Statistical Analysis Plan

Title: A Randomized, Double-Blind, Placebo-Controlled, Phase 2 Study Assessing the Safety, Tolerability and Long-term Efficacy of Bryostatin in the Treatment of Moderately Severe Alzheimer's Disease Subjects Not Receiving Memantine Treatment

Protocol Number: NTRP-101-204

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Previous SAP Versions

New Document

SAP Amendments before database lock

Version	Issue Date	Section	Revision / Addition	Rationale

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1 INTRODUCTION

This document details the planned statistical analyses for Synaptogenix, Inc., protocol NTRP-101-204 study titled "A Randomized, Double-Blind, Placebo-Controlled, Phase 2 Study Assessing the Safety, Tolerability and Long-term Efficacy of Bryostatin in the Treatment of Moderately Severe Alzheimer's Disease Subjects Not Receiving Memantine Treatment".

The proposed analyses are based on the contents of the final version 6.0 of the protocol (dated 07-Sep-2021).

This is a randomized double-blind placebo-controlled, Phase 2 study comparing bryostatin-1 (referred to as 'bryostatin' hereafter) to placebo for long-term efficacy in the treatment of moderately severe Alzheimer's disease (Mini Mental State Examination, 2nd edition [MMSE-2] scores of 10-18 at baseline) in the absence of memantine. Eligible subjects will receive 7 doses of bryostatin (i.v., 20µg) or matching placebo during the first 12 weeks. A second course of treatment consisting of 7 doses of either bryostatin or placebo (consistent with the treatment received in first 12 weeks), will begin 30 days after the final dose of the first treatment period. Cognitive tests will be assessed at intervals during the study and 4 months after the final dose of study drug.

2 STUDY OBJECTIVES

To evaluate the safety, tolerability and long-term efficacy of bryostatin for the treatment of moderately severe Alzheimer's disease in subjects not receiving concurrent memantine treatment, including: 1) determining how long the therapeutic effects will last; and 2) assessing whether a second treatment would be equally effective if the therapeutic effects of the first treatment do not last.

3 ENDPOINTS

3.1 Primary Endpoint

The primary efficacy endpoint is change from baseline in the Severe Impairment Battery (SIB) total score assessment obtained after completion of the second course of treatment (Week 28).

3.2 Secondary Endpoints

Secondary Efficacy Endpoints

- SIB score at the end of the Week 42 follow-up visit
- The change from baseline (screening) SIB total score at Week 13
- The changes from baseline at Weeks 5, 9, 15, 20 and 24 in the SIB total score
- The changes from baseline at Weeks 5, 9, 13, 15, 20 and 24 in the SIB total score for subjects with baseline MMSE-2 scores of 10-14 and 15-18
- SIB trends over time; individual-specific slopes of total SIB scores will be obtained for all patients.

Exploratory Endpoints

- Change from baseline in Alzheimer's Disease Cooperative Study – Activities of Daily Living – Severe Impairment Version (ADCS-ADL-Sev) total score at Weeks 13 and 42
- Change from baseline in MMSE-2 total score at Weeks 13 and 42
- Change from baseline in Neuropsychiatric Inventory (NPI) total score at Weeks 13 and 42

Safety Assessments

- Treatment emergent adverse events (AEs) and serious adverse events (SAEs)
- Vital signs, hematology, blood chemistry, and physical examination including body weight
- Electrocardiogram (ECG) parameters
- Columbia Suicide Severity Rating Scale (C-SSRS)

4 SAMPLE SIZE

The power analysis for the current study is based on a minimum clinically significant difference in mean total SIB scores from baseline of 4 points between the placebo and bryostatin arms at Week 28. Since the analysis of this primary outcome is based on a linear regression model that utilizes the total SIB scores at Weeks 0, 5, 9, 13, 15, 20, 24, and 28, a difference of approximately 0.15 points per week in the slope of total SIB scores between the placebo and bryostatin arms yields the clinically meaningful difference of 4 points at Week 28. General estimating equations (GEE) power analysis is based on the following equation:

$$m = \frac{2\left(z_{\alpha/2}+z_{\beta}\right)^2\sigma^2(1-\rho)}{ns_x^2d^2} \qquad \text{Eq 1}$$

In equation 1, m is the number of patients per group, $z_{a/2}$ is the type I error rate, z_b is the type II error rate, σ is the standard deviations of the error (residual) terms, ρ is the within person correlation of total SIB scores over time within a person, n is the time points where the total SIB score is obtained, s_x^2 is the variance of the X predictor variable (i.e. time in the current scenario), and d is the difference in slopes over time in total SIB measures from baseline between the placebo and bryostatin arms.

Based on the data from the NTRP101-202 study among patients without memantine, we obtained an estimate for σ = 5.3. In addition, the NTRP101-204 study will have SIB total scores measured at n = 8 timepoints, giving a value of s_x^2 = 127. Assuming a total of 100 patients (50 patients per arm), a two-sided type I error rate of 0.05 (i.e. $z_{a/2}$ = 1.96), and moderate within person correlation of outcome measures (i.e. ρ = 0.5), we will have > 99% power to see a difference in slopes over time of d = 0.15 points per week. In addition, we will have > 99% power to see this difference if we experience a 20% drop out.

The GEE methodology is an appropriate approximation for estimating power under the mixed model with repeated measures (MMRM) when time as continuous. The GEE and Mixed model give equal parameter estimates under the conditions of an exchangeable working correlation for the GEE model and a random intercept for the Mixed model. In addition, the GEE was initially proposed for the primary analysis of the trial; however, it was later determined that the MMRM would be generally more commonly used.

