Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Stern RA, Adler CH, Chen K, et al. Tau positron-emission tomography in former National Football League players. N Engl J Med 2019;380:1716-25. DOI: 10.1056/NEJMoa1900757

Supplementary Appendix

Tau PET in Former National Football League Players

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Table of Contents

Description of Control Participants and Study Origins

The Control Group was made up of 36 male research volunteers who were between the ages of 45-69 years, were cognitively asymptomatic, and did not have a history of traumatic brain injury. Exclusion criteria for all participants, including all controls included: any contraindication for magnetic resonance imagining (e.g., metallic implants); history of clinical stroke or brain tumor; visual or hearing impairment severe enough to compromise cognitive testing; and current clinically significant infectious disease, endocrine or metabolic disease, pulmonary, renal or hepatic impairment, or cancer. Additional exclusion criteria specific to the use of the flortaucipir tau PET radioligand included: current clinically significant cardiovascular disease or clinically significant abnormalities on screening ECG (including but not limited to QTc>450 msec); history of additional risk factors for Torsades de Pointes; and current use of medications known to cause QT-prolongation (a specific exclusion criterion for use of the flortaucipir ligand).

The Control Group was comprised of participants from four clinical research studies. The PET procedures for both flortaucipir and florbetapir were the same across all four studies. Although all Control Group participants, across all four studies, met the above entry criteria, there were differences regarding:

- Number of participants included in this study.
- Funding sources.
- Study sites.
- Specific criteria for determining cognitive status.
- Assessment of contact or collision sport history, military service, or other sources of exposure to repetitive head impacts.
- Use of florbetapir amyloid-β PET findings for inclusion into this study.

Each of the four studies will be summarized in the following pages.

Study 1: CTE Study (NCT02079766) (all 26 former NFL players were from this study)

Number of participants included in this study	10 (consecutively enrolled)
Funding sources	• Avid Radiopharmaceuticals investigator-initiated study (AV-1451-A07 Study, "18F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for CTE"; R. Stern, PI) • National Institutes of Health (NIH) grant R01NS078337 (R. Stern, PI) for the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) Study • State of Arizona (E. Reiman, PI)
Study sites	• Boston University School of Medicine (with MRIs conducted at Brigham and Women's Hospital, Boston); N=9 • Mayo Clinic Arizona (with PET scans conducted at Banner Alzheimer's Institute); N=1
Specific criteria for determining cognitive status	Denied any cognitive, mood, or behavioral symptoms during detailed telephone screening interview
Assessment of contact or collision sport history, military service, or other sources of exposure to repetitive head impacts	Detailed standardized interview at telephone screening
Use of florbetapir amyloid-β PET findings for inclusion into this study	No

Study 2. A05 Study (NCT02016560)

Number of participants included in this study	17
Funding sources	AV-1451-A05 Study (funded by Avid Radiopharmaceuticals); "Analysis of 18F-AV-1451 PET Imaging in Cognitively Healthy, MCI and AD"
Study sites	Multi-site study across U.S.
Specific criteria for determining cognitive status	Mini-Mental Status Examination (MMSE) score $\geq$ 29
Assessment of contact or collision sport history, military service, or other sources of exposure to repetitive head impacts	None
Use of florbetapir amyloid-β PET findings for inclusion into this study	Yes; only subjects with negative scans included

Study 3. Alzheimer's Disease Neuroimaging Initiative (NCT01231971 and NCT01687153) (http://adni.loni.usc.edu/data-samples/adni-data-inventory/)

Number of participants included in this study	3
Funding sources	• Alzheimer's Disease Neuroimaging Initiative (ADNI) (funded by NIH, U19 AG024904); N=2 • Department of Defense (DOD)-ADNI (funded by DOD, W81XWH-14-1-0462); N=1
Study sites	Multi-site study across U.S.
Specific criteria for determining cognitive status	MMSE score > 29; Clinical Dementia Rating (CDR) = 0
Assessment of contact or collision sport history, military service, or other sources of exposure to repetitive head impacts	None
Use of florbetapir amyloid-β PET findings for inclusion into this study	Yes; only subjects with negative scans included

Study 4. A01 Study

Number of participants included in this study	1
Funding sources	Avid Radiopharmaceuticals (AV-1451-A01 Study, “An exploratory evaluation of the tau protein binding properties, whole-body biodistribution and safety of 18F-AV-1451 injection in healthy volunteers and cognitively impaired subjects”
Study sites	Multi-site across U.S.
Specific criteria for determining cognitive status	MMSE score > 29
Assessment of contact or collision sport history, military service, or other sources of exposure to repetitive head impacts	None
Use of florbetapir amyloid-β PET findings for inclusion into this study	No

PET Scanner Harmonization Across Sites

As part of site qualification for all studies sponsored by Avid Radiopharmaceuticals, Hoffman phantom data were acquired and analyzed to ensure acceptable levels of image contrast and count uniformity across multiple centers. Image resolution was estimated[1] to be 6-8 mm full width at half maximum (FWHM) transaxially and 6-10 mm FWHM axially.

Site qualification parameters and harmonization procedures for the Alzheimer's Disease Neuroimaging Initiative (ADNI) studies are described at http://adni.loni.usc.edu/methods/documents/.

Image data for all participants in this study, across all sources and sites, were smoothed using an 8 mm Gaussian kernel prior to drawing statistical inference to help mitigate the effects of noise.

Post-Processing and Statistical Analysis for Determining Between-Group Differences in Flortaucipir Uptake

For between-group comparisons of paired helical filament tau burden, the automated brain mapping algorithm software, Statistical Parametric Mapping [SPM] 12, www.fil.ion.ucl.ac.uk/spm/software/), was used to: (1) linearly co-register each participant's PET image to their MRI using normalized mutual information, (2) non-linearly deform the co-registered images into a standard (Montreal Neurological Institute) brain space using 4th degree B-spline interpolation, and (3) generate voxel-wise flortaucipir SUVR images relative to cerebellar grey matter. SUVR images were used to generate age-adjusted statistical parametric maps of SUVR elevations in the former players compared to controls ($P \leq 0.005$, uncorrected for multiple comparisons), and project these brain maps onto the lateral and medial surfaces of the brain.

Description of Monte-Carlo Simulation

Background

Statistical parametric maps (also referred to as statistical brain maps) are created from the analysis of full-brain voxel-based PET images (with thousands of voxels). Statistical methods are commonly used to clarify whether the magnitude and/or spatial extent of regional changes are not solely attributable to type I error due to the number of multiple comparisons. Examples of methods used to address the issue of multiple comparisons include Bonferroni corrections, as well as corrections using Family-Wise Error and False Discovery Rate. The use of these corrections may become too severe, however, leading to a loss of power to detect true group differences, thus increasing the false negative rate and making a type II error. As noted in the main text of our article, findings from our statistical parametric maps were based on an uncorrected $P<0.005$ and need to be replicated in independent studies.

We developed the Monte-Carlo simulation procedure described below to clarify the extent to which the spatial extent of changes in the predicted direction (i.e,. higher tau PET SUVRs in the former NFL player group compared to the control group) is significantly greater than the extent of PET changes in the opposite direction. This approach uses all of the cerebral changes in the brain image, characterizes significant alterations in the spatial extent of changes in one direction, can detect significant alterations even in the absence of particularly large regional changes, and is free from the type I error associated with multiple regional comparisons.

The approach used in this study is conceptually similar to the AlphaSim procedure available from the Analysis of Functional NeuroImages (AFNI) developer (https://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf). AlphSim has been used extensively in the analysis of functional magnetic resonance imaging (fMRI) data, as well as other voxel-based neuroimaging data.[2-6] This procedure provides a method of estimating the overall significance for an entire voxel-based image. This is accomplished by a Monte Carlo simulation of the entire process of image generation, voxel intensity thresholding, masking, and cluster identification. Using a combination of minimum cluster size thresholding and individual voxel probability thresholding, the probability of a per-image false positive detection can be determined.[7]

The purpose of the Monte-Carlo simulation is to examine the overall significance level with regard to the two-group mean difference over the entire brain volume; the brain

volume is defined via the brain mask used in the actual independent two-sample t-test procedure to exclude voxels outside of the brain. That is, the procedure examines the existence of group “directed differences” over the entire brain volume, at the global level, but not at a given voxel. Therefore, the procedure has no localization power.

Similar to the AlphaSim procedure, for the current study we assumed that, at each voxel, the data follows Gaussian distribution, but that the data from different voxels are not independent. In fact, we simulated the correlation among neighboring voxels taking into consideration the additional smoothing generated in the imaging reconstruction as well as the explicit smoothing prior to the statistical analysis. To model the statistical computation process as realistically as possible, our simulation went through a step-by-step independent two-sample t-test, starting from the synthesis of the data from each individual, with the null hypothesis that the two groups have the same mean at each voxel location. We then observed the standard deviation at each voxel to generate: *N1* 3-D images for group 1, and *N2* 3-D images for group 2 (i.e., a Standard Deviation Map).

This approach is based on the following reasoning. If there is no group difference anywhere in the brain (the null hypothesis), then there would be equal probability to observe the measurement from any voxel as either positive or negative. This is due to the symmetricity of the Gaussian distribution, with or without the inter-voxel correlation due to the smoothing. In other words, one should expect an equal number of voxels that are in the positive direction and the negative direction for any given threshold (e.g., 0.005). It is important to note that such a consideration is based on the directed difference, i.e., a one-tailed test. For this study, our hypothesis was that the former NFL player group has higher flortaucipir SUVR than the control group.

Current Study Monte-Carlo Simulation

For our study, the probability was estimated using the Mont-Carlo simulation procedure. For the outcome obtained based on independent two-sample t-tests, we denote the number of voxels at which the hypothesized direction is observed as *p* and the number of voxels in the opposite direction as *o*.

The following is a step-by-step description of the Monte-Carlo simulation used in our study.

1) Determine N, the number of iterations. We used N=1000, set n=0 (see below for its use)
    a. Perform the following for iteration i=1
    b. Synthesize the imaging data based on Gaussian distribution for each of N1 subjects in the former NFL player group and for each of the N2 subjects in the normal control group.
    c. Smooth the imaging using the intrinsic resolution, the smoothing factor in the reconstruction, and the degree of smoothing intentionally performed prior to the statistical analysis.
    d. Calculate the t-score based on the independent two-sample t-test formula at each voxel (resulting in a 3-dimentional t-score image)
    e. Use a pre-specified significance threshold of P<0.005 (note: this is not relevant to the multiple comparison issue) to binarize the t-score image so that voxels that survived the threshold will have voxel intensity equal to 1, and for the rest voxels equal to 0.
    f. Count the number of voxels at which the t-score is positive (in the hypothesized direction) and the number of voxels at which the t-score is negative (in the opposite direction) within the mask generated above.
    g. Express the number of voxels at which the hypothesized direction is observed as p_i, and the number of voxels in the opposite direction as o_i.
    h. Set n→n+1 if $p_i>p$ and $o_i<o$. Otherwise, n does not change.
2) Repeat steps a to f as described above for iteration $i+1$,
3) Repeat steps 1 and 2 above until $i=N$
4) Create the ratio n/N which is the estimated probability (likelihood) under the null hypothesis

Results of Monte-Carlo Simulation Procedure

For this study, with the statistical threshold set at P<0.005, we observed p=5294 voxels in the hypothesized direction (flortaucipir SUVR was higher in the former NFL player group than in the control group) and o=1213 voxels in the opposite direction. This resulted in a corrected significance of P<0.001. This finding is not influenced by the type I error associated with multiple regional comparisons.

Supplementary Figure

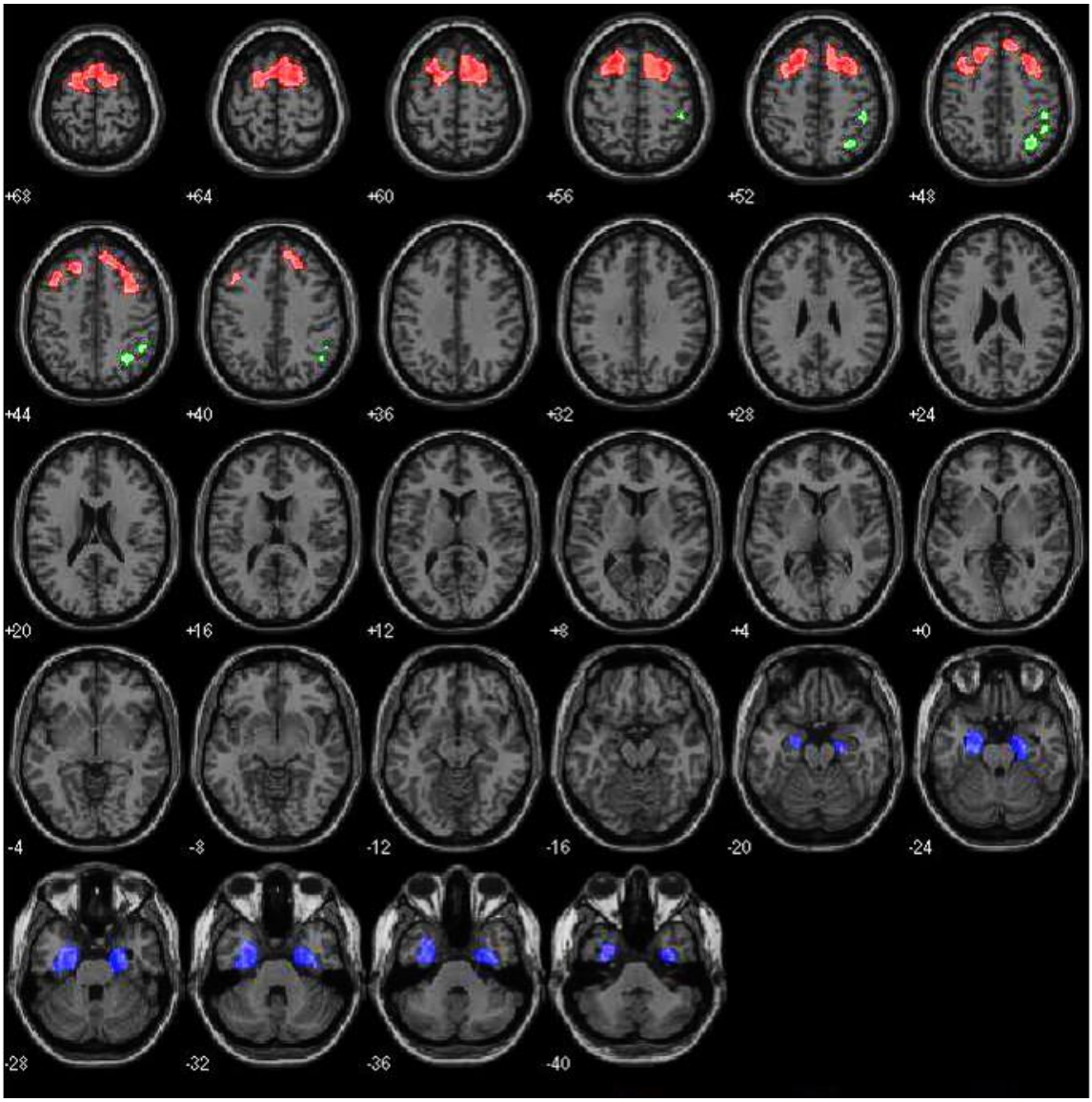

Figure S1. Superior frontal (red), bilateral medial temporal (blue), and left parietal (green) regions with higher flortaucipir tau PET SUVRs in the former NFL player group compared to the control group. The regions correspond to the Statistical Parametric Maps shown in Figure 1 (in main text) after restricting the maps to those clusters of at least 100 contiguous voxels associated with higher regional-to-cerebellar gray matter flortaucipir SUVRs in the former NLF group compared to control group ($P<0.005$, uncorrected for multiple regional comparisons). Numbers at the lower left of each spatially standardized horizontal brain section correspond to the distance in mm above or below a plane through the anterior and posterior commissures. The left hemisphere is on the right.

