STATISTICAL ANALYSIS PLAN
For Exploratory Biomarker

Product Studied: Aducanumab
Protocol Number: 221AD301 & 221AD302

A Phase 3 Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate the Efficacy and Safety of Aducanumab (BIIB037) in Subjects with Early Alzheimer's Disease

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Table of Contents

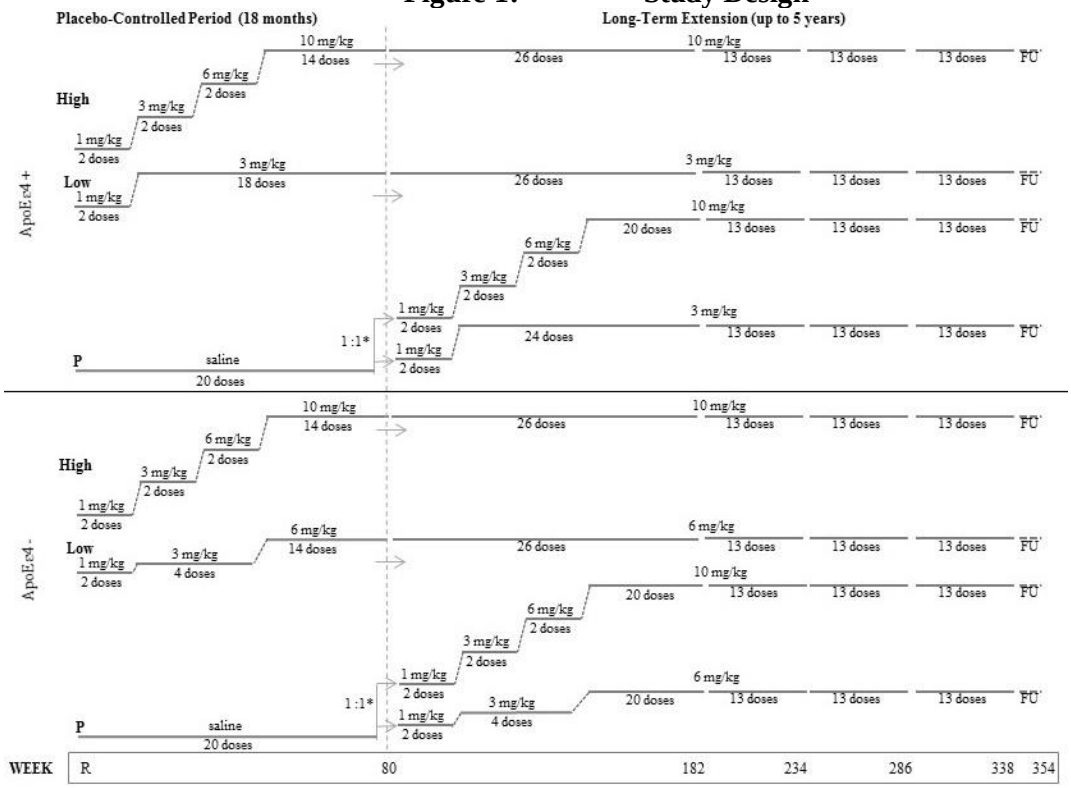
1 OVERVIEW

This statistical analysis plan (SAP) outlines the pre-planned analysis for the exploratory biomarkers, collected in phase III studies 221AD301 and 221AD302, which are identically designed multicenter, randomized, double-blind, placebo-controlled, parallel-group, global studies to evaluate the efficacy and safety of aducanumab in subjects with early AD, including mild cognitive impairment (MCI) due to AD and a subset of mild AD dementia. Each study comprises an 18-month double-blind, placebo-controlled (PC) period, followed by an optional dose-blinded long-term extension (LTE) period of up to 5 years where all subjects will receive aducanumab. Figure 1 presents the study design; for details of the studies, please refer to the study protocols.

On 21Mar2019, Biogen announced the discontinuation of the studies due to futility. All the ongoing subjects will perform an end-of-treatment visit within 4 weeks of the announcement and a safety follow-up visit 18 weeks from the last infusion.

The biomarker analysis will focus on the PC period to assess the treatment effect of aducanumab on exploratory biomarkers; data from LTE period will only be summarized.

