STUDY PROTOCOL

Title:	A Single-Arm, Single-Site, Single-Dose Phase 1 Study Assessing the Safety of Bryostatin in the Treatment of Patients with Multiple Sclerosis
Protocol Number:	NTRP103-301
Study Phase:	1
Date:	28 Oct 2024
Study Drug:	Bryostatin 1
Investigational new Drug Application (IND) Number:	170476
Clinicaltrials.gov	NCT06190912
Version:	v. 4.3
Sponsor-Investigator/PI:	Robert Fox, MD

Regulatory Statement

This study will be performed in compliance with the protocol and in accordance with Good Clinical Practice (GCP) (International Conference on Harmonisation [ICH], Guidance E6, 1996), principles of human subject protection, and applicable country-specific regulatory requirements.

Confidentiality Statement

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be disclosed unless such disclosure is required by applicable laws and regulations. In any event, persons for whom this information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future information supplied to you that is indicated as privileged or confidential.

SIGNATORY PAGES

SPONSOR- INVESTIGATOR'S SIGNATURE PAGE

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

Protocol Number: NTRP103-301

Protocol Version and Date: v4.3; 28Oct2024

Sponsor-Investigator:

Robert J Fox, MD

Signature: Date:

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

Protocol Number: NTRP103-301

Protocol Version and Date: v 4.3; 28Oct2024

Investigational Medicinal Product: Bryostatin 1

Principal Investigator's STATEMENT OF APPROVAL

Confidentiality of all information received or developed in connection with this protocol will be maintained by me, as well as all other personnel involved in the study who are employed by me. By signing this protocol, I confirm that I have read and agree to conduct the study as outlined in the protocol and in compliance with Good Clinical Practice, the Declaration of Helsinki as amended and all other applicable regulatory requirements.

Printed name of PI: Robert J. Fox, MD

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Signature Date

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PROTOCOL SYNOPSIS

Name of Sponsor-Investigator: Robert Fox, MD	**Name of Study Medication:** bryostatin	**Name of Active Ingredient:** bryostatin 1
Title of Study: A Single-Arm, Single-Site, Single-Dose Phase 1 Study Assessing the Safety of Bryostatin-1 in the Treatment of Patients with Multiple Sclerosis		
Study center(s): Cleveland Clinic Neurological Institute		
Protocol Number: NTRP103-301		
Study Duration: 42 weeks	**Study Phase:** 1	
Objective: To evaluate the safety of bryostatin 1 (hereafter referred to as bryostatin) for the treatment of multiple sclerosis (MS).		
Study Drug: bryostatin		
Route of Administration: Intravenous by continuous infusion over 45±5 minutes		
Number of Participants: 20 evaluable participants		

Study Design

This is a single-site, single-arm, single-dose, Phase 1 study of the safety of bryostatin in participants with MS receiving any disease modifying therapy (DMT). The study is 42 weeks in duration, including 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 a total of 14 doses over 26 weeks.

Treatment

Eligible participants will be treated with bryostatin over a 26-week period. Doses 1, 2, 8, and 9 of the study drug will be a loading dose 20% higher (i.e., 24 μg) than the assigned fixed dose and will be administered one week apart. Otherwise, the assigned fixed dose is 20 μg. Drug is administered intravenously (IV) by continuous infusion over 45(±5) minutes. Participants are scheduled to receive 14 doses over 26 weeks.

Primary Objective: Safety and Tolerability

Primary Outcome Measures

Frequency of treatment emergent adverse events (TEAEs) and serious adverse events (SAEs)

Frequency of study medication discontinuation

Potential central nervous system (CNS) inflammatory effects as captured by clinical monitoring and MRI

Name of Sponsor-Investigator: Robert Fox, MD	Name of Study Medication: Bryostatin	Name of Active Ingredient: Bryostatin 1

Exploratory Objectives: Assess Signals of Efficacy

Exploratory Outcome Measures

Changes from baseline to Week 11, Week 28, and Week 40 in the following:

- MRI biomarkers
  - lesion volume and brain parenchymal fraction
  - default mode network node connectivity
  - diffusion MRI measures of tissue integrity- anatomical connectivity of transcallosal motor pathway
  - myelin water fraction
  - measures of myelination derived from magnetization transfer ratio, quantitative T2* and quantitative susceptibility mapping
  - grey matter atrophy, including grey matter fraction, white matter fraction, and cortical atrophy as measured by CLADA
- Expanded Disability Status Scale (EDSS)
- Montreal Cognitive Assessment (MoCA) test
- Controlled Oral Word Association Test (COWAT)
- MS Performance Test Domains
  - lower extremity function
  - upper extremity function
  - cognition
- Quality of Life in Neurological Disorders (Neuro-QoL)

Change from baseline to Week 28 and Week 40 in the following:

- California Verbal Learning Test- 3rd Edition (CVLT 3)
- Brief Visuospatial Memory Test - Revised (BVMT-R)
- Judgement of Line Orientation (JOLO)
- Boston Naming Test (BNT)
- Delis–Kaplan Executive Function System (D-KEFS) Sorting Test

The time-course of changes will be assessed for those outcomes with more frequent assessments (i.e., COWAT, MoCA, and MSPT)

Statistical Considerations:

Treatment emergent adverse events (TEAEs) and other safety data will be analyzed descriptively in all participants who received any dose of study drug (including partial infusions). These data will be summarized by time in study.

All statistical tests for exploratory outcomes (efficacy signals) will be two-sided tests, with $\alpha=0.05$. Since all of the efficacy assessments are exploratory, there will be no adjustment for multiplicity.

Eligibility Criteria:

Inclusion

1. Written informed consent signed by participant
2. English-speaking
3. Hospital Anxiety and Depression Scale <11
4. Male and female participants, 18-65 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 off of a DMT or 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

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. Use of carbamazepine within 7 days prior to screening
10. Use of teriflunomide within 90 days prior to screening
11. Use of dalfampridine within 7 days of screening

12. Current use of acetaminophen, ciprofloxacin, and/or trimethoprim/sulfamethoxazole
13. Use of any potent or moderate inhibitor or inducer of CYP3A4, CYP2C8, or CYP2C9. Concomitant medicines will be examined on a case-by-case basis against the Flockhart Table by study investigator, and if needed, the Medical Monitor, to determine allowability
14. Current use of St. John's Wort, within 2 weeks prior to screening
15. Consumption of grapefruit juice from screening until end of study
16. 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
17. Any screening laboratory values outside the laboratory reference ranges that are deemed clinically significant by the PI
18. Use of an investigational drug within 30 days prior to screening
19. 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
20. 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
21. Diagnosis of alcohol or drug abuse within the last 2 years
22. 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]
23. 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]
24. 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
25. Known to be seropositive for human immunodeficiency virus (HIV)
26. Pregnancy or breastfeeding during the study. A β-hCG serum pregnancy test will be performed at Screening for female patients of child-bearing potential.
27. 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
28. History of significant bleeding disorders.
29. Moderate baseline thrombocytopenia (platelets <100K/uL).
30. Elevated INR (>2.0).
31. Prior exposure to bryostatin, or known sensitivity to bryostatin or any ingredient in the study drug
32. Any other concurrent medical condition, which in the opinion of the PI makes the participant unsuitable for the clinical study

[1] Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018 Feb;17(2):162-73.

