STATISTICAL ANALYSIS PLAN
For plasma phospho-Tau 181

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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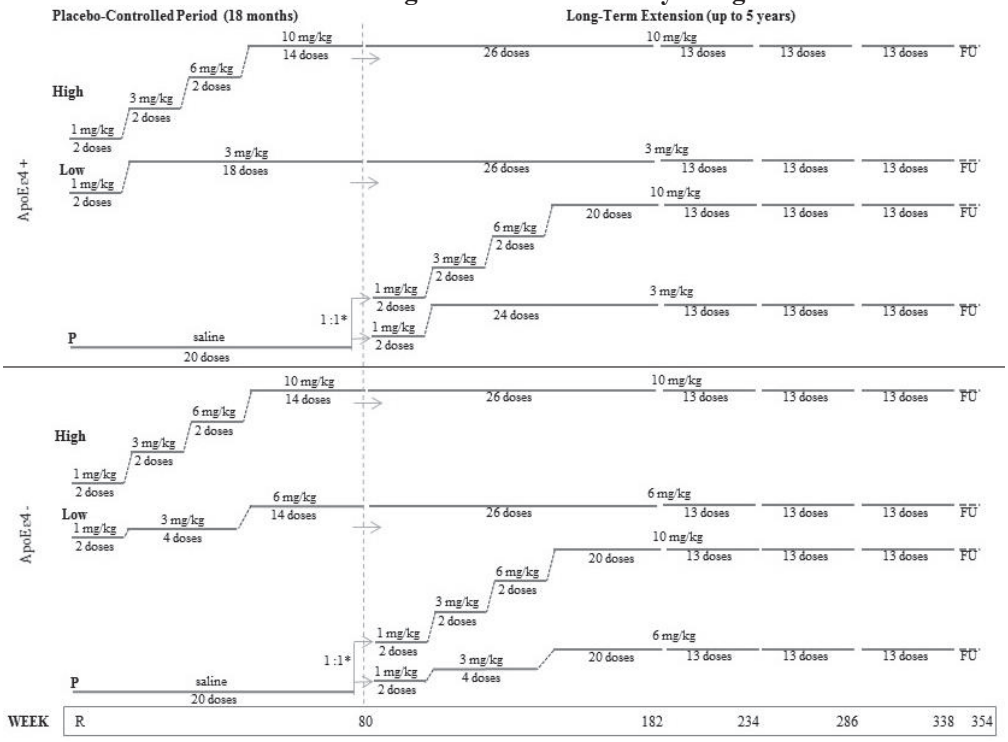
1 OVERVIEW

This statistical analysis plan (SAP) outlines the pre-planned analysis for the exploratory biomarker plasma phospho-Tau 181 (hereafter referred to as plasma pTau181, 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 were to perform an end-of-treatment visit within 4 weeks of the announcement and a safety follow-up visit 18 weeks from the last infusion.

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 biomarker included in this SAP is plasma pTau181. There are 2 objectives in this SAP: (1) assess the natural progression of plasma pTau181 and its association with other endpoints; (2) assess the treatment effect of aducanumab on plasma pTau181.

The analyses covered in this SAP use plasma pTau181 data from the randomized subjects in Studies 221AD301 and 221AD302. No data from screening failure subjects are available at the time of this SAP finalization and therefore this SAP does not cover any analysis that uses screening failure data (e.g., concordance analysis between screening plasma pTau181 and amyloid PET).

The plasma samples were processed after the database lock of the studies (Nov 2019), but this SAP is finalized before the Aducanumab team statisticians see any plasma pTau181 data.

4 SAMPLE COLLECTION AND SELECTION

The scheduled visits for plasma sample collection changed over time for the placebo-controlled period in different protocol versions, as show in Table 1 below. Due to the limit on the number of the samples that can be tested in one batch, only the intent-to-treat (ITT, which is defined as randomized and dosed) subjects with plasma samples available at both screening and Week 78 were selected for testing. For these subjects, their available samples at screening, Week 56 (Week 48 if under protocol versions 1-3), Week 78, Week 104 and Week

128 were tested. Due to the study early termination, the number of samples available for visits after Week 128 is very small and not enough for analysis.

