F-AV-1451 to be used in subjects near end-of-life. ^{18}F-AV-1451 will be administered IV in a radioactive target dose of 370 MBq with a MHD limited to 20 µg of compound by mass. This dose is 150-fold lower than the NOAEL observed in the rat single dose toxicity study and is 50-fold lower than the NOAEL observed in the rat and dog repeat dose toxicity studies.

Human dosimetry has been obtained in nine subjects. The results estimated an Effective Dose of 8.70 mSv for an anticipated 370 MBq (10 mCi) injection and is comparable to the effective dose of approved ^{18}F-labeled compounds such as FDG and Florbetapir F 18 Injection.

The proposed dose has been shown to have acceptable image quality in previous human studies.

5.3. Selection of Subjects

Subjects will be enrolled in the study with the intent of capturing a range of tau neurofibrillary pathology in AD. In order to accomplish this goal in living subjects, cases will be classified based on clinical features that are believed to be related to tau pathology such as cognitive status and age. Enrollment will not be formally stratified, but it is expected that at least 65% of the subjects enrolled will have dementia or MCI. The study sponsor will review enrollment on an ongoing basis to ensure that subjects that represent a broad range of age (including individuals aged > 80 years) and cognitive performance are enrolled. A limited number of subjects with end stage dementia may be enrolled at sponsor's discretion both to ensure appropriate balance across the cognitive spectrum and because these subjects may have difficulty participating in the PET scan. This review will be performed based on clinical data only and the sponsor will remain blinded to PET scan results.

5.3.1. Inclusion Criteria

Only subjects who meet all of the following criteria will be reviewed and will be eligible to enroll in the study:

1. Are males or females ≥ 50 years of age;
2. Have a projected life expectancy of ≤ 6 months as determined by the principal investigator (terminal medical condition such as end-stage congestive heart failure, end-stage chronic obstructive pulmonary disease [COPD], end-stage renal disease, or end-stage cancer);

3. Can tolerate a 20 minute PET scan. The principal investigator will carefully assess each subject and use medical judgment to determine whether the subject can tolerate the PET scan procedure; and
4. Give informed consent or have a legally authorized representative (LAR) to consent for study procedures and brain donation consistent with the legal requirements of the State in which they die.

5.3.2. Exclusion Criteria

Subjects will be excluded from enrollment if they:

1. Are aggressively being treated with life sustaining measures (e.g., receiving chemotherapy, currently on respirator; palliative chemotherapy is allowed);
2. Are known to have a structural brain lesion that would interfere either with PET imaging or pathological assessment. (Such lesions are typically > 2 cm at their greatest extent and may include stroke, primary or metastatic neoplasm, other tumors or cystic lesions. Subjects with a history of major stroke or other structural lesion as well as cases with a history of primary CNS neoplasm or known metastatic cancer must be discussed with the study sponsor prior to enrollment);
3. Have a clinically significant infectious disease, such as Human Immunodeficiency Virus (HIV) infection, hepatitis or prion disease;
4. Are currently receiving any investigational medications except with permission from the study sponsor;
5. Have ever participated in an experimental study with an amyloid or tau targeting agent (e.g., immunotherapy, secretase inhibitor);
6. Have suspected encephalopathy due to alcoholism or end-stage liver disease;
7. Are females of childbearing potential who are pregnant or not using adequate contraception;
8. Subjects who have a history of risk factors for Torsades de Pointes (TdP) (e.g., hypokalemia, family history of Long QT syndrome) or are taking drugs that are known to cause QT-prolongation (a list of prohibited and discouraged medications is provided by the Sponsor).

5.4. Prior and Concomitant Therapy

Except as noted in the exclusion criteria, all medications (prescription or over-the-counter) that have been started prior to screening may be continued during the course of the trial. All medications that are continued from the start of the trial or that are started during the trial (other than the study medication) must be documented in the case record form on the Concomitant Medication Page of the electronic Case Report Form (eCRF).

5.5. Removal of Subjects from Trial

Subjects must be removed from the trial if:

1. Informed consent is withdrawn; or
2. The investigator or the sponsor believes it is in the best interest of the subject to be removed from the trial.

Subjects may be withdrawn from the trial if a serious adverse event occurs. The date and reason for discontinuation should be noted on the eCRF.

5.6. Premature Termination of Trial/Closure of Center

The sponsor may discontinue the trial at any time. Reasons for discontinuation of the trial may include, but are not limited to, new information on safety or efficacy, requests from regulatory authorities, changes in business priorities, or study termination upon review of the recommendations/findings of the DMB. Additional reasons for center closure may include, but are not limited to, excessive protocol violations, inadequate regard for subject safety, failure to follow recommended procedures (e.g., documentation), failure or inability to accommodate Avid/Contract Research Organization (CRO) monitors or to provide required access to data and source documents, staff turnover or inadequate staffing, and inadequate enrollment. Except in cases affecting subject safety, the investigators will be given a minimum of 30 days to complete final study evaluations for ongoing subjects. In all cases of center or study termination, appropriate steps will be taken to ensure the safety of study subjects.

6. PRODUCT RISK ASSESSMENT

The most up-to-date and complete information regarding the use of ^{18}F-AV-1451 Injection can be found in the investigator's brochure.

In brief, ^{18}F-AV-1451 Injection is an experimental imaging agent that will be used at relatively low (tracer) doses. Because ^{18}F-AV-1451 Injection is under clinical investigation, it is recommended that subjects receiving ^{18}F-AV-1451 Injection be followed closely by means of adverse event reporting and vital signs.