[2] Benedict RHB, Amato MP, DeLuca J, Geurts JJG. Cognitive impairment in multiple sclerosis: clinical management, MRI, and therapeutic avenues. Lancet Neurol. 2020 Oct;19(10):860-871. doi: 10.1016/S1474-4422(20)30277-5. Epub 2020 Sep 16. PMID: 32949546.

[3] McCary CA, Yoon Y, Panagabko C, Cho W, Atkinson J, Cook-Mills JM. Vitamin E isoforms directly bind PKCα and differentially regulate activation of PKCα. *Biochem J*. 2012;441(1):189-198. doi:10.1042/BJ20111318

[4] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: DSM-5 – May 27, 2013. American Psychiatric Association. Washington, D.C.

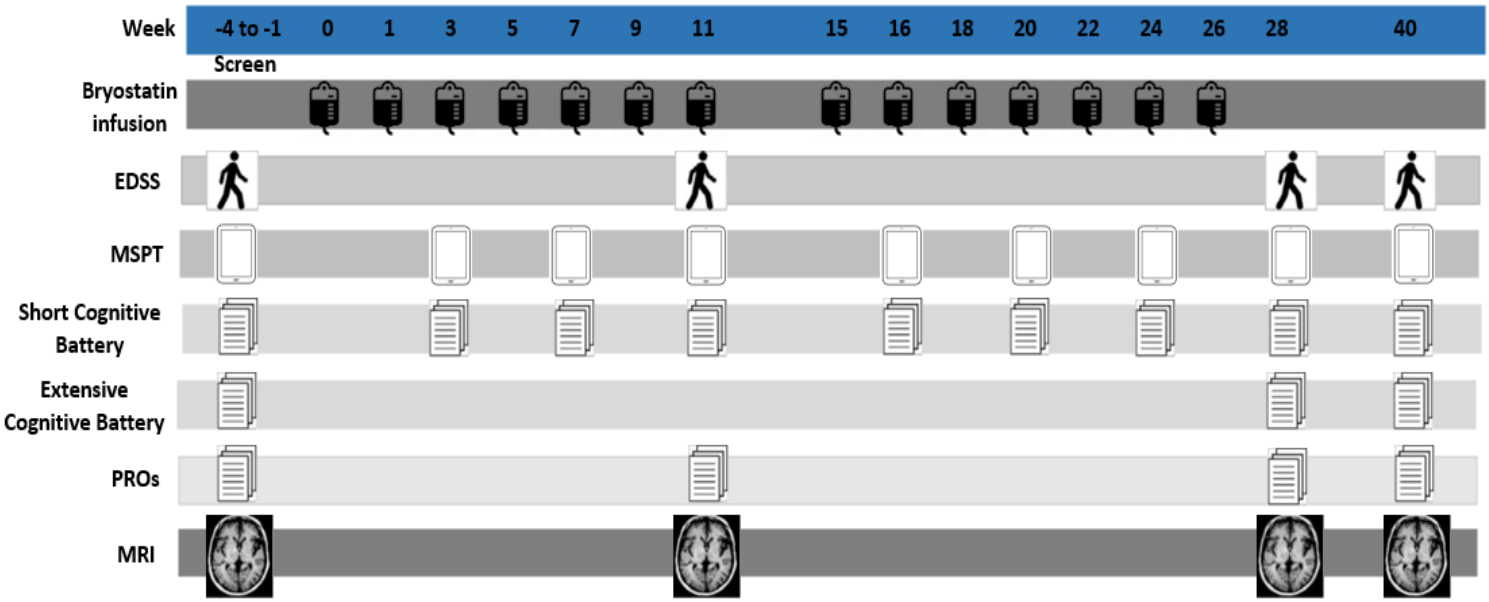
[5] O-Uchi J, Rice JJ, Ruwald MH, Parks XX, Ronzier E, Moss AJ, Zareba W, Lopes CM. Impaired IKs channel activation by Ca(2+)-dependent PKC shows correlation with emotion/arousal-triggered events in LQT1. J Mol Cell Cardiol. 2015 Feb;79:203-11. doi: 10.1016/j.yjmcc.2014.11.020. Epub 2014 Dec 2. PMID: 25479336; PMCID: PMC4302024.

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								NO CLINICAL VISIT												
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				X
Screening Labs[b]	X																				
HADS	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 and Performance Test[d]	X			X		X		X			X		X		X		X		X		X
Extensive Cognitive Battery[e]	X																X		X		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

[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 and Performance Test: MSPT, MoCA, COWAT
[e] Extensive Cognitive Battery: BNT, BVMT-R, CVLT 3, 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.

STUDY FLOW CHART



LIST OF ABBREVIATIONS

9HPT	9-hole Peg Test
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADCS-	Alzheimer's Disease Cooperative Study Activities of Daily Living
ADL-Sev	Inventory for Severe Alzheimer's Disease
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
AST	Aspartate Amino Transferase
AUC	Area under the ROC Curve
β-hCG	Human chorionic gonadotropin-beta subunit
BNT	Boston Naming Test
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
BVMT-R	Brief Visuospatial Memory Test – Revised
CAS	Completer Analysis Set
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression-Improvement Scale
CI	Cognitive Impairment
CL	Clearance
Cmax	Peak Drug Concentration
CNS	Central Nervous System
COWAT	Controlled Oral Word Association Test
CK	Creatine phosphokinase
C-SSRS	Columbia Suicide Severity Rating Scale
CVLT 3	California Verbal Learning Test- 3rd Edition
DMT	Disease Modifying Therapy
DSC	Digit Symbol Coding
DSMB	Data Safety Monitoring Board
D-KEFS	Delis–Kaplan Executive Function System
DTI	Diffusion Tensor Imaging
EAE	Experimental Autoimmune Encephalomyelitis
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDSS	Expanded Disability Status Scale
EOS	End of Study
EMG	Electromyography
ET	Early Termination
FAS	Full Analysis Set
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
FXS	Fragile X Syndrome
GCP	Good Clinical Practice

GCT	gamma glutamyl transferase
GI	Gastrointestinal
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice
HADS	Hospital Anxiety and Depression Scale
HIV	Human Immunodeficiency Virus
HED	Human Equivalent Dose
HVLT-R	Hopkins Verbal Learning Test-Revised
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
INR	International Normalized Ratio
IP	Intraperitoneal Injection
IRB	Institutional Review Board
IU	International Unit
IV	Intravenous
JOLO	Judgement of Line Orientation
LCLA	Low-contrast Letter Acuity
LD50	Dosage causing the death of 50% (one half) of a group of test animals
LDH	Lactate Dehydrogenase
LLOQ	Lower Limit of Quantitation
MDT	Manual Dexterity Test
MedDRA	Medical Dictionary for Regulatory Activities
mGRE	Multi-echo gradient recalled echo
MMRM	Mixed Model for Repeated Measures
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MOG	Myelin Oligodendrocyte Glycoprotein
MP2RAGE	Magnetization Prepared 2 RApid Gradient Echo
MSFC	Multiple Sclerosis Functional Composite
MSPT	Multiple Sclerosis Performance Test
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
MTR	Magnetization Transfer Ratio
MS	Multiple Sclerosis
NCI	National Cancer Institute
Neuro-QoL	Quality of Life in Neurological Disorders Survey
NOAEL	No Adverse Effect Level
NPI	Neuropsychiatric Inventory
NS	Normal Saline
NYHA	New York Heart Association
PASAT	Paced Auditory Serial Addition Test
PBMC	Peripheral Blood Mononuclear Cell
PE	Physical Examination
PET	polyethylene glycol, ethanol, and Tween 80
PI	Principal Investigator
PK	Pharmacokinetics