5 RANDOMIZATION

Once all eligibility criteria for the study have been met, and the site has received approval by the Medical Monitor (MM) and Clinical Assessment Technologies (CAT) group, via the Electronic Data Capture (EDC) system, the subject can be randomized via Interactive Response Technology (IRT) system. Randomization will be stratified by SIB total score at baseline and baseline SIB scores will be balanced at baseline between the treatment groups. Mean values of baseline SIB total scores by group will be monitored in real time during the enrollment process. A randomization number will be assigned, and drug for that randomization number will be shipped to the site for

twelve weeks of treatment. Randomization and scheduling of the first study drug infusion should be timed to allow for receipt of study drug prior to the scheduled study treatment. The drug kits will be shipped to the individual who will be responsible for kit storage and drug preparation and may not be handled by any other study staff member. The study drug kit will not identify the vial containing study drug as either placebo or bryostatin. After completion of Week 13, a second study drug kit will be provided for treatment period two. The IRT system will be used to register subjects for treatment and trigger study drug shipments.

6 PLANNED ANALYSES

All statistical analyses described in this Statistical Analysis Plan (SAP) were finalized and approved by the trial Principal Investigator (PI), Dr. Miao-Kun Sun, Chief Scientific Officer, Dr. Daniel Alkon, Lead Statistician, Richard Thompson, and WCT Statistician, prior to the complete unmasking of the data. In addition, the methods described herein are given precedence over the analytical plans outlined in the clinical protocol. However, no SAP prepared in advance of the data can be final until the data are unmasked. The final Clinical Study Report (CSR) may contain additional tables or statistical tests if warranted by the data obtained. The justification for any such additional analyses will be fully documented in the final CSR.

6.1 Analysis Sets

6.1.1 Enrolled Set

The Enrolled Set includes all those subjects who gave informed consent.

6.1.2 Full Analysis Set

The Full Analysis Set (FAS), consistent with the modified intention-to-treat (mITT) principles, is defined as all randomized subjects who received at least one dose of study medication and who have at least one post-baseline efficacy assessment.

6.1.3 Per-Protocol Set

The Per Protocol Analysis Set (PP) is defined as all patients completing the study without major protocol deviations.

Major protocol deviations will include but not limited to:

- Non eligibility
- Prohibited concomitant medications
- Informed consent withdrawal
- Unable to commit visit schedule
- Missed 3 or more doses

6.1.4 Safety Analysis Set

The Safety Analysis Set (SAS) is defined as all randomized subjects who received any study medication (either partial or completed infusions of bryostatin or placebo).

6.1.5 Completer Analysis Set

The Completer Analysis Set (CAS) is defined as all randomized subjects who completed two courses of treatment, and who have a Week 28 SIB assessment.

6.2 Derived Data

This section describes the derivations required for statistical analysis. Unless otherwise stated, variables derived in the source data will not be re-calculated.

6.2.1 Race

The race will be categorized into two racial groups: White, if only White race selected, and non-White – in all other cases. The listings will reflect the original selected categories.

6.2.2 Baseline

Baseline is defined as the last non-missing value (either scheduled, unscheduled or repeat) before the subject receives the first dose of study drug.

6.2.3 Duration / Study Day / Time

Study day will be calculated as the number of days from first dose of *study drug*.

- date of event – date of first dose of study drug + 1, for events on or after first dose
- date of event – date of first dose of study drug, for events before first dose

6.2.4 Conventions for Missing and Partial Dates

All rules explained below for partial / missing dates will be followed unless contradicted by any other data recorded on the electronic Case Report Form (eCRF).

All dates presented in the individual subject listings will be as recorded on the eCRF (i.e., not completed as per the below rules).

6.2.5 Missing / Partial Start / Stop Date of Adverse Events (AE) and Concomitant Medications

Missing and partial start and stop date will be imputed for analysis purposes as follows.

Partial or missing stop date will be imputed as follows:

If the stop date is completely missing and the event has resolved, or the subject has stopped taking the concomitant medication, the stop date will be imputed as the date of the subject's last clinic visit in the study.

- If only the year is known, the stop date will be imputed as "31-Dec" of that year or as the date of the subject's last clinic visit in the study if in the same year.
- If the month and year are known, the stop date will be imputed as the last day of that month unless the stop date corresponds to the same month as the subject's last clinic visit in which case the date of subject's last clinic visit in the study will be used instead.

Missing start date will be imputed as follows:

- If the stop date occurs on or after the start of study drug or the event / concomitant medication is ongoing, the start date will be imputed as the date of the first dose of study drug.
- If the stop date occurs before the start of study drug, the start date of the event / concomitant medication will be imputed as the subject's screening date or the stop date of the event / concomitant medication whichever the earlier.

Partial start date (year present, but month and day missing)

- If the stop date occurs on or after the start of study drug or the event / concomitant medication is ongoing, and the year is the same as the year of first dosing the start date will be imputed as "01-Jan" of the same year or the date of the first dose of study drug whichever is latest. If the year is different from the year of first dosing "01-Jan" will be used.
- If the stop date occurs before the start of study drug, the start date of the event / concomitant medication will be imputed as the "01-Jan" of the same year.

Partial start date (month and year present, but day missing)

- If the stop date occurs on or after the start of study drug or the event / concomitant medication is ongoing, the start date will be imputed as the first day of the same month and year unless this partial start date is in same month as the first dose of study drug in which case the date of first dose of study drug will be used.
- If the stop date occurs before the start of study drug, the start date will be imputed as the first day of the month and year of the partial stop date.

If the start time is missing it will be imputed only in the case where the start date of the concomitant medication / event corresponds to the date of the first dose of study drug. The time will be imputed as the same time as the first dose of study drug. In all other cases the time will not be imputed.