Figure 1: Study Design



ApoE ε4 +/- = apolipoprotein E4 positive/negative; FU = follow-up; P = placebo; R = randomization date.
*Subjects who are assigned to placebo during the placebo-controlled period are randomized in a 1:1 ratio to high and low dose aducanumab treatment (based upon their ApoE ε4 carrier status) for the long-term extension period on Study Day 1.

2 DESCRIPTION OF ADDITIONAL EXPLORATORY OBJECTIVES AND ENDPOINTS FOR BIOMARKERS LISTED IN PROTOCOL

2.1 Additional Exploratory Objectives

- To assess the effect of aducanumab on various morphometric measures in certain brain areas as measured by magnetic resonance imaging (MRI).
- To assess the effect of aducanumab on functional connectivity by task free functional MRI (tf-fMRI) [where available].
- To assess the effect of aducanumab on cerebral blood flow by arterial spin labeling (ASL)-MRI (in a subset of sites and subjects, where available).
- To assess the effect of aducanumab on disease- or treatment-related biomarkers in cerebrospinal fluid (CSF), which may include, but are not limited to, amyloid and tau proteins (in a subset of subjects).
- To assess the effect of aducanumab on disease- or treatment-related biomarkers in blood, which may include, but are not limited to, amyloid and tau proteins.
- To assess the effect of aducanumab on tau pathology by tau PET imaging (where available, in a subset of subjects).

2.2 Additional Exploratory Endpoints

- Change from baseline in whole brain volume, hippocampal volume, ventricular volume, and cortical gray matter volume measured by MRI over time.
- Change from baseline in functional connectivity as measured by tf-fMRI (where available) over time.
- Change from baseline in cerebral blood flow as measured by ASL-MRI (in a subset of sites and subjects, where available) over time.
- Change from baseline in CSF biomarkers, which may include, but are not limited to, amyloid or tau proteins at Week 78 (in a subset of subjects).
- Change from baseline over time in blood biomarkers, which may include, but are not limited to, amyloid or tau proteins.
- Change from baseline in tau PET signal at Week 78 (where available, in a subset of subjects).

2.3 Additional Exploratory LTE Objective

- To evaluate the long-term efficacy of aducanumab treatment as measured by radiological, clinical, and additional health outcomes.

2.4 Additional Exploratory LTE Endpoints

- Change in the following measures over the placebo-controlled and LTE periods of the study:
  - Whole brain volume, hippocampal volume, ventricular volume, and cortical gray matter volume measured by MRI.
  - Functional connectivity as measured by tf-fMRI (where available).
  - Cerebral blood flow as measured by ASL-MRI (in a subset of sites and subjects, where available).
  - Disease- or treatment-related biomarkers levels in CSF which will include, but are not limited to, amyloid and tau proteins (in a subset of subjects).
  - Disease- or treatment-related biomarker levels in CSF, which may include, but are not limited to, amyloid and tau proteins (in a subset of subjects).
  - Disease or treatment-related biomarker levels in blood, which many include, but not limited to, amyloid and tau proteins.
  - Tau PET signal (where available, in a subset of subjects).

3 DESCRIPTION OF ADDITIONAL EXPLORATORY BIOMARKERS COVERED IN THIS SAP

The exploratory biomarkers included in this SAP come from 3 different modalities: structural MRI imaging, tau PET imaging and CSF. Specific biomarkers for each modality are described in the following subsections. Task-free-MRI, ASL-MRI, serum and plasma biomarker samples have not been analyzed yet so are not covered in this version of the SAP.

4 STATISTICAL ANALYSIS METHODS

4.1 General Considerations

All the analyses will be performed in individual 221AD301 and 221AD302 data respectively unless sample size is too small in individual studies. Some analyses may also be performed in pooled 221AD301 and 221AD302 data in addition to individual studies.

Summary tables will be presented using descriptive summary statistics for each treatment group. For continuous variables, summary statistics will generally include: number of subjects with data, mean, standard deviation, median, 25% percentile, 75% percentile, minimum and maximum. For categorical variables, this will generally include: number of subjects in the analysis population, number with data, and the percent of those with data in each category.

Unless otherwise specified, baseline value is defined as the most recent non-missing measurement collected prior to the first dose. Change from baseline will be defined as post-baseline value minus baseline value.

Unless stated otherwise, all statistical tests will be 2-sided with statistical significance level of 0.05. No multiple comparison adjustment will be used for analysis in this SAP.