Supplementary Tables

Table S1. Clinical Test Performance by Former National Football League Players (N = 26)

Test	Mean±SD	Median	Range	Percent in Impaired Range*
NAB List Learning Delayed Recall[8] (T score)	39.5±13.7	35.0	19-69	35%
Trail Making Test, Part B[9] (T score)	44.1±14.9	46.0	20-71	27%
Category Fluency (Animals)[10] (T score)	45.4±12.7	43.0	18-79	12%
Beck Depression Scale-II[11]	32.3±12.6	31.5	13-63	81%
Beck Hopelessness Scale[12]	6.7±7.1	3.5	0-20	35%
Barratt Impulsivity Scale[13]	65.8±14.9	61.8	45-106	12%

Note: For the three neuropsychological tests with T-Scores, “impaired” is defined as performance lower than 1.5 SD below the mean (i.e., T<35); T-Scores are standardized scores with a mean of 50 and SD of 10. Higher T-Scores represent better performance. For the remaining tests, “impaired” is defined as performance in the clinically impaired range as described in the test manual or related publications. Beck Depression Scale-II has a range of 0-63; higher scores represent more symptoms. Beck Hopelessness Scale has a range of 0-20; higher scores represent more symptoms. Barratt Impulsivity Scale has a range of 0-120; higher scores represent more symptoms.

Table S2. Racial differences in derived regional flortaucipir SUVRs in former NFL player group*

Regional SUVR	Black/African American (N=14)	White (N=11)	T (df)	95% CI
Bilateral Superior Frontal	1.08 ± 0.15	1.08 ± 0.13	0.07 (23)	-0.11, 0.12
Bilateral Mesial Temporal	1.22 ± 0.17	1.22 ± 0.18	0.02 (23)	-0.15, 0.15
Left Parietal	1.13 ± 0.15	1.09 ± 0.16	-0.64 (23)	-0.17, 0.09

*One participant who self-identified as “other” race was not included in this analysis.
Plus-minus values are means ± SD. All comparisons based on T-tests are not significant (P>0.05). Non-parametric Mann-Whitney U Tests were also performed but did not result in any changes in the significance levels.

Table S3. Association between Regional Flortaucipir PET SUVR and Clinical Test Scores in Former National Football League Players (N = 26).

Test	Bilateral Superior Frontal SUVR			Bilateral Medial Temporal SUVR			Left Parietal SUVR		
	Partial corr.	95% CI	P	Partial corr.	95% CI	P	Partial corr.	95% CI	P
NAB List Learning Delayed Recall (T score)	0.12	-0.26, 0.43	0.62	0.04	-0.23, 0.49	0.47	0.03	-0.36, 0.42	0.82
Trail Making Test, Part B (T score)	0.08	-0.26, 0.43	0.62	0.11	-0.23, 0.49	0.47	0.16	-0.18, 0.51	0.31
Category Fluency (Animals) (T score)	-0.39	-0.73, 0.38	0.13	-0.46	-0.74, 0.13	0.06	-0.47	-0.75, 0.18	0.06
Beck Depression Scale-II	-0.17	-0.57, 0.31	0.37	-0.15	-0.57, 0.35	0.58	-0.06	-0.56, 0.40	0.67
Beck Hopelessness Scale	0.19	-0.32, 0.46	0.41	0.08	-0.32, 0.49	0.73	0.24	-0.24, 0.58	0.257
Barrett Impulsivity Scale	0.19	-0.31, 0.51	0.37	0.08	-0.36, 0.42	0.63	0.17	-0.33, 0.46	0.38

Note. Multivariate linear mixed effect models were performed to examine the relationship between SUVRs and the clinical measures. A mixed effect model was conducted for each brain region, resulting in three total models. To reduce type I error and to account for possible non-normality due to the small sample size, 95% confidence intervals and p-values were calculated through a bias-corrected bootstrapping from 1000 re-samples. NAB = Neuropsychological Assessment Battery.

References for Supplementary Appendix

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Protocol Number: ^{18}F-AV-1451-A07

^{18}F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy

Date and Version:
16 December 2013, Final

Name of Compound:
^{18}F-AV-1451 ([F-18]T807)

Sponsor:
Avid Radiopharmaceuticals
Philadelphia, Pennsylvania USA

Approvals/Signatures and Date:

_____

[redacted]
Chief Medical Officer

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451([F-18]T807)	[redacted]

Title of Study: ^{18}F-AV-1451-A07

"^{18}F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy"

Planned number of subjects (Enrolled):
Approximately 30 subjects (20 former NFL players believed to be at high risk of developing chronic traumatic encephalopathy [CTE] and 10 former non-contact athletes) will be recruited from the pool of participants who are participating in the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) study.

Name of compound: ^{18}F-AV-1451([F-18]T807)
Dose*:* 370 MBq (10 mCi)
Route of Administration: Intravenous (IV) bolus

Study Phase: IIa

Study Centers: Approximately 1 center in the United States

Trial Objectives:

The primary objectives of this study are:

- To explore the use of ^{18}F-AV-1451 as a biomarker for CTE; and
- To examine the relationship between clinical presentation and tau deposition as measured by ^{18}F-AV-1451 uptake in subjects with high risk of CTE.

A secondary objective of this study is:

- To expand the ^{18}F-AV-1451 safety database.

Eligibility:

Only subjects duly consented and enrolled in the DETECT study protocol will be considered for participation in this study. (See Section 5.3, Selection of Subjects)

Study Design:

All new DETECT subjects will be contacted to participate and must provide informed consent before starting any ^{18}F-AV-1451-A07 study procedures. Once enrolled, Day One study assessments will occur as defined in the DETECT study protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Additionally, subjects will undergo safety assessments (vital signs and ECG) as part of the DETECT Day One study assessments.

Following Day One study assessments, subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur on the same day as Day

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451([F-18]T807)	[redacted]

One study assessments and the ^{18}F-AV-1451 PET scan occur on Day Two or possibly an additional day should there be issues with dose delivery. The two PET imaging sessions must be performed at least 24 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.

Analyses will explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk with CTE and non-contact athletes (controls).

Assessments and Endpoints:

Day One Study Assessments

Day One study assessments should be completed prior to the PET scans and will occur as defined in the DETECT study protocol with the addition of safety assessments.

Florbetapir F 18 PET Imaging Visit

For the florbetapir F 18 PET imaging visit, an intravenous catheter will be placed for IV administration of Florbetapir F 18 Injection. Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of Florbetapir F 18 Injection followed by a saline flush. A continuous 10-minute brain scan (2 frames of 5 minute duration) will begin immediately following the administration of Florbetapir F 18 Injection. Additionally, at approximately 50 minutes following injection, a continuous 10-minute brain scan (2 frames of 5 minute duration) will begin.

Adverse events will be continuously monitored during the florbetapir F 18 PET imaging session. A physician or physician designee must evaluate the subject for adverse events prior to injection and prior to discharge from the imaging center. Subjects who experience an adverse event will not be discharged from the imaging center until the event has resolved or stabilized.

^{18}F-AV-1451 PET Imaging Visit:

For the ^{18}F-AV-1451 PET imaging visit, an intravenous catheter will be placed for IV administration of ^{18}F-AV-1451 Injection. Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of ^{18}F-AV-1451 Injection followed by a saline flush. At approximately 80 minutes following injection, a continuous 20-minute brain scan (4 frames of 5 minute duration) will begin. If at any point during the imaging session it is determined that the subject is not able to continue, or that it is not in the best interest of the subject to continue, imaging will be discontinued. The image data that has been collected up to that point will be analyzed. Safety assessments will be conducted prior to injection and upon completion of the imaging session.

Adverse events will be monitored continuously during the ^{18}F-AV-1451 imaging session. A physician must evaluate the subject for adverse events prior to injection and prior to discharge from the imaging center. Subjects who experience any adverse event during an imaging session will not be discharged until the event has resolved or stabilized.

Follow-Up Phone Call:

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451([F-18]T807)	[redacted]

A follow-up phone call to the subject, or designated decision maker, will be conducted between 2 or 3 business days of the last 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.

Details of additional assessments that will be performed at each visit are detailed in Section 7.1.

Statistical Methods:

Descriptive Statistics will be applied to describe the ^{18}F-AV-1451 uptake and florbetapir SUVR distribution by subjects at risk with CTE and non-contact athletes (controls). Two sample t-test or Wilcoxon rank sum test when the pre-requisite of t-test is not met, will be applied to test if there are difference of the ^{18}F-AV-1451 uptake and florbetapir SUVR between CTE and control groups. Additional exploratory analyses will be applied to explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk for CTE and controls.

TABLE OF CONTENTS **Page**

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.
Audit	A systematic and independent examination of the trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data were recorded, analyzed, and accurately reported according to the protocol, applicable standard operating procedures (SOPs), good clinical practice (GCP), and the applicable regulatory requirement(s).
Case Report Form (CRF) and electronic Case Report Form (eCRF)	A printed or 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
CTE	Chronic Traumatic Encephalopathy
DETECT	Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests study (also known as R01NS078337 grant)
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
MBq	Megabecquerel
mCi	Millicurie
MHD	Maximum Human Dose
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury
NOAEL	No Observable Adverse Effect Level
PET	Positron Emission Tomography
RBT	Repetitive Brain Trauma
SUVR	Standard Uptake Value Ratio

1. INTRODUCTION

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease marked by widespread accumulation of hyperphosphorylated tau (p-tau), predominantly as neurofibrillary and astrocytic tangles (McKee et al., 2009, 2013). In contrast to Alzheimer's disease (AD), however, CTE does not involve beta amyloid (Aβ) neuritic plaque deposition. CTE ultimately leads to dementia and is believed to be caused, in part, by repetitive mild traumatic brain injury (mTBI), including concussive and subconcussive impacts. CTE has been found most often in professional athletes involved in contact sports (e.g. boxing, American football) who have been subjected to repetitive brain trauma (RBT), including mTBI or even asymptomatic, subconcussive trauma.

CTE, like most neurodegenerative diseases, can only be definitively diagnosed post-mortem. Neuropathological findings have demonstrated that this tauopathy has a unique profile of neurodegeneration and tau deposition that is distinct from AD and other neurodegenerative diseases. To facilitate future CTE research, objective *in vivo* biomarkers must first be discovered.

A currently-funded NIH grant [redacted] will focus on a sample of subjects who are believed to be at high risk of developing CTE and compare them with an age matched control cohort on several potential biomarkers for CTE, selected to detect key variables associated with their hypothesized mechanism for the pathogenesis of CTE, based on their previous neuropathological studies, pilot neuroimaging data collected by their team, and other findings by their team and in the literature regarding the long-term effects of repetitive mTBI. In addition, the relationship among possible biomarkers and pertinent clinical variables, including neurologic, motor, neuropsychological and psychiatric evaluations will be assessed in the "probable CTE" group.

However, a potentially useful biomarker of this tauopathy would be a PET radiotracer that could specifically bind to paired helical filament (PHF) tau. Currently, molecular imaging biomarkers have the potential to aid in the diagnosis of patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) (Dubois, 2010; McKhann, 2011). Positron emission tomography (PET) ligands such as florbetapir F 18 (Wong, 2010) may provide a minimally invasive estimate of cortical Aβ neuritic plaque deposition, a hallmark pathology, and a required element for the evaluation of AD neuropathologic diagnosis (Hyman 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 a., 1987).

^{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). 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. [redacted].

Note: this section of the protocol contained nonclinical pharmacology and toxicology information that was current at the time the protocol was written is out of date at the time of this publication. For up to date information consult the flortaucipir investigator's brochure, available from Avid/Eli Lilly

[redacted]

^{18}F-AV-1451 may be useful as a marker of tau pathology in patients with AD and other neurodegenerative disorders (Figures 1 and 2). Several preliminary studies using ^{18}F-AV-1451 have been completed (e.g., Chien, et al., 2013). Based on this rationale, the goal of this protocol is to perform ^{18}F-AV-1451 PET imaging on subjects enrolled in the DETECT study protocol and explore its potential as a biomarker for CTE.

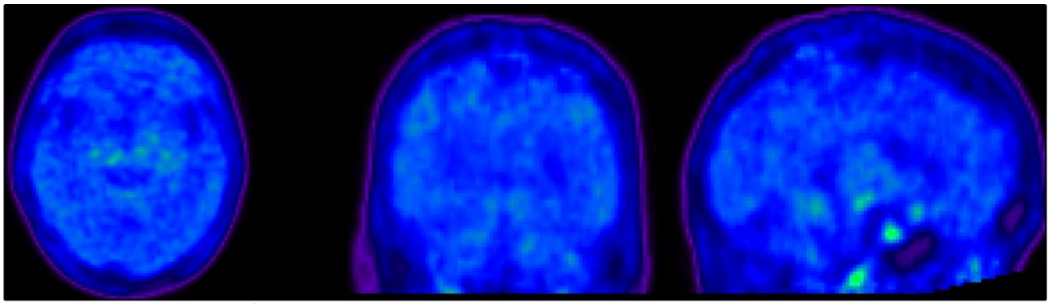


Figure 1: 58 year old female control subject (MMSE = 29)

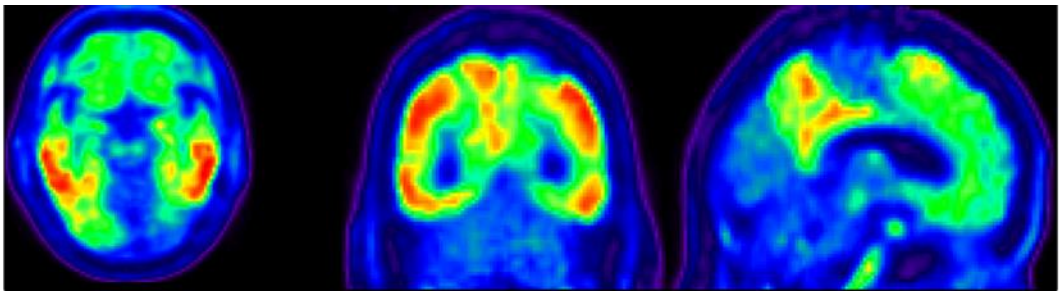


Figure 2: 64 year old male AD subject (MMSE = 18)

2. TRIAL OBJECTIVES

The primary objectives of this study are:

- To explore the use of ^{18}F-AV-1451 as a biomarker for CTE; and
- To examine the relationship between clinical presentation and tau deposition as measured by ^{18}F-AV-1451 uptake in subjects with high risk of CTE.

A secondary objective of this study is:

- To expand the ^{18}F-AV-1451 safety database.

3. SPONSOR, INVESTIGATOR(S) AND OTHER PARTICIPANTS

The trial is sponsored by:

Avid Radiopharmaceuticals

[redacted]

The medical contact is:

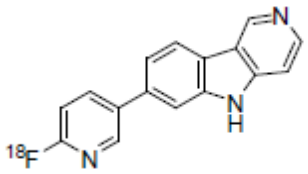
[redacted]

Approximately 1 center in the United States will participate.

4. TEST DRUG AND CONTROL AGENTS

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

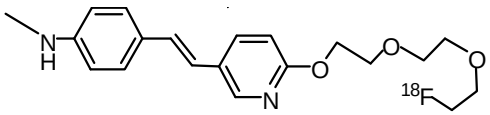
[redacted]



MW = 262.27 amu

4.2 Descriptive Name: Florbetapir F 18

[redacted]



^{18}F-AV-45

MW= 359.4 amu

4.3 Radioactive Labeling

[redacted]

4.4 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.

4.5 Formulation and Dose Florbetapir F 18 Injection

[redacted] Subjects will receive a single IV administration of approximately 370 MBq (10 mCi) of Florbetapir F 18 Injection immediately prior to imaging. [redacted]

Florbetapir F 18 Injection will be supplied from manufacturing facilities approved for commercial distribution under NDA 202-008.

