A Single-Arm, Single-Site, Single-Dose Phase 1 Study Assessing the Safety of Bryostatin in the Treatment of Patients with Multiple Sclerosis

STATISTICAL ANALYSIS PLAN

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ACRONYMS AND ABBREVIATIONS

AE	adverse event
CNS	central nervous system
CP	Creatine Phosphokinase
CTCAE	Common Terminology Criteria for Adverse Events
DMT	disease modifying therapy
GEE	generalized estimating equations
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MS	multiple sclerosis
PI	Principal Investigator
PST	Processing Speed Test
SAE	serious adverse event
SBQ-R	Suicide Behaviors Questionnaire Revised
SD	standard deviation
SOC	System Organ Class
TEAE	treatment emergent adverse event

Contents

1. STUDY SYNOPSIS

This is a single-site, single-arm, single-dose, Phase 1 study of the safety of bryostatin in participants with multiple sclerosis (MS) receiving any disease modifying therapy (DMT). The study is 40 weeks in duration, including a safety and exploratory outcomes evaluation at 30 days after the last full assessment (Week 28) and long-term follow-up at 12 weeks after the last full assessment (Week 28). Participants will receive 14 doses over 26 weeks.

The primary objective of this Phase1 study is to evaluate the safety and tolerability of bryostatin. Primary outcome measures include the frequency of treatment emergent adverse events (TEAEs) and serious adverse events (SAEs), frequency of study medication discontinuation, and potential central nervous system (CNS) inflammatory effects as captured by clinical monitoring and MRI.

Exploratory objectives are to access the preliminary signals of efficacy of bryostatin, through evaluating changes in clinical, imaging, and cognitive measures during and following treatment.

2 GENERAL CONSIDERATIONS

While the primary objectives of this study are to establish the tolerability and safety of bryostatin for patients with MS, we also will monitor measures of MS disease status over time. It is recognized that, in the absence of a control group of participants untreated with bryostatin, this trial will be unable to formally distinguish improvement in disease status from placebo effect or from regression to the mean effects. Moreover, this small Phase I trial is not planned or anticipated to have sufficient power to distinguish small to medium effects of bryostatin over time. Thus, basic descriptive approaches are outlined below, including modeling for descriptive purposes, to be used for the exploratory objectives and in planning future studies, should results of the current study indicate feasibility, safety, and tolerability.

Descriptive statistics will be used to summarize variables as appropriate to the variable's scale (nominal, ordinal, interval, ratio). Numerical summaries of continuous variables will use means, standard deviations, median, interquartile range, and range. Frequency tables will be generated to summarize categorical variables using frequency count and percentage. Graphics, especially boxplots but also potentially QQ-plots, histograms, and empirical cumulative distribution functions will be used as needed to understand and visualize distribution. Outliers will be identified and their influence limited by categorization, Winsorization, or parallel analyses of data including and excluding outliers. The small number of patients also allows easy visualization of data at the individual level to aid interpretation of the numerical and graphical summaries. Thus, spaghetti plots and/or panel plots of individual patient time trends of serial measurements over time will be generated.

3 ANALYSIS SETS

3.1 Inclusion-Exclusion Criteria and General Study Population

People with MS are eligible to enroll. Participants will be permitted to continue current DMTs, as long as they have been stable for 1 year and a change in DMT is not anticipated over the course of this study, but no new treatments can be initiated unless clinically necessary due to safety concerns or significant change in the participant's status.

Inclusion Criteria:

1. Written informed consent signed by participant
2. English-speaking
3. Hospital Anxiety and Depression Scale <11
4. Male and female participants, 18-60 years of age inclusive
5. Established diagnosis of MS, as defined by the 2017 revision of McDonald Diagnostic Criteria (any form of MS).[1] A diagnosis of MS must be confirmed at the time of the screening visit.
6. Processing Speed Test (PST) z-score between -1.0 and -2.5 [2]
7. EDSS between 0.0 and 7.0, inclusive.
8. Adequate vision and motor function to participate in assessment procedures
9. Participants must be on a stable dose of a DMT for at least 1 year prior to entry into the study, and the dose should not change during the study unless a change is required by a clinically significant change in the participant's status.
10. Females participating in the study must meet one the following criteria:
    a. Surgically sterilized (e.g., hysterectomy, bilateral oophorectomy or tubal ligation) for at least 6 months or postmenopausal (postmenopausal females must have no menstrual bleeding for at least 1 year) or
    b. If not postmenopausal, agree to use a double method of contraception, one of which is a barrier method (e.g., intrauterine device plus condom, spermicidal gel plus condom) 30 days prior to dosing until 30 days after last dose and have negative human chorionic gonadotropin (β-hCG) test for pregnancy at screening. Contraception methods resulting in an overall failure rate of <1 % per year are required for women of childbearing potential.
11. Males who have not had a vasectomy must use appropriate contraception methods (barrier or abstinence) from 30 days prior to dosing until 30 days after last dose
12. Participants should be in reasonably good health over the last 6 months and any chronic disease should be stable.