There are no data on the effects of ^{18}F-AV-1451 Injection in human perinatal development. For this reason, females must avoid becoming pregnant. Both females and males must use adequate contraceptive methods for 24 hours after administration of ^{18}F-AV-1451 Injection. ^{18}F-AV-1451 Injection must not be administered to females who are pregnant or lactating. Males with female partners who are pregnant or of childbearing potential must agree to refrain from sexual activity for 24 hours following administration of ^{18}F-AV-1451 Injection. Additionally, males must agree not to donate sperm for 24 hours following administration of ^{18}F-AV-1451 Injection.

7. PROCEDURES AND METHODS

7.1. Assessment Periods

See Section 11.2, Trial Flow Chart

7.1.1. Screening Visit:

Screening may take place over several days and can occur at multiple locations (e.g. hospice, subject's home, research office, or clinic). All screening assessments will preferably be performed within 45 days prior to the PET imaging session. Some screening assessments may be performed on the imaging day prior to injection with sponsor approval. Screening assessments will include:

1. Informed consent (for clinical, imaging and autopsy procedures);
2. Obtain historical brain MRI images if available;
3. Demographics and baseline characteristics (e.g. birth year, gender, race, ethnicity, education);
4. Medical history and concomitant medications;
5. Brief physical and neurological exam;
6. Neurologic disease history (if relevant: date or months since symptom onset, date or months since diagnosis, family history of neurologic disease);
7. Mini-Mental Status Examination (MMSE) may be obtained if the subject is able to cooperate with the test;
8. Caregiver or informant to complete IQCODE;
9. Urine pregnancy test (for females of childbearing potential);
10. Vital signs, including the most recent height and weight which will be collected from subject's previous medical record; and
11. A physician or appropriate designee evaluation and summary of current medical status.

7.1.2. ^{18}F-AV-1451 PET Imaging Visit(s):

1. The subject will be seen by a physician prior to dose administration who will assess their ability to safely tolerate the imaging procedure;
2. Females of childbearing potential will have a urine (or serum if required by local IRB) pregnancy test prior to injection (the result must be negative for the subject to be administered ^{18}F-AV-1451);
3. A target dose of 370 MBq (10 mCi) of ^{18}F-AV-1451 will be administered intravenously and a 20-minute continuous brain PET imaging will begin 80 minutes post injection;
4. Images will be reconstructed and reviewed immediately after completion of the scan;
5. Vital signs will be taken immediately prior to administration of ^{18}F-AV-1451, and after completion of imaging, prior to discharge;
6. Subjects will be observed continuously for signs of adverse events (AE) or serious adverse events (SAE). All AEs and SAEs will be followed until resolution;

7. The injection site will be observed for excessive inflammation or damage to the surrounding tissue where the dose was injected; and
8. A physician will see the subject prior to discharge to evaluate the subject's readiness for discharge.

Follow-Up Phone Call:

A follow-up phone call to the subject, (or caregiver/informant if applicable), will be conducted within 2 or 3 business days of the imaging day, but not before 48 hours post-injection, to confirm subject well-being and to collect information about any new adverse events. If both of these days are not business days, the follow-up phone call can occur the following business day. End of study for the purpose of adverse event reporting is defined as the completion of the follow-up phone call.

7.1.3. Death and Autopsy:

1. The responsible family member or caregiver/informant will be contacted immediately after the time of death to obtain a description of events leading up to death;
2. Subjects will not have an autopsy performed or be included in the primary analyses if death does not occur within nine months of the most recent imaging session or if tissue has not been acquired before the required number of autopsies for the study has been completed.
3. If subject and/or LAR consented, additional research and genetic testing will be performed on brain tissue.

Detailed autopsy procedures are described in the Technical Autopsy Manual.

7.1.4. Additional PET Imaging

If death does not occur within nine months of the ^{18}F-AV-1451 PET scan, the subject may be given the opportunity to undergo a second ^{18}F-AV-1451 PET scan and continue in the protocol at the sponsor's discretion. In order to obtain a second scan before the nine month expiry date, it is recommended the second scan be performed any time between six and nine months after the first scan. If the subject agrees to a second ^{18}F-AV-1451 PET scan, screening assessments will not be repeated. The procedures in Section 7.1.2 will be performed and updated medical history /concurrent medications and an updated neurological disease diagnosis (according to best available information) will be collected. If the subject subsequently comes to autopsy, the second scan (nearest to time of death) will be used in the efficacy analyses. Because cortical tau deposition is expected to occur very slowly, if at all, in cognitively normal (unimpaired) subjects, cognitively normal subjects will not be required to undergo repeat scans. Cognitively normal subjects will remain eligible for autopsy, regardless of time from first scan or second scan to autopsy, and will be included in the primary efficacy cohort if the subject comes to autopsy any time before the trial stopping criteria are met. Subjects of other cognitive status that do not consent to a second ^{18}F-AV-1451 PET scan will not be included in the primary analyses unless death occurs within nine months of the initial scan.

7.2. Observations and Measurements

Informed Consent

Potential subjects and LARs, if applicable, will be allowed to read a written informed consent form. The principal investigator or designee will explain all study procedures, risks, and alternative therapies to the subject and LAR, if applicable. The subject and LAR will have an opportunity to have all questions answered. The appropriate parties will then sign and date the informed consent form, indicating willingness to participate in the study and brain donation (see Section 7.5). A copy of the signed informed consent will be given to the subject and/or LAR.

All informed consent forms must be approved by Avid or designee, and by the appropriate Institutional Review Board (IRB) prior to use.

IQCODE – Short Version

Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE; Jorm 1994) is a tool used to assess cognitive impairment in older people. IQCODE will be administered to the subject's caregiver/informant.

Mini-Mental State Examination (MMSE)

The MMSE (Folstein et al., 1975) is a brief instrument used to assess cognitive function in elderly patients. The instrument is divided into 2 sections. The first section measures orientation, memory, and attention. The maximum score for the first section is 21. The second section tests the ability of the patient to name objects, follow verbal and written commands, write a sentence, and copy figures. The maximum score for the second section is 9. The range for the total MMSE score is 0 to 30. The MMSE will be administered at screening to subjects that are able to cooperate with testing.