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

^{18}F-AV-1451 Injection is a clear solution containing ^{18}F-AV-1451 (drug substance) formulated for intravenous bolus administration. Depending on the manufacturer, ^{18}F-AV-1451 Injection will be formulated in:

[redacted]

[redacted]

Drug product of either formulation is manufactured to meet one common set of specifications.

The expiration time and date of ^{18}F-AV-1451 Injection are provided on the outer label of each dose based on specific activity or strength. ^{18}F-AV-1451 Injection should be stored at room temperature.

4.7 Packaging Florbetapir F 18 Injection

[redacted].

4.8 Packaging ^{18}F-AV-1451 Injection

[redacted]

4.9 Storage and Handling Florbetapir F 18 Injection

Florbetapir F 18 Injection is stored at 25ºC; excursions permitted to 15-30ºC. The product does not contain a preservative. Florbetapir F 18 Injection should be stored within the original container or equivalent radiation shielding. Florbetapir F 18 Injection must not be diluted.

4.10 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. ^{18}F-AV-1451 Injection must not be diluted.

5. INVESTIGATIONAL PLAN

5.1 Overall Design and Plan of Trial

All new DETECT subjects will be contacted to participate and must provide informed consent before starting any ^{18}F-AV-1451-A07 study procedures. Once enrolled, Day One study assessments will occur as defined in the DETECT study protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451

PET scans. Additionally, subjects will undergo safety assessments (vital signs and ECG) as part of the DETECT Day One study assessments.

Following Day One study assessments, subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur on the same day as Day One study assessments and the ^{18}F-AV-1451 PET scan occur on Day Two or possibly an additional day should there be issues with dose delivery. The two PET imaging sessions must be performed at least 24 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.

Analyses will explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk with CTE and non-contact athletes (controls).

5.2 Planned Dosage and Duration of Treatment

5.2.1 Dosage and Administration

Florbetapir F 18:

Subjects will receive a single IV bolus administration of approximately 370 MBq (10 mCi) of Florbetapir F 18 Injection.

^{18}F-AV-1451:

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

5.2.2 Rationale for Dosages

^{18}F-AV-1451 will be administered IV in a radioactive dose of 370 MBq with a maximum human mass dose (MHD) limited to 20 µg of compound by weight. 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.

Preliminary human dosimetry has been obtained in three subjects. The results estimated an Effective Dose of 9.18 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 preliminary human studies. No treatment related adverse events have been reported using this regimen.

5.3 Selection of Subjects

5.3.1 Inclusion Criteria

Subjects who meet all of the following criteria are eligible to enroll in this trial:

1. Male subjects consented and currently enrolled in the DETECT study protocol;
2. Can tolerate up to two PET imaging sessions (one with ^{18}F-AV-1451 and one with florbetapir F 18); and
3. Have the ability to provide informed consent for study procedures (If the subject is ineligible to give informed consent, based on local standards, the subject's legal representative may consent on behalf of the patient but the patient must still confirm assent).

5.3.2 Exclusion Criteria

Subjects will be excluded from enrollment if they:

1. Are claustrophobic or otherwise unable to tolerate the imaging procedure (use of mild sedatives are permitted to manage claustrophobia);

2. Have current clinically significant cardiovascular disease or clinically significant abnormalities on screening ECG (including but not limited to QTc>450 msec);

3. A history of additional risk factors for Torsades de Pointes (TdP) (e.g., heart failure, 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);

4. Have a current clinically significant infectious disease, endocrine or metabolic disease, pulmonary, renal or hepatic impairment, or cancer that the investigator believes would affect study participation or scan results;

5. Do not agree to refrain from sexual activity or to use reliable contraceptive methods for 90 days following administration of ^{18}F-AV-1451 Injection;

6. Have had a non-study related radiopharmaceutical imaging or treatment procedure within 7 days prior to the ^{18}F-AV-1451 imaging session or florbetapir F 18 imaging session; and

7. In the opinion of the investigator, are otherwise unsuitable for a study of this type.

5.4 Prior and Concomitant Therapy

Except as noted below, 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, including investigational 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 on the Concomitant Medication Page of the Case Report Form (CRF).

Subjects who are taking drugs that are known to cause QT-prolongation may not be enrolled in the study (a list of prohibited and discouraged medications is provided by the Sponsor).

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 SAE occurs. The date and reason for discontinuation should be noted on the CRF. Subjects who discontinue prematurely should be seen for a final evaluation.

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, or changes in business priorities. 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 investigator may 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. WARNINGS/PRECAUTIONS

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. However, because ^{18}F-AV-1451 Injection is in the early stages of clinical investigation, it is recommended that subjects receiving ^{18}F-AV-1451 Injection be followed closely by means of adverse event reporting, vital signs, ECGs, and laboratory tests.

There are no data on the effects of ^{18}F-AV-1451 Injection in human perinatal development. For this reason, males enrolled in this study must agree to refrain from sexual activity or use adequate contraceptive methods for 90 days after administration of ^{18}F-AV-1451 Injection.

7. PROCEDURES AND METHODS

7.1 Assessment Periods (See Section 11.2, Trial Flow Chart)

The study will consist of the following sequence of activities:

7.1.1 Day One Study Assessments:

Subjects currently enrolled in the DETECT study protocol will be contacted to participate. Day One study assessments will occur as defined in the DETECT study protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Additional Day One study assessments are as follows:

- Informed Consent will take place before any ^{18}F-AV-1451-A07 study procedures;
- Medical history;
- Safety assessments: Vital signs (pulse rate, respiratory rate, supine blood pressure, height and weight) and ECG (with results reviewed prior to dose administration); and
- A physician will see the subject during the Day One study assessments.

7.1.2 Imaging Visits

Subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur on the same day as Day One study assessments and the ^{18}F-AV-1451 PET scan occur on Day Two or possibly an additional day should there be issues with the dose delivery. The two PET imaging sessions must be performed at least 24 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.

Florbetapir F 18 PET Imaging Session

- A physician or physician designee must see the subject prior to administration of Florbetapir F 18 Injection to determine if they are still suitable to undergo the scan;
- If ^{18}F-AV-1451 Injection has been previously administered, confirm subject well-being and collect information about any new adverse events;
- Vital signs will be taken (pulse rate, respiratory rate, supine blood pressure and weight) immediately prior to injection of florbetapir F 18;
- Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of florbetapir F 18 followed by a saline flush. A continuous 10-minute brain scan (2 frames of 5 minute duration) will begin immediately following the administration of Florbetapir F 18 Injection;
- At approximately 50 minutes following injection, a continuous 10-minute brain scan (2 frames of 5 minute duration) will begin;
- Adverse events will be continuously monitored during the Florbetapir F 18 PET imaging visit. Subjects who experience an adverse event will not be discharged from the imaging center until the event has resolved or stabilized;
- A physician or physician designee will see the subject prior to discharge from the imaging center to evaluate the subject's readiness for discharge; and
- If the florbetapir F 18 imaging visit occurs second, a follow-up phone call to the subject, or designated decision maker, 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.

^{18}F-AV-1451 PET Imaging Visit

- A physician must see the subject prior to administration of ^{18}F-AV-1451 Injection to determine if they are still suitable to undergo the scan;
- If florbetapir F 18 Injection has been previously administered, confirm subject well-being and collect information about any new adverse events;
- Vital signs will be taken (pulse rate, respiratory rate, supine blood pressure) at the following time points:
  - Immediately prior to administration of ^{18}F-AV-1451 Injection (Weight and Temperature will also be collected)
  - Within 5 minutes after completion of injection of ^{18}F- AV-1451 Injection
  - After completion of the PET scan prior to discharge. (Temperature will also be collected);
- Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of ^{18}F-AV-1451 Injection followed by a saline flush. At

approximately 80 minutes following injection, a continuous 20-minute brain scan (4 frames of 5 minute duration) will begin;

- The injection site will be observed for excessive inflammation or damage to the surrounding tissue where the dose was injected;
- Adverse events will be continuously monitored during the ^{18}F-AV-1451 PET imaging visit. Subjects who experience an adverse event will not be discharged from the imaging center until the event has resolved or stabilized;
- A physician will see the subject prior to discharge from the imaging center to evaluate the subject's readiness for discharge; and
- If the ^{18}F-AV-1451 imaging visit occurs second, a follow-up phone call to the subject, or designated decision maker, 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.

7.2 Observations and Measurements

Informed Consent

Potential subjects and legally authorized representatives, 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 subject. The subject 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 (see Section 7.5). A copy of the signed informed consent will be given to the subject or legally authorized representative.

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

Medical History

The investigator or designee will obtain an updated history at the screening visit.

- Relevant demographic information
- Review of body systems
- Social history
- Medical and surgical history, including medical care for head trauma
- Concurrent medications

Whenever possible, the medical history will be confirmed by medical records.

Vital Signs

Vital signs (pulse rate, respiratory rate, supine blood pressure) will be taken as part of the DETECT Day One study visit and at the following time points:

- Florbetapir F18 Imaging Visit
  - immediately prior to injection of florbetapir F 18
- ^{18}F-AV-1451 Imaging Visit
  - Immediately prior to the administration of ^{18}F-AV-1451 Injection
  - Within 5 minutes after completion of injection of ^{18}F-AV-1451 Injection
  - After the completion of imaging prior to discharge.

Temperature will also be obtained at the following time point:

- ^{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 both the DETECT Day One study visit and imaging visits (immediately prior to ^{18}F-AV-1451 and florbetapir F 18 administration) body weight will be measured, lightly clothed. Height will only be measured at screening.

Electrocardiogram (ECG)

A resting 12-lead electrocardiogram will be recorded as part of the DETECT Day One study visit.

Physician Visit

A physician must see the subject at baseline, prior to drug administration and at study end, prior to discharge from the ^{18}F-AV-1451 imaging session. A physician or physician designee must see the subject at baseline, prior to drug administration and at study end, prior to discharge from the florbetapir F 18 imaging session. At discharge, the physician (or designee for the florbetapir F 18 imaging visit) should review all safety data and briefly examine/query the subject regarding potential adverse events or other treatment issues.

7.3 Protocol for Image Collection

The sponsor will prepare and distribute imaging manuals for ^{18}F-AV-1451 and florbetapir F 18 image acquisition parameters and transmission procedures prior to site initiation.

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/IEC approval: An investigation will be initiated at a study site only after the IRB/IEC 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 legally authorized representative (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 legally authorized representative 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.

If the subject is capable of giving informed consent then the subject should sign on the consent line of the informed consent form. When applicable, the legally authorized representative should sign as well, indicating that they have witnessed the subject's consent, and further agree to participate as an informant.

If the subject is not capable of giving consent, consent may be given by a legally authorized representative. However, it is expected that all subjects entering this study should at least have the capacity to understand that they are engaging in a research study and should affirm that they do not object to participating, by signing on the Subject Assent line of the consent form.

All informed consent forms must be approved by Avid or designee, and by the appropriate Institutional Review Board (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 patient and/or their legally authorized representative for their records.

7.6 Documentation

^{18}F-AV-1451 and florbetapir F 18 PET scans will be saved in an appropriate electronic format as specified in the imaging manuals. A copy of all scans, including the MRI/MRS scans conducted per DETECT study protocol, will be

saved at the site/imaging center and a copy of each will be forwarded to the sponsor or to the designated imaging core lab as described in the imaging manuals. All other data required by the protocol will be recorded in Boston University's REDCap study database. All data in the eCRFs will be substantiated by "source documents," which consist of the subject's medical files, laboratory result sheets, ECG tracings, etc. All source documentation must be available to Avid and designees. Completed source documents and eCRFs may need to be made available and complete for an audit by the FDA or other international regulatory authorities or Avid at any time. CRFs 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 eCRFs. Investigators will be instructed to report to Avid or its designee their assessment of the potential relatedness of each AE to study drug or protocol procedure via electronic data entry. If a patient'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 patient, 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").

Laboratory test abnormalities considered by the Investigator to be clinically relevant should be reported on the adverse event eCRFs. Signs and symptoms of each AE should be described in detail (e.g., start and stop dates/time, severity/intensity, relationship to study drug, action taken, and outcome). Additionally, any clinically significant findings from laboratory evaluations, 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 patient must be carefully monitored for adverse events. This includes clinical laboratory test variables. An assessment must be made of the severity/intensity and relationship to the administration of the study drug.

7.7.2 Adverse Event Definitions

Adverse Events

An adverse event is any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the study drug.

For reporting purposes, Avid will distinguish among pre-existing conditions, trial-emergent adverse events and treatment-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 eCRF pages. During the study, site personnel will record any change in the condition(s) and occurrence and nature of any AEs. Signs and symptoms that are believed to be due to the pre-existing condition under study (started prior to dose of study medication) do not have to be recorded in the AEs section of the eCRF, unless there is an increasing in frequency and severity.

Trial-emergent adverse events are undesirable experiences, signs or symptoms that begin, or worsen in intensity or frequency, after the informed consent, and prior to administration of the first study drug (either florbetapir F 18 or ^{18}F-AV-1451) at the imaging visit. These will be recorded on the adverse event eCRFs.

Treatment-emergent adverse events are undesirable experiences, signs, or symptoms associated with the use of a study drugs. For the purposes of this study an adverse event will be considered associated with the use of ^{18}F-AV-1451 or Florbetapir F18 Injection if it begins or worsens in intensity or frequency within 24 hours after the administration of the respective drug. Adverse experiences that occur after administration of study drug but outside the 48 hour reporting window will not be reported unless the investigator believes they are attributable to the drug.

The end of study for the purpose of adverse event reporting is defined as 48 hours after the administration of ^{18}F-AV-1451 Injection or Florbetapir F 18 Injection or the time of the next visit in the case that florbetapir F 18 imaging visit occurs first.

<u>Serious Adverse Event (SAE)</u>

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 patient 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 Study Drug

Investigators will be instructed to report their assessment of the potential relatedness of each adverse event to protocol procedure or study drug. The assessment of the relationship of an adverse event to the administration of the study drug 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 study drug 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 eCRFs. Documentation must be supported by an entry in the subject file. Laboratory test, vital signs and ECG abnormalities considered by the Investigator to be

clinically relevant should be reported on the adverse event eCRFs. Signs and symptoms of each AE should be described in detail (e.g., start and stop dates, severity/intensity, relationship to study drug, action taken, and outcome).

Adverse events and laboratory test abnormalities 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 receive a dose of study drug will be collected until 48 hours after the dosing of the study drug, regardless of the investigator's opinion of causation. Therefore, SAEs that occur later than 48 hours after the dosing of the study drug are not required to be reported unless the investigator feels the events were related to either study drug or a protocol procedure.

If a patient experiences an SAE after signing informed consent, but prior to receiving study drug, the event will NOT be reported unless the investigator feels the event may have been caused by a protocol procedure. 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 study data collected under DETECT protocol, such as but not limited to subjects' demographic and baseline characteristics, history taking, CSF, portable EEG, neurological and motor, neuropsychological, psychiatric evaluations, and MRI will be transferred to Avid for analysis purpose.

Data will be summarized using descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, quartiles, minimum, and maximum) for continuous variables and using frequency count and percentage for discrete variables. The demographic and baseline characteristics data will be summarized for all subjects in the safety population according to clinical group (subjects at

risk with CTE and non-contact athletes [controls]). Safety data will be summarized for all patients.

Subject listings of all data from the electronic case report forms (eCRFs) as well as any derived variables will be presented.

Descriptive Statistics will be applied to describe the ^{18}F-AV-1451 uptake and florbetapir SUVR distribution by subjects at risk with CTE and non-contact athletes (controls). Two sample t-test or Wilcoxon rank sum test when the pre-requisite of t-test is not met, will be applied to test if there are difference of the ^{18}F-AV-1451 uptake and florbetapir SUVR between CTE and control groups. Additional exploratory analyses will be applied to explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk for CTE and controls.

Additional details concerning statistical analyses will be included in the Statistical Analysis Plan (SAP) to be completed prior to the end of enrollment into the study.

8.2 Safety Analysis

Safety laboratory test results and 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. Subjects whose laboratory values are outside the pre-determined upper and lower limits of normal will be identified and tabulated.