Exclusion Criteria:

1. Evidence of significant CNS vascular disease on previous neuroimaging, including but not limited to cortical stroke, multiple infarcts, localized single infarcts in the thalamus, angular gyrus, multiple lacunar infarcts, or extensive white matter injury
2. Clinically significant neurologic disease or condition other than MS, such as cerebral tumor, chronic subdural fluid collections, Huntington's Disease, Parkinson's Disease, normal pressure hydrocephalus, or any other diagnosis that could interfere with assessment of safety and efficacy
3. Previous history of seizures or seizure disorders.
4. Evidence of clinically significant unstable cardiovascular, pulmonary, renal, hepatic, gastrointestinal, neurologic, or metabolic disease within the 6 months prior to enrollment. If

there is a history of cancer, the participant should be clear of cancer for at least 2 years prior to screening. More recent history of basal cell or squamous cell carcinoma and melanoma *in situ* (Stage 0) may be acceptable after review by the Medical Monitor.

5. Estimated Glomerular Filtration Rate (eGFR) of <45ml/min
6. Poorly controlled diabetes (at the discretion of the Principal Investigator)
7. Use of vitamin E > 400 International Units (IU) per day within 14 days prior to screening[3]
8. Use of valproic acid and/or lithium within 14 days prior to screening
9. Routine or intermittent use of benzodiazepines in the last year (rare use in the last year is allowed as long as use during the study is not expected).
10. Use of carbamazepine within 7 days prior to screening
11. Use of teriflunomide within 90 days prior to screening
12. Use of dalfampridine within 7 days of screening
13. Current use of acetaminophen, ciprofloxacin, and/or trimethoprim/sulfamethoxazole
14. At the discretion of the PI, any medical or psychiatric condition that is unstable or may require the initiation of additional medication or surgical intervention during the course of the study
15. Any screening laboratory values outside the laboratory reference ranges that are deemed clinically significant by the PI
16. Use of an investigational drug within 30 days prior to screening
17. Suicidality defined as active suicidal thoughts during the 6 months prior to screening or at Baseline [SBQ-R], or history of suicide attempt in previous 2 years, or at serious suicide risk in study neurologist or PI's judgment
18. Major psychiatric illness such as currently uncontrolled major depression according to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition[4], current or past diagnosis of bipolar disorder, schizophrenia, or any other psychiatric disorder that might interfere with the assessments of safety or efficacy at the discretion of the PI
19. Diagnosis of alcohol or drug abuse within the last 2 years
20. History of prolonged QT or prolonged QT on screening ECG [QT correction with Bazett formula (QTcB) or QT correction by Fridericia (QTcF)>499 per central reader][5]
21. Acute or poorly controlled medical illness: blood pressure >180 mmHg systolic or 100 mmHg diastolic; myocardial infarction within 6 months; uncompensated congestive heart failure [New York Heart Association (NYHA) Class III or IV]
22. Known to be seropositive for Hepatitis B or C, unless successful curative treatment for Hepatitis C (e.g., Harvoni) has been received, and there is documentation that there is no Hepatitis B/C virus detected 3 months after completion of treatment
23. Known to be seropositive for human immunodeficiency virus (HIV)
24. Pregnancy or breastfeeding during the study. A β-hCG serum pregnancy test will be performed at Screening for female patients of child-bearing potential.
25. Aspartate Amino Transferase (AST) or Alanine Aminotransferase (ALT) >3x upper limit of normal (ULN) and total bilirubin >2x ULN or International Normalized Ratio (INR) >1.5
26. History of significant bleeding disorders.
27. Moderate baseline thrombocytopenia (platelets <100K/uL).
28. Elevated INR (PTT >2.0).
29. Prior exposure to bryostatin, or known sensitivity to bryostatin or any ingredient in the study drug
30. Any other concurrent medical condition, which in the opinion of the PI makes the participant unsuitable for the clinical study

3.2 Randomization and Blinding

This study is open-label and all study participants receive the same administration.