Table 1. Scheduled visits for plasma sample collection in placebo-controlled period

	SV1	WK12	WK16	WK24	WK32	WK48	WK56	WK72	WK78 (EOT)
PV 1	Y			Y		Y		Y	Y
PV 3	Y		Y	Y		Y		Y	Y
PV 4-6	Y	Y	Y	Y	Y		Y		Y

Abbreviations: SV1 = screening visit 1; WK = week; PV = protocol version; EOT = end of treatment; FU = follow-up.

Approximately 7000 samples were tested, as shown in Table 2 below. It is anticipated that there will be tubes that do not have adequate volume or have quality issues. Therefore, the actual sample sizes will be smaller than this estimation.

Table 2. Counts of subjects and samples for testing

	221AD301	221AD302	Total
ITT subjects with samples at screening and Week 78	922	882	1804
Subjects with sample at screening (a)	922	882	1804
Subjects with sample at Week 48 or 56 (b)	861	834	1695
Subjects with sample at Week 78 (c)	922	882	1804
Subjects with sample at Week 104 (d)	517	402	919
Subjects with sample at Week 128 (e)	252	210	462
Total number of samples (a+b+c+d+e)	3474	3210	6684

Note: Due to the limit on the number of the samples that can be tested in one batch, only the ITT subjects with plasma samples available at both screening and Week 78 were selected for testing.

5 STATISTICAL ANALYSIS METHODS

5.1 General Considerations

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. For MMRM analysis, adjusted means for each treatment group and the

standard errors, pairwise adjusted differences with placebo, 95% confidence intervals for the differences and associated p-values will be presented for each post-baseline visit, if applicable. For correlation analysis, the Pearson and Spearman correlation coefficients, 95% CIs and p-values will be presented.

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.

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

5.2 Considerations on data distribution and normality

Data distribution will be assessed before conducting the analyses. If skewness or outliers in the distribution of the endpoint used in the analysis are observed, which violates the normality assumption required for that analysis, data transformation may be used to normalize the data, or the pre-specified approach may be replaced with non-parametric approach that is more appropriate in such case.

5.3 Analysis population and summary of the population

The plasma pTau181 analysis population will include all the ITT subjects whose samples were selected for testing per Section 4 and had an evaluable baseline plasma pTau181 value. According to how the samples for testing was selected (Section 4), all the subjects should also have the Week 78 plasma pTau181 value, unless missing due to sample issues.

Demography and baseline characteristics will be summarized for the plasma pTau181 analysis population by treatment group and also in total for each study.

5.4 Analysis to assess the natural progression

This section covers the analyses to assess the natural progression of plasma pTau181 and its association with other biomarkers. Only data collected without active treatment will be used (data from baseline/screening visits, or longitudinal data from placebo subjects in the PC period). Analyses will be done on each study as well as pooled, unless otherwise specified.

5.4.1 Summary statistics of the baseline plasma pTau181

Summary statistics of the baseline plasma pTau181 data will be provided, as well as stratified by sex, age group, and ApoE carrier status.

5.4.2 Subject-level correlation between plasma pTau181 and CSF pTau181

The following analyses will be conducted to assess the biological correlation between plasma pTau181 and CSF pTau181:

(1) Correlation between baseline plasma pTau181 values and baseline CSF pTau181 values. Pearson/Spearman correlation will be assessed in the randomized subjects with baseline data for both biomarkers. Scatterplots of the 2 biomarkers will be provided for visualization.

(2) Correlation between plasma pTau181 change from baseline at Week 78 and CSF pTau181 change from baseline at Week 78 in placebo subjects. Pearson/Spearman correlation, both unadjusted and adjusted for age and the baseline values of the 2 biomarkers, will be assessed in the overlapping subjects. Scatterplots of the 2 biomarkers will be provided for visualization.

