Protocol No. 18F-AV-1451-A20

Flortaucipir (18F) PET Imaging in the BIOCARD Study

Statistical Analysis Plan

Prepared by:
Avid Radiopharmaceuticals, Inc

Final Version 1.0
14 March 2019

Revision History

Version	Date	Author	Revisions
0.1	04MAR2019	[redacted]	Initial Draft
1.0	14MAR2019		Finalized document

Table of Contents

1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Table 1: Abbreviations and Definitions of Terms

AAL	Automated anatomical labeling
Aβ	amyloid-β
AD	Alzheimer's disease
AE	adverse event
BIOCARD	Biomarkers of Cognitive Decline Among Normal Individuals
CRF	Case report form
CSF	cerebrospinal fluid
CSR	clinical study report
ECG	electrocardiogram
IV	intravenous
MBq	megabecquerel
mCi	millicuries
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini-Mental State Examination
MUBADA	Multiblock Barycentric Discriminant Analysis
PERSI	parametric estimated signal reference intensity
PET	positron emission tomography
PiB	Pittsburgh compound B
PT	preferred term
SAE	Serious adverse event
SAP	statistical analysis plan
SAS	Statistical analysis software
SOC	system organ class
SUVr	standardized uptake value ratio
TEAE	treatment-emergent adverse event
WHO	World Health Organization

2 INTRODUCTION

Of the 2 pathological protein deposits that are the hallmark of Alzheimer's disease (AD), beta- amyloid (Aβ) is thought to accumulate very early in the disease process and is frequently observed in elderly subjects with no clinical symptoms, a condition that may represent a preclinical phase of the disease. Furthermore, as a biomarker, Aβ plaques as seen on PET amyloid imaging appear to plateau in intensity early in the symptomatic phase of the disease.

In contrast to Aβ deposits, the density and distribution in the brain of phosphorylated tau, in the form of neocortical tangle pathology, has been shown in autopsy studies to be increased with AD-related cognitive impairment and appears to correlate with overall evidence of neurodegeneration across the entire spectrum of the illness (Duyckaerts et al. 1987; Nelson et al. 2012). Thus, an understanding of the onset and progression of tau pathology in relation to amyloid pathology and cognitive change may be critical to understanding the course of the disease.

It is important to evaluate cognitive normal subjects with elevated brain amyloid to obtain information regarding the presence/onset of tau pathology because clinically normal amyloid positive subjects have been hypothesized to represent the earliest preclinical stage of AD (Sperling et al., 2011). The present study planned to obtain flortaucipir (^{18}F) tau positron emission tomography (PET) scans in up to 100 clinically normal subjects from the Biomarkers of Cognitive Decline Among Normal Individuals (BIOCARD) study that have been identified as having elevated brain amyloid by PET or cerebrospinal fluid (CSF) assays.

3 STUDY OBJECTIVES

The primary objectives of this study are:

- To evaluate imaging characteristics of flortaucipir (^{18}F) with respect to brain amyloid status in subjects who are enrolled in the BIOCARD study
- To examine the relationship between cognitive and function performance, other biomarker data, and tau deposition as measured by flortaucipir (^{18}F) uptake in clinically normal subjects

A secondary objective of this study is:

- To expand the flortaucipir safety database.

4 STUDY DESIGN

4.1 General Design

This is a phase I study that will evaluate imaging characteristics of flortaucipir in subjects enrolled in the BIOCARD study. Subjects enrolled in the BIOCARD study were contacted to participate and must have provided informed consent before starting any AV-1451-A20 study procedures. In addition to consenting to study procedures, participants consented to have Magnetic Resonance Imaging (MRI) images/data,

laboratory data, medical and neuropsychological assessments, as well as amyloid status, and lumbar puncture results made available to this study to allow for analysis and comparison. This study hoped to recruit roughly 80 percent amyloid positive subjects, and 20 percent amyloid negative subjects from the BIOCARD study. Amyloid positivity (Aβ+/Aβ-) was determined based on a visual read of the BIOCARD Pittsburgh compound B (PiB) amyloid PET scan. A list of subjects to be targeted for recruitment, including appropriate numbers of both amyloid positive and amyloid negative cases, was provided to the site. The subjects' amyloid status were not revealed to the site or subjects as part of recruitment or inclusion for this study.