PKC	Protein Kinase C
PPMS	Primary Progressive Multiple Sclerosis
PRN	As Needed
PST	Processing Speed Test
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PVC	Polyvinylchloride
QTcB	QT correction with Bazett formula
QTcF	QT correction by Fridericia
RBANS	Repeatable Battery of Assessments for Neuropsychological Status
RMS	Relapsing Multiple Sclerosis
RRMS	Relapsing Remitting Multiple Sclerosis
rs-fMRI	Resting State-Functional Magnetic Resonance Imaging
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SD	Standard Deviation
SDMT	Symbol Digit Modality Test
SIB	Severe Impairment Battery
SOC	System Organ Classes
SOP	Standard Operating Procedures
SPMS	Secondary Progressive Multiple Sclerosis
SBQ-R	Suicide Behaviors Questionnaire-Revised
SUSAR	Suspected Unexpected Serious Adverse Reactions
T25FW	Timed 25-Foot Walk
TEAE	Treatment Emergent Adverse Events
TSH	Thyroid Stimulating Hormone
Tmax	Time-to-Peak Measure
ULN	Upper Limit of Normal
USP	United States Pharmacopeia
US	United States
ViSTa	Visualization of Short Transverse
WST	Walking Speed Test

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1. INTRODUCTION

1.1. Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system (CNS) that often leads to severe neurologic disability and, most typically, begins in early adulthood. Approximately 2.8 million people worldwide are living with MS,[1] with the disease affecting young adults primarily (mean age at diagnosis of ~30 years) and women affected at least twice as often as men in most types of MS.[2, 3]

Clinical signs of MS can occur alone or in combination and include weakness, spasticity, gait and coordination disturbances, sensory dysfunction, visual loss, sexual dysfunction, fatigue, depression, chronic pain, sleep disorders, and cognitive impairment.[4] MS is degenerative, and assistance in walking is required usually within 15 years of disease onset [Expanded Disability Status Scale (EDSS) score, 6.0].[5] Thus, irreversible disease progression is an important factor in selecting appropriate treatment by patients and their physicians. While the disease is devastating physically, MS also creates substantial economic burden on patients, their families, and society as a whole, with total annual costs of approximately $85 Billion in the US alone (2019).[6]

MS is classified into three clinical phenotypes: relapsing remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS) based on current course. Each of these phenotypes can be classified as active or non-active, based on the presence or absence of clinical relapses and the presence and character of lesions on MRI. The term “Relapsing MS” (RMS) includes RRMS and early stages of SPMS, where patients experience progression with superimposed relapses.[7]

Demyelination and potential remyelination

Immunologic changes that occur in MS can lead to the infiltration of immune cells into the CNS and activation of specific cell types in the brain that surround neurons, including microglia, and the destruction of myelin-producing oligodendrocytes. The inflammatory response in the CNS leads to damage to the myelin sheath surrounding neurons, with eventual demyelination and, in some cases, axon loss.[8, 9] The demyelinated lesions appear as plaques or lesions on magnetic resonance imaging (MRI), often surrounding blood vessels. While the demyelination process continues, other mechanisms attempt to repair the damage. Oligodendrocyte precursor cells migrate to the lesions and differentiate into myelin-producing oligodendrocytes, which can re-wrap the damaged axons with myelin, although the new sheath is usually not as thick as the original.[10] Remyelination appears to occur more consistently in early stages of the disease, with over 20% of demyelinated lesions observed to have completely remyelinated.[11] This demonstrates that repair is possible and provides hope that if the inflammatory processes could be halted and/or the repair mechanisms could be potentiated, the tissue damage due to neuroinflammation may be reduced and reversed. *In vitro* studies indicate that protein kinase C (PKC) modulation by bryostatin can decrease the expression of pro-inflammatory cytokines and promote the expression of anti-inflammatory ones, implicating PKC as a key target in regulation of the innate immune system.[12] Murine experimental autoimmune encephalomyelitis (EAE) studies demonstrated that bryostatin was “highly effective at attenuating the neurologic deficits associated with” myelin oligodendrocyte glycoprotein (MOG)-induced EAE [12]. The effect on neuroinflammation and innate immunity is through the activity of bryostatin as a PKC modulator, as in vitro experiments demonstrated that the activation of microglia and macrophages occurs in a PKC-dependent manner.[13]

Structural impairment in MS

Functional degeneration in MS is reflected in the substantial inflammation, demyelination, and neurodegeneration that occurs during the course of the disease and results in the progressive loss of brain gray and white matter. These structural changes can be detected using MRI data, an important tool in the diagnosis of MS, in monitoring disease progression, and in determining the efficacy of disease modifying

therapies.[14] Inflammation, axon demyelination (remyelination), changes in the appearance of plaques or lesions, brain atrophy, and, possibly, changes in brain functional status, all of which are consistent with a diagnosis of MS, can be assessed through the use of different types of MRI. Conventional T2- and T1-weighted MRI can detect focal inflammation, but quantitative analysis methods are required to detect global and regional brain volume loss, changes in lesions and plaques, and changes in myelination. MRI measurements of gray matter atrophy (linked to neurodegeneration in progressive MS) and magnetization transfer ratio (MTR) (a marker of myelin density) have been associated with long-term disability accumulation and cognitive decline.[15-17] A relatively new MRI sequence, Magnetization Prepared 2 RApid Gradient Echo (MP2RAGE), has been shown to be superior to other types of MRI (2D fluid-attenuated inversion recovery and conventional MP-RAGE) in localizing MS lesions due to its superior gray matter to white matter contrast.[18-20] Together, these MRI methods can detect sensitive and reliable biomarkers of MS (inflammation, brain volume changes, myelination changes, and lesion changes) that enable monitoring of MS disease progression and assessing the efficacy of new therapies. As described previously, studies indicate that bryostatin can modulate PKC and affect neuroinflammation and innate immunity to create a less inflammatory tissue environment.[12, 13, 21] Thus, bryostatin treatment may result in reduced inflammation, a slowing of demyelination or increased remyelination, fewer or resolved lesions, and reduced brain volume changes, all of which can be quantitated by MRI.