5.4.3 Baseline plasma pTau181 association with other baseline factors

This analysis is to assess the association between plasma pTau181 and other demographic or disease characteristic factors at baseline (see the list below). Pearson/Spearman correlation will be assessed between the baseline plasma pTau181 and each of the factors of interest. Scatterplots will be provided for visualization.

(1) Baseline age
(2) Baseline amyloid PET SUVR
(3) Baseline Tau PET
(4) Baseline clinical scores (primary and 3 secondary endpoints)

5.4.4 Baseline plasma pTau181 as a predictor of biomarker and clinical progression

(1) This analysis is to assess if the baseline plasma pTau181 predicts the post-baseline plasma pTau181 progression. Only the placebo-controlled period data from the placebo subjects will be used. Scatterplots of baseline pTau181 vs change from baseline in pTau181 at each post-baseline visits (Week 56 and 78) will be created to visualize the pattern. Correlation between baseline and change from baseline will be assessed. Depending on the pattern, an MMRM model may be used to analyze change from baseline value in plasma pTau181, with fixed effects of visit, baseline plasma pTau181 value and its interaction with visit, baseline age and its interaction with visit, and laboratory ApoE ε4 status. More covariates may be explored in addition to the ones listed.

(2) This analysis is to assess if the baseline plasma pTau181 predicts the post-baseline clinical progression (primary and 3 secondary endpoints). Only the placebo-controlled period data from placebo subjects will be used. Scatterplots of baseline pTau181 vs change from baseline in clinical endpoint at each post-baseline visits (Week 26, 50 and 78) will be created to visualize the pattern. Correlation between baseline and change from baseline will be assessed. Depending on the pattern, an MMRM model may be used to analyze change from baseline value in each of the clinical endpoints, with fixed effects of visit, baseline plasma pTau181 value, baseline plasma pTau181 value by visit interaction, baseline clinical endpoint value, baseline clinical endpoint value by visit interaction, baseline MMSE, symptomatic AD med use at baseline, laboratory ApoE ε4 status, and baseline age. More covariates may be explored in addition to the ones listed.

(3) If the analysis in (2) provides the evidence that baseline plasma pTau181 is a predictor of the clinical progression, additional efficacy analyses including baseline plasma pTau181 may be conducted. The possible analyses could be:

(a) Include baseline plasma pTau181 as an additional continuous covariate in the primary efficacy MMRM model for the clinical endpoints.

(b) Categorize subjects by their baseline plasma pTau181 values and then conduct the primary efficacy MMRM models for the clinical endpoints within each category.

5.5 Analysis to assess the longitudinal treatment effect

This section covers the analyses to assess the treatment effect of Aducanumab on plasma pTau181. Most of the analyses will be performed on the placebo-controlled period data, if not specified otherwise. A small set of analyses will be performed on the data including LTE timepoints. By default, all the analyses will be done for each study separately, unless otherwise specified.

5.5.1 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 plasma pTau181 up to Week 78.

An MMRM model will be used to analyze change from baseline value as the primary analysis for pTau181. Fixed effects of the model will include treatment groups (placebo, low dose and high dose), visit (Week 56 and Week 78), treatment group-by-visit interaction, baseline value (continuous), baseline value by visit interaction, 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 56 and Week 78.

5.5.2 By visit summary and MMRM model for PC+LTE data

Analyses will be presented by LTE treatment group (early start low dose, early start high dose, late start low dose, late start high dose). The following two comparisons will be evaluated for plasma pTau181 in the LTE period:

- The early start low dose compared with the late start low dose;
- The early start high dose compared with the late start high dose.

The baseline and change from baseline plasma pTau181 values will be summarized by LTE treatment groups by visit for all the visits in the placebo-controlled and LTE period. An MMRM model similar to the one for PC period will be used to analyze the cumulative data from both PC and LTE period. In this model the visits include baseline, Week 56, Week 78, Week 104 and Week 128. LTE treatment groups will be used.