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.

Laboratory Data

Subjects whose laboratory values are outside threshold values will be identified and tabulated.

Vital Signs

Changes in vital signs from baseline will be summarized.

ECG

Any subjects showing QTc > 500 or change from baseline of more than 60 msec will be highlighted.

8.3 Image Analysis

All ^{18}F-AV-1451 PET images obtained starting at 80 minutes post injection will be analyzed. The ^{18}F-AV-1451 PET images will be spatially normalized to standard stereotactic atlas space using MNI brain atlas. The uptake in tau protein rich brain regions will be assessed with regions of interest (ROI, designed in MNI brain atlas) in terms for standard uptake value ratio (SUVR, normalized by cerebellar uptake). The spatially normalized images and the measured SUVR values will be used to accordingly for the following objectives.

Mean, standard deviation, minimum and maximum of the SUVR values for all groups will be calculated.

Exploratory analysis will include co-registration of MRI to ^{18}F-AV-1451 PET images and partial volume correction of ^{18}F-AV-1451 PET images using anatomical information from MRI data.

Florbetapir F 18 images will be analyzed as described previously (Clark et al. 2011, 2012).

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 single center study. A single center publication, reporting the primary analysis data set, 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 patient information/informed consent form and any advertising material used to recruit subjects must be submitted to the clinical investigator's local IRB/IEC 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/IEC approval must be obtained and also forwarded to Avid**.**

10.2 Informed Consent

A signed, written informed consent must be obtained from each patient. A copy of the signed informed consent should be given to the patient for their records. A copy of the local IRB/IEC's approved version of the informed consent form must be forwarded to Avid or designee for review prior to being used to obtain patient 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/IEC and radiation safety committee approval (if applicable);
- Copy of the IRB/IEC 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 florbetapir F 18 or ^{18}F-AV-1451.

10.5 Study Drug Control

The receipt of clinical supplies (i.e. starting material for ^{18}F-AV-1451) 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 Injection 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 patient files should include appropriate source documents, including but not limited to patient's medical records and laboratory test results. 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 Avid or a designated Imaging Core Lab as specified in the Imaging Manual.

Each patient'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.

All original laboratory reports must be available for review in each patient's file. It is important that the original reports be available for review because of the possibility of inaccuracies or errors in transcribing data from original records to the eCRF.

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 patient enrolled in the trial and signed by the investigator. This should be done as soon as possible after completion of the patient's participation in the trial. 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/IEC within 24 hours of receipt.

10.8 Records Retention

All correspondence (e.g., with Avid, IRB/IEC, 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 2 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 2 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	Day One Study Assessments[a]	Florbetapir F 18 Imaging Visit[b]	End of Florbetapir F 18 Imaging (prior to discharge)	^{18}F-AV- 1451 Imaging Visit[b]	End of ^{18}F-AV- 1451 Imaging (prior to discharge)	Follow-up Phone Call[h]
Signed Informed Consent	X					
Medical History	X					
ECG	X					
Vital Signs	X[c]	X[d]		X [d,e]	X[f]	
PET Brain Scan		X		X		
Evaluation by a physician	X	X[g]	X[g]	X	X	
Adverse Events	X	X	X	X	X	X
Serious Adverse Events	X	X	X	X	X	X

a. Day One study assessments will occur as defined in the DETECT study protocol (with the addition of safety assessments listed in this protocol) and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans.
b. It is preferable that the florbetapir F 18 PET scan occur on the same day as Day One study assessments and the ^{18}F-AV-1451 PET scan occur on Day Two or possibly an additional day should there be issues with dose delivery. The two PET imaging sessions must be performed at least 24 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.
c. Day One vital signs include pulse rate, respiratory rate, supine blood pressure, height and weight.
d. Vital signs (pulse rate, respiratory rate, supine blood pressure and weight) and temperature (for the ^{18}F-AV-1451 imaging visit) will be taken immediately prior to injection.
e. Vital signs (pulse, respiratory rate, and supine blood pressure) within 5 minutes after completion of injection of ^{18}F-AV-1451 Injection.
f. Vital signs (pulse, respiratory rate, supine blood pressure, and temperature).
g. A physician or physician designee.
h. Will be conducted between 2 or 3 business days of the last 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.

INVESTIGATOR'S AGREEMENT TO PROTOCOL

Protocol ^{18}F-AV-1451-A07: "^{18}F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy"

Date and Version: 16 December 2013, Final

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

^{18}F-AV-1451-A07 Amendment 1

Summary of Changes from Protocol dated 16 December 2013

1. **Synopsis: Study Design, page 2**

Changed sentence from:
"Additionally subjects will undergo safety assessments (vital signs and ECG) as part of the DETECT Day One study assessments"

To:
"Additionally subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the Mini-Mental State Examination (MMSE) as part of the DETECT Day One study assessments."

Same change made to the following:
page 14, Section 5.1: Overall Design and Plan of Trial

2. **Synopsis: Study Design, page 3**

For when Florbetapir F 18 Injection is administered first, changed sentence from:
"The two PET imaging sessions must be performed at least 24 hours apart."

To:
"The two PET imaging sessions must be performed at least 12 hours apart."

Same change made to the following:
page 15, Section 5.1: Overall Design and Plan of Trial
page 18, Section 7.1.2: Imaging Visits

3. **Synopsis: Assessments and Endpoints, page 3**

Added "and MMSE" to the end of the sentence under Day One Study Assessments.

Same change made to the following:
page 34, Section 11.2: Trial Flow Chart

4. **Synopsis: Assessments and Endpoints, page 3**

For the florbetapir F 18 PET imaging visit, changed 10-minute brain scan acquisition time from:
"2 frames of 5 minute duration"

To:

"10 frames of 1 minute duration"

Same change made to the following:
page 19, Section 7.1.2: Imaging Visits, Florbetapir F 18 PET Imaging Session

5. **Section 1, Introduction, page 9**

Deleted reference "Dickson et al., 1997"

Same change made to the following:
page 32, Section 11.1: References

6. **Section 1, Introduction, page 9**

Added references "Braak et al., 2011; Nelson et al., 2012)."

Same change made to the following:
page 32, Section 11.1: References

7. **Section 1, Introduction, page 10**

Changed from:
"Preliminary human dosimetry has been obtained in three 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 (0.107 ± 0.009 mSv/MBq), followed by the small intestine and the liver. The Effective Dose was 0.0248 ± 0.0011 mSv/MBq. This results in an estimated Effective Dose of 9.18 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."

To:
"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 8.92 mSv for an anticipated 370 MBq (10 mCi) injection and is comparable to the effective dose of approved ^{18}F-labeled compounds such as fluorodeoxyglucose (FDG) and florbetapir F 18 injection."

Same change made to the following:
page 15, Section 5.2.2: Rationale for Dosages

8. **Section 5.3.1, Inclusion Criteria, page 16**

Inclusion Criterion #3, deleted "(If the subject is ineligible to give informed consent, based on local standards, the subject's legal representative may consent on behalf of the patient but the patient must still confirm assent)."

9. **Section 7.1.1, Day One Study Assessments, page 18**

Added "and safety labs (hematology, chemistry and urinalysis);" to bullet # 3 and added bullet #4 "MMSE; and".

10. **Section 7.2, Observations and Measurements, page 20**

Deleted "and legally authorized representatives, if applicable" from Informed Consent section.

11. **Section 7.2, Observation and Measurements, page 21**

Changed language from:
"Height will only be measured at screening."

To:
Height will only be measured as part of the Day One study visit."

12. **Section 7.2, Observation and Measurements, page 21**

Added language:

"Clinical Laboratory Tests

Clinical laboratory evaluation will be performed as part of the Day One study visit.

Tests will include:

- **Hematology** (3 mL EDTA): hemoglobin, hematocrit, RBC, WBC, MCH, MCHC, neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, platelets, morphology, MCV, and RBC morphology.

- **Chemistry** (5 mL blood): total bilirubin, alkaline phosphatase, ALT (SGPT), AST (SGOT), urea nitrogen, creatinine, glucose, uric acid, calcium, phosphorus, total protein, albumin, total cholesterol, triglycerides, sodium, potassium, bicarbonate, chloride, magnesium, globulin, GGT.

- **Urinalysis** (10 mL, urine): Samples will be used to assess glucose, RBC, WBC, specific gravity, pH, protein, ketones, urobilinogen, blood, nitrite, microscopic, color, bilirubin, casts, epithelial cells, leukocyte, esterase, and bacteria.

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."

13. **Section 7.4, Good Clinical Practice and Monitoring, 21**

Deleted "/IEC" in #1 and throughout protocol.

14. **Section 7.4, Good Clinical Practice and Monitoring, 21**

Deleted "and/or their legally authorized representative (as appropriate)."

Same change made to the following:
page 22, Section 7.5: Informed Consent and Subject Information

15. **Section 7.5, Informed Consent and Subject Information, page 22**

Deleted the following paragraphs:
"If the subject is capable of giving informed consent then the subject should sign on the consent line of the informed consent form. When applicable, the legally authorized representative should sign as well, indicating that they have witnessed the subject's consent, and further agree to participate as an informant.

If the subject is not capable of giving consent, consent may be given by a legally authorized representative. However, it is expected that all subjects entering this study should at least have the capacity to understand that they are engaging in a research study and should affirm that they do not object to participating, by signing on the Subject Assent line of the consent form."

And added: "If the subject is incapable of giving informed consent then the subject will be removed from the study."

16. **Section 7.6, Documentation, page 22**

Changed sentence from:
"All other data required by the protocol will be recorded in Boston University's REDCap study database."

To:
"All other data required by the protocol will be recorded in the eCRFs."

17. **Section 10, Investigator's Regulatory Obligations, page 29**

Deleted “EMA”.

18. **Section 10.6, Data Collection, page 30**

Added paragraph:

“Researchers would like to save and store all of the information gathered from each subject in this study (as well as the DETECT study) for future studies about the effects of repetitive head trauma. Information from the DETECT study will be coded with a patient number, not the subject’s name, and kept in a secure database maintained by the BU Data Coordinating Center and for this protocol, a separate secure database maintained by Avid Radiopharmaceuticals. Scientists at BU and researchers at other places would be able to use the subject’s information for future research on the effects of repetitive head trauma. If, in the future subjects decide that they don't want the researchers to keep their information, the subjects could let BU know and the information would be discarded.”

19. **Section 11.2, Trial Flow Chart, page 34**

Added rows for “Clinical Lab Tests (safety)” and “MMSE” to be done during Day One Study Assessments.

20. **Table of Contents, page 5**

Revised Table of Contents to reflect updated page numbers.

^{18}F-AV-1451-A07 Protocol Amendment 2

Summary of Changes from Protocol Amendment 1 dated 27 February 2014

1. **Synopsis, Planned number of subjects (Enrolled), page 2**

Changed from:

"Approximately 30 subjects (20 former NFL players believed to be at high risk of developing chronic traumatic encephalopathy [CTE] and 10 former non-contact athletes) will be recruited from the pool of participants who are participating in the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) study."

To:

"Approximately 50 subjects (30 former NFL players believed to be at high risk of developing chronic traumatic encephalopathy [CTE] and 20 former non-contact athletes) will be recruited from the pool of participants who are participating in the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) study being conducted at Boston University or Long-Term Consequences of Repetitive Brain Injury in Athletes: A Longitudinal Study with Eventual Brain Donation study being conducted at Mayo Clinic College of Medicine, Banner Sun Health Research Institute and Banner Alzheimer's Institute."

2. **Synopsis: Study Centers, page 2**

Revised:
From 1 to 5 centers, specifically: "Up to 5 centers in the United States."

Same revision made to the following:
- page 12, Section 3, Sponsor, Investigator(s) and Other Participants

3. **Synopsis, Eligibility, page 2**

Changed from:

"Only subjects duly consented and enrolled in the DETECT study protocol will be considered for participation in this study."

To:

"Only subjects duly consented and enrolled in the site's companion study protocol (either DETECT or Long-Term Consequences of Repetitive Brain Injury in Athletes) will be considered for participation in this study."

4. **Synopsis, Study Design, page 2**

First two paragraphs revised from:

"**Study Design:**

All new DETECT subjects will be contacted to participate and must provide informed consent before starting any ^{18}F-AV-1451-A07 study procedures. Once enrolled, Day One study assessments will occur as defined in the DETECT study protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Additionally, subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the Mini-Mental State Examination (MMSE) as part of the Day One study assessments.

Following Day One study assessments, subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur on the same day as Day One study assessments and the ^{18}F-AV-1451 PET scan occur on Day Two or possibly an additional day should there be issues with dose delivery. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor."

To:

"**Study Design:**

New subjects being enrolled in the site's companion protocol will be contacted to participate and must provide informed consent before starting any ^{18}F-AV-1451-A07 study procedures. Once enrolled, Day One/Screening study assessments will occur as defined in the companion protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Additionally, subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the Mini-Mental State Examination (MMSE) if MMSE is not administered per the site's companion protocol as part of the Day One/Screening companion protocol study assessments or at a study visit prior to the first PET scan (either florbetapir F 18 or ^{18}F-AV-1451).

Following Day One/Screening study assessments, subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur prior to the ^{18}F-AV-1451 PET scan. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor."

Same changes made to the following:

- page 14, Section 5.1, Overall Design and Plan of Trial
- page 18, Section 7.1.1, Day One Study Assessments
- page 18, Section 7.1.2, Imaging Visits

5. **Synopsis, Assessments and Endpoints, Day One Study Assessments, page 3**

Changed from:
"Day One study assessments should be completed prior to the PET scans and will occur as defined in the DETECT study protocol with the addition of safety assessments and MMSE."

To:
"Day One/Screening study assessments should be completed prior to the PET scans and will occur as defined in the site's companion protocol. Additionally, subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the Mini-Mental State Examination (MMSE) if MMSE is not administered per the site's companion protocol as part of the Day One/Screening companion protocol study assessments or at a study visit prior to the first PET scan (either florbetapir F 18 or ^{18}F-AV-1451)."

6. **Synopsis, Assessments and Endpoints, ^{18}F-AV-1451 PET Imaging Visit, page 3**

Changed from:
"At approximately 80 minutes following injection, a continuous 20-minute brain scan (4 frames of minute duration) will begin."

To:
At approximately 75 minutes following injection, a continuous 30-minute brain scan (6 frames of 5 minute duration) will begin.

Same changes made to the following:
- page 19, Section 7.1.2, Imaging Visits

7. **Synopsis, Assessments and Endpoints, ^{18}F-AV-1451 PET Imaging Visit, page 3**

Changed from:
"Adverse events will be monitored continuously during the ^{18}F-AV-1451 imaging session. A physician must evaluate the subject for adverse events prior to injection and prior to discharge from the imaging center. Subjects who experience any adverse event during an imaging session will not be discharged until the event has resolved or stabilized."

To:
"Adverse events will be monitored continuously during the ^{18}F-AV-1451 imaging session. A physician or a licensed/credentialed medical professional (i.e., a PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator must assess or evaluate the subject for adverse events prior to injection and prior to discharge from the imaging

center. If a designee performs this activity, a physician must be available to provide medical consultation. Subjects who experience any adverse event during an imaging session will not be discharged until the event has resolved or stabilized."

8. **Table of Contents, pages 5-6**

Table of Contents revised to reflect current page numbers and section titles.

9. **Section 1, Introduction, page 9**

Added the following paragraph after the 3rd paragraph:
"Additionally, a similar trial is being funded by the Arizona Alzheimer's Consortium called Long-Term Consequences of Repetitive Brain Injury in Athletes: A Longitudinal Study with Eventual Brain Donation. This study aims to examine the relationship between head impact exposure and later life neurological consequences, as well as genetic modifiers of this relationship. This is a pilot project designed to develop preliminary data."

10. **Section 1, Introduction, page 11**

Changed last sentence of last paragraph from:
"Based on this rationale, the goal of this protocol is to perform ^{18}F-AV-1451 PET imaging on subjects enrolled in the DETECT study protocol and explore its potential as a biomarker for CTE."