6.2.6 Missing Date of Study Drug Dosing

In this trial, patients will receive their assigned treatment via infusion under the direction of a clinician. Any missing date on the administration of the study drug will occur if the clinician neglects to record the date the infusion was given. If a date of dosing is missing, efforts will be made to determine the date of infusion from additional sources of information including healthcare facility visitation logs, contact with health care providers, or family members who would have scheduled the infusion visit. If alternative records on are not available to determine an infusion date, then for all administration visits, excepting 3rd and 8th doses, the missing infusion date will

be imputed as 7 days plus the date of the previously weeks' infusion since the doses are given on a weekly basis. For 3rd and 8th doses, the missing infusion date will be imputed as respectively 14 and 28 days plus the date of the previously weeks' infusion, according to the Schedule of Activities.

6.2.7 Missing Diagnosis Dates

If the month and year are present but the day is missing, the diagnosis date will be set to first day of the relevant month. If only the year is recorded the diagnosis date will be set as "01-Jan" for that year.

6.2.8 Exposure to Study Drug

Total volume of bryostatin infused (mg) during the study will be calculated as:

$$\sum_i [0.024 \cdot L_i + 0.020 \cdot (1 - L_i)] \cdot \left[C_i + (1 - C_i) \cdot \frac{TVI_i}{59.6 \cdot L_i + 58.0 \cdot (1 - L_i)}\right] \quad \text{Eq 2}$$

0.024 (mg) – loading dose of bryostatin, 0.020 (mg) – maintenance dose of bryostatin, according to Section 4.1 of the Protocol,

59.6 (mL) – total solution volume for loading dose, 58.0 (mL) – total solution volume for maintenance dose, according to Section 5.3 of the Protocol,

L_i – sign for loading dose, equals to 1 if dose is loading and equals to 0 if dose is maintenance,

C_i – sign for completed dose, equals to 1 if subject received the full intended infusion and equals to 0 otherwise,

TVI_i – total volume infused (mL), in case of incomplete infusion

i – number of an infusion. The sum should be calculated for all performed infusions.

Planned dose of bryostatin is the sum of doses of full infusions for whole study: for each of 2 courses there are 2 infusions of loading dose 24 mcg and 5 infusions of maintenance dose 20 mcg. Totally planned dose equals to $2 \cdot (2 \cdot 54 + 5 \cdot 20) = 416$ mcg.

This value will be used for calculation of fraction of subjects received at least 80% of planned dose.

6.2.9 Inexact Values

In the case where a variable is recorded as "> x", "≥ x", "< x" or "≤ x", a value of x will be taken for analysis purposes.

6.2.10 Electrocardiogram Data

For ECG data recorded on continuous scales, if more than one value is recorded at a time point, the mean value rounded to the integer will be presented. For overall interpretation if more than one value is recorded, the most severe (worst case) of the respective readings will be taken.

6.2.11 Columbia-Suicide Severity Rating Scale (C-SSRS)

The following outcomes are C-SSRS categories and have binary responses (yes / no). The categories have been re-ordered from the actual scale to facilitate the definition of composite endpoints:

Category 1	Wish to be Dead
Category 2	Non-specific Active Suicidal Thoughts
Category 3	Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
Category 4	Active Suicidal Ideation with Some Intent to Act, without Specific Plan
Category 5	Active Suicidal Ideation with Specific Plan and Intent
Category 6	Preparatory Acts or Behavior
Category 7	Aborted Attempt
Category 8	Interrupted Attempt
Category 9	Actual Attempt (non fatal)
Category 10	Completed Suicide

Suicidal Ideation since baseline – A "yes" answer at any time during double blind treatment to any one of the 5 suicidal ideation questions (categories 1-5) on the C-SSRS.

Suicidal Behavior since baseline – A "yes" answer at any time during double blind treatment to any one of the 5 suicidal behavior questions (categories 6-10) on the C-SSRS.

There will be no imputation of missing data for C-SSRS.

6.2.12 Unscheduled Visits

Only scheduled post-baseline laboratory, electrocardiogram, physical examination, and vital signs values will be tabulated. Post-baseline repeat / unscheduled assessments will only be listed in the relevant appendices to the CSR.

6.3 Conventions

All data listings, summaries, figures, and statistical analyses will be generated using SAS version 9.3 or higher[1].

Summaries will be presented by treatment group for efficacy endpoints and overall. Treatment group labels will be displayed as follows:

Bryostatin (0.20 mg)	Placebo

Overall columns are to be included within the table shells as follows:

Parameters	Columns
Demography	Treatment and overall
Baseline	Treatment and overall
Disposition	Treatment and overall
Efficacy	Treatment
AEs	Treatment
Other safety	Treatment

Listings will be sorted in the following order: treatment group, subject, parameter, and visit unless otherwise stated. All data will be listed, subjects who were not randomized will be displayed after the randomized treatment groups.

Continuous variables will be summarized by the number of non-missing observations, mean, median, inter-quartile range, standard deviation, and minimum and maximum.

Categorical variables will be summarized by presenting the frequency and percent. Percentages will be based on the number of non-missing observations or the subject population unless otherwise specified. For each variable, all categories will be shown. Zero frequencies (but not the percent) within a category will be presented.

6.3.1 Decimal Places

Decimal places for derived data described in Section 6.1.4 will be determined by the scale of measurement unless otherwise stated. No decimal places will be displayed if the smallest calculated value is ≥ 100; 1 decimal place will be displayed when the smallest value is within the interval (10, 100), with 10 being inclusive; 2 decimal places will be displayed when the smallest value is within (1, 10), with 1 being inclusive; and so on for even smaller scales of measurement.

Derived data known in advance of the results will be given as an integer. For example day, month, year, number of days and total scores (for rating scales) will be presented with zero decimal places.

Means, medians and percentiles will be displayed to one more decimal place than the data**,** dispersion statistics (e.g. standard deviation) will have two more decimal places, and the minimum and maximum will be displayed to the same number of decimal places as reported in the raw data. Percentages will be displayed with one decimal place.

P- Values will be quoted to 3 decimal places.