Medical History, Neurologic Disease History

The investigator or designee will obtain a case history at the screening visit. Whenever possible, the medical history will be confirmed by medical records.

- Relevant demographic information (e.g. birth year, gender, race, ethnicity, education);
- Social history/baseline characteristics (e.g. history of alcohol, drug use, and smoking, prior CSF or genetic testing results);
- Medical and surgical history;
- Concurrent medications;
- Neurologic Disease history (if relevant: date or months since symptom onset, date or months since diagnosis, family history of neurologic disease);

After the 48 hour follow-up phone call has been completed, additional medical history and concurrent medications will not be collected until death or unless the subject consents to a second ^{18}F-AV-1451 PET scan.

Documentation of the events leading up to death will be collected.

Physical Examination

A brief physical examination will be conducted by a medically qualified study team member at the screening visit. Clinically significant changes from screening will be recorded as adverse events for the relevant study period.

Neurological Examination

A brief neurological examination will be performed by a medically qualified study team member at the screening visit to evaluate cranial nerves, gait, sensory, and motor function, coordination and tendon reflexes.

MRI

Historical brain MRIs that have been obtained within 12 months of the ^{18}F-AV-1451 PET imaging visit and are available electronically will be sent to the designated imaging core lab if the subject provides consent.

Vital Signs

Vital signs (pulse rate, respiratory rate, blood pressure) will be taken in the supine position at the following time points:

- Screening Visit
- ^{18}F-AV-1451 Imaging Visit
  - Immediately prior to the administration of ^{18}F-AV-1451 Injection;
  - After the completion of imaging prior to discharge.

Height and Weight

At the screening visit the most recent body weight and height will be collected from the subject's medical record.

Optional Brain Tissue Research/Genetic Testing

Additional research and genetic testing is optional, and may be performed on brain tissue and will be outlined in the Neuropathology Analysis Plan or other vendor manual. Where local regulations allow, samples will be stored and analysis may be performed on genetic variants thought to play a role in dementia or related diseases. Samples will only be used for investigations related to disease and drug or class of drugs under study in the context of this clinical program. They will not be used for broad exploratory unspecified disease or population genetic analysis.

Samples will be identified by the subject number and stored for up to 15 years after the last subject visit for the study at a facility selected by the sponsor. The sample and any data generated from it can only be linked back to the subject by investigator-site personnel. The results of this testing will not be shared with the principal investigator or subjects' families.

Pregnancy Testing

A urine beta-hCG test will be performed at screening and at the ^{18}F-AV-1451 imaging visit(s) prior to injection for females of childbearing potential (defined as pre-menopausal, less than 2 years post-menopausal or not surgically sterile). A serum pregnancy test may also be obtained prior to injection at the ^{18}F-AV-1451 imaging visit(s) if required by the local site.

Physician Visit

At screening, a physician or designee must see the subject to evaluate their current medical status.

A physician must see the subject prior to drug administration and prior to discharge from the ^{18}F-AV-1451 imaging session. At this time, the physician should review all safety data and briefly examine/query the subject regarding potential adverse events or other treatment issues.

Brain Autopsy

For those subjects who come to autopsy, brain harvesting will be performed locally by the site in accordance with the Technical Autopsy Manual and tissue will be sent to a pathology core lab(s) for neuropathological examination according to the Neuropathology Analysis Plan, which will be written prior to the first subject neuropathology analysis in this study. Autopsies will not be performed on subjects that live longer than nine months after the ^{18}F-AV-1451 PET scan.

Brain tissue will be under custody of Avid Radiopharmaceuticals for up to 15 years for research purposes. During this time Avid may choose to transfer the brain tissue to another laboratory for additional study related research, or to a long term storage facility, or back to the clinical site or brain bank at which the subject was previously enrolled. Autopsy reports will be sent to the principal investigators to share with the subjects' families after the study has been completed.

7.3. Protocol for Image Collection

The designated imaging core lab will prepare and distribute a PET Technical Operations Manual for ^{18}F-AV-1451. Historical brain MRI transmission procedures will be outlined in a separate document.

7.4. Good Clinical Practice and Monitoring

All clinical studies performed under the direction of Avid/CRO will be conducted in accordance with applicable regulatory requirements and International Conference on Harmonization (ICH) Good Clinical Practice (GCP) and Avid/CRO Standard Operating Procedures (SOP).

This includes:

1. IRB approval: An investigation will be initiated at a study site only after the IRB for that study site has given their written approval of the protocol and informed consent;

2. Informed Consent: Study procedures will not be initiated until the subject and/or their legally authorized representative (as appropriate) signs the informed consent form;
3. Recording and monitoring of adverse events as outlined in Section 7.7.3 including the notification of study site clinical investigators, local IRBs and the FDA regarding serious adverse event;
4. Avid RP's obligation to monitor the participating center on a regular basis; and
5. The termination of a center or the trial if conditions apply, as outlined in Section 5.6.

7.5. Informed Consent and Subject Information

Potential subjects, or their LAR (as appropriate), will be allowed to read a written informed consent form. The principal investigator or designee will explain all study procedures, risks, and alternative therapies. The subject and LAR will have an opportunity to have all questions answered by a physician. The subject will then sign and date the informed consent form, indicating willingness to participate in the study and brain donation.

End-of-life, as well as, AD subjects are potentially a vulnerable population with compromised mental capacity. Investigators should take extra care to evaluate a subject's ability to give consent. If the subject is capable of giving informed consent then the subject should sign on the consent line of the informed consent form. If the subject is not capable of giving consent, consent may be given by a LAR, consistent with the requirements of the State where the subject resides and local IRB guidance. Subjects with the capacity to understand that they are engaging in a research study should affirm that they do not object to participating. Subjects lacking the capacity to assent and/or dissent will not be precluded from participation.