To:
"Based on this rationale, the goal of this protocol is to perform ^{18}F-AV-1451 PET imaging on subjects enrolled in the DETECT or Long-Term Consequences of Repetitive Brain Injury in Athletes companion study protocols and explore its potential as a biomarker for CTE."

11. **Section 5.3.1, Inclusion Criteria, page 16**

Revised first criterion from:
1. Male subjects consented and currently enrolled in the DETECT study protocol;

To:
1. Male subjects consented and currently enrolled in the site's companion protocol;

12. **Section 6, Warnings/Precautions, page 18**

Deleted "ECGs, and laboratory tests" from the last sentence in the second paragraph.

13. **Section 7.1.1, Day One Study Assessments, page 18**

Added to 4th bullet: "(if not administered per the site's companion protocol)".

14. **Section 7.1.2, Imaging Visits, ^{18}F-AV-1451 PET Imaging Visit, page 19**

Revised first bullet from:

- A physician must see the subject prior to administration of ^{18}F-AV-1451 Injection to determine if they are still suitable to undergo the scan;

To:

- A physician or a licensed/credentialed medical professional (i.e., a PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator must assess or evaluate the subject prior to administration of ^{18}F-AV-1451 Injection to determine if they are still suitable to undergo the scan. If a designee performs this activity, a physician must be available to provide medical consultation;

15. **Section 7.1.2, Imaging Visits, ^{18}F-AV-1451 PET Imaging Visit, page 19**

Revised 7th bullet from:

- A physician will see the subject prior to discharge from the imaging center to evaluate the subject's readiness for discharge; and

To:

- A physician or a licensed/credentialed medical professional (i.e., a PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator must assess or evaluate the subject prior to discharge from the imaging center to evaluate the subject's readiness for discharge. If a designee performs this activity, a physician must be available to provide medical consultation; and

16. **Section 7.2, Observations and Measurements, page 20**

Changed "Day One study visit" to "Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan" under Vital Signs, Height and Weight, Electrocardiogram, and Clinical Laboratory Tests.

17. **Section 7.2, Observations and Measurements, Clinical Laboratory Tests, page 21**

Deleted amount of blood/urine collected: 3 mL EDTA, 5 mL blood and 10 mL urine.

Deleted "morphology" for Hematology and made "RBC morphology" optional.

Deleted "casts" for Urinalysis.

18. **Section 7.2, Observations and Measurements, MMSE, page 21**

Added:

"If the MMSE is not administered per the site's companion protocol, the MMSE will be administered as part of the Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan."

19. **Section 7.2, Observations and Measurements, Physician Visit, page 22**

Added "or a licensed/credentialed medical professional" after "physician".

20. **Section 7.6, Documentation, page 23**

Deleted "or to the designated imaging core lab" and replaced "DETECT" with "companion.

Same changes made to the following:

- page 30, Section 10.6, Data Collection

21. **Section 8.2, Safety Analysis, page 27**

First paragraph changed from:

"Safety laboratory test results and 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. Subjects whose laboratory values are outside the pre-determined upper and lower limits of normal will be identified and tabulated."

To:

"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."

22. **Section 8.2, Safety Analysis, page 28**

Deleted:

"Laboratory Data

Subjects whose laboratory values are outside threshold values will be identified and tabulated.

ECG

Any subjects showing QTc > 500 or change from baseline of more than 60 msec will be highlighted."

23. **Section 8.3, Image Analysis, page 28**

Changed “80 minutes” to “75 minutes” and deleted “and partial volume correction of ^{18}F-AV-1451 PET images using anatomical information from MRI data.”

24. **Section 9, Use of Data and Publication, page 29**

Changed last paragraph from:

“This is a single center study. A single center publication, reporting the primary analysis data set, should precede any other publications.”

To:

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

25. **Section 10.6, Data Collection, page 30**

Replaced “BU Data Coordinating Center” to “local site” and replaced “BU” with “these participating investigative centers”.

26. **Section 11.2, Trial Flow Chart, page 35**

Updates made to trial flow chart to reflect changes made in protocol amendment.

^{18}F-AV-1451-A07 Protocol Amendment 3

Summary of Changes from Protocol Amendment 2 dated 29 October 2014

1. Synopsis, Planned number of subjects (Enrolled), page 2

Changed from:

"Approximately 50 subjects (30 former NFL players believed to be at high risk of developing chronic traumatic encephalopathy [CTE] and 20 former non-contact athletes) will be recruited…"

To:

"Approximately 50 subjects (30 former NFL players believed to be at high risk of developing chronic traumatic encephalopathy [CTE] and 20 age-matched male subjects with no history of head trauma)…"

Same revision regarding control cohort made to the following:

- Synopsis, Study Design, page 3, third paragraph
- Synopsis, Statistical Methods, page 4
- Section 5.1, Overall Design and Plan of Trial, page 15, third paragraph
- Section 8.1, General Statistical Considerations, page 27, third and fifth paragraph

2. Table of Contents, pages 5-6

Table of Contents revised to reflect current page numbers.

3. Abbreviations and Definitions, page 8

Added:

LAR	Legally Authorized Representative

4. Section 5.3.1, Inclusion Criteria, page 16

Changed from:

3. Have the ability to provide informed consent for study procedures.

To:

3. Have the ability to provide informed consent, or have a Legal Authorized Representative (LAR), who can provide informed consent on behalf of the subject if allowed by the site's companion protocol, for study procedures.

5. Section 7.2, Observations and Measurements, Informed Consent, page 20

Added to paragraph one:

"If specified in the site's companion protocol, a LAR may consent on behalf of the subject as determined necessary by the investigator."

Same addition made to the following:

- Section 7.5, Informed Consent and Subject Information, page 23, paragraph one
- Section 10.2, Informed Consent, page 30

Protocol Number: ^{18}F-AV-1451-A07

^{18}F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy

Date and Version:
26 January 2015, Amendment 3

Name of Compound:
^{18}F-AV-1451 ([F-18]T807)

Sponsor:
Avid Radiopharmaceuticals
Philadelphia, Pennsylvania USA

Approvals/Signatures and Date:

_____

[redacted]
Chief Medical Officer

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451([F-18]T807)	[redacted]

Title of Study: ^{18}F-AV-1451-A07

"^{18}F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy"

Planned number of subjects (Enrolled):
Approximately 50 subjects (30 former NFL players believed to be at high risk of developing chronic traumatic encephalopathy [CTE] and 20 age-matched male subjects with no history of head trauma) will be recruited from the pool of participants who are participating in the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) study being conducted at Boston University or Long-Term Consequences of Repetitive Brain Injury in Athletes: A Longitudinal Study with Eventual Brain Donation study being conducted at Mayo Clinic College of Medicine, Banner Sun Health Research Institute and Banner Alzheimer's Institute.

Name of compound: ^{18}F-AV-1451([F-18]T807)
Dose: 370 MBq (10 mCi)
Route of Administration: Intravenous (IV) bolus

Study Phase: IIa

Study Centers: Up to 5 centers in the United States

Trial Objectives:

The primary objectives of this study are:

- To explore the use of ^{18}F-AV-1451 as a biomarker for CTE; and
- To examine the relationship between clinical presentation and tau deposition as measured by ^{18}F-AV-1451 uptake in subjects with high risk of CTE.

A secondary objective of this study is:

- To expand the ^{18}F-AV-1451 safety database.

Eligibility:

Only subjects duly consented and enrolled in the site's companion study protocol (either DETECT or Long-Term Consequences of Repetitive Brain Injury in Athletes) will be considered for participation in this study. (See Section 5.3, Selection of Subjects)

Study Design:

New subjects being enrolled in the site's companion protocol will be contacted to participate and must provide informed consent before starting any ^{18}F-AV-1451-A07 study procedures. Once enrolled, Day One/Screening study assessments will occur as defined in the companion protocol and should be completed prior to the florbetapir F 18 and ^{18}F-AV-1451 PET scans. Additionally,

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451([F-18]T807)	[redacted]

subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the Mini-Mental State Examination (MMSE) if MMSE is not administered per the site's companion protocol as part of the Day One/Screening companion protocol study assessments or at a study visit prior to the first PET scan (either florbetapir F 18 or ^{18}F-AV-1451).

Following Day One/Screening study assessments, subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur prior to the ^{18}F-AV-1451 PET scan. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.

Analyses will explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk with CTE and age-matched male subjects with no history of head trauma (controls).

Assessments and Endpoints:

Day One/Screening Study Assessments

Day One/Screening study assessments should be completed prior to the PET scans and will occur as defined in the site's companion protocol. Additionally, subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the Mini-Mental State Examination (MMSE) if MMSE is not administered per the site's companion protocol as part of the Day One/Screening companion protocol study assessments or at a study visit prior to the first PET scan (either florbetapir F 18 or ^{18}F-AV-1451).

Florbetapir F 18 PET Imaging Visit

For the florbetapir F 18 PET imaging visit, an intravenous catheter will be placed for IV administration of Florbetapir F 18 Injection. Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of Florbetapir F 18 Injection followed by a saline flush. A continuous 10-minute brain scan (10 frames of 1 minute duration) will begin immediately following the administration of Florbetapir F 18 Injection. Additionally, at approximately 50 minutes following injection, a continuous 10-minute brain scan (2 frames of 5 minute duration) will begin.

Adverse events will be continuously monitored during the florbetapir F 18 PET imaging session. A physician or physician designee must evaluate the subject for adverse events prior to injection and prior to discharge from the imaging center. Subjects who experience an adverse event will not be discharged from the imaging center until the event has resolved or stabilized.

^{18}F-AV-1451 PET Imaging Visit:

For the ^{18}F-AV-1451 PET imaging visit, an intravenous catheter will be placed for IV administration of ^{18}F-AV-1451 Injection. Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of ^{18}F-AV-1451 Injection followed by a saline flush. At approximately 75 minutes following injection, a continuous 30-minute brain scan (6 frames of 5 minute duration) will begin. If at any point during the imaging session it is determined that the subject is not able to

Sponsor:	**Name of Compound:**	**Active Ingredient(s):**
Avid Radiopharmaceuticals	^{18}F-AV-1451([F-18]T807)	[redacted]

continue, or that it is not in the best interest of the subject to continue, imaging will be discontinued. The image data that has been collected up to that point will be analyzed. Safety assessments will be conducted prior to injection and upon completion of the imaging session.

Adverse events will be monitored continuously during the ^{18}F-AV-1451 imaging session. A physician or a licensed/credentialed medical professional (i.e., a PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator must assess or evaluate the subject for adverse events prior to injection and prior to discharge from the imaging center. If a designee performs this activity, a physician must be available to provide medical consultation. Subjects who experience any adverse event during an imaging session will not be discharged until the event has resolved or stabilized.

Follow-Up Phone Call:

A follow-up phone call to the subject, or designated decision maker, will be conducted between 2 or 3 business days of the last 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.

Details of additional assessments that will be performed at each visit are detailed in Section 7.1.

Statistical Methods:

Descriptive Statistics will be applied to describe the ^{18}F-AV-1451 uptake and florbetapir SUVR distribution by subjects at risk with CTE and age-matched male subjects with no history of head trauma (controls). Two sample t-test or Wilcoxon rank sum test when the pre-requisite of t-test is not met, will be applied to test if there are difference of the ^{18}F-AV-1451 uptake and florbetapir SUVR between CTE and control groups. Additional exploratory analyses will be applied to explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk for CTE and controls.

TABLE OF CONTENTS **Page**

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.
Audit	A systematic and independent examination of the trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data were recorded, analyzed, and accurately reported according to the protocol, applicable standard operating procedures (SOPs), good clinical practice (GCP), and the applicable regulatory requirement(s).
Case Report Form (CRF) and electronic Case Report Form (eCRF)	A printed or 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
CTE	Chronic Traumatic Encephalopathy
DETECT	Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests study (also known as R01NS078337 grant)
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
MHD	Maximum Human Dose
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury
NOAEL	No Observable Adverse Effect Level
PET	Positron Emission Tomography
RBT	Repetitive Brain Trauma
SUVR	Standard Uptake Value Ratio

1. INTRODUCTION

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease marked by widespread accumulation of hyperphosphorylated tau (p-tau), predominantly as neurofibrillary and astrocytic tangles (McKee et al., 2009, 2013). In contrast to Alzheimer's disease (AD), however, CTE does not involve beta amyloid (Aβ) neuritic plaque deposition. CTE ultimately leads to dementia and is believed to be caused, in part, by repetitive mild traumatic brain injury (mTBI), including concussive and subconcussive impacts. CTE has been found most often in professional athletes involved in contact sports (e.g. boxing, American football) who have been subjected to repetitive brain trauma (RBT), including mTBI or even asymptomatic, subconcussive trauma.

CTE, like most neurodegenerative diseases, can only be definitively diagnosed post-mortem. Neuropathological findings have demonstrated that this tauopathy has a unique profile of neurodegeneration and tau deposition that is distinct from AD and other neurodegenerative diseases. To facilitate future CTE research, objective *in vivo* biomarkers must first be discovered.

A currently-funded [redacted] will focus on a sample of subjects who are believed to be at high risk of developing CTE and compare them with an age matched control cohort on several potential biomarkers for CTE, selected to detect key variables associated with their hypothesized mechanism for the pathogenesis of CTE, based on their previous neuropathological studies, pilot neuroimaging data collected by their team, and other findings by their team and in the literature regarding the long-term effects of repetitive mTBI. In addition, the relationship among possible biomarkers and pertinent clinical variables, including neurologic, motor, neuropsychological and psychiatric evaluations will be assessed in the "probable CTE" group.

Additionally, a similar trial is being funded by the Arizona Alzheimer's Consortium called Long-Term Consequences of Repetitive Brain Injury in Athletes: A Longitudinal Study with Eventual Brain Donation. This study aims to examine the relationship between head impact exposure and later life neurological consequences, as well as genetic modifiers of this relationship. This is a pilot project designed to develop preliminary data.

However, a potentially useful biomarker of this tauopathy would be a PET radiotracer that could specifically bind to paired helical filament (PHF) tau. Currently, molecular imaging biomarkers have the potential to aid in the diagnosis of patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) (Dubois, 2010; McKhann, 2011). Positron emission tomography (PET) ligands such as florbetapir F 18 (Wong, 2010) may provide a minimally invasive estimate of cortical Aβ neuritic plaque deposition, a hallmark pathology, and a required element for the evaluation of AD neuropathologic diagnosis (Hyman 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 (Duyckaerts et al., 1987; Brack et al., 2011; Nelson et al., 2012).

^{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). 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 [redacted]

Note: this section of the protocol contained nonclinical pharmacology and toxicology information that was current at the time the protocol was written is out of date at the time of this publication. For up to date information consult the flortaucipir investigator's brochure, available from Avid/Eli Lilly

[redacted]

[redacted]

^{18}F-AV-1451 may be useful as a marker of tau pathology in patients with AD and other neurodegenerative disorders (Figures 1 and 2). Several preliminary studies using ^{18}F-AV-1451 have been completed (e.g., Chien et al., 2013). Based on this rationale, the goal of this protocol is to perform ^{18}F-AV-1451 PET imaging on subjects enrolled in the DETECT or Long-Term Consequences of Repetitive Brain Injury in Athletes companion study protocols and explore its potential as a biomarker for CTE.