6.4 Subject Disposition

Subject disposition will be summarized and listed as follows:

- The number of subjects, who entered the study, were randomized, and who are in each analysis set will be summarized by treatment group and overall for the Enrolled Set.
- The number of subjects who failed screening and the reasons for failure will be tabulated for the Enrolled Set.
- Eligibility Criteria vioaltions will be presented for Enrolled Set.

- The number of early withdrawals and the reasons for withdrawals will be tabulated by treatment group and overall for the Enrolled Set.
- Number of subjects in the SAS by site
- Number of subjects by visit for the SAS

6.5 Protocol Deviations

Protocol deviations will be summarized by treatment group and overall.

Also a listing of protocol deviations will be provided.

6.6 Baseline Comparability

The comparability of treatment groups with respect to subject demographics and baseline characteristics will be assessed in a descriptive manner, but no formal statistical testing will be performed.

Standard continuous or categorical variable summaries will be presented by treatment group determined by the treatment actually received for the following variables based on the SAS and on the FAS.

- Demographic data
- Disease history
- Medical history
- Neuroimaging
- Neuropsychological assessments at screening:
    - Severe Impairment Battery (SIB)
    - Mini Mental State Examination, 2nd edition (MMSE-2)
    - Alzheimer's Disease Cooperative Study – Activities of Daily Living - Severe Impairment Version (ADCL-ADL-Sev)
    - Neuropsychiatric Inventory (NPI) metrics
    - Columbia Suicide Severity Rating Scale (C-SSRS)
- Physical examination by body system at screening
- Rosen-modified Hachinski Scale

All Baseline data will also be listed.

6.7 Medical History

Separate tabulations of previous and ongoing conditions at screening will be presented by randomized treatment group and overall for the SAS and the FAS. Medical History will be considered as previous if the start date and end date is less than date of first dose. Medical History will be considered as ongoing if the start date is greater than or equal to date of first dose or if end date is greater than or equal to date of first dose. Conditions will be coded using Medical Dictionary of Regulated Activities (MedDRA, version 23.0 or later) primary system organ class (SOC) and preferred term (PT).

All Medical History data will also be listed.

6.8 Prior and Concomitant Medications

Separate tabulations will be produced for prior and concomitant medications presented by randomized treatment group and overall for the SAS and the FAS. Prior medications are defined as all medications starting before the date of first dose of study drug. Concomitant medications are defined as medications taken on or after the date of first dose of study drug. Concomitant medications will be coded using WHO Drug dictionary version March 2020 (or later) and summarized using Anatomic Therapeutic Chemical (ATC) Level 2.

6.9 Exposure to Study Drug

Data on exposure will be presented by randomized treatment group, overall, and by visit/whole study for the Safety Analysis Set. Following parameters will be presented in a table:

- For each visit:
  - Subjects who had an infusion
  - Subjects that received full infusion
  - Subjects that had at least 1 interruption
  - Subjects that received partial infusion
- For whole study

- Subjects that received all full infusions
- Subjects that had at least 1 interruption
- Subjects that received at least 1 partial infusion
- Subjects who received 1 unfusion, >=2 infusion and so on.
- Total volume of bryostatin infused
- Subjects who had >=80% of planned dose

All data on exposure will also be listed.

6.10 Treatment Compliance

Number and percentage of subjects receiving at least 80% of planned dose will be presented.

6.11 Efficacy Analyses

All statistical tests will be performed using a two-tailed 5% overall significance level, unless otherwise stated. All comparisons between treatments will be reported with 95% confidence intervals for the difference.

6.11.1 Primary Endpoint

The primary efficacy endpoint is the change from the pre-treatment baseline in the SIB total score at Week 28, after two 12-week courses of treatment.

6.11.2 Primary Efficacy Analysis

The primary efficacy analysis will be performed using linear mixed model with repeated measures (MMRM) with SIB measured at baseline as covariate, visit treated as a continuous as well as categorical variable, treatment and treatment by visit interaction. Categorical visit will be used to identify the ordering of measurements within a subject, while continuous visit will be used as covariate alone as well as in interaction with treatment.

Week of planned visits will be used as values of categorical variable of visit. Actual study day of visits (calculated in weeks) will be used as values of continuous variable of visit. The dependent

variable will be the change from baseline in SIB total score at weeks 5, 9, 13, 15, 20, 24, 28, and 30.

Correlation between repeated observations within a subject will be accounted for by specifying an unstructured correlation. If this model does not converge then the following covariance matrices will be used in this particular order until the model converges: Toeplitz, compound symmetry, and first-order autoregressive.

Analysis will be performed using PROC MIXED in SAS, and the resulting F-tests will be based on using Kenward-Roger's adjusted degrees of freedom.

The treatment groups will be compared at all timepoints using a two-sided test at a 5% significance level, with the primary comparison being the difference in group means of SIB from baseline at Week 28.

The sample SAS code below implements the model detailed above with an unstructured covariance matrix (TYPE = UN in the REPEATED statement). The code assumes that AVISITN has 8 values (one for each post-baseline visit), and TRT01PN representing treatment is coded as 1 = bryostatin, 2 = placebo.

```
proc mixed data = <input-dataset>;
  class subjid trt01pn avisitn;
  model sibdiff = sibbase trt01pn avisitd trt01pn* avisitd /
ddfm = kr outpred = pred;
  repeated avisitn / type = un subject = subjid;
  estimate "Bryostatin vs Placebo at week 28" trt01pn 1 -1
trt01pn* avisitd 28 -28 / cl;
run;
```

Variables names:

subjid – unique number of subject,

avisitn – visit as categorical variable,

avisitd – visit as continuous variable,

sibbase – SIB at baseline,

sibdiff – SIB difference from baseline,

trt01pn – treatment group.