All informed consent forms must be approved by Avid or designee, and by the appropriate IRB. No study-related procedures shall be performed prior to completion of the informed consent process, and signing of the consent form. A copy of the signed informed consent should be given to the subject and/or their legally authorized representative for their records.

7.6. Documentation

^{18}F-AV-1451 PET scans, as well as historical MRI scans, will be saved in an appropriate electronic format as specified in the imaging manuals. A copy of all scans will be saved at the site/imaging center and a copy of each will be forwarded to the designated imaging core lab as described in the imaging manuals. All other data required by the protocol will be recorded in the eCRF. All data in the eCRF will be substantiated by "source documents," which consist of the subject's medical files, etc. All source documentation must be made available to Avid and designees. Completed source documents and eCRFs may need to be made available and complete for an inspection by the FDA, other international regulatory authorities, or Avid at any time. A ballpoint pen should be used

to ensure that all copies are legible. eCRFs and all other records must be filed in accordance with applicable laws and regulations (see Section 10.6).

7.7. Adverse Events (AE)

Avid's standards for recording and reporting adverse events (AEs) are to be followed regardless of applicable regulatory requirements that may be less stringent. All AEs must be fully recorded on the Adverse Event Page of the eCRF. Investigators will be instructed to report to Avid or its designee their assessment of the potential relatedness of each AE to investigational product or protocol procedure via electronic data entry. If a subject's treatment is discontinued as a result of an AE, study site personnel must clearly report to Avid or its designee via electronic data entry the circumstances and data leading to any such discontinuation of treatment. In cases where the investigator notices an unanticipated benefit to the subject, study site personnel should report "unexpected benefit" with the actual event term to Avid or its designee (for example, the complete actual term would be "unexpected benefit- sleeping longer").

Signs and symptoms of each AE should be described in detail (e.g., start and stop dates/time, severity/intensity, relationship to investigational product, action taken, and outcome). Additionally, any clinically significant findings from vital sign measurements or other study procedures including those that result in a diagnosis should be reported as an AE to Avid or its designee.

7.7.1. Adverse Event Monitoring

Each subject must be carefully monitored for adverse events. An assessment must be made of the severity/intensity and relationship to the administration of the investigational product.

7.7.2. Adverse Event Definitions

Adverse Events

For reporting purposes, Avid will distinguish among pre-existing conditions, treatment - emergent adverse events and trial-emergent adverse events. Pre-existing conditions (i.e., undesirable experiences, signs or symptoms that begin prior to the Screening Visit) will be recorded on the medical history and/or physical exam eCRF pages. Signs and symptoms that are believed to be due to the pre-existing condition (started prior to dose of investigational product) do not have to be recorded in the AEs section of the eCRF, unless there is an increase in frequency or severity. Additionally, signs or symptoms or changes in pre-existing conditions that occur outside the trial-defined adverse event reporting will be recorded in medical history.

An adverse event is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. For the purposes of this study, untoward medical occurrences will be considered associated with the use of ^{18}F-AV-1451, and thus be reported as treatment-emergent adverse events, if they occur within 48 hours after ^{18}F-AV-1451 administration.

In the context of this study, adverse experiences including hospitalizations, medical complications and death are expected, since terminal illness is a requirement for entry into the study. Thus, if the investigator becomes aware of such events that occur after administration of the drug but outside the 48 hour reporting window, these will only be reported as adverse events if the investigator believes they are attributable to the investigational product or protocol procedure.

In order to capture possible adverse effects of trial participation, any untoward medical occurrences occurring after the informed consent until the administration of ^{18}F-AV-1451 will be recorded in the CRF for reporting as trial-emergent adverse events. If the subject consents to a second ^{18}F-AV-1451 PET scan, trial-emergent adverse events will be collected in a similar period, from either date of additional consent (if applicable) or the date the dose request for the second scan is submitted to Avid, up to the second administration of ^{18}F-AV-1451.

Serious Adverse Event (SAE)

An SAE is an AE that results in one of the following outcomes or constitutes one of the following events:

- Death;
- Initial or prolonged inpatient hospitalization (other than that required by protocol; "social hospitalization" or any hospitalization for non-medical reasons does not constitute an SAE);
- A life-threatening experience (that is, immediate risk of dying);
- Persistent or significant disability/incapacity;
- Congenital anomaly/birth defect;
- Considered significant by the investigator for any other reason.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug events when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unexpected Adverse Event

An unexpected adverse event is an adverse event not previously reported or an adverse event that occurs with specificity, severity or frequency that is not consistent with the current investigator's brochure.

Relationship to Investigational Product

Investigators will be instructed to report their assessment of the potential relatedness of each adverse event to protocol procedure and/or investigational product. The assessment of the relationship of an adverse event to the administration of the investigational product is a clinical decision based on all available information at the time of the completion of the eCRF.

Intensity/Severity of an Adverse Event

In addition to assessing the relationship of the administration of the investigational product to adverse events, an assessment is required of the intensity (severity) of the event.

The following classifications should be used:

Mild:

A mild adverse event is an adverse event, usually transient in nature and generally not interfering with normal activities.

Moderate:

A moderate adverse event is an adverse event that is sufficiently discomforting to interfere with normal activities.

Severe:

A severe adverse event is an adverse event that incapacitates the subject and prevents normal activities. Note that a severe event is not necessarily a serious event. Nor must a serious event necessarily be severe.