Flortaucipir PET Imaging Session:

For the flortaucipir PET imaging session, an intravenous (IV) catheter was placed for administration of flortaucipir injection. Subjects received a single IV bolus injection target dose of 370 megabecquerel (MBq) (10 millicuries (mCi)) of flortaucipir injection followed by a saline flush. At approximately 80 minutes post dose, a continuous 20-minute brain scan (4 acquisitions of 5-minute duration) was obtained. If at any point during the imaging session it was determined that the subject was not able to continue, or that it was not in the best interest of the subject to continue, the protocol specified that imaging would be discontinued, and the image data that had been collected up to that point would be analyzed. Clinical laboratory tests were obtained prior to injection and upon completion of each imaging session. Adverse events (AE) were monitored continuously during the imaging session. Subjects who experienced any AE during an imaging session were not discharged until the event had resolved or stabilized.

4.2 Method of Assignment of Subjects to Treatment Groups

Subjects who qualify for the study will return to the clinic at a later date for the flortaucipir (^{18}F) PET imaging visit. For the flortaucipir PET imaging visit, an intravenous catheter will be placed for IV administration of flortaucipir (^{18}F) injection. Subjects will receive a single IV bolus injection with a target dose of 370 MBq (10 mCi) of flortaucipir (^{18}F) injection followed by a saline flush.

4.3 Blinding

The study recruited roughly 80 percent Aβ+ subjects and 20 percent Aβ- subjects from the BIOCARD study. Amyloid positivity was determined by an Avid expert reader's visual interpretation. A list of subjects for recruitment was randomly selected, stratifying by amyloid status to maintain an approximately 80% Aβ+ subjects. The list was provided to the site with amyloid status blinded. The subjects' amyloid status were not revealed as a part of recruitment or inclusion for this study.

4.4 Determination of Sample Size

No power or sample size calculation was done for this exploratory study. Twenty-three (23) subjects were dosed with flortaucipir and had an accompanying visual read or imaging quantification.

5 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

5.1 Changes in the Conduct of the Study

There were no changes in the conduct of the study at the time of preparing this statistical analysis plan (SAP).

6 BASELINE, EFFICACY AND SAFETY EVALUATIONS

6.1 Schedule of Evaluations

Evaluations	Screening Assessments[a]	^{18}F-AV- 1451 Imaging Visit	End of ^{18}F-AV- 1451 Imaging (prior to discharge)	Follow-up Phone Call[b]
Signed Informed Consent	X			
Demographics	X			
Medical History	X			
Concomitant Medications	X			
ECG	X[c]			
PET Brain Scan		X[d, e]		
Evaluation by a physician	X	X[f]	X[g]	
Pregnancy Test	X[h]	X[i]		
Adverse Events	X	X	X	X
Serious Adverse Events	X	X	X	X

a. Screening may take place over several days. All assessments must be performed within 90 days prior to the flortaucipir (^{18}F) imaging session. Subjects may be permitted to return for the flortaucipir (^{18}F) PET scan after the 90 day window with sponsor approval if the investigator does not recognize any significant medical changes.
b. A follow-up phone call to the subject, or information where applicable, will be conducted within 2 or 3 business days of each 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.
c. ECG (with results reviewed prior to flortaucipir (^{18}F) administration).
d. Subjects will receive a single IV bolus injection target dose of 370 MBq (10 mCi) of flortaucipir (^{18}F) injection followed by a saline flush.
e. At approximately 75 minutes following flortaucipir (^{18}F) injection, a continuous 30-minute brain scan will begin.
f. 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, the physician must be available to provide medical consultation.
g. Or physician designee.
h. Serum pregnancy test
i. Urine pregnancy test prior to flortaucipir (^{18}F) injection

6.2 Time Point Algorithms

6.2.1 Windows

For all analyses, results will be summarized at the planned study visit they were obtained.

6.3 Screening and Baseline Assessments

Screening assessments took place over several days, within 90 days of the flortaucipir PET scan, and included demographics, medical history and concomitant medications, a physical exam, and a safety evaluation. Additionally, participants had an electrocardiogram (ECG) with results reviewed prior to the initial flortaucipir imaging visit.

6.4 Efficacy Variables

6.4.1 Primary Efficacy Variable(s)

6.4.1.1 Tau Status Based on Flortaucipir

The ^{18}F-AV-1451 PET scan image was visually interpreted according to the following criteria:

Read Outcome		Objective Image Features
Not consistent with AD pattern (τAD-)		No increased neocortical activity, or increased neocortical activity isolated to the mesial temporal, anterolateral temporal, and/or frontal regions.
AD pattern (τAD)	τAD+	In either hemisphere, increased neocortical activity in the posterolateral temporal (PLT) or occipital region(s).
	τAD++	In either hemisphere, increased neocortical activity in the parietal/precuneus region(s), or frontal region(s) with increased uptake in the PLT, parietal, or occipital region(s).

