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

Protocol Version: Version 6.0
Date of Protocol: 28 Jun 2018

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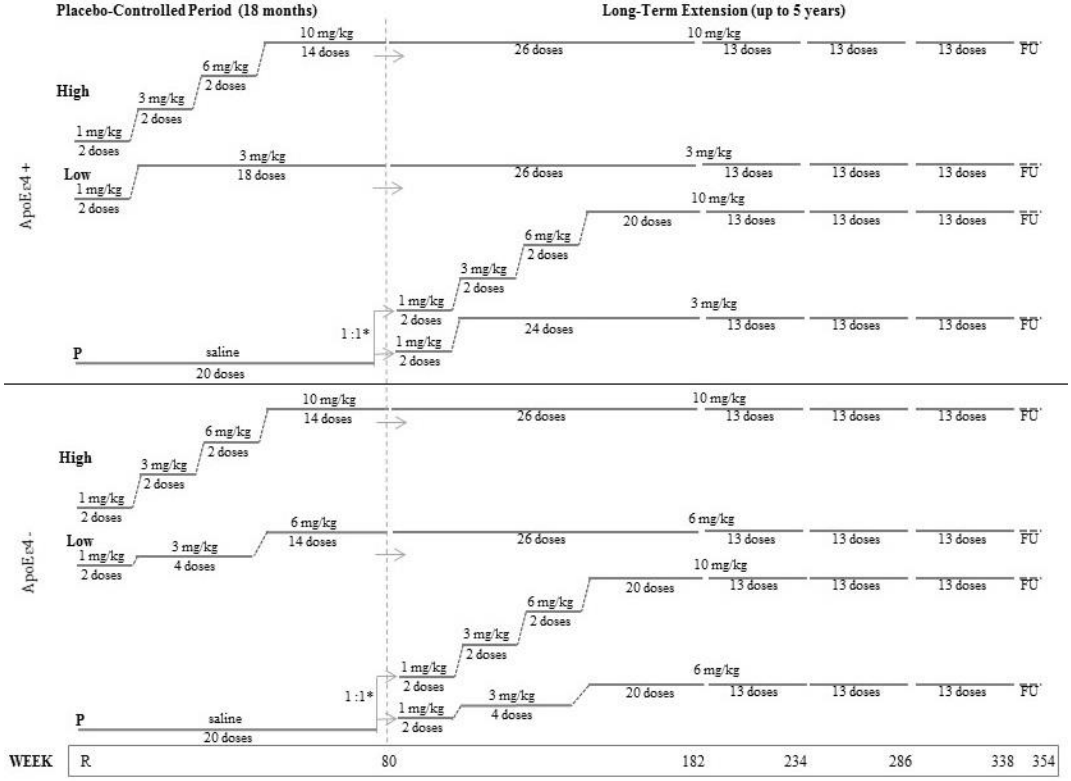
1 OVERVIEW

This document 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 period, followed by an optional dose-blinded 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 placebo-controlled period (PCP) to assess the treatment effect of aducanumab on exploratory biomarkers; data from long-term extension (LTE) 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 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 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 cerebrospinal fluid (CSF). Specific biomarkers for each modality are described in the following subsections. Task-free-MRI, ASL-MRI, serum and plasma biomarkers 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. Certain analyses may also be performed in pooled 221AD301 and 221AD302 data if the sample size is small for each individual study.

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 magnetic resonance imaging (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 PCP

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.

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 PCP will also be performed.

4.2.5 By visit summary for LTE

The baseline and change from PCP 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 volumetric 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. Since all the tau PET subjects were still in PCP at the time of the early termination of the studies due to futility, no LTE tau PET data were collected, and no LTE analysis will be performed.

Table 3. Summary of ROI

ROI Description	Location
Middle Frontal Gyrus	FRONTAL LOBE
Precentral Gyrus	FRONTAL LOBE, PRECENTRAL
Straight Gyrus	FRONTAL LOBE, STRAIGHT GYRUS
Anterior Orbital Gyrus	ORBITOFRONTAL CORTEX
Inferior Frontal Gyrus	FRONTAL LOBE
Superior Frontal Gyrus	FRONTAL LOBE

Medial Orbital Gyrus	ORBITOFRONTAL CORTEX
Lateral Orbital Gyrus	ORBITOFRONTAL CORTEX
Posterior Orbital Gyrus	ORBITOFRONTAL CORTEX
Subgenual Frontal Cortex	CINGULATE, SUBGENUAL
Subcallosal Area	SUBCALLOSAL AREA
Pre-Subgenual Frontal Cortex	CINGULATE, PRESUBGENUAL
Hippocampus	HIPPOCAMPUS
Anterior Temporal Lobe Medial Part	TEMPORAL LOBE
Anterior Temporal Lobe Lateral Part	TEMPORAL LOBE
Parahippocampal And Ambient Gyri	PARAHIPPOCAMPAL
Superior Temporal Gyrus Posterior Part	TEMPORAL LOBE
Middle and Inferior Temporal Gyrus	TEMPORAL LOBE
Fusiform Gyrus	FUSIFORM GYRUS
Posterior Temporal Lobe	TEMPORAL LOBE
Superior Temporal Gyrus Anterior Part	TEMPORAL LOBE
Postcentral Gyrus	PARIETAL LOBE, POSTCENTRAL
Superior Parietal Gyrus	PARIETAL LOBE
Inferiolateral Remainder of Parietal Lobe	PARIETAL LOBE
Lateral Remainder of Occipital Lobe	OCCIPITAL LOBE
Lingual Gyrus	OCCIPITAL LOBE, LINGUAL GYRUS
Cuneus	OCCIPITAL LOBE, CUNEUS
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, 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, will be used for the analyses on tau PET.

4.3.3 By visit summary and ANCOVA model for PCP

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

An ANCOVA model will be used to analyze change from baseline value for each ROI at Week 78. 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 week 78.

For PCP analysis, all the post-baseline tau PET data (either at the planned Week 78 visit or at early termination visit) will be used for summary and modeling for the analysis visit Week 78. In addition, a window of 12 months or greater may be applied to Week 78 analysis visit if a big distance to the target Week 78 is observed in a large proportion of Tau PET subjects.

A listing of individual tau PET data by treatment group will be provided.

4.4 CSF Biomarker Analysis

4.4.1 Background

CSF samples are supposed to be collected 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 will be used for analysis are listed in Table 4 below.

Table 4. CSF biomarkers

Total Tau
Phospho-181 Tau (pTau)
Neurofilament Light (NfL)
Phospho Neurofilament Heavy (pNfH)
Neurogranin (Ng)
PSD-95
sTREM

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 overlap between the CSF analysis population and other analysis populations will be presented. Baseline characteristic in the CSF analysis population will be assessed.

4.4.3 By visit summary and ANCOVA model for PCP

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

The baseline and change from PCP 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 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 Analysis on screening data from screen failure subjects

CSF data at screening are also available for a subset of screen failure subjects. The screening data for both randomized and screen failure subjects will be summarized.

4.4.7 Correlation between CSF biomarkers and efficacy endpoints

Summary of the primary and secondary efficacy endpoints will be performed in the subjects that are in the CSF analysis population. More analyses on exploring the correlation between CSF biomarkers and efficacy endpoints may be performed.