As a sensitivity analysis the same model but using only categorical visit will be used. The following SAS code implements this model (the variable designations are the same):

```
proc mixed data = <input-dataset>;
  class subjid trt01pn avisitn;
  model sibdiff = sibbase trt01pn avisitn trt01pn* avisitn /
ddfm = kr outpred = pred;
  repeated avisitn / type = un subject = subjid;
  estimate "Bryostatin vs Placebo at week 28" trt01pn 1 -1
trt01pn* avisitn 0 0 0 0 0 0 1 0 0 0 0 0 0 0 -1 0 / cl;
run;
```

Patients that have missing SIB outcome data will be included in the primary analysis if they have complete SIB data for at least one follow-up timepoint (i.e., available case analysis). Although there is no universally acceptable, adequate method for analyzing longitudinal clinical trial data under ITT with missing primary endpoints, the available case analysis using a generalized linear mixed model approach such as MMRM will produce unbiased estimates of the treatment effect if the missingness is ignorable (i.e. missing completely at random or missing at random)[2,3].

6.11.3 Supportive Analysis

Supportive sensitivity analysis for primary endpoint will performed in the same manner as primary analysis, for PP and CAS populations.

Using MMRM for available cases as described in section 6.11.2 assumes that missingness in the SIB outcome measures is non-ignorable. However, at least some missing data at a given timepoint may be dependent on outcome values that are unobserved and therefore unmeasured, giving rise to non-ignorable missingness or missingness not at random (MNAR). Reasons for missing data will be recorded to help determine if missingness not at random is present. Determining the timing,

frequency, and possible reasons for missing data is critical in helping to determine the underlying mechanism of the missed data, particular since all methods for handling missing data are based on unverifiable assumptions.[3]

If the reasons for missingness provides clinical evidence that MNAR is present, then sensitivity analyses that make reasonable assumptions about missing data will be performed. In the proposed sensitivity analyses, intermediate missed SIB values will be replaced with a 'best' case and 'worst' case, where the 'best' and 'worst' case SIB scores will be determined for each patient based on their observed data. In these sensitivity analyses, only intermediate missed SIB data will be filled in with the best- and worst-case values, giving rise to a monotonic missing data set. Under this scenario, it is reasonable to assume that data lost to follow-up is ignorable[3].

6.11.4 Other Secondary Endpoints

Analysis of secondary efficacy endpoints will be conducted on the FAS population.

6.11.4.1 SIB total score at the Week 42 follow-up visit

This endpoint will be analyzed using an Analysis of Covariance (ANCOVA) model with SIB score at Week 42 as dependent variable; treatment group as factor, and baseline SIB total score, baseline MMSE-2 score, age and gender as covariates.

6.11.4.2 The change from baseline in SIB total score at Week 13

This endpoint will be analyzed using the same ANCOVA model as described in Section 6.11.6.1.

6.11.4.3 SIB total scores from baseline at Weeks 9, 20, 24 and 30

This endpoint will be analyzed using the same MMRM model as described in Section 6.11.2 with corrections for obtaining results on corresponding visits.

6.11.4.4 SIB total scores from baseline at Weeks 9, 20, 24 and 30for subjects with baseline MMSE-2 scores of 10-14 and 15-18

This endpoint will be analyzed using the same MMRM model as described in Section 6.11.2 with additional fixed effect for grouped baseline MMSE-2 scores (10-14 and 15-18) and corrections for obtaining results on corresponding visits.

6.11.4.5 Individual patient's SIB trends over time

Assessment of slopes in treatment groups and comparison of these slopes will be performed using the MMRM model for primary efficacy analysis, as it described in Section 6.11.2. This approach will be used for absolute SIB values collected from baseline over Weeks 0 to 28 as well as for changes from baseline for these time points:

```
proc mixed data = <input-dataset>;
  class subjid trt01pn avisitn;
  model sibdiff = VAR trt01pn avisitd trt01pn* avisitd / ddfm =
kr outpred = pred;
  repeated avisitn / type = cs subject = subjid;
  estimate "Slope, Bryostatin" time 1 time*trt_no 1 0 / cl;
  estimate "Slope, Placebo" time 1 time*trt_no 0 1 / cl;
  estimate "Slope Difference" time*trt_no 1 -1 / cl;
run;
```

Variables names:

subjid – unique number of subject,

avisitn – visit,

VAR=sib – SIB absolute total score for the first model,

VAR=sibdiff – SIB difference from baseline for the second model,

trt01pn – treatment group.

6.11.5 Exploratory Endpoints

Analysis of exploratory endpoints will be conducted on the FAS population.

The following exploratory endpoints will be analyzed:

6.11.5.1 • Change from baseline in ADCS-ADL-Sev total score at Week 13 and Week 42

Analysis of this endpoint will be similar to analysis of primary efficacy endpoint. Analysis will be performed using the same MMRM model as described in Section 6.11.2.

6.11.5.2 • Change from baseline in MMSE-2 total score at Week 13 and Week 42

Analysis of this endpoint will be similar as described in Section 6.11.7.1 but for post-baseline changes in MMSE-2 total score.

6.11.5.3 • Change from baseline in NPI total score at Week 13 and Week 42

Analysis of this endpoint will be similar as described in Section 6.11.7.1 but for post-baseline changes in MMSE-2 total score.

6.12 Safety Analyses

The safety analyses will be presented by the treatment received for the Safety Analysis Set.

6.12.1 Adverse Events

Adverse events will be coded using Medical Dictionary of Regulated Activities (MedDRA, version 23.0 or later) primary system organ class (SOC) and preferred term (PT).

Treatment Emergent AEs (TEAEs) are defined as events with an onset on or after the first randomized treatment.

Severity grade will be defined by variable “Severity/Intensity” of the CRF. Maximum severity will be assumed for an AE with missing severity.

Relationship to study drug will be defined by variable “Causality” of the CRF. Maximum relationship will be assumed for an AE with missing causality.

An AE will be considered as resulting in discontinuation of study treatment if variable “Action taken with Study Treatment“ equals to “DRUG WITHDRAWN”.

