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Study Statistical Analysis Plan: 18F-AV-1451-A13

Official Title: A High Resolution Autopsy Study Evaluating the Relationship of 18F-AV-1451 PET Imaging and Tau Pathology

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Title Page

^{18}F-AV-1451-A13 Study Efficacy Statistical Analysis Plan

Protocol Title: A High-Resolution Autopsy Study Evaluating the Relationship of ^{18}F-AV-1451 PET Imaging and Tau Pathology

Protocol Number: ^{18}F-AV-1451-A13

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Version history

This Statistical Analysis Plan (SAP) for study 18F-AV-1451-A13 is based on the protocol dated 14NOV2016.

Table 1 SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1.0		Not Applicable	Initial version

1. Introduction

Molecular imaging biomarkers hold great potential in aiding the diagnosis of individuals undergoing evaluation for cognitive impairment and Alzheimer's disease (AD) (Dubois et. al., 2010; McKhann et. al., 2011). One such biomarker is ^{18}F-Flortaucipir (commonly referred to as flortaucipir or ^{18}F-AV-1451, or initially known as [F-18]T807 by Siemens Molecular Imaging Biomarker Research group), which has been developed as a positron emitting radiopharmaceutical for *in vivo* imaging of tau protein aggregates (Xia et al., 2013).

Preliminary analyses of flortaucipir PET scans have shown minimal tracer accumulation in young subjects. In contrast, flortaucipir accumulation is predominately observed in the medial temporal lobe of amyloid-negative older adults with clinically normal cognition and is more pronounced in neocortical regions of amyloid-positive patients with mild cognitive impairment (MCI) and AD dementia (Johnson et. al., 2016, Scholl et. al., 2016, Schwarz et. al., 2016). These observations align with the tau pathology pattern previously documented in autopsy studies (Braak and Braak, 1991), suggesting that ^{18}F-Flortaucipir may serve as a valuable marker for tau pathology in AD.

Several studies have reported a correlation between *in vivo* ^{18}F-Flortaucipir PET signal and the density of tau pathology observed at autopsy (Lowe et al., 2020; Pontecorvo et.al., 2020; Smith et al., 2019; Gatto et al., 2023; Martersteck et al., 2022, Freiburghaus et. al., 2024). The present study, ^{18}F-AV-1451–A13, aims to investigate the relationship between the regional patterns observed in the 18F-Flortaucipir PET scan signal and the high-resolution 3D brain tau pathology as observed postmortem (Ushizima et. al., 2022).

A statistical analysis plan (SAP) focused on safety analysis was approved on July 16, 2018, and can be found in the AVID Biostat shared folder under the title "^{18}F-AV-1451-A13 SAP" (safety SAP). Safety SAP did not include efficacy analyses as it was decided that efficacy analyses would be deferred until digitalized autopsy and imaging data became available.

The current SAP, now addressing efficacy analyses per study protocol, is titled "^{8}F-AV-1451-A13 Efficacy SAP" (efficacy SAP) to differentiate it from the safety SAP. While the Efficacy SAP is comprehensive, it is recommended to review it alongside the protocol and the safety SAP to understand study fully.

Throughout this document, as well as in the safety SAP and the study protocol, the terms "Flortaucipir", ^{18}F-Flortaucipir", "18F-AV-1451", and "AV1451" are used interchangeably. It is important to note that the analyses outlined in the protocol represent broad objectives, and the specifics detailed in this SAP align with those high-level objectives.

1.1. Objectives, Endpoints, and Estimands

The primary objective of this study is to investigate the association at global, composite and regional levels between a flortaucipir PET scan and the corresponding global and regional signals derived from the high-resolution 3D histological maps of brain tau pathology observed at autopsy.