7.7.3. Adverse Event Documentation

All adverse events must be fully recorded on the Adverse Event Page via the Electronic Data Capture (EDC) system. Documentation must be supported by an entry in the subject file. AEs considered by the Investigator to be clinically relevant should be reported on the Adverse Event page of the eCRF. Signs and symptoms of each AE should be described in detail (e.g., start and stop dates, severity/intensity, relationship to investigational product, action taken, and outcome).

Adverse events fulfilling the definition of a serious adverse event should, in addition, be reported on the Serious Adverse Event Reporting Form.

7.7.4. Reporting of Serious Adverse Events

Study site personnel must alert Eli Lilly or its designee of any SAE within 24 hours of their awareness of the event via a sponsor-approved method. Alerts issued via telephone are to be immediately followed with official notification on study-specific SAE forms.

Serious adverse events occurring after a subject receives a dose of investigational product will be collected until 48 hours after the dosing of the investigational product, regardless of the investigator's opinion of causation. Therefore, SAEs that occur later than 48 hours after the dosing of the investigational product are not required to be reported unless the investigator feels the events were related to either investigational product or a protocol procedure.

If a subject experiences an SAE after signing informed consent(s), but prior to receiving investigational product, the event will be reported on the eCRF and to Eli Lilly or its designee. Previously planned (prior to signing the ICF) surgeries should not be reported

as SAEs unless the underlying medical condition has worsened during the course of the study.

8. STATISTICAL ANALYSIS

8.1. General Statistical Considerations

All statistical analyses will be performed using SAS® version 8.2 or higher. The specific analyses to address the objectives will be described in the Statistical Analysis Plan (SAP).

8.2. Populations for Analysis

Invalid Data

Subject specimens for which technical errors result in invalid or uninterpretable autopsy specimens (e.g. lost tissue, tissue processed improperly by the site or tissue irretrievably missing key identifying information), as determined by the pathology core lab(s), in accordance with criteria set in the Technical Autopsy Manual and Neuropathology Analysis Plan will be excluded from primary analyses.

Subjects with invalid or unevaluable PET data will be excluded from analyses. Criteria for declaring an image invalid or not evaluable will be specified in advance in the Image Review Charter. If an image is considered invalid by the designated imaging core lab, the subject will not be followed to autopsy. Valid images will be considered unevaluable only if 3 out of 5 independent readers declare the image unevaluable for the same reason. Subjects with unevaluable images will be excluded from the Primary Efficacy Population.

8.2.1. Primary Objective Analyses

This study is designed to test the relationship between ante-mortem ^{18}F-AV-1451 PET imaging and tau neurofibrillary pathology associated with AD, as measured at autopsy. To accomplish this goal, two primary analyses will be performed:

Primary Analysis 1

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to NFT Score of B3 (Hyman et al., 2012; Montine et al., 2012) at autopsy will be evaluated; and if success criteria are met,

Primary Analysis 2

The diagnostic performance (sensitivity/specificity) of 5 independent readers' interpretations of ante-mortem ^{18}F-AV-1451 PET imaging for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by NIA-AA criteria (Hyman et al., 2012) will be

evaluated. For individuals with cognitive impairment, high levels of AD neuropathologic change are considered adequate to explain cognitive impairment or dementia symptoms.

The trial will be considered successful if both co-primary endpoints are met according to the Statistical Analysis Plan.

For Primary Analysis 1, the hypothesis to be tested is that for the same 3 out of 5 independent readers, the lower bound of the two-sided 95% CI for both sensitivity and the specificity of ^{18}F-AV-1451 PET reading results will be ≥ 50%. As detailed in Section 8.5 Table 7, NFT scores for autopsy cases will be categorized as either B0/B1/B2 or B3 level and will serve as the NFT Score TS for this analysis. ^{18}F-AV-1451 PET imaging will be classified as either neocortical uptake not consistent with AD (AD-) or neocortical uptake consistent with AD (AD+) and consistent with AD and likely to progress (AD++) by each reader. Sensitivity and specificity will be calculated for each reader as the proportions of TP and TN cases correctly identified as such, according to Table 5 below:

Table 5: Primary Analysis 1: Diagnostic Performance Calculations for AV-1451 PET Scan Reader Interpretation vs. Autopsy NFT Score TS

	Autopsy NFT Score TS	
Physician Reader Interpretation	**NFT Score B3 [Truth Positive]**	**NFT Score B0–B2 [Truth Negative]**
AV-1451 Neocortical uptake; AD pattern (AD+/AD++)	True Positive (TP)	False Positive (FP)
AV-1451 neocortical uptake not consistent with AD (AD-)	False Negative (FN)	True Negative (TN)

Two-sided 95% CIs for sensitivity and specificity will be calculated using the Wilson score method. The first primary endpoint is considered to be met if for the same 3 out of 5 readers, the lower bound of the 95% CIs for both sensitivity and specificity are ≥ 50% (i.e., statistically significant at two-sided significance level of 0.05).

Like Primary Analysis 1, the hypothesis to be tested for Primary Analysis 2 is that for the same 3 out of 5 readers, the lower bound of the 95% CIs for both sensitivity and the specificity will be ≥ 50%. For Primary Analysis 2, autopsy cases will be categorized as either [not, low, or intermediate] vs. [high] level of AD neuropathologic change according to NIA-AA criteria, considering Braak stage, Thal plaque score, and CERAD amyloid neuritic plaque score, and this level of AD neuropathologic change will be used as the NIA-AA Autopsy Diagnosis TS as detailed in Section 8.5 Table 8.