6.4.1.2 Flortaucipir Quantitation

Standard uptake value ratios (SUVr) were calculated to estimate tau load globally and in individual regions for the flortaucipir images. A target region derived statistically with a Multiblock Barycentric Discriminant Analysis (MUBADA) method will be used for the global measurement.

Voxels of interest determined in the automated anatomical labeling (AAL) atlas masked to exclude white matter and CSF for amygdala, anterior fusiform, posterior fusiform, anterior hippocampus, posterior hippocampus, anterior parahippocampus, posterior parahippocampus, caudate, frontal, parietal, precuneus, temporal, occipital, left frontal, right frontal, left occipital, right occipital, left parietal, right parietal, left putamen, right putamen, left temporal, right temporal will be applied at the individual region level. A

selected white matter region derived using a parametric estimated signal reference intensity (PERSI) method will be used as reference region for all SUVr calculations.

MUBADA SUVr will be the variable used in any efficacy analysis involving flortaucipir quantitation. All regional SUVrs will be presented in listings.

6.4.1.3 Amyloid Status

Aβ is thought to accumulate very early in the AD process and is frequently observed in elderly subjects with no clinical symptoms. Furthermore, as a biomarker, Aβ plaques as seen on PET amyloid imaging appear to plateau in intensity early in the symptomatic phase of the disease. Amyloid positivity (Aβ+ or Aβ-) was determined by an Avid expert reader's visual interpretation.

6.4.2 Additional Efficacy Variables

6.4.2.1 Cognitive Assessments

Mini-Mental State Examination (MMSE)

The MMSE (Folstein et al., 1975) is a brief instrument used to assess cognitive function in elderly patients. The range for the total MMSE score is 0 to 30, the sum of each correct answer, with higher scores indicating better cognition. The score on a continuous scale will be used for primary objective analysis.

MMSE was recorded in the BIOCARD data. The MMSE measurement collected closest to the flortaucipir scan will be used for the purposes of the correlation analysis.

6.4.2.2 Functional Assessments

Pfeffer Functional Activities Questionnaire (FAQ), (Pfeffer et al. 1982)

Functional status is conceptualized as the "ability to perform self-care, self-maintenance and physical activities." The FAQ was developed to assess instrumental activities of daily living involving higher level functional skills such as shopping alone, writing checks, remembering appointments, etc. The FAQ asks the informant to rate the patient's ability using the following scoring system: Dependent = 3; Requires assistance = 2; Has difficulty but does by self = 1; Normal = 0; Never did [the activity] but could do now = 0; Never did and would have difficulty now = 1. The sum scores ranges from 0-30, where higher scores indicate greater functional impairment. FAQ sum score will be used as a continuous variable in the primary objective analysis.

FAQ was recorded in the BIOCARD data. The FAQ measurement collected closest to the flortaucipir scan will be used for the purposes of the correlation analysis.

6.5 Drug Concentration Measurements and Pharmacokinetic Parameters

6.5.1 Handling of Pharmacokinetic Parameter Outliers

No pharmacokinetic parameters or drug concentration measurements will be collected during this study.

6.6 Safety Assessments

6.6.1 Extent of Exposure and Compliance to Study Treatment

During the flortaucipir imaging sessions, all subjects received a single IV bolus administration target dose of 370 MBq (10 mCi) of flortaucipir injection.

All exposure information will be summarized in a table and presented in a listing.

6.6.1.1 Unit Conversion for Radioactive Dose

All exposure tables and listings will display volume in MBq. Volume collected in mCi will be converted to MBq as follows:

$$MBq = 37 \times mCi$$

6.6.2 Adverse Events

An AE is any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the study drug. AEs are classed by severity and seriousness.

Treatment-emergent adverse events (TEAE) are any untoward medical occurrences associated with the use of a drug in humans, whether or not considered drug related. For the purposes of this study, untoward medical occurrences will be considered associated with the use of flortaucipir, and thus be reported as TEAEs if they occur within 48 hours after administration of the PET tracer. The end of study for the purpose of AE reporting is defined as 48 hours after the administration of flortaucipir injection.

The investigator's verbatim term of both serious and non-AEs will be mapped to system organ class (SOC) and preferred terms (PT) using the Medical Dictionary for Regulatory Activities (MedDRA) Version 19.1.

Severity is classified as mild/moderate/severe (increasing severity). If a subject reports a TEAE more than once within that SOC/PT, the TEAE with the worst case severity will be used in the corresponding severity summaries.