Cognitive impairment in MS

Cognitive impairment (CI) is a common feature of MS, occurring in approximately 50% of patients.[22] Cognitive domains shown to be affected predominantly in MS include recent/episodic working memory and information processing speed, with lesser effects in executive functioning and spatial perception [22]. Cognitive impairment negatively impacts quality of life, the ability to engage in daily activities, and continued employment for people with MS.[23] Information processing speed is measured routinely in MS clinics and clinical trials through the use of a variety of tests such as the Paced Auditory Serial Addition Test (PASAT),[24] the Symbol Digit Modality Test (SDMT),[25] and the newer iPad-based Processing Speed Test (PST).[26] Currently, no effective pharmaceutical interventions have been demonstrated to improve cognitive impairment in MS,[27] and cognitive rehabilitation is the only available intervention to improve CI; however, its effectiveness is controversial.[23] Trials of bryostatin in memantine-free Alzheimer's disease (AD) patients suggest that the agent may improve cognitive ability as some participants demonstrated improvement in the Severe Impairment Battery (SIB) compared to those who received placebo.[28] Additionally, in another randomized, placebo-controlled, AD trial, an increase in PKC activity was measured following bryostatin administration, and participants who received the study drug demonstrated a temporary improvement on the Mini Mental Status Examination (MMSE) compared to those receiving placebo.[29]

Neurogenesis stimulation

The potential mechanism underlying the observed cognitive improvement in the bryostatin AD trials may be a result of neuroprotection and synaptogenesis, primarily in the hippocampus.[30, 31] In an animal model, bryostatin prevented neuronal damage and the restoration of dendritic spines and their synapses.[32] It also decreased the level of apoptosis and necrosis of neurons in an ischemic brain injury model.[33] In cortical cultures, bryostatin increases synaptogenesis and decreases immature dendritic spine density in a PKC-dependent manner.[30] The PKC effect may be a result of a PKC-dependent increase in protein level and cellular distribution of HuD, a mRNA-binding protein which is important in post-transcriptional regulation.[34] The up-regulation of HuD leads to an increase in mRNA stability, and subsequently increased expression, of several neurotrophic factors in cultured hippocampal neurons. These effects make bryostatin an attractive candidate for cognitive restoration in people with MS.

1.2. Rationale for the use of bryostatin in the treatment of MS

Bryostatin has demonstrated pre-clinical efficacy for anti-inflammation, synaptogenesis associated with induction of synaptic growth factors (BDNF, NGF, IGF) and anti-apoptosis (prevention of neuronal death). Bryostatin has also been shown to be safe and well-tolerated in the clinic with demonstrated improvements in various measures of cognition in moderate to severely impaired AD patients.

Bryostatin was shown to act on antigen-presenting cells to promote differentiation of lymphocytes into Th2 cells, an action that might benefit Th1-driven inflammation in MS and to provide benefit in mice with experimental autoimmune encephalomyelitis (EAE), a well-established experimental MS animal model. In that study, preventative treatment with bryostatin abolished the onset of neurologic deficits. Most remarkably, bryostatin reversed neurologic deficits 28 days after EAE.[21]

These results, and others, suggest that bryostatin may improve inflammation in MS, demyelination via oligodendrocyte-mediated, growth factor-dependent remyelination, and cognition by way of the prevention and amelioration of synaptic loss in brain tissues.[35]

2. NONCLINICAL STUDIES

2.1. Toxicology

Single dose toxicity of bryostatin has been determined in four studies conducted under the National Cancer Institute's (NCI) former investigational new drug (IND), including three IV toxicity studies in mice and one IV toxicity study in rats. These studies were conducted using two different dosing formulations: bryostatin in ethanol/saline or bryostatin in polyethylene glycol, ethanol, and Tween 80 (PET) diluent. LD50 ranged from a low of 38 µg/kg to 75 µg/kg.

A 21-day repeat-dose toxicity study was performed in rats at doses of 0, 10, 15, and 25 µg/m^2 with the PET formulation resulting in no relevant toxicology findings. The maximum tolerated dose (MTD) and no adverse effect level (NOAEL) were noted to be 25 µg/m^2, the highest dose studied. However, since there were no toxicologically relevant findings, the report suggested that the MTD and NOAEL are greater than 25 µg/m^2.

Two non-GLP dose range-finding IV toxicology studies were performed in the rat and dog to characterize the toxicity of bryostatin and to estimate the MTD when administered by bolus injection and continuous 1-hour IV infusion. In the Sprague-Dawley rat, bryostatin was tolerated at up to 10 µg/kg (infusion) and 15 µg/kg (bolus) with no significant differences in toxicity noted between the two routes of administration. In the beagle dog, bryostatin was tolerated at doses up to 15 µg/kg (infusion and bolus). There were no significant differences in toxicity between the two routes of administration.

Detailed results can be found in the Investigator's Brochure, v 8.0.

2.2. Pharmacokinetics (PK)

Limited PK data are available in animals. The pharmacokinetics of bryostatin was analyzed in female CD1/F2 mice using [C26-3H]-labeled bryostatin by IV and IP administration.[36] Following IV administration of 40 µg/kg (120 mg/m^2), the plasma disappearance curve for bryostatin could be described by a 2-compartment model, with a distribution t½ of 1.05 hours and an elimination t½ of 22.96 hours. In contrast, following IP administration, the plasma disappearance curve was better described by a first-order absorption one-compartment model, with an absorption t½ of 0.81 hours and an elimination t½ of 28.76 hours. Urinary excretion represented the major pathway of elimination in the first 12 hours after IV administration, with 23.0 ± 1.9% (mean ± standard deviation) of the administered dose excreted. Approximately equal amounts of radioactivity (40%) were excreted in feces compared with urine within 72 hours after IV administration. A greater area under the curve, longer mean resident time, and lower clearance were observed with IP administration compared with IV administration. Bryostatin was widely distributed to various tissues

following both IV and IP administration. However, accumulation was observed in the lungs, liver, gastrointestinal (GI) tract, and fatty tissue.[36]

2.3. Adsorption, Distribution, Metabolism, Excretion (ADME)

A pharmacokinetic, excretion, mass balance, metabolism and distribution study was assessed in male, Sprague-Dawley rats by administering a single dose of [C26-3H]-labeled bryostatin by IV infusion over 45 minutes. Tritiated bryostatin was shown to have a short distribution half-life, a long terminal half-life, low clearance, and a high-volume of distribution. In rat plasma tritiated bryostatin was predominant while only a single minor metabolite (M16) was detected. In rat feces tritiated bryostatin and 17 metabolites were observed. The structures of metabolites were not characterized by liquid chromatography-high resolution MS due to low concentrations. A more detailed summary is found in the Investigator's Brochure, v 8.0.

2.4. Drug-drug Interactions (DDI)

In consideration of potential drug-drug interactions (DDI), the metabolism of bryostatin by seven CYP isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) was investigated.[37, 38, 39]

Cytochrome P450 Enzyme	Is Bryostatin a Substrate?	Is Bryostatin an Inhibitor?[1]	Is Bryostatin an Inducer?[2]
3A4	Yes	No	Yes, but not at physiologic doses[3]
2C8	Yes	No	
2C9	Yes	No	
2B6	No	No	Yes, but not at physiologic doses[3]
1A2	No	No	No
2C19	No	No	
2D6	No	No	

[1]Decreases activity of CYP450 enzymes resulting in slower metabolism and longer systemic exposure

[2]Increases activity of CYP450 enzymes resulting in faster drug metabolism and reduced systemic exposure

[3]Only at a concentration of 150nM. Bryostatin 40μg fixed dose or 20μg/m^2.is anticipated to produce a concentration of approximately 1nM.