^{18}F-AV-1451 PET images will be interpreted by visual examination as either AD- or AD+/AD++ as for Primary Analysis 1. Sensitivity and specificity will then be calculated for each reader as the proportions of TP and TN cases correctly identified as such, according to Table 6 below:

Table 6: Primary Analysis 2: Diagnostic Performance Calculations for AV-1451 PET Scan Reader Interpretation vs. NIA-AA Autopsy Diagnosis TS

	NIA-AA Autopsy Diagnosis TS	
Physician Reader Interpretation	**High AD Neuropathologic Change [Truth Positive]**	**Not, Low, or Intermediate AD Neuropathologic Change [Truth Negative]**
AV-1451 Neocortical uptake; AD pattern (AD+, AD++)	True Positive (TP)	False Positive (FP)
AV-1451 Neocortical uptake; Inconsistent with AD pattern (AD-)	False Negative (FN)	True Negative (TN)

Two-sided 95% CIs will be calculated using the Wilson score method. The second primary endpoint is met if for the same 3 out of 5 readers, the lower bounds of the 95% CIs for both sensitivity and specificity are ≥ 50%.

8.2.2. Secondary Objective Analyses

Secondary Analysis 1: To assess diagnostic performance of ante-mortem ^{18}F-AV-1451 PET imaging, based on majority interpretation of 5 independent readers, for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to Neurofibrillary Tangle (NFT) Score of B3 and for detection of a pattern of ^{18}F-AV-1451 neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by National Institute on Aging - Alzheimer's Association (NIA-AA) criteria:

Majority scan interpretation will be derived using individual readers' scan interpretation results. Diagnostic performance of this majority scan interpretation will be assessed relative to the Autopsy NFT score TS as per Primary Analysis 1 and again relative to the NIA-AA Autopsy Diagnosis TS as per Primary Analysis 2. In each case (image), a majority reading result will be derived using each individual reader's scan interpretations results. Sensitivity and specificity, and two-sided 95% CI thereof, will be calculated for the majority interpretation results relative to the appropriate TS. The hypotheses to be tested are:

1a: the majority reading result will have the lower bounds of two-sided 95% CI ≥ 55% for both sensitivity and specificity for detection of tau neurofibrillary pathology that corresponds to NFT Score of B3, and

1b: the majority reading results will have the lower bounds of two-sided 95% CI ≥ 55% for both sensitivity and specificity for detection of a pattern of ^{18}F-AV-1451

neocortical uptake that corresponds to high levels of AD neuropathologic change as defined by NIA-AA criteria.

Secondary Analysis 2: Assess agreement among readers of ^{18}F-AV-1451 PET scans:

Fleiss' Kappa statistics will be used to assess inter-reader agreement for the diagnostic decisions associated with Primary Analyses 1 and 2. For evaluation of agreement in the context of Primary Analysis 1, kappa will be calculated according to the reader interpretation classification in Table 5. If Primary Analysis 1 is successful, a second kappa will be performed using the reader interpretation classification in Table 6 . The hypothesis to test is the observed kappa values are ≥ 0.64 and the lower bound of the two-sided CIs for this assessment are ≥ 0.55.

8.3. Safety Analysis

Safety vital signs measurements will be summarized by subject and by evaluation time point. Change from baseline (pre-dose time point) values will be determined and summarized.

Adverse events including injection site reactions will be summarized in terms of number and percentage of subjects experiencing an AE. The summary will be further broken down by system organ class (SOC) and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) terms. Adverse events will also be presented by severity, relationship to treatment and seriousness. All subjects who experience SAEs or who discontinue due to AEs will be summarized.

Discontinuation

All subjects who discontinued participation prior to completing the study will be listed and their discontinuation reasons will be tabulated.

Vital Signs

Changes in vital signs from baseline will be summarized.

8.4. ^{18}F-AV-1451 Image Analysis

^{18}F-AV-1451 retention on PET images from subjects with valid autopsy data will be interpreted by visual examination as either

- Neocortical Uptake Not Consistent with an AD Pattern (AD-: no increased neocortical activity in any region or increased activity is isolated to the mesial temporal, anterolateral temporal or frontal region(s);
- Neocortical Uptake Consistent with an AD Pattern (AD+: increased neocortical tracer activity in the posterolateral temporal (PLT), parietal (including precuneus), or occipital region(s)); or
- Neocortical Uptake Consistent with an AD Pattern and with Likelihood of Disease Progression (AD++, which is a subset of AD+ (Neocortical uptake consistent with an AD pattern) where increased tracer activity is found in the parietal (including precuneus) region(s) regardless of uptake elsewhere, or in the frontal region in addition to PLT, occipital, or parietal region(s)).

For the purpose of testing the primary analyses, a scan interpretation will be considered positive (hypothesized to correspond to a B3 neuropathology score in Primary Analysis 1 and an NIA-AA score of high AD pathology in Primary Analysis 2) if the scan is interpreted as at least consistent with an AD pattern (AD+ or AD++).
Details of the visual PET image review methods will be provided in the Blinded Read Manual.

Images will also be assessed quantitatively (semi-automated computerized measurement of SUVRs) for exploratory analysis purposes. Details of the analysis methods will be provided in the Quantitative Methodology Document.

The ^{18}F-AV-1451 PET imaging results will not be shared with the investigator sites or subjects and their families prior to the end of the study.

8.5. Neuropathology Analysis

All neuropathological measurements on brain tissue in this trial will be evaluated in a standardized and blinded (with respect to PET image results and subject identifying/clinical information) fashion in a qualified laboratory. A Technical Autopsy Manual and Neuropathology Analysis Plan will be developed for this study containing all methods and measurement procedures. Neuropathological assessment will be derived from the NIA-AA guidelines (Hyman et al., 2012; Montine et al., 2012). Sections from brain regions recommended for pathological assessment of AD, as well as additional, pre-specified neocortical regions will be sampled. Staining will follow the procedures recommended in the NIA-AA practical approach guidelines. NFT extent will be assessed using standard procedures and NFT scores (B0-B3) will be recorded (Hyman et al., 2012; Montine et al., 2012). The TS for Primary Analysis 1 will be constructed from NFT scores as shown in Table 7.