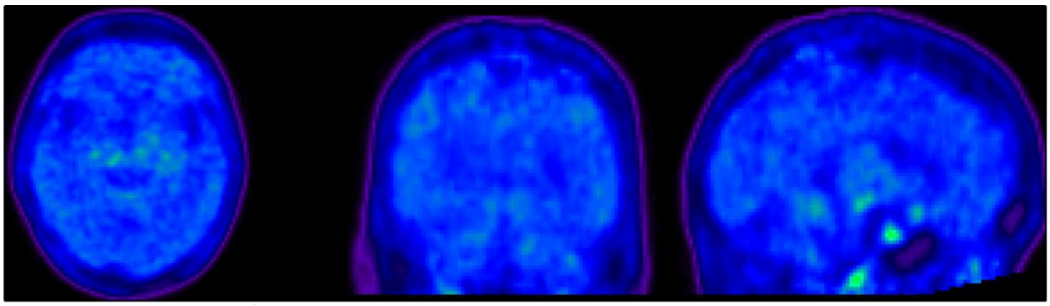


Figure 1: 58 year old female control subject (MMSE = 29)

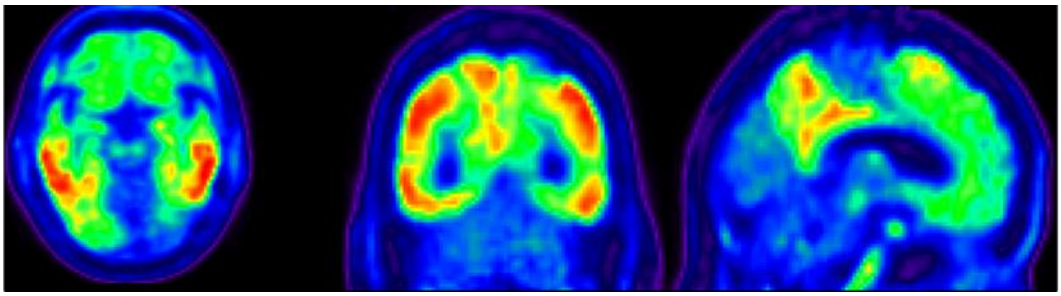


Figure 2: 64 year old male AD subject (MMSE = 18)

2. TRIAL OBJECTIVES

The primary objectives of this study are:

- To explore the use of ^{18}F-AV-1451 as a biomarker for CTE; and
- To examine the relationship between clinical presentation and tau deposition as measured by ^{18}F-AV-1451 uptake in subjects with high risk of CTE.

A secondary objective of this study is:

- To expand the ^{18}F-AV-1451 safety database.

3. SPONSOR, INVESTIGATOR(S) AND OTHER PARTICIPANTS

The trial is sponsored by:

Avid Radiopharmaceuticals

[redacted]

The medical contact is:

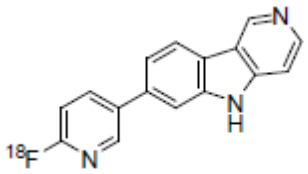
[redacted]

Up to 5 centers in the United States will participate.

4. TEST DRUG AND CONTROL AGENTS

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

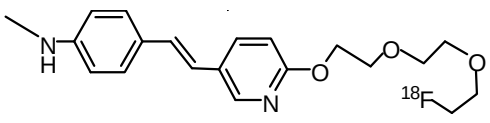
[redacted]



MW = 262.27 amu

4.2 Descriptive Name: Florbetapir F 18

[redacted]



^{18}F-AV-45

MW= 359.4 amu

4.3 Radioactive Labeling

[redacted]

4.4 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.

4.5 Formulation and Dose Florbetapir F 18 Injection

[redacted] Subjects will receive a single IV administration of approximately 370 MBq (10 mCi) of Florbetapir F 18 Injection immediately prior to imaging. [redacted]

Florbetapir F 18 Injection will be supplied from manufacturing facilities approved for commercial distribution under NDA 202-008.

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

^{18}F-AV-1451 Injection is a clear solution containing ^{18}F-AV-1451 (drug substance) formulated for intravenous bolus administration. Depending on the manufacturer, ^{18}F-AV-1451 Injection will be formulated in:

[redacted]

Drug product of either formulation is manufactured to meet one common set of specifications.

The expiration time and date of ^{18}F-AV-1451 Injection are provided on the outer label of each dose based on specific activity or strength. ^{18}F-AV-1451 Injection should be stored at room temperature.

4.7 Packaging Florbetapir F 18 Injection

[redacted]

4.8 Packaging ^{18}F-AV-1451 Injection

[redacted]

4.9 Storage and Handling Florbetapir F 18 Injection

Florbetapir F 18 Injection is stored at 25ºC; excursions permitted to 15-30ºC. The product does not contain a preservative. Florbetapir F 18 Injection should be stored within the original container or equivalent radiation shielding. Florbetapir F 18 Injection must not be diluted.

4.10 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. ^{18}F-AV-1451 Injection must not be diluted.

5. INVESTIGATIONAL PLAN

5.1 Overall Design and Plan of Trial

New subjects being enrolled in the site's companion protocol will be contacted to participate and must provide informed consent before starting any ^{18}F-AV-1451-A07 study procedures. Once enrolled, Day One/Screening study assessments will occur as defined in the companion study protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Additionally, subjects will undergo safety assessments (vital signs, safety labs and ECG) and be administered the MMSE if MMSE is not administered per the site's companion protocol as part of the Day One/Screening companion protocol study assessments or at a study visit prior to the first PET scan (either florbetapir F 18 or ^{18}F-AV-1451).

Following Day One/Screening study assessments, subjects will receive ^{18}F-AV-1451 and florbetapir F 18 on two separate days. It is preferable that the florbetapir F 18 PET scan occur prior to the ^{18}F-AV-1451 PET scan. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.

Analyses will explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk with CTE and age-matched male subjects with no history of head trauma (controls).

5.2 Planned Dosage and Duration of Treatment

5.2.1 Dosage and Administration

Florbetapir F 18:

Subjects will receive a single IV bolus administration of approximately 370 MBq (10 mCi) of Florbetapir F 18 Injection.

^{18}F-AV-1451:

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

5.2.2 Rationale for Dosages

^{18}F-AV-1451 will be administered IV in a radioactive dose of 370 MBq with a maximum human mass dose (MHD) limited to 20 µg of compound by weight. 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 8.92 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 preliminary human studies. No treatment related adverse events have been reported using this regimen.

5.3 Selection of Subjects

5.3.1 Inclusion Criteria

Subjects who meet all of the following criteria are eligible to enroll in this trial:

1. Male subjects consented and currently enrolled in the site's companion protocol;
2. Can tolerate up to two PET imaging sessions (one with ^{18}F-AV-1451 and one with florbetapir F 18); and
3. Have the ability to provide informed consent, or have a Legal Authorized Representative (LAR), who can provide informed consent on behalf of the subject if allowed by the site's companion protocol, for study procedures.

5.3.2 Exclusion Criteria

Subjects will be excluded from enrollment if they:

1. Are claustrophobic or otherwise unable to tolerate the imaging procedure (use of mild sedatives are permitted to manage claustrophobia);

2. Have current clinically significant cardiovascular disease or clinically significant abnormalities on screening ECG (including but not limited to QTc>450 msec);

3. A history of additional risk factors for Torsades de Pointes (TdP) (e.g., heart failure, 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);

4. Have a current clinically significant infectious disease, endocrine or metabolic disease, pulmonary, renal or hepatic impairment, or cancer that the investigator believes would affect study participation or scan results;

5. Do not agree to refrain from sexual activity or to use reliable contraceptive methods for 90 days following administration of ^{18}F-AV-1451 Injection;

6. Have had a non-study related radiopharmaceutical imaging or treatment procedure within 7 days prior to the ^{18}F-AV-1451 imaging session or florbetapir F 18 imaging session; and

7. In the opinion of the investigator, are otherwise unsuitable for a study of this type.

5.4 Prior and Concomitant Therapy

Except as noted below, 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, including investigational 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 on the Concomitant Medication Page of the Case Report Form (CRF).

Subjects who are taking drugs that are known to cause QT-prolongation may not be enrolled in the study (a list of prohibited and discouraged medications is provided by the Sponsor).

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 SAE occurs. The date and reason for discontinuation should be noted on the CRF. Subjects who discontinue prematurely should be seen for a final evaluation.

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, or changes in business priorities. 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 investigator may 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. WARNINGS/PRECAUTIONS

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. However, because ^{18}F-AV-1451 Injection is in the early stages of 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, males enrolled in this study must agree to refrain from sexual activity or use adequate contraceptive methods for 90 days after administration of ^{18}F-AV-1451 Injection.

7. PROCEDURES AND METHODS

7.1 Assessment Periods (See Section 11.2, Trial Flow Chart)

The study will consist of the following sequence of activities:

7.1.1 Day One/Screening Study Assessments:

New subjects being enrolled in the site's companion protocol will be contacted to participate. Day One/Screening study assessments will occur as defined in the companion study protocol and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Additional study assessments are as follows and should be completed as part of Day One/Screening companion protocol study assessments or at a study visit prior to the first PET scan (either florbetapir F 18 or ^{18}F-AV-1451):

- Informed Consent will take place before any ^{18}F-AV-1451-A07 study procedures;
- Medical history;
- Safety assessments: Vital signs (pulse rate, respiratory rate, supine blood pressure, height and weight), ECG (with results reviewed prior to dose administration), and safety labs (hematology, chemistry and urinalysis);
- MMSE (if not administered per the site's companion protocol); and
- A physician will see the subject during these study assessments.

7.1.2 Imaging Visits

It is preferable that the florbetapir F 18 PET scan occur prior to the ^{18}F-AV-1451 PET scan. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET

imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.

Florbetapir F 18 PET Imaging Session

- A physician or physician designee must see the subject prior to administration of Florbetapir F 18 Injection to determine if they are still suitable to undergo the scan;
- If ^{18}F-AV-1451 Injection has been previously administered, confirm subject well-being and collect information about any new adverse events;
- Vital signs will be taken (pulse rate, respiratory rate, supine blood pressure and weight) immediately prior to injection of florbetapir F 18;
- Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of florbetapir F 18 followed by a saline flush. A continuous 10-minute brain scan (10 frames of 1 minute duration) will begin immediately following the administration of Florbetapir F 18 Injection;
- At approximately 50 minutes following injection, a continuous 10-minute brain scan (2 frames of 5 minute duration) will begin;
- Adverse events will be continuously monitored during the Florbetapir F 18 PET imaging visit. Subjects who experience an adverse event will not be discharged from the imaging center until the event has resolved or stabilized;
- A physician or physician designee will see the subject prior to discharge from the imaging center to evaluate the subject's readiness for discharge; and
- If the florbetapir F 18 imaging visit occurs second, a follow-up phone call to the subject, or designated decision maker, 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.

^{18}F-AV-1451 PET Imaging Visit

- A physician or a licensed/credentialed medical professional (i.e., a PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator must assess or evalaute the subject prior to administration of ^{18}F-AV-1451 Injection to determine if they are still suitable to undergo the scan. If a designee performs this activity, a physician must be available to provide medical consultation;
- If florbetapir F 18 Injection has been previously administered, confirm subject well-being and collect information about any new adverse events;

- Vital signs will be taken (pulse rate, respiratory rate, supine blood pressure) at the following time points:
  - Immediately prior to administration of ^{18}F-AV-1451 Injection (Weight and Temperature will also be collected)
  - Within 5 minutes after completion of injection of ^{18}F- AV-1451 Injection
  - After completion of the PET scan prior to discharge. (Temperature will also be collected);
- Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of ^{18}F-AV-1451 Injection followed by a saline flush. At approximately 75 minutes following injection, a continuous 30-minute brain scan (6 frames of 5 minute duration) will begin;
- The injection site will be observed for excessive inflammation or damage to the surrounding tissue where the dose was injected;
- Adverse events will be continuously monitored during the ^{18}F-AV-1451 PET imaging visit. Subjects who experience an adverse event will not be discharged from the imaging center until the event has resolved or stabilized;
- A physician or a licensed/credentialed medical professional (i.e., a PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator must assess or evaluate the subject prior to discharge from the imaging center to evaluate the subject's readiness for discharge. If a designee performs this activity, a physician must be available to provide medical consultation; and
- If the ^{18}F-AV-1451 imaging visit occurs second, a follow-up phone call to the subject, or designated decision maker, 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.

7.2 Observations and Measurements

Informed Consent

Potential subjects 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 subject. The subject 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 (see Section 7.5). If specified in the site's companion protocol, a LAR may consent on behalf of the subject as determined necessary by the investigator. A copy of the signed informed consent will be given to the subject.

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

Medical History

The investigator or designee will obtain an updated history at the screening visit.

- Relevant demographic information
- Review of body systems
- Social history
- Medical and surgical history, including medical care for head trauma
- Concurrent medications

Whenever possible, the medical history will be confirmed by medical records.

Vital Signs

Vital signs (pulse rate, respiratory rate, supine blood pressure) will be taken as part of the Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan and at the following time points:

- Florbetapir F18 Imaging Visit
  - immediately prior to injection of florbetapir F 18
- ^{18}F-AV-1451 Imaging Visit
  - Immediately prior to the administration of ^{18}F-AV-1451 Injection
  - Within 5 minutes after completion of injection of ^{18}F-AV-1451 Injection
  - After the completion of imaging prior to discharge.

Temperature will also be obtained at the following time point:

- ^{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 both the Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan and imaging visits (immediately prior to ^{18}F-AV-1451 and florbetapir F 18 administration) body weight will be measured, lightly clothed. Height will only be measured as part of the Day One/Screening companion protocol study visit or at a visit prior to the first PET scan.

Electrocardiogram (ECG)

A resting 12-lead electrocardiogram will be recorded as part of the Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan.

Clinical Laboratory Tests

Clinical laboratory evaluation will be performed at the site's local laboratory as part of the Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan.

Tests will include:

- **Hematology**: hemoglobin, hematocrit, RBC, WBC, MCH, MCHC, neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, platelets, and MCV. Optional: RBC morphology

- **Chemistry**: total bilirubin, alkaline phosphatase, ALT (SGPT), AST (SGOT), urea nitrogen, creatinine, glucose, uric acid, calcium, phosphorus, total protein, albumin, total cholesterol, triglycerides, sodium, potassium, bicarbonate, chloride, magnesium, globulin, GGT.

- **Urinalysis**: Samples will be used to assess glucose, RBC, WBC, specific gravity, pH, protein, ketones, urobilinogen, blood, nitrite, microscopic, color, bilirubin, epithelial cells, leukocyte esterase, and bacteria.

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.

If the MMSE is not administered per the site's companion protocol, the MMSE will be administered as part of the Day One/Screening companion protocol study visit or at a study visit prior to the first PET scan.

Physician Visit

A physician or a licensed/credentialed medical professional must see the subject at baseline, prior to drug administration and at study end, prior to discharge from the ^{18}F-AV-1451 imaging session. A physician or physician designee must see the subject at baseline, prior to drug administration and at study end, prior to discharge from the florbetapir F 18 imaging session. At discharge, the physician or a licensed/credentialed medical professional (or designee for the florbetapir F 18 imaging visit) should review all safety data and briefly examine/query the subject regarding potential adverse events or other treatment issues.

7.3 Protocol for Image Collection

The sponsor will prepare and distribute imaging manuals for ^{18}F-AV-1451 and florbetapir F 18 image acquisition parameters and transmission procedures prior to site initiation.

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 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 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 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. If the subject is incapable of giving informed consent then the subject will be removed from the study unless the site's companion protocol allows a LAR to consent on behalf of the subject as determined necessary by the investigator.

All informed consent forms must be approved by Avid or designee, and by the appropriate Institutional Review Board (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 patient for their records.

7.6 Documentation

^{18}F-AV-1451 and florbetapir F 18 PET scans will be saved in an appropriate electronic format as specified in the imaging manuals. A copy of all scans, including the MRI/MRS scans conducted per companion study protocol, will be saved at the site/imaging center and a copy of each will be forwarded to the sponsor as described in the imaging manuals. All other data required by the protocol will be recorded in the eCRFs. All data in the eCRFs will be substantiated by "source documents," which consist of the subject's medical files, laboratory result sheets, ECG tracings, etc. All source documentation must be available to Avid and designees. Completed source documents and eCRFs may need to be made available and complete for an audit by the FDA or other international regulatory authorities or Avid at any time. CRFs 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 eCRFs. Investigators will be instructed to report to Avid or its designee their assessment of the potential relatedness of each AE to study drug or protocol procedure via electronic data entry. If a patient'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 patient, 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").