The statistical software, SAS® will be used for all summaries and analyses.

4.2 Structural MRI Analysis

4.2.1 Background

Longitudinal structural MRI is performed on all the randomized subjects. Measurements for local cortical thickness and volume are generated according to the MRI imaging study protocol for each region of interest (ROI). Table 1 summarizes the ROI measurements taken and the corresponding units. Scans for brain MRI at screening visit, Week 30, Week 78, Week 134, Week 182 and early termination visits were used for structural MRI data processing and therefore will be used in the analysis.

Table 1. Summary of ROI measurements and units in structural MRI

Region of interest and measurement	Unit
Whole cortex thickness	mm
Frontal cortex thickness	mm
Parietal cortex thickness	mm
Lateral temporal cortex thickness	mm
Sensorimotor cortex thickness	mm
Ant. cingulate cortex thickness	mm
Post. cingulate cortex thickness	mm
Medial temporal cortex thickness	mm
Occipital cortex thickness	mm
Whole cortical volume	mm3
Frontal cortical volume	mm3
Parietal cortical volume	mm3
Lateral temporal cortical volume	mm3
Sensorimotor cortical volume	mm3
Anterior cingulate cortical volume	mm3
Posterior cingulate cortical volume	mm3
Medial temporal cortical volume	mm3

Occipital cortical volume	mm3
Left hippocampus volume	cm3
Right hippocampus volume	cm3
Sum of the HCL and HCR volume	cm3
Lateral ventricle volume	cm3
Whole brain volume	cm3

4.2.2 Analysis population

The intent-to-treat (ITT) population, defined as all randomized subjects who received at least one dose of study treatment (aducanumab or placebo), will be used for the analyses on structural MRI.

4.2.3 By visit summary and MMRM model for PC period

The baseline and change from baseline values will be summarized by treatment groups (placebo, low dose and high dose) and by visit for each of the ROIs up to Week 78.

An MMRM model will be used to analyze change from baseline value for each ROI. Fixed effects of the model will include treatment groups (placebo, low dose and high dose), visit (Week 30 and Week 78), treatment group-by-visit interaction, baseline value (continuous), baseline value by visit interaction, baseline MMSE (continuous), laboratory ApoE ε4 status (carrier and non-carrier), and baseline age (continuous). Visit and treatment group will be treated as categorical variables in the model along with their interactions. An unstructured covariance matrix will be used to model the within-patient variance-covariance errors. If the unstructured covariance structure matrix results in a lack of convergence in any of the parameters, the heterogeneous Toeplitz covariance structure followed by the heterogeneous first-order autoregressive covariance structure will be used for all the parameters, and the sandwich estimator will be used to get the variance of the treatment effect estimator. The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. Adjusted means for each treatment group, pairwise adjusted differences with placebo, 95% confidence intervals for the differences and associated p-values will be presented at week 30 and week 78.

Due to the early termination of the studies and a large proportion of the subjects did not have the opportunity to complete Week 78, a sensitivity analysis using the same MMRM model but only includes subjects who have had the opportunity to complete Week 78 will be conducted.

4.2.4 Subgroup analysis

Subgroup analysis by laboratory ApoE ε4 status will be performed using the same MMRM model as the primary analysis except that ApoE will not be used as a covariate in the model since the subgroup analysis will be done in ApoE ε4 carrier and non-carrier separately. Subgroup analysis by ARIA status during PC period will also be performed.

4.2.5 By visit summary for LTE period

The PC baseline and change from PC baseline values will be summarized by LTE treatment groups (early start low dose, early start high dose, late start low dose, late start high dose) and by visit for each of the ROIs up to Week 182.

4.2.6 Visit windows for by visit analysis

For by visit summaries and MMRM models, the analysis visit should be defined using visit windows (see Table 2 below). If there is more than 1 value in the same analysis visit window (>1 value for the same analysis visit), the closest record to the target visit day will be used for the by visit analysis. If they are with the same distance from the target visit day, then the record with the later time will be used for the by visit analysis.