It was determined that bryostatin at its expected concentration for this study does not induce or inhibit any of the tested CYP isoforms. Further study found that bryostatin is a substrate for 3 of the enzymes – CYP2C8, CYP3A4, and CYP2C9.[39] The relationship between these 3 CYP enzymes and medications commonly used by patients with MS, including the FDA approved disease modifying therapies (DMTs) for MS and medications commonly used in symptomatic management, were evaluated to determine potential DDIs with bryostatin. Medications considered included the following: disease modifying therapies teriflunomide, monomethyl fumarate, dimethyl fumarate, diroximel fumarate, cladribine, fingolimod, siponimod, ponesimod, ozanimod, interferon-β1a/b, glatiramer acetate, natalizumab, ublituximab, alemtuzumab, rituximab, ocrelizumab, ofatumumab; fampridine, baclofen, tizanidine, valacyclovir, acyclovir, methylprednisolone, prednisone, clonazepam, diazepam, amantadine, armodafinil, modafinil, methylphenidate, dextroamphetamine, sertraline, citalopram, escitalopram, fluoxetine, paroxetine, venlafaxine, duloxetine, mirtazapine, buproprion, amitriptyline, nortriptyline, imipramine, oxybutynin, tolterodine, solifenacin, darifenacin, sulfamethoxazole/trimethorprim, ciprofloxacin, sildenafil, tadalafil, vardenafil, dextromethorphan hydrobromide/quinidine sulfate, gabapentin, pregabalin, carbamazepine, and levetiracetam.

Of these medications, only *teriflunomide, carbamazepine, ciprofloxacin, and sulfamethoxazole* were moderate or greater inducers or inhibitors of relevant CYP enzymes. Teriflunomide (an MS disease modifying therapy), is a moderate inhibitor of CYP2C9 and has a half-life of 18-19 days; therefore, teriflunomide is excluded from use during the trial starting 90 days before screening. Carbemazepine (an antiepileptic sometimes used for dysesthesias and/or mood) is a strong inducer of CYP3A4 and a moderate

inducer of CYP2C9, with a half-life of approximately 35 hours; therefore, carbemazepine is excluded from use during the trial starting 7 days before screening. Ciprofloxacin (an antibiotic often used for treatment of infections, particularly bladder infections) is a moderate inhibitor of CYP3A4 and has a half-life of 4 hours. Because ciprofloxacin is generally used only for short periods and bryostatin has a very short half-life, ciprofloxacin is excluded from use the day before bryostatin infusion and on the day of bryostatin infusion. Sulfamethoxazole, which is a component of the antibiotic Bactrim (often used to treat infections, particularly bladder infections), is a moderate inhibitor of CYP2C9 and has a half-life of 6-12 hours. Similar to ciprofloxacin, Bactrim (trimethoprim/sulfamethoxazole) is generally used only for short periods and bryostatin has a very short half-life, Bactrim is excluded from use the day before bryostatin infusion and on the day of bryostatin infusion.

In consideration of transporters, bryostatin was tested for an effect on the common human transporters (P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K, OCT1 and BSEP).[40, 41] Bryostatin was found to not be a substrate for OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K, OCT1 and BSEP. One cannot come to a firm conclusion if bryostatin is or is not a substrate for P-gp or BCRP. Bryostatin was found to inhibit the activity of some transporters including P-gp but not BCRP, OATP1B1, OAT3, MATE2-K, and BSEP. The physiologic concentrations of bryostatin expected at the dose used during the trial (20μg) is expected to result in a C_{max} cellular concentration ranging between 0.622 and 0.722 nM [Investigator's Brochure, v8.0, 14 Sep 2023]. The percent inhibition of each transporter ranges from 22% to 48% at 1μM. Therefore, at the cellular concentrations anticipated, significant inhibition of these transport proteins are not expected.

2.5. Genotoxicity

Bryostatin was evaluated using the bacterial reverse mutation assay (i.e., the Ames test) with no positive responses observed. Bryostatin was evaluated as negative (non-clastogenic) in the micronucleus assay; it was also evaluated as negative (non-deoxyribonucleic acid damaging) in the Comet Assay. Overall, based on the results of the Ames test and the combined micronucleus/comet assay, bryostatin is not considered to be genotoxic.

2.6. Reproductive Toxicology

A developmental toxicity study of bryostatin was evaluated in SD rats by a group at the Department of Toxicology, College of Basic Medical Science, Secondary Military Medical University, Shanghai, China. The control article and bryostatin (test article) were administered intravenously on gestation days 6-15 at 0, 4, 8 and 16 μg/kg. Results of the study showed no teratogenic effects at the doses studied. Maternal, embryo and fetal toxicities were observed at 8, 16 and 4 μg/kg, respectively.[42] The source, purity and quality of the bryostatin used in this study were not reported, nor is it known if the study was performed pursuant to FDA's Good Laboratory Practice for Nonclinical Laboratory Studies (GLP) regulations (21 CFR Part 58).

2.7. Safety Pharmacology

Effect of bryostatin 1 on Cloned hERG Potassium Channels Expressed in Human Kidney Cells

The objective of this study was to examine the in vitro effects of bryostatin on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for IKr, the rapidly activating delayed rectifier cardiac potassium current) at near-physiological temperature. Two concentrations of bryostatin (0.3 and 1 μM) were tested. Results show that bryostatin inhibited hERG current by (Mean ± SEM; n = 3) -0.6 ± 0.9% at 0.3 μM and -0.7 ± 1.2% at 1 μM versus 0.8 ± 0.4% (n = 3) in control. hERG inhibition at 0.3 and 1 μM was not statistically significant ($P < 0.05$) when compared to vehicle control values. The IC_{50} for the inhibitory effect of bryostatin on hERG potassium current was not calculated since no net current inhibition was observed.

Cardiovascular and Respiratory Evaluation of Intravenously Administered bryostatin in the Beagle Dog

This study was conducted to evaluate the potential cardiovascular and respiratory effects of the test article, bryostatin, in conscious freely moving beagle dogs. Animals (groups of 4) were administered the control and test article treatments via intravenous injection at doses of 1.5, 5 and 10 µg/kg. Assessments of cardiovascular and respiratory effects and general toxicity were based on mortality, clinical observations, body temperature, blood pressure (systolic, diastolic, and mean arterial), heart rate, the electrocardiogram (QRS duration and the RR, PR, QT and QTc intervals), and respiratory function (respiratory rate, tidal volume, and minute volume). As evaluated in this study, bryostatin, at doses up to 10 µg/kg, did not produce mortality, clinical signs, or effects on the cardiovascular or respiratory parameters in beagle dogs.