Laboratory test abnormalities considered by the Investigator to be clinically relevant should be reported on the adverse event eCRFs. Signs and symptoms of each AE should be described in detail (e.g., start and stop dates/time, severity/intensity, relationship to study drug, action taken, and outcome). Additionally, any clinically significant findings from laboratory evaluations, 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 patient must be carefully monitored for adverse events. This includes clinical laboratory test variables. An assessment must be made of the severity/intensity and relationship to the administration of the study drug.

7.7.2 Adverse Event Definitions

<u>Adverse Events</u>

An adverse event is any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the study drug.

For reporting purposes, Avid will distinguish among pre-existing conditions, trial-emergent adverse events and treatment-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 eCRF pages. During the study, site personnel will record any change in the condition(s) and occurrence and nature of any AEs. Signs and symptoms that are believed to be due to the pre-existing condition under study (started prior to dose of study medication) do not have to be recorded in the AEs section of the eCRF, unless there is an increasing in frequency and severity.

Trial-emergent adverse events are undesirable experiences, signs or symptoms that begin, or worsen in intensity or frequency, after the informed consent, and prior to administration of the first study drug (either florbetapir F 18 or ^{18}F-AV-1451) at the imaging visit. These will be recorded on the adverse event eCRFs.

Treatment-emergent adverse events are undesirable experiences, signs, or symptoms associated with the use of a study drugs. For the purposes of this study an adverse event will be considered associated with the use of ^{18}F-AV-1451 or Florbetapir F18 Injection if it begins or worsens in intensity or frequency within 24 hours after the administration of the respective drug. Adverse experiences that occur after administration of study drug but outside the 48 hour reporting window will not be reported unless the investigator believes they are attributable to the drug.

The end of study for the purpose of adverse event reporting is defined as 48 hours after the administration of ^{18}F-AV-1451 Injection or Florbetapir F 18 Injection or the time of the next visit in the case that florbetapir F 18 imaging visit occurs first.

<u>Serious Adverse Event (SAE)</u>

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 patient 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 Study Drug

Investigators will be instructed to report their assessment of the potential relatedness of each adverse event to protocol procedure or study drug. The assessment of the relationship of an adverse event to the administration of the study drug 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 study drug 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 eCRFs. Documentation must be supported by an entry in the subject file. Laboratory test, vital signs and ECG abnormalities considered by the Investigator to be clinically relevant should be reported on the adverse event eCRFs. Signs and symptoms of each AE should be described in detail (e.g., start and stop dates, severity/intensity, relationship to study drug, action taken, and outcome).

Adverse events and laboratory test abnormalities 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 receive a dose of study drug will be collected until 48 hours after the dosing of the study drug, regardless of the investigator's opinion of causation. Therefore, SAEs that occur later than 48 hours after the dosing of the study drug are not required to be reported unless the investigator feels the events were related to either study drug or a protocol procedure.

If a patient experiences an SAE after signing informed consent, but prior to receiving study drug, the event will NOT be reported unless the investigator feels the event may have been caused by a protocol procedure. 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 study data collected under the companion protocol, such as but not limited to subjects' demographic and baseline characteristics, history taking, CSF, portable EEG, neurological and motor, neuropsychological, psychiatric evaluations, and MRI will be transferred to Avid for analysis purpose.

Data will be summarized using descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, quartiles, minimum, and maximum) for continuous variables and using frequency count and percentage for discrete variables. The demographic and baseline characteristics data will be summarized for all subjects in the safety population according to clinical group (subjects at risk with CTE and age-matched male subjects with no history of head trauma [controls]). Safety data will be summarized for all patients.

Subject listings of all data from the electronic case report forms (eCRFs) as well as any derived variables will be presented.

Descriptive Statistics will be applied to describe the ^{18}F-AV-1451 uptake and florbetapir SUVR distribution by subjects at risk with CTE and age-matched male subjects with no history of head trauma (controls). Two sample t-test or Wilcoxon rank sum test when the pre-requisite of t-test is not met, will be applied to test if there are difference of the ^{18}F-AV-1451 uptake and florbetapir SUVR between CTE and control groups. Additional exploratory analyses will be applied to explore the relationship among ^{18}F-AV-1451 uptake measurements, florbetapir SUVR, cognitive and neuropsychiatric measurements, and other collected biomarkers in subjects at risk for CTE and controls.

Additional details concerning statistical analyses will be included in the Statistical Analysis Plan (SAP) to be completed prior to the end of enrollment into the study.

8.2 Safety Analysis

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.

8.3 Image Analysis

All ^{18}F-AV-1451 PET images obtained starting at 75 minutes post injection will be analyzed. The ^{18}F-AV-1451 PET images will be spatially normalized to standard stereotactic atlas space using MNI brain atlas. The uptake in tau protein rich brain regions will be assessed with regions of interest (ROI, designed in MNI brain atlas) in terms for standard uptake value ratio (SUVR, normalized by cerebellar uptake). The spatially normalized images and the measured SUVR values will be used to accordingly for the following objectives.

Mean, standard deviation, minimum and maximum of the SUVR values for all groups will be calculated.

Exploratory analysis will include co-registration of MRI to ^{18}F-AV-1451 PET images.

Florbetapir F 18 images will be analyzed as described previously (Clark et al. 2011, 2012).

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 analysis will include data from all centers. A multi-center publication, reporting the primary analysis 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 or international regulatory authorities) at any time.

10.1 Institutional Review Board (IRB)

The intent of the research program, the trial protocol, the patient 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 must be obtained from each patient or the patient's LAR, if allowed by the site's companion protocol. A copy of the signed informed consent should be given to the patient 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 patient 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 florbetapir F 18 or ^{18}F-AV-1451.

10.5 Study Drug Control

The receipt of clinical supplies (i.e. starting material for ^{18}F-AV-1451) 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 Injection 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

Researchers would like to save and store all of the information gathered from each subject in this study (as well as the companion study) for future studies about the effects of repetitive head trauma. Information from the companion study will be coded with a patient number, not the subject's name, and kept in a secure database maintained by the local site and for this protocol, a separate secure database maintained by Avid Radiopharmaceuticals. Scientists at these participating investigative centers and researchers at other places would be able to use the subject's information for future research on the effects of repetitive head trauma. If, in the future subjects decide that they don't want the researchers to keep their information, the subjects could let these participating investigative centers know and the information would be discarded.

Electronic case report forms (eCRFs) will be used for this trial. Individual patient files should include appropriate source documents, including but not limited to patient's medical records and laboratory test results. 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 Avid as specified in the Imaging Manual.

Each patient'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.

All original laboratory reports must be available for review in each patient's file. It is important that the original reports be available for review because of the possibility of inaccuracies or errors in transcribing data from original records to the eCRF.

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 patient enrolled in the trial and signed by the investigator. This should be done as soon as possible after completion of the patient's participation in the trial. 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 2 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 2 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	Day One/Screening Study Assessments[a]	Florbetapir F 18 Imaging Visit[b]	End of Florbetapir F 18 Imaging (prior to discharge)	^{18}F-AV- 1451 Imaging Visit[b]	End of ^{18}F-AV- 1451 Imaging (prior to discharge)	Follow-up Phone Call[j]
Signed Informed Consent	X					
Medical History	X					
ECG	X					
Vital Signs	X[c]	X[d]		X [d,e]	X[f]	
Clinical Lab Tests (safety)	X					
MMSE	X[g]					
PET Brain Scan		X		X		
Evaluation by a physician	X	X[h]	X[h]	X[i]	X[i]	
Adverse Events	X	X	X	X	X	X
Serious Adverse Events	X	X	X	X	X	X

a. Day One/Screening study assessments will occur as defined in the companion study protocol (with the addition of safety assessments and MMSE listed in this protocol) and should be completed prior to the florbetapir F18 and ^{18}F-AV-1451 PET scans. Assessments checked in this column can also be performed at a study visit prior to the first PET scan (either florbetapir or ^{18}F-AV-1451).
b. It is preferable that the florbetapir F 18 PET scan occur prior to the ^{18}F-AV-1451 PET scan. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the two PET imaging sessions must be performed at least 48 hours apart unless discussed and approved by Sponsor.
c. Include pulse rate, respiratory rate, supine blood pressure, height and weight.
d. Vital signs (pulse rate, respiratory rate, supine blood pressure and weight) and temperature (for the ^{18}F-AV-1451 imaging visit) will be taken immediately prior to injection.
e. Vital signs (pulse, respiratory rate, and supine blood pressure) within 5 minutes after completion of injection of ^{18}F-AV-1451 Injection.
f. Vital signs (pulse, respiratory rate, supine blood pressure, and temperature).
g. If not administered per the site's companion protocol.
h. Or physician designee.
i. Or a licensed/credentialed medical professional (i.e. PET technologist, imaging center nurse or a regional equivalent) designated by the site principal investigator. If a designee performs this activity, a physician must be available to provide medical consultation.
j. Will be conducted between 2 or 3 business days of the last 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.

INVESTIGATOR'S AGREEMENT TO PROTOCOL

Protocol ^{18}F-AV-1451-A07: "^{18}F-AV-1451 and Florbetapir F 18 PET Imaging in Subjects with Repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy"

Date and Version: 26 January 2015, Amendment 3

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

TEMPLATE REVISON HISTORY

DATE	VERSION	AUTHOR/ UPDATED BY	COMMENTS
29-Oct-2015	0.1	[redacted]	
21-Dec-2015	0.2	[redacted]	
20-Jan-2016	1.0	[redacted]	

Statistical Analysis Plan

Protocol No.: ^{18}F-AV-1451-A07 (Amendment 3)

^{18}F-AV-1451-A07 and Florbetapir F 18 PET Imaging in Subjects with repetitive Brain Trauma at High Risk for Chronic Traumatic Encephalopathy

Sponsor:	Avid Radiopharmaceuticals Philadelphia, Pennsylvania USA
Version:	Final, Version 1.0
Issue Date:	20 January 2015

STATISTICAL ANALYSIS PLAN APPROVAL

Author:

_____ Date: _____/_____/_____

[redacted]
Biostatistician, Biostatistics, FMD K&L Inc.

Reviewed and approved by*:*

_____ Date: _____/_____/_____

[redacted]
Sr. Director, Biostatistics, FMD K&L Inc.

Sponsor Review:

_____ Date: _____/_____/_____

[redacted]
Director, Statistics
Avid Radiopharmaceuticals Inc.

_____ Date: _____/_____/_____

[redacted].
Vice President, Clinical Development
Avid Radiopharmaceuticals Inc.

_____ Date: _____/_____/_____

[redacted]
Senior Medical Director, Clinical Development
Avid Radiopharmaceuticals Inc.

_____ Date: _____/_____/_____

[redacted]
Vice President, Imaging
Avid Radiopharmaceuticals Inc.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Term
AD	Alzheimer's disease
AE	adverse event
ANOVA	analysis of variance
Aβ	Beta amyloid
BMI	body mass index
CI	confidence interval
CNS	Central Nervous System
CT	Computed Tomography
CTE	Chronic Traumatic Encephalopathy
FDA	US Food and Drug Administration
FDG	^{18}F - Fluorodeoxyglucose
IV	Intravenous
Kd	Dissociation Constant
MBq	Megabecquerel
mCi	Millicurie
MedDRA	Medical Dictionary for Regulatory Activities
MHD	Maximum Human Dose
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury
PET	Positron Emission Tomography
RBT	Repetitive Brain Trauma
SAE	serious adverse event
SAP	statistical analysis plan
SUVR	Standard Uptake Value Ratio
TEAE	treatment-emergent adverse event
TLF	tables, listings, and figures
WHO	World Health Organization

1. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures and the statistical methods that will be used to analyze and report results for Protocol ^{18}F-AV-1451-A07 (Amendment 3).

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective(s)

The primary objectives of this study were:

- To explore the use of ^{18}F-AV-1451 as a biomarker for chronic traumatic encephalopathy (CTE);
- To examine the relationship between clinical presentation and tau deposition as measured by ^{18}F-AV-1451 uptake in subjects with high risk of CTE.

1.1.2 Secondary Objective(s)

The secondary objective of this study was to expand the ^{18}F-AV-1451 safety database.

1.2 STUDY ENDPOINTS

1.2.1 Efficacy Endpoint(s)

- Florbetapir F 18 PET results will be recorded as a binary visual interpretation, i.e. "amyloid positive" or "amyloid negative". Readers will also note any focal regions of uptake
- The overall brain uptake score for ^{18}F-AV-1451will be recorded as an ordinal value with one of the four levels, i.e. 0= "No uptake", 1="Mild uptake", 2="Moderate uptake", and 3="Intense uptake".
- Mean/median brain uptake score of 18F-AV-1451 and Florbetapir F 18 will be compared between study groups (CTE high risk vs. Control)

1.2.2 Safety will be assessed on the basis of the following:

- Adverse events (AEs)
- Vital Signs (temperature, pulse rate, respiratory rate, and supine blood pressure)
- Clinical laboratory parameters (chemistry, hematology, and urinalysis)
- 12 lead ECG

1.3 SUMMARY OF THE STUDY DESIGN

1.3.1 General Study Design and Plan

The purpose of the study was to explore the potential of ^{18}F-AV-1451, i.e. a PET radiotracer that could specifically bind to paired helical filament (PHF) tau, as a biomarker for tauopathy in CTE. Two groups of subjects were enrolled to the study, i.e. Group 1, former NFL players (male) who were hypothesized to have high risk of developing CTE, and Group 2, age-matched male subjects with no history of head trauma (controls). These subjects were from the pool of participants enrolled in the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) or Long-Term Consequences of Repetitive Brain Injury in Athletes companion study conducted at Mayo Clinic College of medicine, Banner Sun Health Research Institute and Banner Alzheimer's Institute.

Once enrolled, on Day One/Screening, all subjects received study assessments, including medical history assessment, safety assessment including vital signs, safety labs, ECG, adverse events and physician evaluation, and Mini-Mental State Examination (MMSE).

Following Day One/Screening study assessments, all subjects received ^{18}F-AV-1451 labeled or Florbetapir F 18 labeled PET imaging on two separate days. Florbetapir F 18 is a different PET radiotracer that could specifically bind to cortical Aβ neuritic plaque deposition, a hallmark for Alzheimer's disease (AD). It was preferable that the Florbetapir F 18 PET scan occurred prior to the ^{18}F-AV-1451 PET scan. The two PET imaging sessions must be performed at least 12 hours apart. If the ^{18}F-AV-1451 imaging visit occurs first, the Florbetapir F 18imaging must be performed at least 48 hours later unless discussed and approved by Sponsor. For Florbetapir F 18 labeled scan, two imaging sessions were performed, i.e. a continuous 10-minute brain scan (10 frames of 1 minute duration) immediately following the IV and a continuous 10-minute brain scan (2 frames of 5 minute duration) at approximately 50 minutes after the IV. For ^{18}F-AV-1451 labeled scan, a single imaging session was performed, i.e. a continuous 30-minute brain scan (6 frames of 5 minute duration) at approximately 75 minutes following the IV. A professional PET scan reader who was masked to the study groups evaluated the imaging and assigned an overall brain uptake score for each radiotracer. Safety assessments were also performed on the imaging day. A follow-up phone call was conducted between 2 or 3 business days of the last imaging day (after 48 hours post-injection) to confirm subject well-being and to collect any new adverse events.

The PET imaging results of ^{18}F-AV-1451 and Florbetapir F 18 will be compared between the two groups of subjects, along with the correlation analysis with cognitive assessment (MMSE), to evaluate the performance of ^{18}F-AV-1451 as a potential biomarker for CTE.

The study design is depicted in Figure 1 below.