3.3 Study Assessments

All participants will be treated with the study drug bryostatin-1 for infusion. A follow-up visit will take place 30 days after the last full assessment (Week 28) for all participants, including participants that have discontinued treatment before completion of the study. Assessments will be performed according to the Schedule of Activities (Table 1).

We anticipate the collection of study assessment data to be within 3 days of the scheduled visit day, but will include all available data to the closest visit day. For multiple measurements that occur within the same assessment time window, the closest measurement to the visit day will be included in analysis.

3.4 Sample Size

Based on feasibility, twenty participants will be included in this open label, single-arm, single-dose, Phase 1 study of the safety of bryostatin for the treatment of MS in participants receiving any DMT.

No formal power analyses were conducted, however with 20 patients we can estimate the rate of SAEs to within an exact 95% binomial confidence interval width of 0.146 if the proportion of participants with SAEs is 5% (1/20).

3.5 Enrollment and Analysis Populations

The full analysis population will be based on intention to treat and will include all participants who consented and received any dose of study drug. This will include all participants with a baseline visit (Week 0) who received at least the initial dose (including one partial dose).

For the exploratory analyses of signals of efficacy, the analysis population will include all participants from the full analysis population for whom any efficacy data was collected.

A per-protocol analysis will additionally be conducted to include only those patients who complete the full treatment plan through Week 40.

4 PATIENT DESCRIPTION

Demographic and medical history data at study entry will be summarized using descriptive statistics. Frequency count and percentage will be used for categorical variables, and mean, standard deviation, quartiles, minimum and maximum will be used for continuous variables. Graphical displays of data such as boxplots and histograms will be constructed for visual interpretation where needed.

Participant disposition will be summarized and will include numbers screened, dosed, and withdrawn with reason for withdrawal. The number of subjects reaching the various stages of the trial will be summarized, including how many dropped out and for what reasons (death, toxicity, treatment failure, withdrew consent). A CONSORT flow diagram will be generated following published guidelines: http://www.consort-statement.org/.

5 STUDY ENDPOINTS

Primary outcomes include the frequency of treatment emergent adverse events (TEAEs) and serious adverse events (SAEs), the frequency of bryostatin discontinuation, and potential central nervous system (CNS) inflammatory effects. Changes in CNS inflammatory effects will be evaluated based on physical examination, vital signs, 12-lead ECG, the Suicide Behaviors Questionnaire Revised (SBQ-R), clinical chemistry, hematology, coagulation lab tests, and MRI.

Exploratory signals of efficacy outcomes include:

1. Changes from baseline to Week 11, 28, and 40 in the following:
    a. MRI biomarkers
        i. lesion volume and brain parenchymal fraction
        ii. default mode network
        iii. microstructural complexity of dendrites and axons
        iv. anatomical connectivity of transcallosal motor pathway
        v. axon density and dispersion
        vi. myelin water fraction
        vii. measures of myelination
        viii. grey matter atrophy, including grey matter fraction, white matter fraction, and cortical atrophy as measured by CLADA
    b. Expanded Disability Status Scale (EDSS)
    c. Montreal Cognitive Assessment (MoCA) test
    d. Controlled Oral Word Association Test (COWAT)
    i. MS Performance Test Domains
        i. lower extremity function (WST)
        ii. upper extremity function (MDT)
        iii. cognition (PST)
    ii. Quality of Life in Neurological Disorders (Neuro-QoL)

2. Changes from baseline to Week 28 and 40 in the following:
    a. California Verbal Learning Test- 2nd Edition (CVLT-II)
    b. Brief Visuospatial Memory Test - Revised (BVMT-R)

c. Judgement of Line Orientation (JOLO)
d. Boston Naming Test (BNT)
e. Delis–Kaplan Executive Function System (D-KEFS) Sorting Test

6 SAFETY AND TOLERABILITY OF BRYOSTATIN (PRIMARY OBJECTIVE)

Overall safety and tolerability will be assessed by the incidence of treatment emergent AEs and SAEs and by evaluations of change from baseline in physical examination, vital signs, 12-lead ECG, the SBQ-R, clinical chemistry, hematology, coagulation lab tests, and MRI.

AEs, safety laboratory, ECGs, physical exam, vital signs, and MRI data will be presented in tabular format and summarized descriptively. Descriptive summaries of the safety data including number of evaluable participants will be provided by scheduled visit.