Table 2. Visit Windows for structural MRI data

Analysis visit *	Target visit day	Analysis visit window
Baseline	1	Most recent non-missing pre-dose value
Week 30	211	[92, 379]
Week 78	547	[380, the end day of the placebo-controlled period[1]]
Week 134	939	[the start day of the LTE period[2], 1107]
Week 182	1275	>=1108

* Analysis visit is the visit at which the specific week started.
1. The end day of the placebo-controlled period is the last day before the first infusion in LTE, and is the last day in study for subjects who do not enter LTE.
2. The start day of the LTE period is the day of first infusion in LTE period.

4.3 Tau PET Analysis

4.3.1 Background

To evaluate aducanumab treatment effect on tau pathology, tau PET imaging is performed on a subset of sites and subjects using ^{18}F-MK-6240 ligand. Standardized Uptake Values Ratios (SUVRs) are calculated for each region of interest (ROI) sampled for the brain regions listed in Table 3 as compared to the reference region (RR) of cerebellar cortex. Each subject participating in tau PET substudy is supposed to undergo scans at screening, Week 78, Week 132, Week 182 and early termination visit. Due to the early termination of the studies, all the post-baseline tau PET assessments were performed within the timeframe of PC period, with a range of 9 to 20 months post-baseline. No assessments were performed in the long-term extension period. The distribution of the study time when the post-baseline assessments were performed will be presented. Due to the small sample size, all the analyses will be conducted using pooled data from the 2 studies.

For each ROI, the SUVRs from the corresponding left and right-side brain regions will be averaged to get a single SUVR for that ROI. If one side SUVR is missing, then the SUVR of the other side will be used as the SUVR for that ROI.

SUVRs of 6 composite regions (Table 3) will be calculated by averaging the SUVRs from all the ROIs that belong to that specific composite region.

Table 3. Summary of ROI

ROI Description	Location	Composite region
Middle Frontal Gyrus	FRONTAL LOBE	Frontal composite
Precentral Gyrus	FRONTAL LOBE, PRECENTRAL	Frontal composite
Straight Gyrus	FRONTAL LOBE, STRAIGHT GYRUS	Frontal composite
Anterior Orbital Gyrus	ORBITOFRONTAL CORTEX	Frontal composite
Inferior Frontal Gyrus	FRONTAL LOBE	Frontal composite
Superior Frontal Gyrus	FRONTAL LOBE	Frontal composite
Medial Orbital Gyrus	ORBITOFRONTAL CORTEX	Frontal composite
Lateral Orbital Gyrus	ORBITOFRONTAL CORTEX	Frontal composite
Posterior Orbital Gyrus	ORBITOFRONTAL CORTEX	Frontal composite
Subgenual Frontal Cortex	CINGULATE, SUBGENUAL	Cingulate composite
Subcallosal Area	SUBCALLOSAL AREA	Cingulate composite
Pre-Subgenual Frontal Cortex	CINGULATE, PRESUBGENUAL	Cingulate composite
Hippocampus	HIPPOCAMPUS	Medial temporal composite
Anterior Temporal Lobe Medial Part	TEMPORAL LOBE	Medial temporal composite
Anterior Temporal Lobe Lateral Part	TEMPORAL LOBE	Medial temporal composite
Parahippocampal And Ambient Gyri	PARAHIPPOCAMPAL	Medial temporal composite
Superior Temporal Gyrus Posterior Part	TEMPORAL LOBE	Temporal composite
Middle and Inferior Temporal Gyrus	TEMPORAL LOBE	Temporal composite
Fusiform Gyrus	FUSIFORM GYRUS	Temporal composite
Posterior Temporal Lobe	TEMPORAL LOBE	Temporal composite
Superior Temporal Gyrus Anterior Part	TEMPORAL LOBE	Temporal composite

Postcentral Gyrus	PARIETAL LOBE, POSTCENTRAL	Parietal composite
Superior Parietal Gyrus	PARIETAL LOBE	Parietal composite
Inferiolateral Remainder of Parietal Lobe	PARIETAL LOBE	Parietal composite
Lateral Remainder of Occipital Lobe	OCCIPITAL LOBE	Occipital composite
Lingual Gyrus	OCCIPITAL LOBE, LINGUAL GYRUS	Occipital composite
Cuneus	OCCIPITAL LOBE, CUNEUS	Occipital composite
Caudate Nucleus	CAUDATE NUCLEUS	
Putamen	PUTAMEN	
Thalamus	THALAMUS	
Cingulate Gyrus Anterior Part	CINGULATE	
Gyrus Cinguli Posterior Part	CINGULATE	
Subcortical White Matter	SUBCORTICAL WHITE MATTER	
Pons	PONS VAROLII	
Cerebellar White Matter	CEREBELLAR WHITE MATTER	
Cerebellum	CEREBELLUM	
Cerebellum Eroded	CEREBELLAR CORTEX	