Evaluations	Day One/Screening Study Assessments[a]	Florbetapir F 18 Imaging Visit[b]	End of Florbetapir F 18 Imaging (prior to discharge)	^{18}F-AV- 1451 Imaging Visit[b]	End of ^{18}F-AV- 1451 Imaging (prior to discharge)	Follow-up Phone Call[j]
Signed Informed Consent	X					
Medical History	X					
ECG	X					
Vital Signs	X[c]	X[d]		X[d,e]	X[f]	
Clinical Lab Tests (safety)	X					
MMSE	X[g]					
PET Brain Scan		X		X		
Evaluation by a physician	X	X[h]	X[h]	X[i]	X[i]	
Adverse Events	X	X	X	X	X	X
Serious Adverse Events	X	X	X	X	X	X

Figure 1 Study Diagram

1.3.2 Randomization and Blinding

There was no randomization performed in this study.

A blind visual reading was performed in evaluating the PET scan to avoid bias, i.e. the expert reader was blinded to the subject's group and other clinical assessments while evaluating the scan images.

1.3.3 Sample Size and Statistical Power Considerations

Approximately fifty male subjects were planned to participate in the study, including:

- Group 1: thirty former NFL players believed to be at high risk of developing CTE.
- Group 2: twenty age-matched male subjects with no history of head trauma.

This sample size is based on clinical considerations rather than a priori statistical requirements.

2. STATISTICAL METHODS

2.1 GENERAL CONSIDERATIONS

In general, continuous variables will be summarized by number of subjects, mean, standard deviation, median, minimum, and maximum values. Categorical variables will be summarized by number and percentage of subjects in each category. If applicable, hypothesis testing will be carried out at the two-sided α=0.05 level unless otherwise specified; 2-sided 95% confidence intervals (CIs) will be presented, where specified.

In general, summary tables will present data by study group and overall, where appropriate. Source data for the summary tables and statistical analyses will be presented as subject data listings, which include data collected on the eCRFs as well as any derived variables, e.g. age, body mass index (BMI), etc. for all enrolled subjects. All analyses will be based on observed data only, and no missing values will be imputed.

2.1.1 Reporting Precision

Summary statistics will be presented to the following degree of precision, unless otherwise specified:

Statistics	Degree of Precision
Mean, Geometric mean, Median, Quartiles, Confidence limit boundaries	One decimal place more than the raw data.
Standard deviation, Standard error	Two decimal places more than the raw data.
Minimum, Maximum	The same as the raw data.
p-value	Rounded to 4 decimal places and therefore presented as 0.xxxx; p-values smaller than 0.0001 as '<0.0001'; p-values greater than 0.9999 as '>0.9999'.
Percentage	One decimal place. A percentage of 100% will be reported as 100%. Percentages of zero will be reported as 0.

For weight, height, and body mass index (BMI), one decimal place will be used for summary statistics. Fractional numeric values will be presented with a zero to the left of the decimal point (for example, 0.12 – 0.30).

2.2 DEFINITIONS OF ANALYSIS POPULATIONS (ANALYSIS SETS)

2.2.1 Enrolled Population

The enrolled population will consist of all subjects in the clinical database. Subjects who signed informed consent but failed screening were not included in the clinical dataset. All baseline information will be summarized using the enrolled population.

2.2.2 Safety Population

The safety population will consist of all subjects that received an injection of ^{18}F-AV-1451or an injection of Florbetapir F 18. All safety endpoints will be summarized and listed using the safety population.

2.2.3 Efficacy Population

The efficacy population will include all subjects for whom image data are available from the ^{18}F-AV-1451 imaging session. All efficacy endpoints will be summarized and listed using the efficacy population.

2.3 TIME WINDOWS FOR ANALYSIS

For all analyses of this study, the scheduled visit and/or time point from the CRF (i.e., CRF visit) will be used as the analysis visit and/or time point.

2.4 POOLING OF CENTERS

This is a multi-center study. The data from all sites will be pooled by study group.

2.5 HANDLING OF MISSING DATA

Missing data will not be imputed for items with no rules for handling missing data specified.

2.6 ANALYSIS SOFTWARE

All statistical analyses will be performed using SAS® version 9.2 or higher for Windows [SAS Institute Inc., USA].

3. STUDY SUBJECTS

3.1 DISPOSITION OF SUBJECTS

The number and percent of subjects enrolled, completed and withdrawn early will be summarized by study group and overall. Primary reasons for early withdrawal will also be summarized.

The subject disposition will include the following:

- Subjects who are in the enrolled population
- Subjects who are in the safety population
- Subjects who are in the efficacy population
- Subjects who complete the study
- Subjects who complete the imaging session
- Subjects who complete the ^{18}F-AV-1451imaging session
- Subjects who complete the Florbetapir F imaging session
- Subjects who discontinue the study
- Primary Reasons for discontinuation

Subjects will be considered to have completed imaging session if they completed the last scheduled PET scan. Subjects will be considered to have completed the study if they completed the follow-up phone call. A listing of dispositions will be provided for the enrolled population.

3.1.1 Informed Consent and Subject Eligibility

The date of informed consent and subject eligibility will be listed for all subjects.

3.2 PROTOCOL DEVIATIONS

Protocol deviation/violation will be listed for the enrolled population.

4. DEMOGRAPHICS AND BASELINE CHARACTERISTICS

4.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics including age, gender (male), race (and sub-race if Asian), ethnicity, height, weight, the highest level of education and MMSE scores. The data will be summarized by study group and overall for the enrolled population.

Age will be calculated as: Age =year of consent-Birth Year +1.

A t-test or Fisher's exact test will be performed to assess the comparability of the two study groups, where appropriated. p-value will be included in the summary tables.

- A t-test will be used to compare the two study groups on age and MMSE scores.
- A Fisher's exact test will be used to compare the two study groups on race, ethnicity and the highest level of education.

A listing of demographics and baseline characteristics will be provided.

4.2 MEDICAL HISTORY

Medical history will be summarized by study group and overall using number and percentage of subjects of each category for the enrolled population. Medical history recorded on the eCRF will be coded using MedDRA (version 17.1), and presented in the data listings.

5. STUDY DRUG AND EXPOSURE

5.1 TREATMENT COMPLIANCE

Treatment compliance will not be summarized, as each subject will receive a single dose of study drug in an inpatient setting.

5.2 EXTENT OF EXPOSURE

Exposure, i.e. the actual dose (mCi) of ^{18}F-AV-1451 and Florbetapir F 18 received will be summarized by study group and overall, and listed for each subject by study group for the safety population.

5.3 PRIOR AND CONCOMITANT THERAPY

Prior and concomitant medications will be coded to indication-specific ATC (Anatomic Therapeutic Chemical classification) and preferred term using the World Health Organization Drug Dictionary (WHODD, March 2013 Format B2). Prior medications are defined as

medications that started prior to the day of the first dose of study drug. Concomitant medications are defined as medications (other than the study drug) taken on or after the day of administration of the first dose of study drug. Medications started before start of study drug and continuing after the study drug administration are considered both prior and concomitant medication.

Partial start dates of prior and concomitant medications will be assumed to be the earliest possible date consistent with the partial date. Partial stop dates of concomitant medications will be assumed to be the latest possible date consistent with the partial date. In the case of completely missing stop date, medication will be assumed to be ongoing.

Prior and concomitant medications will be summarized by level 4 ATC and preferred term using the number and percentage of subjects by study group for the enrolled population. Medications will be sorted alphabetically by ATC and preferred term within ATC. Subjects with multiple occurrences of a medication in ATC and preferred term will only be counted once within each ATC and preferred term. Since medications are coded to ATC by indication, preferred term may appear under multiple ATCs.

Investigator verbatim as well as coded terms will be listed for each subject by study group.

5.4 PRIMARY EFFICACY ANALYSIS

All efficacy analysis will be performed based on the efficacy population.

The number and percentage of subjects at each level of the overall brain uptake will be summarized by study group for ^{18}F-AV-1451 and Florbetapir F 18 separately. A Fisher's exact test will be performed to evaluate the association between the overall brain uptake and the study group, for each of the two radiotracers, and p-value will be presented.

A supportive listing will be provided.

5.4.1 Adjustments for Covariates

No adjustments for covariates are planned for this study.

5.4.2 Multiple Comparisons/Multiplicity

No adjustments for multiple comparisons or multiplicity will be made.

5.4.3 Examination of Subgroups

No subgroup analyses are planned for this study.

5.4.4 Handling of Missing Data, Drop-outs, and Outliers

Dropout subjects will not be replaced in this study. For items with no rules for handling missing data defined, missing data will not be imputed.

5.5 EXPLORATORY EFFICACY ANALYSES

The relationship between ^{18}F-AV-1451 and Florbetapir F 18 overall brain uptake will be presented by a2x4 contingency table, for group 1 subjects (CTE high risk) in the efficacy population. A Fisher's exact test will be performed to evaluate the association between the two radiotracers in terms of overall brain uptake, and p-value will be presented.

The relationship between clinical presentation and tau deposition will also be examined by ANOVA for group 1 subjects (CTE high risk) in the efficacy population. In the ANOVA, the MMSE score is the response variable and the ^{18}F-AV-1451 overall brain uptake score (4 levels) is the fixed effect. The point estimate (and 95% CI) of MMSE score will be presented for each level, and p-value will be presented to show the significance of the fixed effect. A log-transformation of MMSE scores will be performed if the normality assumption is violated.

6. SAFETY ANALYSIS

All safety analyses will be based on the Safety Population. Only observed data will be used for safety analyses, and missing data will not be imputed. Safety data will be presented by study group and overall.

6.1 ADVERSE EVENTS

Adverse events (AEs) will be categorized into trial-emergent AEs and treatment-emergent AEs (TEAEs), defined as:

- Trial-emergent AEs: AEs that occur or worsen in intensity or frequency after the informed consent and prior to administration of the first study drug (either Florbetapir F 18 or ^{18}F-AV-1451) at the imaging visit.
- Treatment-emergent AEs (TEAE): AEs that occur or worsen in intensity or frequency within 48 hours after the administration of the study drug (either Florbetapir F 18 or ^{18}F-AV-1451.

Adverse experiences that occur after administration of study drug but outside the 48 hour reporting window will not be reported as AE (but will be recorded in medical history) unless the investigator believes they are attributable to the drug.

All AEs will be coded by System Organ Class and Preferred Term using the Medical Dictionary for Regulatory Activities (MedDRA®, version 18.0/AECODE). All AEs will be listed by subject. Only TEAEs will be included in the summary tables and be presented by study drug and by study group. The number and percentage of subjects with TEAEs will be presented for the following summarizes:

- An overview summary, including number (and %) of subjects with TEAEs, serious AEs, AEs leading to study discontinuation, study drug related TEAEs/SAE, study procedure related TEAEs/SAE, and TEAEs by severity
- TEAE by System Organ Class and Preferred Term
- TEAE by System Organ Class, Preferred Term, and Severity
- Drug Related TEAEs by System Organ Class, Preferred Term
- Protocol Procedure Related TEAEs by System Organ Class, Preferred Term

All AE summary tables will be sorted by System Organ Class and then Preferred Term in decreasing frequency of the number and percentage of subjects in the ^{18}F-AV-1451 column.

A subject having the same AE (as determined by the coded MedDRA preferred term) more than once will be counted only once in the number and percentage of subjects' calculation for that AE. Similarly, if a subject had more than one AE in a System Organ Class, the subject will be counted only once in the number of subjects with an AE for that System Organ Class. If a subject has the same AE on multiple occasions, the highest severity (severe > moderate > mild) recorded for the event will be presented in the AEs by severity table.

6.1.1 Deaths, Serious and Other Significant Adverse Events

Serious AEs, AE leading to study discontinuation, and subjects who died during the study will be listed for each subject by study group.

6.2 CLINICAL LABORATORY PARAMETERS

Clinical laboratory evaluation were performed on Day One/Screening visit or at a study visit prior to the first PET scan. Tests include:

- Hematology: hemoglobin, hematocrit, RBC, WBC, MCH, MCHC, neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, platelets, and MCV. Optional: RBC morphology.

- Chemistry: total bilirubin, alkaline phosphatase, ALT (SGPT), AST(SGOT), urea nitrogen, creatinine, glucose, uric acid, calcium, phosphorus, total protein, albumin, total cholesterol, triglycerides, sodium, potassium, bicarbonate, chloride, magnesium, globulin, GGT.

- Urinalysis: Samples will be used to assess glucose, RBC, WBC, specific gravity, pH, protein, ketones, urobilinogen, blood, nitrite, microscopic, color, bilirubin, epithelial cells, leukocyte esterase, and bacteria.

The sample collection status for clinical laboratory evaluation will be listed for each subject by study group.

6.3 VITAL SIGNS

Vital signs were measured on Day One/Screening visit, immediately prior to each study drug injection, within 5 minutes after completion of ^{18}F-AV-1451 imaging session, and prior to discharge after completion of ^{18}F-AV-1451 imaging session.

Vital signs measurements include pulse rate, respiratory rate, supine blood pressure, height (for screening visit only), weight (for screening and predose), and temperature (for ^{18}F-AV-1451predose and pre-discharge).

Baseline and change from baseline will be summarized using descriptive statistics by study group. When the Florbetapir F 18 imaging was performed prior to 18F-AV-1451 imaging, the change from baseline for Florbetapir F 18 imaging/dosing will be calculated with pre-Dose of Florbetapir F 18 as the baseline and pre-dose of 18F-AV-1451 as the post-baseline. The changes from baseline for 18F-AV-1451 imaging/dosing include two post-baseline endpoints, i.e. 5 minutes post-dose of 18F-AV-1451 and pre-discharge of 18F-AV-1451 imaging, and the baseline is defined as the pre-dose of 18F-AV-1451. When the 18F-AV-1451 imaging was performed first, the change from baseline for 18F-AV-1451 dosing will be derived the same as above, and no change from baseline will be derived for Florbetapir F 18 imaging/dosing.

Data of vital signs will be listed for each subject by study group.

6.4 PHYSICIAN VISIT

A physician or a licensed/credentialed medical professional visit was scheduled on Day One/Screening visit, prior to each study drug injection and before discharge after completion of each PET imaging session. Physician visit status will be listed for each subject by study group.

6.5 ECG

A resting 12-lead electrocardiogram (ECG) was scheduled on Day One/Screening visit or at a study visit prior to the first PET scan. The assessment status will be listed for each subject by study group, including the assessment date, time and if the results were reviewed prior to dose administration.

7. INTERIM ANALYSES AND DATA MONITORING COMMITTEE (DMC)

No interim analyses are planned for this study.

8. SUMMARY OF MAJOR CHANGES IN THE PLANNED ANALYSES

Per Protocol ^{18}F-AV-1451-A07 Amendment 3 (26 January 2015), quantitative assessments of the Florbetapir F18 and 18F-AV-1451 PET scans will be conducted and calculated as standard uptake value ratio (SUVR). Two sample t-test or Wilcoxon rank sum test will be applied to exam if there are differences of the ^{18}F-AV-1451 uptake and Florbetapir F18 SUVR between study groups.

During the study, the expert reader who conducted imaging review suggested that a visual reading on ^{18}F-AV-1451scans would be more appropriate for the study objectives of this early phase pilot study, i.e., explore the use of AV1451 scans as a biomarker for CTE. Therefore, a visual interpretation of ^{18}F-AV-1451scans in ordinal scales is to be conducted, and the associated statistical analyses are planned accordingly.

Per the same protocol, additional exploratory analyses will be applied to explore the relationship among the brain uptake of the radiotracer and other biomarkers collected. Per sponsor update, there will be no additional biomarkers assessment collected, thus no corresponding analysis designed in the SAP.

9. REFERENCES

1. Avid Study Protocol: ^{18}F-AV-1451-A07, Amendment 3, 2015-01-26.
2. Clark CM, Pontecorvo MJ, Beach TG, etc. (2012) Cerebral PET with florbetapircompared with neuropathology at autopsy for detection of neuriticamyloid-β plaques: a prospective cohort study. *Lancet Neurol* 11:660-678.

10. MOCK TABLES, LISTINGS AND FIGURES (TLFS)

The study TLF shells will be provided in a separate document, which will show the content and format of all tables, listings, and figures in detail.

11. PROGRAMMING SPECIFICATIONS

The rules for programming derivations and dataset specifications will be provided in separate documents.