3. CLINICAL TRIAL DATA

3.1 Oncology Data

Safety data are available from published clinical studies of bryostatin for the treatment of cancer. Altogether, over 1400 oncology participants received bryostatin, mainly under NCI's IND # 42,780, with exposures to bryostatin in both single and combination agent studies. About 584 participants received bryostatin as monotherapy, with dose levels ranging from 5 µg/m^2 to >180 µg/m^2. Most participants in both the monotherapy and combination therapy studies received bryostatin at doses >25 µg/m^2, most often as 1-hour infusions administered at various time intervals from weekly infusion to continuous infusions for 72 hours. Most studies were repeated dose studies where participants received treatment for several weeks (See Investigators Brochure, v 8.0).

Adverse events, occurring in the single agent studies that resulted in discontinuation from the studies, were myalgia (28 participants), acute transient reaction (dyspnea, flushing, hypotension, and bradycardia; 4 participants each), phlebitis (attributed to 60% ethanol concentration in earlier formulations, 6 participants), fatigue (3 participants), and 1 participant each with bacteremia, chest pain, dehydration, dysphagia, hematuria, nausea, skin rash, subclavian vein thrombosis, thrombocytopenia, and vomiting. The following AEs were associated with death in the single agent trials: cardiac arrest (2), hypotensive with evidence of renal and hepatic failure (1), perforated gastric ulcer (1), renal function decline with cardiac arrest and perforated gastric ulcer (1; PIs considered not related to bryostatin), and sudden death (1; PIs considered likely to be cardiovascular event). Relatedness to bryostatin treatment was not assessed except where noted. Other severe (Grade 3 or higher) AEs were reported in the single agent clinical studies in cancer participants including alkaline phosphatase (ALP) (elevated; participant had pre-existing liver metastases), allergic reaction, anemia, anorexia, arthralgia, ataxia, cardiac arrhythmias, cardiovascular, coagulation, community-acquired pneumonia, congestive heart failure, constipation, dermatitis, dermatologic, diarrhea, dyspnea, edema/weight gain, fever, gastrointestinal, genitourinary, granulocytopenia, headache, hepatic, hyperbilirubinemia, hyperglycemia, hypokalemia, hyponatremia, infection, leg weakness, lymphedema, lymphocytopenia, myocardial infarction, neurotoxicity, neutropenia, pain (abdominal, back, eye, site not specified), pulmonary, pulmonary embolus from inferior vena cava tumor thrombus, sepsis and pneumonia without neutropenia, syncope, and urinary frequency. The absence of a Placebo-control group in bryostatin oncology studies makes it difficult to determine the extent to which underlying disease or concomitant medications may have contributed to this safety profile.

As a single agent and in combination with chemotherapeutic agents, bryostatin's outcomes were not very compelling; consequently, on August 19, 2011, in its Annual Report to FDA, the NCI indicated that it chose not to pursue further studies and requested that its IND be withdrawn.

3.2 Alzheimer's Disease Data

3.2.1 Study NTRP101-204

This multicenter, double-blind, parallel-group, placebo-controlled, randomized clinical study was designed to evaluate bryostatin versus placebo for subjects with moderate and moderately severe (MMSE-2 score of 10 to 18, inclusive) AD in subjects who were not receiving memantine treatment. The study included a Screening period (approximately 4 weeks), 2 treatment periods, and a safety follow-up visit 30 days after the final dose of study drug. The first treatment period of the study was 15 weeks in duration (11 weeks of dosing with study drug plus follow-up with safety and efficacy evaluation 30 days after the last treatment). One hundred twenty-two eligible subjects were stratified based on Severe Impairment Battery (SIB) total scores at Baseline and were randomized 1:1 to treatment with bryostatin 20 μg or placebo for 2 identical courses of treatment. Study drug was administered by intravenous infusion over 45 minutes (±5 minutes). The first 2 doses of study drug were a loading dose 20% higher (i.e., 24 μg) than the assigned dose and were administered 1 week apart. Thereafter, the assigned dose of 20 μg was commenced with the third dose and administered every other week for 5 doses. The second treatment period, beginning at Week 15, was identical to the first, including the loading doses. Assessments performed during visits included the SIB, the Mini Mental State Examination (Second Edition; MMSE-2), Alzheimer's Disease Cooperative Study - Activities of Daily Living - Severe Impairment Version (ADCS-ADL-Sev), Neuropsychiatric Inventory (NPI), Columbia Suicide Severity Rating Scale (C-SSRS), laboratory testing, electrocardiograms (ECGs), physical examinations, and vital signs. Findings are as follows:

- Bryostatin was generally well tolerated, and there were no new safety findings. The overall treatment-emergent adverse event (TEAE) profile was similar between the placebo and bryostatin groups. A similar number of subjects had treatment-related TEAEs in the placebo and bryostatin groups (13 events in 8 [13.8%] subjects and 15 events in 10 [16.9%] subjects, respectively). Most TEAEs were not considered treatment related. There were no treatment-emergent adverse events of special interest (*e.g.*, myalgia) and no fatal TEAEs.
- The primary efficacy endpoint of the study was not met; the LS mean difference in change from Baseline in SIB total score for bryostatin versus placebo was not statistically significant at Week 28 (0.8 [95% CI: -1.9, 3.6]; $P = 0.563$). Sensitivity and supportive analyses were broadly consistent with the primary efficacy endpoint analysis.
- A subgroup of subjects with moderately severe AD may derive some benefit from treatment with bryostatin. Statistically significant differences in change from Baseline in SIB total score were noted for bryostatin versus placebo in the subgroup of subjects with Baseline MMSE-2 score of 10 to 14 at Week 20 (LS mean difference: 5.7 [95% CI: 0.5, 11.0]; $P = 0.033$), Week 24 (LS mean difference: 6.6 [95% CI: 0.5, 12.7]; $P = 0.036$), and Week 30 (LS mean difference: 7.9 [95% CI: 0.3, 15.5]; $P = 0.042$).

Exploratory analyses that referenced additional SIB endpoints specified in the clinical study protocol provided additional support for the potential benefit of bryostatin in the cohort of subjects with Baseline MMSE-2 score of 10 to 14; there is consistent and significant divergence of the bryostatin group from placebo when the mean change from Baseline in SIB total scores at time points from Week 13 through Week 42 is considered. These differences are further reinforced by the exploratory trend analysis showing a significant downward trend for the placebo group ($P < 0.001$) and the absence of such a trend for the bryostatin group ($P > 0.05$).

3.2.2 Study NTRP-101-203

NTRP-101-203 was a randomized, double-blind, placebo-controlled, Phase 2 trial that compared bryostatin to placebo for the treatment of moderately severe to severe AD in participants not receiving memantine treatment. The study was 15 weeks in duration, including a safety and efficacy evaluation at 30

days after the last dose of study drug. Seven fixed doses of the study drug (20 μg) and placebo control were administered by a 45-minute continuous IV infusion over 12 weeks. The primary efficacy endpoint was defined as the change from baseline to Week 13 in the SIB total score.

Methodology

Eligible participants were stratified based on MMSE-2 scores 4-9 and 10-15. Stratified participants were randomized 1:1 to one of two treatment arms, 20 μg bryostatin or placebo, for 12 weeks (7 doses). Trial drug was administered IV by continuous infusion. The first two doses (24 μg) for the active treatment arm were loading doses (20% higher than the assigned dose of 20 μg) and were administered one week apart. Thereafter, the assigned dose (20 μg) commenced with the third dose and was administered every other week. Participants were scheduled to receive 7 doses over 12 weeks, with the primary efficacy measure being at Week 13, and a follow-up visit scheduled 30 days from the last dose of trial drug administration.