4.3.2 Tau PET analysis population

The tau PET analysis population is defined as all randomized subjects who received at least one dose of study treatment (aducanumab or placebo) and had an evaluable baseline tau PET SUVR value. The tau PET modified analysis population is defined as all randomized subjects who received at least one dose of study treatment (aducanumab or placebo), had an evaluable baseline tau PET SUVR value and a post-baseline tau PET SUVR value. The tau PET modified analysis population will be used for ANCOVA model.

4.3.3 By visit summary and ANCOVA model for PC period

All the available post-baseline tau PET assessments will be used as one single post-baseline timepoint for PC analysis purpose. No tau PET data were collected in the LTE period, and thus no LTE analysis will be performed.

The baseline and change from baseline values will be summarized by treatment groups (placebo, low dose and high dose) for each of the 6 composite regions and each of the ROIs at baseline and at post-baseline timepoint.

An ANCOVA model will be used to analyze change from baseline value for each composite region and each ROI at post-baseline timepoint. The model will include treatment groups (placebo, low dose and high dose), baseline SUVR value (continuous) and laboratory ApoE ε4 status (carrier and non-carrier) as covariates. Adjusted means for each treatment group, pairwise adjusted differences with placebo, 95% confidence intervals for the differences and associated p-values will be presented at post-baseline timepoint.

A listing of individual tau PET data for the composite regions by treatment group will be provided. All the subjects in the tau PET analysis population will be included.

4.3.4 Dosing related analysis for tau PET

A summary table of the dosing information in PC period by treatment group will be provided, including the cumulative dose, number of doses at each dose level. A scatterplot of the cumulative dose in PC period versus Tau PET change from baseline at post-baseline assessment time will be provided for composite regions. These analyses will be done using the tau PET modified analysis population.

4.3.5 Correlation analysis between tau PET and other endpoints

Correlation analysis between tau PET composite regions and amyloid PET composite SUVR will be performed. The latest available amyloid PET assessment in PC period will be used for subjects with a post-baseline tau PET for the correlation, regardless of the analysis visit window in amyloid PET analysis. Partial Pearson/Spearman correlation adjusting for baseline tau values and baseline amyloid PET values will be used. Due to the very small sample size, the correlation analysis between tau PET and efficacy endpoints will not be conducted.

4.4 CSF Biomarker Analysis

4.4.1 Background

CSF sample collection was included at screening, Week 78, Week 132, Week 182 and early termination visit in a subset of subjects who consented to the CSF substudy. The CSF biomarkers that may be used for analysis are listed in Table 4 below.

Table 4. CSF biomarkers

Biomarker	Assay	Lab/Assay Validation Status
pTau 181	Innotest Phospho-Tau (181P)	Exploratory
Total Tau	Quanterix Simoa Total Tau 2.0	Exploratory
sTREM2	Biogen sTREM2 ELISA	Exploratory
Neurogranin	Euroimmun Neurogranin ELISA	Exploratory
β-Amyloid 1-42	Lumipulse G β-Amyloid 1-42	GCLP
β-Amyloid 1-40	Lumipulse G β-Amyloid 1-40	GCLP
pTau 181	Lumipulse G pTau 181	GCLP
Total Tau	Lumipulse G Total Tau	GCLP

Abbreviation: GCLP = good clinical laboratory practice.

4.4.2 CSF analysis population

The CSF analysis population for a specific biomarker is defined as all randomized subjects who received at least one dose of study treatment (aducanumab or placebo) and had an evaluable baseline value for that specific biomarker. Each CSF biomarker will have its own analysis population. The CSF modified analysis population for a specific biomarker is defined as all randomized subjects who received at least one dose of study treatment (aducanumab or placebo), had an evaluable baseline value as well as a post-baseline value at Week 78 for that specific biomarker. The overlap between the CSF modified analysis population and other analysis populations will be presented. Baseline characteristic in the CSF modified analysis population will be assessed. The CSF modified analysis population will be used for ANCOVA analysis.