Table 7: Autopsy NFT Score Truth Standard (Primary Analysis 1)

Braak Stage	NFT Score[a]	Truth Standard (TS)
0 (no NFTs)	B0	Negative
I-II	B1	
III-IV	B2	
V-VI	B3	Positive

[a] adapted from Hyman et al., 2012

Amyloid pathology will be evaluated using Thal and CERAD rating systems. Distribution of amyloid (Thal plaque score A0-A3; Montine et al., 2012) will be recorded. The frequency of neuritic amyloid plaques will be evaluated semi-quantitatively (CERAD score: none, sparse, moderate or frequent). Potential alternative neuropathologies contributing to cognitive impairment will also be considered as recommended by Montine et al., 2012, and level of AD neuropathologic change will be recorded as none, low, intermediate or high per NIA-AA guidelines (Hyman et al., 2012;

Montine et al., 2012). The TS for Primary Analysis 2 will be constructed from levels of AD neuropathologic change as shown in Table 8.

Table 8: NIA-AA Autopsy Diagnosis Level of AD Neuropathologic Change Truth Standard (Primary Analysis 2)

Level of AD Neuropathologic Change Present[a]	Truth Standard (TS)
Not	Negative
Low	
Intermediate	
High	Positive

8.6. Sample Size Calculation and Trial Stopping Criteria

Assuming sensitivity/specificity of approximately 80%, a minimum of 14 autopsy cases in each of the truth standard groups (pathological positive or negative) is required for the lower bound of the two-sided 95% CI to be > 50% for each individual reader. The sponsor is blinded to the pathology results (truth standard) until after the study is completed and the database is locked. Therefore, in order to ensure that an adequate number of subjects are recruited and come to autopsy for each truth standard group, the sponsor will assume that subjects diagnosed on clinical grounds as having dementia due to AD will be truth standard positive, subjects diagnosed as having no cognitive impairment (cognitively normal) will be truth standard negative. Based on these assumptions, the study sample size requirement and trial stopping criteria will be deemed to have been met when

- the number of subjects diagnosed as having impairment due to AD that have come to autopsy, is greater than or equal to 14, AND
- the number of subjects diagnosed as having no cognitive impairment that have come to autopsy is greater than or equal to 14

The sponsor recognizes that there may be some errors in clinical diagnosis such that some patients diagnosed clinically as having impairment due to AD may have a nonAD pathology, or limited amounts of AD tau pathology, and thus be truth standard negative, and, less often, subjects considered clinically normal may have AD pathology and be truth standard positive. However, given the excess frequency of cases with AD diagnoses relative to clinically normal subjects already recruited in this trial population, any diagnostic errors will likely help ensure a minimum of 14 truth standard negative subjects without jeopardizing the criterion for truth standard positive subjects.

In addition to the above subjects diagnosed as clinically normal or AD patients, it is expected that approximately 10-15 subjects with nonAD impairments will be enrolled and come to autopsy in this trial. However, the proportion of these that will have truth standard positive/negative AD tau pathology is very difficult to predict and will depend on the precise cases enrolled. Thus, it is not expected that these cases will be useful in

estimating when a sufficient number of cases have occurred in each truth standard group and are not employed in the stopping criteria.

No new autopsies will begin once the sample size requirement/trial stopping criteria are met. However, some subjects may have died and had autopsy material removed at the time the sample size requirement is met. Histology analysis will be completed in subjects for whom autopsies are ongoing at the time the sample size requirement is met and these subjects will also be included in the Primary Efficacy Population.

9. USE OF DATA AND PUBLICATION

Avid adheres to the Pharmaceutical Research and Manufacturers of America (PhRMA) Principles on Conduct of Clinical Trials and Communication of Clinical Trial Results. A complete copy of these principles is available from Avid and can also be found at the PhRMA website (http://www.phrma.org). Our policy is briefly summarized below:

- We commit to timely communication of meaningful results of controlled clinical trials, regardless of outcome.
- As a sponsor, we may recommend that the Investigator(s) delay or decline publication in cases where the study design, conduct, or data are insufficient to allow meaningful interpretation. Avid and the Investigator(s) will discuss the study design and data in advance of the study, and again after completion, and will strive, through appropriate scientific debate, to reach a consensus regarding the potential merits of publication.
- Avid retains the right to review any manuscripts, presentations, or abstracts before they are submitted for publication. Where differences of opinion or interpretation exist regarding data planned for publication, the parties (Avid and the Investigator) should try to resolve them through appropriate scientific debate. Avid retains the right to delay publication for up to 60 days to protect intellectual property.
- Anyone who provides substantial contributions should receive appropriate recognition as an author or contributor when the manuscript is published.

This is a multi-center study. The primary analyses will include data from all centers. A multicenter publication, reporting the primary analyses data set, with authorship from all contributing centers, should precede any other publications.

10. INVESTIGATOR'S REGULATORY OBLIGATIONS

All clinical work conducted under this protocol is subject to Good Clinical Practice regulations; this may include an inspection by Avid and/or Health Authority representatives (FDA, EMA or international regulatory authorities) at any time.

10.1. Institutional Review Board (IRB)

The intent of the research program, the trial protocol, the subject information/informed consent form and any advertising material used to recruit subjects must be submitted to the clinical investigator's local IRB and its approval must be obtained prior to its use. A copy of the approval must be forwarded to Avid. When necessary, an extension or renewal of IRB approval must be obtained and also forwarded to Avid**.**

10.2. Informed Consent

A signed, written informed consent for study procedures and brain donation must be obtained from each subject and/or LAR. A copy of the signed informed consent should be given to the subject and/or LAR for their records. A copy of the local IRB's approved version of the informed consent form must be forwarded to Avid or designee for review prior to being used to obtain subject consent.