5.5.3 Visit windows for by visit analysis

For by visit summaries and MMRM models, the analysis visit should be defined using visit windows (see Table 3 below). The lower bound of day 92 for Week 24 is inherited from the analysis window for Week 26 in the efficacy analysis of the main SAP, to avoid including visits that happened shortly after day 1 into post-baseline analysis. For the same reason, the lower bound of day 645 for Week 104 is inherited from the analysis window for Week 106 in the efficacy analysis of the main LTE SAP. The analysis window for Week 56 is picked as from day 281 (visit for Week 40) to day 448 (the day before the visit for Week 64), in order to cover both the Week 56 visits under protocol versions 4-6 and the Week 48 visits under protocol versions 1-3.

If there is more than one record in the same analysis visit window, the record closest to the target visit day will be used for the by visit analysis. If the records have the same distances from the target visit day, the later record will be used for the by visit analysis.

Table 3. Visit Windows

Analysis visit	Target visit day	Analysis visit window
Baseline	1	Most recent non-missing pre-dose value
Week 24	169	[92, 280]
Week 56	393	[281, 448]
Week 78	547	[449, the end day of the placebo-controlled period[1]]
Week 104	729	[645, 812]
Week 128	897	>= 813
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.		

5.5.4 Subgroup analysis by baseline factors

Subgroup analysis by baseline factors will be performed using the same MMRM model as the primary analysis but within each subgroup category. The baseline factors include:

- Age (<=64, 65-74, >=75)
- Sex (female or male)
- ApoE ε4 status (carrier or non-carrier)
- Baseline clinical stage (MCI due to AD or mild AD)
- Symptomatic AD medication use at baseline (yes or no)
- Baseline amyloid PET composite SUVR (by quartile)
- Baseline plasma pTau181 (by quartile)
- Consenting to protocol version 4 (PV4) by Week 13 (pre-PV4 or post-PV4), if the sample size is big enough for post-PV4 group.

5.5.5 Dosing related analysis for plasma pTau181

A summary table of the dosing information in PC period by treatment group will be provided, including the cumulative dose and the number of doses at each dose level. A scatterplot of the cumulative dose in PC period versus biomarker change from baseline at Week 78 will be provided.
Analysis following the same primary MMRM model but done in subsets defined by the actual dosing received will be conducted.

5.5.6 Subject-level correlation between plasma pTau181 and amyloid

Correlation analysis between the change from baseline at Week 78 in plasma pTau181 and change from baseline at Week 78 in amyloid PET composite SUVR will be conducted. Pearson/Spearman correlation, both unadjusted and adjusted for age and the baseline values of the 2 biomarkers, will be assessed in each study by treatment groups and pooled (placebo, low dose, high dose, active total and total) in the overlapping subjects who have the Week 78 measure for both biomarkers.

5.5.7 Subject-level correlation between plasma pTau181 and clinical endpoints

Correlation analysis between the change from baseline at Week 48/56 or Week 78 in plasma pTau181 vs change from baseline at Week 78 in each of the 4 clinical endpoints (primary and the 3 secondary endpoints) will be conducted. Pearson/Spearman correlation, both unadjusted and adjusted for age and the baseline values of pTau181 and the clinical endpoint, will be assessed in each study by treatment groups and pooled (placebo, low dose, high dose, active total and total) in the overlapping subjects who have the Week 78 measure for both pTau181 and clinical.

5.5.8 Group level correlation between plasma pTau181 and clinical endpoints

Treatment effects (difference vs placebo obtained from MMRM analysis) of plasma pTau181 at Week 48/56 or Week 78 vs treatment effects of clinical endpoints in low dose and high dose groups at Week 78 for each of the studies will be examined to see if on a group level they are correlated. Bubble plots will be produced to help visualization and correlation coefficient will be calculated.