Ideally, this relationship would be assessed at a finer resolution than FreeSurfer ROIs, e.g. voxel-wise. However, due to challenges in acquiring a complete set of pathology images from collaborators, as well as technical challenges related to image processing, we will conduct the analysis at the resolution of FreeSurfer ROIs. The availability of autopsy-based pathology data at the FreeSurfer ROIs level still is a unique advancement in PET-to-Autopsy analysis design, providing a rich dataset for efficacy analysis.

An analysis dataset will include 82 FreeSurfer-defined regions of interest (ROIs), normalized to the brain stem for PET data. This dataset will incorporate average regional flortaucipir PET signal and the corresponding immunohistology results from three specific antibodies, AT8 (pSer202 + Thr205, Thermo), AT100 (pThr212 + Ser214, Thermo), and MC1 (MC1, gift of Peter Davies). The mapping of 3D histological immunohistology signals to FreeSurfer ROIs and the corresponding flortaucipir SUVR were estimated, quality-controlled and shared by a team at UCSF led by Duygu Tosun-Turgut.

Available ROIs will be considered for global analysis, then focusing on macrolobar and/or composite brain regions deemed most relevant based on AD pathophysiology, also considering possible exclusions due to expected off-target flortaucipir PET binding (e.g. medial temporal regions). Composite regions of interest will include a combination of selected cortical and subcortical ROIs from the frontal, temporal, parietal, occipital regions.

For each patient, separately and/or combined, with and without patient level data centering, Spearman's rank correlation analysis will be applied to quantify the strength and direction of the relationship between tau levels measured by PET scan and tau pathology at autopsy based on the three different antibodies. The results of these correlation analyses will be presented graphically and in tabularly form, both at regional/composite and global levels.

Objectives	Endpoints
Primary	
The primary objective of this study is to investigate the relationship between flortaucipir PET scan signals and brain pathology observed at autopsy using tau antibody AT8.	Correlation coefficients between flortaucipir PET quantitation and AT8 antibody staining in respective regions • Spearman correlation coefficients • Global and regional/composite levels
Tertiary/Exploratory	
The exploratory objective is to evaluate the relationship among three antibodies (AT8, AT100, MC1) targeting different tau epitopes and evaluate differential correlative patterns between flortaucipir PET scan and these antibodies	Pairwise correlation coefficients among AT8, AT100, MC1 antibody staining and flortaucipir PET quantitation • Spearman correlation coefficient • Global and regional/composite levels

1.2. Study Design

The study was designed to investigate the correlation between imaging findings obtained through a flortaucipir PET scan and the pathological observations found at autopsy, using a high-resolution approach, all of which occurred within a six-month window following the imaging procedure. Initially, multiple clinical centers were anticipated to enroll subjects of various levels of cognitive status, ranging from individuals with normal cognitive function to those with dementia. However, due to shifting priorities, only two clinical centres ultimately participated in this study. As a result, the study enrolled and completed just two participants, one cognitively normal and the other cognitively impaired.

The inclusion of a cognitively normal participant in the study holds significance as it enables a comparison of the patterns of tracer distribution between normal and impaired.

Participants who qualified for the study received a single intravenous (IV) bolus of flortaucipir followed by brain PET imaging for 20 minutes, beginning approximately 80 minutes post-injection. Vital signs were obtained before the administration of flortaucipir and after the imaging session. Adverse events were continuously monitored during the imaging session. Participants who experienced an adverse event were released when the event was resolved or stabilized. After the 48-hour safety follow-up was completed, subject participation in the clinical portion of the study was considered to have ended.

Please refer to table 2 on page 11 of safety SAP for the visit schedule, safety data collection, and safety analysis.

2. Statistical Hypotheses

The primary objective of the study is to explore the relationship between signals detected on flortaucipir PET scan and tau pathology from immunohistochemistry observed postmortem. We aim to confirm a positive correlation between the *in vivo* flortaucipir PET signal and density of tau pathology at autopsy. We anticipate that the strength of this correlation will vary across different brain regions, either taken individually or as composite, while also depending on the antibodies used to target specific tau protein epitopes, as well as individual patient characteristics.