Serious AEs (SAEs) are events that result in one of the following outcomes or constitute one of the following events:

- Death

- Initial or prolonged 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

7 STATISTICAL METHODS

7.1 Definitions and Conventions

All analysis will be performed using SAS version 9.2 or higher.

Data will be summarized using descriptive statistics (number of subjects [n], mean, standard deviation [SD], median, minimum [min], and maximum [max]) for continuous variables and using frequency count and percentage for discrete variables. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.

The tables and listings will be numbered using a decimal system to reflect main levels of unique tables and listings and sub-levels of replicate tables and the listings with maximum two digits per level (e.g., Table XX.YY.ZZ…). Tables will be presented in clinical study report (CSR) section 14, and thus will be numbered as 14.YY.ZZ. Baseline analysis will be reported in table series 14.1, efficacy analysis in series 14.2, and safety analysis in series 14.3. Listings will be presented in in CSR section 16, and thus will be numbered as 16.YY.ZZ.

Unless otherwise specified, hypothesis testing will be two-sided with type I error rate of 0.05.

7.2 Handling of Dropouts or Missing Data

Dropout subjects will not be replaced in this study. For situations with no rules for handling missing data the default will be no imputation.

7.3 Interim Analyses and Data Monitoring

No interim analysis or data monitoring is planned for this study.

7.4 Multi-center Studies and Pooling of Centers

This is a single center study. The pooled data will be analyzed and presented.

7.5 Multiple Comparisons/Multiplicity

No multiple comparisons/multiplicity adjustment is planned.

7.6 Examination of Subgroups

No subgroup analysis will be conducted for this study.

8 STATISTICAL ANALYSIS

8.1 Analysis Populations

8.1.1 Enrolled Population

The enrolled population will consist of all BIOCARD study subjects who have signed the consent form for AV-1451-A20 and data exists in the electronic data capture system set for this AV-1451-A20. Disposition will be summarized using the enrolled population.

8.1.2 Safety Population

The safety population will consist of all subjects who received at least one injection of flortaucipir. All baseline and safety data will be summarized and listed using the safety population. All efficacy variables described in section 6.4 will be presented in listings for the safety population.

8.1.3 Efficacy Population

The efficacy population will include all subjects with a valid, interpretable baseline PET image (visual read and/or SUVr). All efficacy endpoints will be summarized using the efficacy population.

8.2 Disposition and Withdraws

The enrolled population will be represented in the disposition table. The disposition table will summarize the analysis populations in section 8.1, completed, and discontinued subjects.

Discontinued subjects will be defined as any safety subject who fail to complete the 24-48 hour safety follow-up. Termination status (i.e. ‘Completed’ and ‘Discontinued’) percentages will be based on the safety population. Percentages outlining discontinuation reasons will be based on the number of discontinued subjects.

8.3 Baseline Subject Data

8.3.1 Demographic and Other Baseline Characteristics

All baseline summaries will be based on the safety population. Age (years), gender, race, ethnicity, education, ApoE genotyping, alcohol, drug use, and smoking will all be summarized in a table and presented in a listing.

8.3.2 Medical and Surgical History

Medical and surgical histories were coded using MedDRA version 19.1, and will be presented in a listing for the safety population.

8.3.3 Concomitant Therapy

Concomitant therapies were coded using WHODRUG 3Q 2016. All concomitant medications data will be presented in listing.

8.3.4 Pregnancy Test

Pregnancy testing information captured on the case report form (CRF) will be presented in a listing for the safety population.

8.3.5 Electrocardiogram

ECG data captured on the CRF will be presented in a listing for the safety population.

8.4 Analysis of Efficacy Parameters

8.4.1 Analysis of Primary Efficacy Variable

8.4.1.1 Qualitative Assessment of Images

The visual read results as described in section 6.4.1.1 will be summarized for the efficacy population using descriptive statistics by amyloid status (Aβ+, Aβ-) in a 2x3 table.

8.4.1.2 Quantitative Assessment of Images

The MUBADA SUVr as described in section 6.4.1.2 will be summarized for the efficacy population using descriptive statistics by amyloid status (Aβ+, Aβ-).

8.4.1.3 Correlation between MMSE and MUBADA SUVr in Clinically Normal Subjects

Spearman's correlation coefficient will be used to measure the relationship between baseline MMSE and MUBADA SUVr in clinically normal subjects. This relationship will also be presented in a scatter plot.

8.4.1.4 Correlation between FAQ and MUBADA SUVr in Clinically Normal Subjects

The same analysis as described in section 8.4.1.3 will be applied, correlating FAQ with MUBADA SUVr.