Demographics

A total of 111 participants were screened and randomized on a 1:1:1 basis to a treatment group. Of those, 3 participants withdrew prior to receiving treatment.

Safety

A total of 108 participants were analyzed (53 receiving 20 μg bryostatin, 55 receiving placebo). In the study there was 1 death, in the placebo arm. SAEs were fairly equivalent across arms with 3 occurring in the bryostatin arm (9.43%) and 6 in the placebo arm (10.91%). SAEs included gastrointestinal (GI) disorders (1 bryostatin), death (1 placebo, cause unknown), infections and infestations (1 bryostatin, 2 placebo), injury, poisoning and procedural complications (2 bryostatin, 1 placebo), neoplasms benign/malignant/unspecified (1 placebo), nervous system disorders (1 bryostatin), psychiatric disorders (3 placebo), and renal and urinary disorders (1 bryostatin). Non-serious AEs were more common in the bryostatin arm (16; 30.19%) than in the placebo arm (4; 7.27%). Common AEs included urinary tract infections (3 bryostatin, 2 placebo), falls/skin abrasions (7 bryostatin, 1 placebo), and agitation (6 bryostatin, 1 placebo).

Efficacy

The efficacy of treatment, as specified in the trial, was evaluated primarily using SIB score at baseline compared to the final readout at Week 13. Lower scores indicate greater cognitive impairment. There was no difference in the modulation of SIB score in subjects treated with bryostatin or placebo. The primary efficacy endpoint for the change in SIB over the 13-week treatment period was not significantly different from placebo at Week 13. The observation of lack of benefit in SIB score was observed in both the full analysis set, the completer analysis set and multiple imputations set. SIB improvement was significant for the Week 9 measurement.

There were two secondary efficacy outcomes specified in the trial. One was change in performance in the SIB over time, in which lower scores indicate greater cognitive impairment. For this outcome, differences were noted between the bryostatin and placebo groups. The placebo group out-performed the bryostatin group at Weeks 5 (0.7 placebo vs -0.1 bryostatin) and 15 (2.1 placebo vs 1.6 bryostatin) and the bryostatin group out-performed the placebo group at Week 9 (2.7 bryostatin vs 1.4 placebo). The second efficacy outcome was performance on the MMSE at specific timepoints, lower scores indicate greater cognitive impairment. Only 18 participants from each group were administered the MMSE exams. At each timepoint, the bryostatin group was outperformed by the placebo group, except at Week 9, where the group scores were equivalent.

Overall, the combined data did not support the efficacy of bryostatin on cognitive improvement.

Post Hoc Analysis

SIB score, often considered a useful outcome measure in advanced stages of AD, showed that at baseline, subjects in the placebo group had a higher SIB score average, indicative of moderately severe disease. In comparison subjects in the bryostatin group had a lower average baseline SIB score, indicative of more severe disease. This imbalance had almost a five-point difference observed between baseline SIB score in the two treated populations. This suggests the SIB grouping of more severe cases in the bryostatin treatment group compared to the placebo control group with less severe impairment may have obscured the real treatment effect and efficacy benefits. In the moderate stratum, baseline SIB imbalance was less marked which may have contributed to the observation of SIB improvement of 4.8 SIB points by week 13 in the bryostatin-treated patients. For the severe stratum, the baseline imbalance was even more marked (6.1 points in favor of the placebo patients and no SIB improvement observed in the bryostatin treated group. Given this inadvertent imbalance, another statistical analysis was conducted using each patient as his (her) own control. In this post-hoc analysis, patients treated with bryostatin in the Moderately Severe Cohort (10 - 15) showed a statistically significant benefit when compared to their individual baseline Severe Impairment Battery (SIB) values ($p<0.0076$), 2-tailed t-tests. While Placebo patients improved to a lesser degree ($p<0.014$), Trend Analyses (measurements of slopes) were significant for the bryostatin-treatment group patients, but not for the Placebo patients.

3.2.3 Study NTRP101-202

NTRP 101-202 was a randomized, double-blind, placebo-controlled, Phase 2 trial assessing the safety and tolerability (primary objective) and efficacy (secondary objective) of bryostatin in the treatment of moderately severe to severe AD participants. Exploratory objectives characterize the PK and pharmacodynamics of bryostatin in this subject population. Seven doses of study drug were administered by a 45-minute continuous IV infusion over 12 weeks.

Methodology

Eligible participants with moderately severe to severe AD were stratified based on MMSE-2 scores 4-9 vs. 10-15 and randomized 1:1:1 to one of three treatment arms: 20 μg, 40 μg, or placebo for 12 weeks. Trial drug was administered IV by continuous infusion. The first two doses (24 μg, 48 μg) of each of the two active treatment arms were a loading dose, 20% higher than the assigned dose (20 μg, 40 μg), and were administered one week apart. Thereafter, the assigned dose commenced with the third dose and was administered every other week. Participants were scheduled to receive 7 doses over 12 weeks, with the primary efficacy measure being at Week 13, and a follow-up visit scheduled 30 days from the last dose of trial drug administration.

Demographics

A total of 264 participants were screened in the trial. Of these, 147 participants were randomized on a 1:1:1 basis to a treatment group. There were 50 participants randomized to the placebo treatment group, 49 randomized to the 20 μg bryostatin treatment group, and 48 randomized to the 40 μg bryostatin treatment group. A total of 141 of the randomized participants were treated with investigational product and were included in the Safety Analysis Set (SAS). A total of 135 participants provided a post-baseline efficacy assessment and were analyzed as the Full Analysis Set (FAS). A total of 113 participants out of 147 randomized (76.9%) performed a Week 13 evaluation of the SIB and were analyzed as the Completer Analysis Set (CAS). A total of 106 participants in the SAS completed the trial (75.2%). A total of 35 participants in the SAS and 29 participants in the FAS withdrew early from the trial. The most common reason for early withdrawal was withdrawal of consent (18 participants), followed by AE (11 participants).

Safety

Overall, the 20 μg bryostatin treatment group showed minimal differences from the placebo treatment

group in safety assessments. The 40 µg bryostatin treatment group had significantly greater AEs than the other treatment groups. There was also no clear difference in SAEs between the 20 µg bryostatin group and placebo treatment groups, and again the 40 µg bryostatin treatment group had significantly greater SAEs than the other treatment group.

Similarly, the placebo and 20 µg group had similar numbers of TEAEs (28 events vs 30 events, respectively). The TEAEs observed more often in the 20 µg treatment group vs placebo were infusion site reaction (8 events vs 3 events in placebo) and diarrhea (5 events vs 1 event in placebo). The 40 µg treatment group was associated with more TEAEs than either the 20 µg or placebo treatment groups across multiple system organ classes (SOC).

The most common treatment related TEAEs were grouped infusion reactions, diarrhea, headache, fatigue, and myalgia. Myalgia was seen in 5 participants; 4 of those participants were given the higher dose of bryostatin. Myalgia observed was mostly mild and managed with analgesics. There were also more treatment related TEAEs of diarrhea, headache, and fatigue in the higher dose bryostatin group. Both bryostatin treatment groups reported higher rates of infusion site TEAEs, and in particular the 40 µg bryostatin treatment group reported 4 events of infusion site cellulitis.