An AE will be considered as lead to study discontinuation if the subject didn’t complete the study with primary reason for termination equals to “ADVERSE EVENT“ and the AE has number equals to value of variable “Adverse Event Number” on page “End Of Study” of the CRF.

An AE will be considered as AE of Special Interest (AESI) if variable "Was this event considered Adverse Event of Special Interest (AESI)?" equals to "YES".

The following tables will be presented, by SOC and PT, both sorted alphabetically, with summaries for each treatment group and overall:

- Overview of AEs
- TEAEs
- TEAEs by maximal severity grade
- TEAEs by maximal relationship to study treatment
- Serious TEAEs (TESAEs)
- TEAEs resulting in discontinuation of study treatment
- TEAEs resulting in discontinuation of study treatment by maximal relationship to study treatment
- TEAEs that lead to study discontinuation by maximal relationship to study treatment
- TESAEs by maximal relationship to study treatment
- Treatment Emergent Adverse Events of Special Interest (TEAESI)

Following listings on AEs will be provided:

- Pre-treatment AEs
- TEAEs
- Serious TEAEs
- TEAEs resulting in discontinuation of study treatment
- TEAE with fatal outcome

6.12.2 Laboratory Data

Laboratory data will be collected at Screening, Weeks 2, 7, 13, 18, 26 and 42.

Hematology tests will include CBC with differential, platelet count and coagulation (prothrombin time (PT) and partial prothrombin Time (PTT)) studies.

Clinical chemistry tests will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, estimated creatinine CL, glucose, calcium, CO2, total protein, albumin, ALP,

ALT, AST, gamma glutamyl transferase (GGT) lactate dehydrogenase (LDH), uric acid and bilirubin.

A serum creatine phosphokinase (CPK) will be done at screening and in the event of myalgia. A Thyroid-Stimulating Hormone (TSH) analysis will be done at screening, and B12, T-3 and T-4 will be done if TSH result is abnormal.

Test for βhCG will be performed if indicated.

Test for HbA1C will be performed if clinically indicated.

Descriptive statistics of the observed values and change from baseline (continuous data) will be presented by treatment group and visit **only for hematology tests and clinical chemistry tests**. Each measurement (continuous data) will be classed as below, within, or above normal range, based on ranges supplied by the laboratory used. Shift tables in relation to the normal range from baseline to each follow-up visit will be presented.

Data on CPK, TSH (and corresponding tests), βhCG and HbA1C will not be tabulated.

All laboratory data will be presented in listings.

6.12.3 Vital Signs

Vital signs will be collected at Screening and each study visit. At dosing visits, collection will be performed prior to infusion and at 30, 60 and 90 minutes from start of the infusion (+/- 5 minutes) Descriptive statistics for observed values and changes from baseline in the following vital signs will be presented by treatment group and visit:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Pulse rate (bpm)
- Respiration rate (breath / min)
- Body temperature

For the calculation of descriptive statistics the values of body temperature measured in Fahrenheit will be transformed using the following formula: [value in °C] = ([value in °F] − 32) × 5/9.

All data on vital signs will be listed.

6.12.4 Electrocardiogram Data

Electrocardiogram (ECG) will be performed at Screening (triplicate), Weeks 2, 7, 13, 18 and 26. Descriptive statistics for ECG interpretation results will be presented in a table. Interpretation results and changes from baseline will be presented in a shift table.

Electrocardiogram data will also be presented in a listing.

6.12.5 Physical Examination

Physical examination (PE) will be performed at Screening, Weeks 7 (abbreviated), 13, 18, 26 and 42. It will include, but is not limited to the following:

- General appearance
- Weight
- Height (screening only)
- Ears
- Eyes
- Nose
- Throat
- Neck
- Respiratory system
- Cardiovascular system
- Abdomen
- Musculoskeletal /Neurologic
- Extremities
- Skin
- Lymph nodes

An abbreviated PE will include but is not limited to the following:

- General appearance
- Weight
- Respiratory system
- Cardiovascular system

- Abdomen

For the calculation of descriptive statistics, the values of weight measured in LB will be transformed using the following formula: [value in °KG] = [value in °LB] × 0.45359.

Values of weight and changes from baseline will be tabulated with descriptive statistics.

Assessment results and changes from baseline for each body system will be presented in shift tables.

Results of physical examinations will be listed.

6.12.6 Columbia Suicide Severity Rating Scale

Columbia Suicide Severity Rating Scale (C-SSRS) will be collected at screening, Weeks 2, 7, 13, 18, 26 and 42. Results will be presented in a table with descriptive statistics and in a listing.

7 INTERIM ANALYSIS

No interim analyses are planned.

8 DATA SAFETY MONITORING BOARD ANALYSIS

Safety data will be reviewed by Data Safety Monitoring Board (DSMB) when 30 subjects have received four doses of study drug, followed by additional safety analyses when 60 subjects have received a similar number of doses. Following tables and listings will be prepared for DSMB review:

Tables:

- Demographic data
- Disease history
- Medical history
- Summary of TEAEs
- Summary of TEAEs by relationship to clinical trial treatment
- Summary of Serious TEAEs
- Summary of Serious TEAEs by relationship to clinical trial treatment

- Summary of Adverse Events of Special Interest (AESI)
- Summary of AESI by relationship to clinical trial treatment

Listings:

- Demographic data
- Disease history
- Medical history
- Pre-treatment AEs
- TEAEs
- Serious TEAEs
- TEAEs resulting in discontinuation of study treatment
- TEAE with fatal outcome

9 CHANGES TO PLANNED PROTOCOL ANALYSIS

1. The control of false discovery rate (FDR) excluded because it was planned for the secondary (not primary) endpoints and this is a phase 2 study.
2. For the 4th secondary endpoint: <The changes from baseline at Weeks 5, 9, 13, 15, 20 and 24 in the SIB total score for subjects with baseline MMSE-2 scores of 10-14 and 15-18> the list of visits changed from Weeks 9, 15, 20 and 24 to Weeks 9, 20, 24 and 30.
3. For efficacy analysis the GEE models replaced with MMRM models as more suitable approach for analysis of longitudinal data in neurology studies.