AEs will be coded using standard guidelines determined by the sponsor and are defined as events with an onset on or after the first treatment, however the AE and SAE reporting period starts from the time of consent to the last study visit. Tabulations of the incidences, severities, and mean durations of AEs and SAEs with initial symptoms/signs occurring at any time following enrollment through week 40 will be classified as probably, possibly, or unlikely treatment-related. These will be summarized overall, and by MedDRA System Organ Class (SOC) and preferred term.

AEs leading to premature discontinuation of clinical trial treatment, AEs that lead to study discontinuation, AEs that lead to death and SAEs will also be summarized.

Myalgia is an AE of special interest and has been reported as the dose limiting toxicity in oncology studies, whereby the incidence of myalgia appears to be dose dependent and cumulative. For all cases of myalgia, a narrative will be created documenting onset, severity, treatment, and outcome. Muscle enzymes, CK, will be collected for all severe cases or (in the case of mild or moderate severity) at the investigators discretion and compared to baseline values.

7 SIGNALS OF EFFICACY ANALYSES (EXPLORATORY OBJECTIVES)

Descriptive summaries of exploratory data including number of evaluable participants, mean, standard deviation, quartiles, maximum, and minimum for continuous variables will be provided by scheduled visit. Categorical variables will be presented showing number evaluable, frequencies, and percentages. Plots of the data will also be created, including box plots and histograms, in order to determine the distribution of these data as well as to identify any unusual or outlying observations.

The same descriptive statistics will be provided for changes from baseline at each post-baseline visit through Week 40. For change from baseline summaries, participants with an undefined change from baseline, because of missing data, will be excluded. Bootstrap confidence intervals for standard errors of

the mean values and mean change from baseline will be calculated, to augment standard descriptive statistics and provide unbiased point estimates for potential use in planning future studies. Cohen's d effect sizes for one-group pre-post test design will be calculated. Paired t-test or nonparametric equivalent will be used to evaluate significant change from baseline, however due to the limited sample size, greater emphasis will be placed on magnitudes of effect when interpreting the data.

To evaluate changes across all time points, mixed effects models or generalized estimating equations (GEEs) will be constructed. Linear mixed effects models will be constructed for continuous dependent variables that meet model assumptions. Marginal and conditional residual plots will be examined for compatibility with model assumptions. For other dependent variables, GEEs will be constructed with the appropriate distribution and link functions. Random effects will include intercept and fixed effects will include time, treated categorically. Change over time will be estimated and graphically presented along with measure of variability at each time point. Contrasts will estimate the change from baseline to follow-up time point and durability of effects between Week 28 and Week 40 will be estimated for the applicable endpoints.

For NeuroQoL domains, a responder analysis will be conducted by summarizing meaningful change at the individual level [1]. The percentage of participants who meet the minimum clinically important difference of +3.5 T-score points at each visit after baseline will be summarized using frequency count and percentage [2].

All statistical tests for efficacy will be two-sided tests, with α=0.05. Since all efficacy assessments are exploratory, there will be no adjustment for multiplicity.

7.1 Lab Values, Physical Examinations, Vital Signs, ECGs, and MRI findings

All available results of the clinical laboratory evaluations, physical examinations, vital signs, and ECG and MRI findings will be listed and summarized by scheduled visit. For change from baseline summaries, participants with an undefined change from baseline, because of missing data, will be excluded.

Summary statistics of raw data and change from baseline values for each continuous measure, including laboratory parameter, physical examination including vital signs, and MRI findings will be presented by time point. Data will be summarized as appropriate for the variable type.

For ordinal data, including physical examination and ECG findings, shift tables will be presented to show any abnormality shifts from baseline to post-baseline visits [3].

8 DATA MONITORING

An independent Data Safety Monitoring Board (DSMB) will provide consultation to Synaptogenix to assess the implementation and progress of the study and will review accumulating trial data to monitor the safety of bryostatin administered to participants. Safety data will be reviewed when 10 participants have reached Week 11 of the trial (and have received 7 doses of the study drug), followed by additional

safety analyses when 20 participants have reached Week 26. Medical decisions relevant to this study will be made by the PI in consultation with the DSMB.

Prior to each safety review, a cut-off date for the data to be provided will be established by the PI in consultation with the Study Statistician.

Data review documents, safety data tables, and listings will be provided to the DSMB at least 7 business days prior to scheduled reviews. SAEs reported during the 7 days before the scheduled review and, therefore, not included in the provided documentation will be provided by the Medical Safety Monitor as soon as possible before the review date. The reviews of the safety data will be summarized for the participant group.