10.3. Protocol Adherence

The protocol must be read thoroughly and the instructions must be followed exactly. Where a deviation occurs, it must be documented, the sponsor/monitor informed, and a course of action agreed upon.

10.4. Documents Necessary for Initiation of the Trial

Avid must be provided with the following documents prior to the enrollment of any subjects:

- Original signed and dated Statement of Agreement page;
- Copy of the IRB and radiation safety committee approval (if applicable);
- Copy of the IRB stamped approved consent form;
- Name and location of the laboratory utilized for laboratory assays, and other facilities conducting tests, including laboratory certification number and date of certification if available. Avid may be responsible for supplying these to the investigator if a central laboratory is used;
- List of reference range laboratory values. Avid may be responsible for this if a central laboratory is used; and
- Any additional licenses required in order to order to use ^{18}F-AV-1451.

10.5. Investigational Product Control

The receipt of clinical supplies must be documented at the site.

All drug supplies for this trial should be retained in a safe and secure place at all times during the trial. ^{18}F-AV-1451 should be prepared by a qualified PET manufacturing site and administered by a qualified individual under the investigator's supervision. An up-to-date drug inventory/dispensing record must be maintained. All drug supplies must be

accounted for. After completion of the trial, all remaining clinical supplies must be returned to the sponsor or their representative.

10.6. Data Collection

Electronic case report forms (eCRFs) will be used for this trial. Individual subject files should include appropriate source documents, including but not limited to subject's medical records. The files should include information such as visit dates, records of medical history, examinations administered, laboratory, concomitant treatment, any adverse event encountered and other notes as appropriate. These constitute "source data". All entries on the eCRFs must be backed up by source data. Original electronic versions of imaging studies are also considered source data and should be kept on file by the site/imaging center, and appropriate copies should be forwarded to the designated imaging core lab as specified in the PET Technical Operations Manual.

Each subject's source file should include an original signed informed consent form. When the trial is completed, the informed consent form should be kept on file with other trial related records.

The eCRFs must be kept in order and up-to-date so that they always reflect the latest observations on the subjects that are enrolled in the trial. The eCRFs must be completed for each subject enrolled in the trial and signed by the investigator. A monitor will verify the source data for all information on the eCRF.

10.7. Adverse Events

All adverse events encountered during the clinical trial must be documented on the eCRF, whether or not considered drug-related.

Eli Lilly must be notified immediately (as soon as possible, and in all cases within 24 hours) of a drug experience, condition, development, or event, which is considered serious. Eli Lilly must be notified immediately of any findings with the use of the drug that may suggest significant hazards, contraindications, adverse drug reactions (ADRs) and precautions pertinent to the safety of the drug. The investigator will be requested to complete a separate report form in addition to the information on the CRF. See section 7.7.4 for reporting serious adverse events.

If an SAE is determined to be unexpected (not previously reported or described by Avid), and study drug-related, Eli Lilly will notify the investigator in writing. The investigator should forward this notification to the IRB within 24 hours of receipt.

10.8. Records Retention

All correspondence (e.g., with Avid, IRB, etc.) relating to this clinical trial should be kept in appropriate file folders. Records of subjects, source documents, and drug inventory sheets pertaining to the trial must be kept on file. Records must be retained until the date a marketing application (NDA) is approved for the drug for the indication for which it is being investigated, or until 3 years following the date of clinical trial termination or completion, whichever is later. If no application is to be filed or if the application is not

approved for such indication, records should be kept until 3 years following the date of clinical trial termination or completion.

If an investigator moves, withdraws from an investigation, or retires, the responsibility for maintaining the records may be transferred to another person who will accept the responsibility. Notice of transfer must be made to and agreed upon by Avid.

11. APPENDICES

11.1. References

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11.2. Trial Flow Chart

Evaluations	Screening Visit	Pre-Dose	^{18}F-AV-1451 Dose	PET Imaging (20 minutes)	End of Imaging	Follow-Up Phone Call	Autopsy
Signed Consent[e]	X						
Demographics and baseline characteristics	X						
Medical History/Neurologic Disease History	X						X[c]
Concomitant Medication	X						X[c]
Physical Exam/ Neurological Exam	X						
Vital Signs[a]	X	X			X		
IQCODE	X						
Urine Pregnancy test	X	X[b]					
MMSE	X						
^{18}F-AV-1451 Injection			X				
PET Imaging				X			
Evaluation by a physician	X[d]	X			X		
Adverse Events	X	X	X	X	X	X	
Serious Adverse Events	X	X	X	X	X	X	
Death							X

[a] Vital signs will be performed in the supine position. Height and weight will be collected from subject's medical records.

[b] A urine or serum pregnancy test is acceptable on imaging day.

[c] Documentation of the events leading up to death will be collected including concomitant medications, significant medical events, and cause(s) of death.

[d] Evaluation by physician or designee at screening.

[e] Subjects may consent to a second [18]F-AV-1451 PET scan 6 months after the initial scan. Cognitively normal subjects will not be required to undergo repeat scans. Cognitively normal subjects will remain eligible for autopsy, regardless of time from first scan or second scan to autopsy.

INVESTIGATOR'S AGREEMENT TO PROTOCOL

Protocol: A Clinico-Pathological Study of the Correspondence Between ^{18}F-AV-1451 PET Imaging and Post-Mortem Assessment of Tau Pathology

Date and Version: 18Dec2017 – Amendment 2

I agree to conduct the study according to this protocol and to comply with its obligations, subject to ethical and safety considerations and all applicable regulations (ICH, CFR).

I shall not disclose the confidential information contained in this protocol or any results obtained from the study, except for publication in accordance with Section 9 of this protocol, without written authorization from Avid.

_____

Printed Name Date

_____

Signature