In this study, we employed three antibodies that target different epitopes on tau protein. MC1 identifies the earliest known structural conformational change in tau, primarily staining neurofibrillary tangles (NFT) pathology in its early maturity stages, including the pretangle phase, and to a lesser extent, more mature tangles. AT8, a widely used antibody, detects earlier stages NFT maturation, showing less affinity for pretangle compared to more mature tangles, and also reacts to neuritic pathology, contributing to the overall signal. AT100 shares a similar profile to AT8 but is more likely to detect later phosphorylation events. Given that AT8 and AT100 exhibit similar profiles and are more likely to capture mature tangles compared to MC1, and considering AT8's widespread use as a tau pathology marker, we selected AT8 for the primary objective analysis to assess the relationship between flortaucipir PET signal and tau staining at autopsy.

Based on the antibodies differences and similarities described above, we hypothesize that AT8 vs. PET correlations will show relatively higher similarity to AT100 vs. PET correlations as compared to MC1 vs. PET correlations. To further understand these relationships, we will also explore the association between flortaucipir PET signal and both AT100 and MC1 in exploratory analyses.

2.1. Multiplicity Adjustment

Considering the exploratory nature of this study, no multiple testing adjustment is planned.

3. Analysis Sets

Data Set	*Description*
Participant and analysis data set	• *Participants 1181-0001 and 1183-0002 will be included in the analyses* • *Flortaucipir and immunochemistry data from the two participants will be included in the analyses*

Data points include mean Flortaucipir-PET signal in each of 82 Freesurfer atlas ROI, normalized against the brainstem ROI, along with the mean immunochemistry signals for AT8, AT100, and MC1 in the same regions. These ROIs will be used for global correlation analyses.

The selection of ROIs for regional/composite analysis will be based on well-established AD regions documented in the Study A16 and referenced publications. The objective is to fully capture the dynamic range of flortaucipir PET binding in AD, as highlighted in studies by Devous et. al. (2018), Pontecorvo et.al. (2020), and other relevant literature. Given the exploratory nature of this study, ROIs for regional/composite analyses will not be pre-specified.

4. Statistical Analyses

4.1. General Considerations

All analysis will be performed using R Software, Version 4.3 or SAS 9.4.

Participant demographics will be presented for the two participants individually and correlation analysis will be conducted for each participant individually and for the two participants combined, globally and regionally, with and without individual level data centering. Among the three antibodies AT8, AT100 and MC1, AT8 will be used to draw conclusion as primary outcome based on binding properties and frequent utilization in past Flortaucipir PET to Autopsy studies. Analyses on AT100 and MC1 will be included in the exploratory objective.

4.2. Participant Dispositions

Two participants enrolled and completed the study.

4.3. Primary Endpoint Analysis

Spearman correlation coefficients will be computed to quantify relationships between flortaucipir PET quantification and AT8 antibody staining quantification globally and regional/composite.

Mean signals of antibody AT8 staining and flortaucipir PET signals from 82 ROIs will be used for the global correlation analyses, followed by regional/composite analyses based on selected ROIs based on AD pathophysiology and possibly expected off-target binding. Correlation analyses will be performed for each subject, and two subjects combined, with and without subject level data centering.

4.4. Secondary Endpoint(s)/Estimands(s)] Analysis

4.5. Tertiary/Exploratory Endpoint Analysis

The same analysis as for the primary endpoint will be conducted, but with AT8, AT100 and MC1, focusing on exploring the differential patterns among the three antibodies.

4.6. (Other) Safety Analyses

Safety analyses were defined in a Safety SAP.

4.7. Other Analyses

4.8. Interim Analyses

4.9. Changes to Protocol-Planned Analyses

5. Sample Size Determination

The sample size was determined outside of statistical consideration.

6. Supporting Documentation

No supporting documentation.

7. References

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