8.5 Analysis of Safety

8.5.1 Exposure

The total dose administered (MBq) of flortaucipir will be summarized in a table using descriptive statistics by imaging visits and collectively, and presented in a listing.

8.5.2 Treatment Emergent Adverse Events

A summary of TEAEs will be reported in the tables including number of all TEAEs and number of subjects who experienced at least one TEAE. The summary of TEAEs will be broken down further in descending frequency by SOC and PT, and by PT only in separate tables. A subject will be counted once if the subject reported one or more events in a given level of summarization.

All AEs will be presented in a listing.

8.5.3 Severity

TEAE severity will be reported in a table in the same manner as outlined in 8.5.2. For the summarization of TEAEs by intensity, events recorded with missing intensity will be summarized as Severe. If a subject reports the same AE more than once within an SOC/PT, the AE with the worst case severity will be summarized in the table.

8.5.4 Relationship to Flortaucipir

TEAE relationship to flortaucipir will be reported in a table in the same manner as outlined in 8.5.2. TEAEs with a missing relationship to flortaucipir will be regarded as related to flortaucipir. If a subject reports the same AE more than once within an SOC/PT, the AE most related to flortaucipir will summarized in the table.

8.5.5 Relationship to Study Procedure

TEAE relationship to study procedure will be reported in a table in the same manner as outlined in 8.5.2. TEAEs with a missing relationship to study procedure will be regarded

as related to study procedure. If a subject reports the same AE more than once within that SOC/PT, the AE with the worst case relationship to study procedure will be used in the corresponding relationship summaries.

8.5.6 Serious Adverse Events

Serious TEAEs will be summarized in a similar manner as described in Section 8.5.2. If a subject reported more than one serious TEAE with the same SOC or PT, the TEAE will be counted only once in that SOC or PT. For the summarization of TEAEs by seriousness, events recorded with missing seriousness will be summarized as Serious.

8.5.7 Adverse Events Leading to Study Discontinuation

TEAEs leading to study discontinuation will be summarized in a similar manner as described in section 8.5.2.

8.5.8 Adverse Events Leading to Death

TEAEs leading to death will be summarized in a similar manner as described in section 8.5.2.

8.5.9 Missing and Partial AE Onset Dates

See appendix 1.1 for specific algorithms to impute missing start and stop dates.

8.5.10 Follow-up Contact Data

The follow-up contact information captured on the CRF will be presented in a listing for the safety population.

APPENDIX I: ALGORITHMS TO HANDLE MISSING AND PARTIAL DATES

1.1. MISSING AND PARTIAL AE ONSET AND END DATES

If the AE onset dates are missing, then the most conservative approach will be used to decide if the AE is TEAE or not, as detailed in the table below:

AE ONSET DATE/TIME	AE STOP DATE/TIME	ACTION
Partial, but known components show that it cannot be on or after an injection date/time and within 48 hours post-injection	Known	Not TEAE
	Partial	Not TEAE
	Missing	Not TEAE
Partial, could be on or after an injection date/time and within 48 hours post-injection	Known	If stop date/time < flortaucipir injection date/time, then not TEAE If stop date/time >= flortaucipir injection date/time, then TEAE
	Partial	Impute stop date as latest possible date (i.e. 59 if minutes unknown or 23:59 if hours and minutes unknown; last day of month if day unknown or 31st December if day and month unknown), then: If stop date/time < flortaucipir injection date/time, then not TEAE If stop date/time >= flortaucipir injection date/time, then TEAE
	Missing	Assumed TEAE
Missing	Known	If stop date/time < flortaucipir injection date/time, then not TEAE If stop date/time >= flortaucipir injection date/time, then TEAE
	Partial	Impute stop date as latest possible date (i.e. 59 if minutes unknown or 23:59 if hours and minutes unknown; last day of month if day unknown or

AE ONSET DATE/TIME	AE STOP DATE/TIME	ACTION
		31st December if day and month unknown), then: If stop date/time < flortaucipir injection date/time, then not TEAE If stop date/time >= flortaucipir injection date/time, then TEAE
	Missing	Assumed TEAE

Leo Document ID = b9e6f9ff-92af-4092-bb6e-571c13a139ff

Approver: [redacted]
Approval Date & Time: 14-Mar-2019 17:30:12 GMT
Signature meaning: Approved

Approver: [redacted]
Approval Date & Time: 14-Mar-2019 18:18:43 GMT
Signature meaning: Approved

Approver: [redacted]
Approval Date & Time: 14-Mar-2019 18:23:12 GMT
Signature meaning: Approved

Approver: [redacted]
Approval Date & Time: 14-Mar-2019 19:08:13 GMT
Signature meaning: Approved