CSF data at screening are also available for a subset of screen failure subjects. The CSF screening population is defined as all subjects (including randomized and screen failed) who had an evaluable screening value for the specific biomarker. The CSF screening population will be used for the analyses that the screening failure data are included.

4.4.3 By visit summary and ANCOVA model for PC period

The baseline and change from baseline values will be summarized by treatment groups (placebo, low dose and high dose) for each CSF biomarker at baseline and Week 78.

An ANCOVA model will be used to analyze change from baseline value for each CSF biomarker at Week 78. The model will include treatment groups (placebo, low dose and high dose), baseline biomarker value (continuous), laboratory ApoE ε4 status (carrier and non-carrier) and baseline age as covariates. Adjusted means for each treatment group, pairwise adjusted differences with placebo, 95% confidence intervals for the differences and associated p-values will be presented at week 78.

4.4.4 By visit summary for LTE period

The PC baseline and change from PC baseline values will be summarized by LTE treatment groups (early start low dose, early start high dose, late start low dose, late start high dose) for each CSF biomarker at baseline and each of the post-baseline visit.

A listing of individual CSF biomarker data by LTE treatment group will be provided for each of the CSF biomarkers. A spaghetti plot for longitudinal data over time by treatment group will be generated.

4.4.5 Visit windows for by visit analysis

For by visit summaries and ANCOVA models, the analysis visit should be defined using visit windows (see Table 5 below). If there is more than 1 value in the same analysis visit window (>1 value for the same analysis visit), the closest record to the target visit day will be used for the by visit analysis. If they are with the same distance from the target visit day, then the record with the later time will be used for the by visit analysis. In addition, a window of 12 months or greater may be applied to Week 78 analysis visit if big distance to the target Week 78 is observed in a large proportion of subjects in this analysis.

Table 5. Visit Windows for CSF biomarker data

Analysis visit *	Target visit day	Analysis visit window
Baseline	1	Most recent non-missing pre-dose value
Week 78	547	[2, the end day of the placebo-controlled period[1]]
Week 132	925	[the start day of the LTE period[2], 1107]
Week 182	1275	>=1108

* Analysis visit is the visit at which the specific week started.
1. The end day of the placebo-controlled period is the last day before the first infusion in LTE, and is the last day in study for subjects who do not enter LTE.
2. The start day of the LTE period is the day of first infusion in LTE period.

4.4.6 Dosing related analysis for CSF biomarkers

A summary table of the dosing information in PC period by treatment group will be provided, including the cumulative dose, number of doses at each dose level. A scatterplot of the cumulative dose in PC period versus CSF biomarker change from baseline at Week 78 will be provided. These analyses will be done using the CSF modified analysis population.

4.4.7 Correlation analysis between CSF biomarkers and other endpoints

Correlation analysis between certain CSF biomarkers and amyloid PET SUVR will be performed. Partial Pearson/Spearman correlation between the change from baseline at Week 78 in CSF biomarker and amyloid PET composite SUVR adjusting for baseline CSF and baseline amyloid PET composite SUVR values will be conducted. The correlation analysis between CSF biomarker and efficacy endpoints may be conducted.

4.4.8 Analysis on screening data from screen failure subjects

The screening CSF biomarker data for both randomized and screen failure subjects will be summarized.

4.4.9 Concordance analysis between CSF biomarkers and amyloid PET scan visual read at screening

The concordance between amyloid status based on CSF biomarkers and amyloid PET scan visual read at screening will be analyzed. Subjects in the CSF screening population with an amyloid PET visual read at screening will be used for this analysis. The demographic and baseline characteristics of the subjects used in this analysis will be presented.

The CSF amyloid status will be assessed using different CSF biomarkers (Aβ42, Aβ40, tTau and pTau) and their ratios (Aβ42/Aβ40, tTau/Aβ42 and pTau/Aβ42). Receiver operating characteristic (ROC) plot will be presented for each biomarker or ratio and the area under the curve (AUC) will be calculated. The positive percent agreement (PPA or sensitivity), negative percent agreement (NPA or specificity), and the Youden J index (PPA + NPA – 1) will be plotted for each biomarker or ratio. The biomarker or ratio value that maximizes the Youden J index will be identified and the corresponding 2-by-2 agreement table will be presented. In addition, plots for visualizing the biomarker values in subgroup of amyloid PET scan visual positive subjects and negative subjects will be performed.