10 REFERENCES

1. SAS Institute Inc., Cary, NC, 27513, USA
2. Chakraborty H, Gu H. A Mixed Model Approach for Intent-to-Treat Analysis in Longitudinal Clinical Trials with Missing Values. RTI Press, Research Triangle Park (NC); 2009. PMID: 30896910.
3. Committee for Medicinal Products for Human Use. *Guideline on Missing Data in Confirmatory Clinical Trials*. London: European Medicines Agency; 2010.

11 LIST OF TABLES, FIGURES AND LISTINGS

Table Number	Table Title	Validation Method	Shell Number (if repeat)
14.1	**Demographics Data**		
14.1.1	**Disposition**		
14.1.1.1	Subject Disposition, Analysis Sets	IP	
14.1.1.2	Screen Failures	IP	
14.1.1.3	Inclusion/Exclusion Criteria not met, Screen Failures	IP	
14.1.1.4	Subject Disposition, Early Withdrawal	IP	
14.1.1.5	Safety Analysis Set by Site	IP	
14.1.1.6	Subject Visits	IP	
14.1.1.7	Protocol Deviations	IP	
14.1.2	**Demographics**		
14.1.2.1	Demographics. Safety Analysis Set	IP	
14.1.2.2	Demographics. Full Analysis Set	IP	
14.1.3	**Baseline Characteristics**		
14.1.3.1	Prior Medical History. Safety Analysis Set	IP	
14.1.3.2	Prior Medical History. Full Analysis Set	IP	
14.1.3.3	Ongoing Medical History. Safety Analysis Set	IP	
14.1.3.4	Ongoing Medical History. Full Analysis Set	IP	
14.2	**Efficacy Data**		
14.2.1	**Primary Efficacy Endpoint**		
14.2.1.1	Severe Impairment Battery. Full Analysis Set	Stat IP	
14.2.1.2	Severe Impairment Battery. Per-Protocol Analysis Set	Stat IP	
14.2.1.3	Severe Impairment Battery. Completers Analysis Set	Stat IP	

Table Number	Table Title	Validation Method	Shell Number (if repeat)
14.2.1.4	Mixed Model with Repeated Measures Analysis of Change from Baseline at week 28 in Severe Impairment Battery. Main Analysis	Stat IP	
14.2.1.5	Mixed Model with Repeated Measures Analysis of Change from Baseline at week 28 in Severe Impairment Battery. Sensitivity Analysis: Visit included as categorical predictor	Stat IP	
14.2.1.6	Mixed Model with Repeated Measures Analysis of Change from Baseline at week 28 in Severe Impairment Battery. Supportive Analysis: Best Case Approach	Stat IP	
14.2.1.7	Mixed Model with Repeated Measures Analysis of Change from Baseline at week 28 in Severe Impairment Battery. Supportive Analysis: Worst Case Approach	Stat IP	
14.2.1.8	Mixed Model with Repeated Measures Analysis of Change from Baseline at week 28 in Severe Impairment Battery. Supportive Analysis: Per-Protocol Analysis Set	Stat IP	
14.2.1.9	Mixed Model with Repeated Measures Analysis of Change from Baseline at week 28 in Severe Impairment Battery. Supportive Analysis: Completers Analysis Set	Stat IP	
14.2.2	**Secondary Efficacy Endpoints**		
14.2.2.1	Analysis of Covariance model for Severe Impairment Battery at Week 42 follow-up visit	Stat IP	
14.2.2.2	Analysis of Covariance model for Change from Basleine of Severe Impairment Battery at Week 13	Stat IP	
14.2.2.3	Mixed Model with Repeated Measures Analysis of Changes from Baseline at weeks 9, 20, 24 and 30 in Severe Impairment Battery	Stat IP	
14.2.2.4	Mixed Model with Repeated Measures Analysis of Changes from Baseline at weeks 9, 20, 24 and 30 in Severe Impairment Battery for subjects with baseline MMSE-2 scores of 10-14 and 15-18	Stat IP	
14.2.2.5	Individual subject's trends of SIB over time	Stat IP	
14.2.2.6	Individual subject's trends of SIB changes from baseline over time	Stat IP	
14.2.3	**Exploratory Endpoints**		

Table Number	Table Title	Validation Method	Shell Number (if repeat)
14.2.3.1	Mixed Model with Repeated Measures Analysis of Change from Baseline at weeks 13 and 42 in Alzheimer's Disease Cooperative Study – Activities of Daily Living - Severe Impairment Version	Stat IP	
14.2.3.2	Mixed Model with Repeated Measures Analysis of Change from Baseline at weeks 13 and 42 in Mini Mental State Examination, 2nd edition	Stat IP	
14.2.3.3	Mixed Model with Repeated Measures Analysis of Change from Baseline at weeks 13 and 42 in Neuropsychiatric Inventory	Stat IP	
14.3	**Safety Data**		
14.3.1	**Displays of Adverse Events**		
14.3.1.1	Overview of Adverse Events	IP	
14.3.1.2	Treatment Emergent Adverse Events	IP	
14.3.1.3	Patients with Treatment Emergent Adverse Events by Maximal Severity	IP	
14.3.1.4	Patients with Treatment Emergent Adverse Events by Maximal Relationship to Study Treatment	IP	
14.3.1.5	Serious Treatment Emergent Adverse Events	IP	
14.3.1.6	Treatment Emergent Adverse Events Resulting in Discontinuation of Study Treatment	IP	
14.3.1.7	Patients with Treatment Emergent Adverse Events Resulting in Discontinuation of Study Treatment by Maximal Relationship to Study Treatment	IP	
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14.3.1.9	Patients with Serious Treatment Emergent Adverse Events by Maximal Relationship to Study Treatment	IP	
14.3.1.10	Treatment Emergent Adverse Events of Special Interest	IP	
14.3.4	**Abnormal Laboratory Values**		
14.3.4.1	Hematology, Measured Values and Changes from Baseline by Visit	IP	
14.3.4.2	Hematology, Shifts from Baseline to Post-Baseline Visits	IP	
14.3.4.3	Clinical chemistry, Measured Values and Changes from Baseline by Visit	IP	