8.1 Stopping Rules

Results will be examined for potential early stopping after every participant has been followed for >1 month after infusion. Discontinuation of the trial will be considered if 2 participants within the first 6 of 20 planned experience significant AEs (bryostatin-related AE ≥severe as defined in 7.4) or any bryostatin-related SAE at any point during follow-up or if 3 participants experience significant AEs (bryostatin-related AE ≥ severe as defined in 7.4) or a bryostatin-related SAE at any time.

The DSMB will review the safety data and determine the safety of the bryostatin regimen tested in this trial.

Criteria for permanent discontinuation of study drug:

Study drug treatment may be discontinued for the following reasons:

- if sponsor or regulatory authorities discontinue study
- if the sponsor-investigator/PI believes that discontinuing treatment is in the best interest of the participant

Participants will stop study medication if they experience any of the following serious adverse events:

- Pregnancy
- Systemic infusion-related reactions which are categorized as moderate or severe
- Infusion site reactions which are categorized as moderate or severe
- One or more seizure
- Clinically significant weight loss as defined as 5% or more of baseline body weight
- Myalgias which are categorized as moderate or severe

Study discontinuation:

The sponsor-investigator in collaboration with the sponsor (Synaptogenix) has the right to discontinue this clinical study at any time.

The PI has the right to discontinue participation in this clinical study at any time for any reason.

Should the clinical study be discontinued prematurely, all participants should be brought in for Early Termination procedures as outlined in Table 1. All clinical study materials should be returned to Synaptogenix or designee.

A low frequency of AEs is anticipated for this study based on accumulating experience with prior bryostatin clinical trials. While a number of infusions have been performed, little information can be considered directly applicable to participants with MS. We anticipate a reasonable chance of statistically distinguishing a relatively low toxicity rate (AEs occurring in 1 of 20 participants) from a rate of 4-5/20 participants, while at the same time giving good protection against rejecting this therapeutic approach prematurely due to a sample with uncharacteristically frequent AEs. Early study discontinuation will be considered for accumulating evidence of toxicity, but not for results relating to efficacy.

TABLE 1- SCHEDULE OF ACTIVITIES

Week	Screening	0	1	3	5	7	9	11	13	15	16	18	20	22	24	26	28	32	40		ET
Day (+/- 3 days)[g]	-28 to -2	0	7	21	35	49	63	77	91	105	112	126	140	154	168	182	196	224	280		
Informed Consent	X																				
Confirm Eligibility	X																				
Demographics	X																				
Medical & MS history	X																				
Physical Examination	X				X			X					X				X				X
Vital signs[a]	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X				X
Weight	X				X								X				X				X
Screening Labs[b]	X																				
Routine Labs[c]				X				X				X				X	X				X
Urine Pregnancy[g]		X		X		X		X		X		X		X		X					
Biobank	X							X									X				
EDSS	X							X									X		X		X
SBQ-R	X			X		X		X			X		X		X		X				X
Neuro-QoL[d]	X							X									X		X		X
Short Cognitive Battery[d]	X			X		X		X			X		X		X		X		X		X
Extensive Cognitive Battery[e]	X																X		X		X
AMNART	X																				
ECG	X							X									X				X
MRI	X							X									X		X		X
Study Drug Dose		X	X	X	X	X	X	X		X	X	X	X	X	X	X					
Adverse Events		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X			X
Con meds	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X

NO CLINICAL VISIT

[a] Vital signs prior to infusion, and 30, 60 and 90 minutes from start of the infusion (+/-5 min)

[b] Screening labs: Complete blood count (CBC)/hematology including differential, coagulation, clinical chemistry, gamma glutamyl transferase, lactate dehydrogenase, uric acid, bilirubin, thyroid stimulating hormone (TSH), β-hCG serum pregnancy test, creatine phosphokinase (CK) and B12

[c] Routine labs: Complete blood count (CBC)/hematology including differential, coagulation, clinical chemistry, gamma glutamyl transferase, lactate dehydrogenase, uric acid, bilirubin

[d] Short Cognitive Battery: MSPT, MoCA, COWAT

[e] Extensive Cognitive Battery: BNT, BVMT-R, CVLT-II, D-KEFS Sorting, and JOLO

[f] ±3 Days does not apply to the Screening period, which is a maximum of 28 days
ET, early termination.
[g] Urine pregnancy test to be performed prior to study drug infusion.
AMNART - American National Adult Reading Test

References

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