Table Number	Table Title	Validation Method	Shell Number (if repeat)
14.3.4.4	Clinical chemistry, Shifts from Baseline to Post-Baseline Visits	IP	
14.3.5	**Extent of Exposure, Dosage Information and Compliance**		
14.3.5.1	Exposure	IP	
14.3.6	**Vital Signs and Physical Examination**		
14.3.6.1	Vital Signs, Measured Values and Changes from Baseline by Visit	IP	
14.3.6.2	Weight and BMI, Measured Values and Changes from Baseline by Visit	IP	
14.3.6.3	Physical Examination	IP	
14.3.7	**Other Safety**		
14.3.7.1	Electrocardiogram, Measured Values and Changes from Baseline by Visit	IP	
14.3.7.2	Electrocardiogram, Shift in Overall interpretation from Baseline to Post-Baseline Visits	IP	
14.3.7.3	Columbia-Suicide Severity Rating Scale (C-SSRS) at Screening	IP	
14.3.7.4	Columbia-Suicide Severity Rating Scale (C-SSRS) at Post-baseline Visits	IP	
14.3.8	**Concomitant Medication**		
14.3.8.1	Prior Medications. Safety Analysis Set	IP	
14.3.8.2	Prior Medications. Full Analysis Set	IP	
14.3.8.3	Concomitant Medications. Safety Analysis Set	IP	
14.3.8.4	Concomitant Medications. Full Analysis Set	IP	

Figure Number	Figure Title	Validation Method	Shell Number (if repeat)
14.2.1	**Primary Efficacy Endpoint**		
14.2.1.1	Mean Values with 95% Confidence Intervals of Severe Impairment Battery. Full Analysis Set	IP	
14.2.1.2	Mean Values with 95% Confidence Intervals of Severe Impairment Battery. Per-Protocol Analysis Set	IP	14.2.1.1
14.2.1.3	Mean Values with 95% Confidence Intervals of Severe Impairment Battery. Completers Analysis Set	IP	14.2.1.1
14.2.1.4	LS Mean Values with 95% Confidence Intervals of Change from Baseline in Severe Impairment Battery. Main Analysis	IP	14.2.1.1
14.2.1.5	LS Mean Values with 95% Confidence Intervals of Change from Baseline in Severe Impairment Battery. Sensitivity Analysis	IP	14.2.1.1

Listing Number	Listing Title	Validation Method	Shell Number (if repeat)
16.2	**Subject Data Listings**		
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16.2.1.2	Subject Visits	IP	
16.2.1.3	Screen Failures		
16.2.1.4	Eligibility Criteria		
16.2.2	**Protocol Deviations**		
16.2.2.1	Protocol Deviations	IP	
16.2.3	**Subjects Excluded from The Efficacy Analyses**		
16.2.3.1	Analysis Sets	IP	
16.2.4	**Demographic Data**		
16.2.4.1	Demographic Data	IP	
16.2.4.2	Prior and Ongoing Medical History	IP	
16.2.4.3	Prior and Concomitant Medications	IP	
16.2.4.4	Non-Pharmacological Procedures	IP	
16.2.4.5	Rosen-modified Hachinski lschemic score	IP	
16.2.5	**Compliance**		
16.2.5.1	Exposure	IP	
16.2.6	**Individual Efficacy Response Data**		
16.2.6.1	Severe Impairment Battery. Questions 1-20	IP	
16.2.6.2	Severe Impairment Battery. Questions 21-40	IP	
16.2.6.3	Severe Impairment Battery. Total Scores	IP	
16.2.6.4	Alzheimer Disease Cooperative Study - Activities of Daily Living - Severe Impairment Version	IP	
16.2.6.5	Alzheimer Disease Cooperative Study - Activities of Daily Living - Severe Impairment Version. Total Scores	IP	
16.2.6.6	Mini Mental State Examination Version 2	IP	
16.2.6.7	Mini Mental State Examination Version 2. Total Scores	IP	
16.2.6.8	Neuropsychiatric Inventory	IP	
16.2.6.9	Neuropsychiatric Inventory. Total Scores	IP	
16.2.7	**Adverse Event Listings**		
16.2.7.1	Pre-Treatment Adverse Events	IP	
16.2.7.2	Treatment Emergent Adverse Events	IP	
16.2.7.3	Serious Treatment Emergent Adverse Events	IP	

Listing Number	Listing Title	Validation Method	Shell Number (if repeat)
16.2.7.4	Treatment Emergent Adverse Events resulting in discontinuation of study treatment	IP	
16.2.7.5	Treatment Emergent Adverse Events with Fatal Outcome	IP	
16.2.8	**Individual Laboratory Measurements and Other Safety**		
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16.2.8.2	Clinical Chemistry Results	IP	
16.2.8.3	Screening Clinical Chemistry Results	IP	
16.2.8.4	Vital Signs	IP	
16.2.8.5	Temperature	IP	
16.2.8.6	Weight and BMI	IP	
16.2.8.7	Physical Examinations	IP	
16.2.8.8	Electrocardiogram Results	IP	
16.2.8.9	Pregnancy Urine Test	IP	
16.2.8.10	Columbia-Suicide Severity Rating Scale (C-SSRS)	IP	