Although IV infusion-related reactions were also reported more often in the higher dose bryostatin group, none were reported following WebEx-based training on IV infusion and universal precautions, suggesting that this AE can be prevented. The higher dose bryostatin group also reported more TEAEs of seizure and fall compared to the lower dose bryostatin and placebo groups.

A total of 97 (68.8%) participants in all treatment groups reported 287 separate TEAEs. Of these, 49 (34.8%) participants reported 107 separate treatment-related TEAEs. There were 8 (16.7%) participants with 20 treatment-related TEAEs in the placebo treatment group, 17 (37.0%) participants with 30 events in the 20 µg bryostatin treatment group, and 24 (51.1%) participants with 57 events in the 40 µg bryostatin treatment group. Six participants had a TEAE that lead to trial drug discontinuation, 2 in the placebo treatment group, 1 in the 20 µg bryostatin treatment group, and 3 in the 40 µg bryostatin treatment group. Overall, 43 (30.5%) participants had a TEAE that was mild in intensity, 46 (32.6%) participants had a TEAE that was moderate in intensity, and 8 (5.7%) participants had a TEAE that was severe in intensity. There was 1 death in the trial, a subject in the 40 µg bryostatin treatment group who suffered a severe TEAE of worsening of AD that was unrelated to the IP.

There were 12 (8.5%) participants who had 14 treatment emergent non-fatal SAEs during the trial; 4 participants with 4 events in the placebo treatment group, 2 participants with 2 events in the 20 µg bryostatin treatment group, and 6 participants with 8 SAEs in the 40 µg bryostatin treatment group. A total of 4 participants in the 40 µg bryostatin treatment group had 4 events that were judged as possibly or probably related to the IP: 3 events of cellulitis, and 1 event of seizure.

There were no apparent differences between treatment groups in laboratory assessments over time. No differences were apparent between treatment groups when examining most vital signs, physical examinations (PEs), and ECG. However, there was a decline in weight in the bryostatin groups, which was more prominent in the higher dose group: 20 µg bryostatin treatment group (mean loss of -1.65±2.77 kg) and the 40 µg bryostatin treatment group (-2.98±2.10 kg), while there was a slight weight gain in the placebo treatment group (0.442±2.52 kg). Further, 5 participants in the 40 µg bryostatin treatment group had 5 TEAEs of weight decreased, 3 of which were judged to be related to the investigational product. No weight related TEAEs were observed in the 20 µg bryostatin dose group.

There were no differences between treatment groups on the Columbia Suicide Severity Rating Scale (C-SSRS) as most participants did not have suicidal thoughts during the trial, and there were no attempts at suicide by any participant during the trial.

Efficacy

Baseline scores on the SIB for the FAS were similar across all treatment groups. In the FAS, the treatment difference between the placebo treatment group and the 20 µg bryostatin treatment group was

statistically significant only at Week 5 (treatment difference 3.0[0.6, 5.3], 80%CI, P=0.056). There were no statistically significant differences between the placebo treatment group with either the 20 µg bryostatin treatment group or 40 µg bryostatin treatment group at Week 9 or Week 13. Combining the bryostatin treatment groups and comparing with the placebo treatment group also did not produce a statistically significant difference at any time point.

For the CAS, pre-specified along with FAS to assess primary and secondary endpoints, mean scores on the SIB followed the same pattern seen in the FAS were similar across all treatment groups. There was a statistically significant difference, however, between the placebo treatment group and the 20 µg bryostatin treatment group at Week 5 (P=0.016). However, the treatment difference at Week 13 (primary outcome measure) was not met with statistical significance (P=0.070). When both bryostatin treatment groups were pooled, there was a statistically significant difference between the placebo treatment group and pooled group at Week 5 (P=0.039); however, statistically significant treatment difference at Week 13 was not met (P=0.094). There were no statistically significant differences between the placebo treatment group and the 40 µg bryostatin treatment group at any time point.

In the CAS there were significant differences in the ADCS-ADL-Sev at Week 13 between the placebo treatment group and the 20 µg bryostatin treatment group (P=0.082), and between the placebo treatment group and pooled bryostatin treatment group (P=0.087). There were no statistically significant differences between treatment groups for the ADCS-ADL-Sev, MMSE-2, Neuropsychiatric Inventory (NPI), or Clinical Global Impression- Improvement Scale (CGI-I) assays for any time point in the FAS.

In multiple exploratory analyses adjusting for covariates for the primary endpoint, there were additional significant differences between the placebo treatment group and bryostatin treatment groups. In a Mixed Model for Repeated Measures (MMRM) analysis of change from baseline in SIB total score, excluding all participants from sites that recruited 2 or fewer participants during the trial, there was a significant difference between the placebo treatment group and the 20 µg bryostatin treatment group at Week 5 (P=0.068). There was a significant difference between the placebo treatment group and the 20 µg bryostatin treatment group (P=0.031), and between the placebo treatment group and the pooled bryostatin treatment group in an MMRM analysis of change from baseline in SIB total score at the 30-day follow up visit (P=0.041). When an Analysis of Covariance (ANCOVA) was performed to assess site effect on SIB, there was a significant difference between the placebo treatment group and 20 µg bryostatin treatment group (P=0.032), and between the placebo treatment group and pooled bryostatin treatment groups (P=0.049) at Week 5. In an ANCOVA of change from baseline in SIB total score, with use of acetylcholinesterase inhibitor (AChEI) or memantine at baseline as an additional covariate, at Week 5 the 20 µg bryostatin treatment group and pooled bryostatin treatment groups had statistically significant treatment differences from the placebo treatment group (P=0.024 and P=0.027, respectively).

In multiple exploratory analyses adjusting for covariates for secondary endpoints, there were statistically significant differences between the placebo treatment group and the 20 µg bryostatin treatment group at Week 5 (P=0.062), and the placebo treatment group and pooled bryostatin treatment group at Week 5 (P=0.041) when performing an ANCOVA of change from baseline in ADCS-ADL-Sev total score for participants with > median baseline ADCS-ADL-Sev total score.

Statistically significant difference between the placebo and 40 µg bryostatin treatment groups at Week 5 (P=0.099) in an ANCOVA of change from baseline in NPI Total score to assess site affects was not met. When performing an ANCOVA of change from baseline in NPI total score for participants with > median baseline NPI total score, there was a near statistically significant difference between the placebo treatment group and the 40 µg bryostatin treatment group at Week 9 (P=0.054) but not at Week 13 (P-0.096).

No clear differences were apparent between treatment groups for participant distress on the NPI, each NPI subscore at baseline, or on the NPI 10-item score.

The results for SIB score from the MSSE-2 Stratum 1 analysis show the 20 µg bryostatin treatment group had a more positive response than any treatment group at Week 5 and Week 9 and the 40 µg bryostatin treatment group had a slightly better response at Week 13. Results for SIB score in the MSSE-2

Stratum 2 analysis show the positive response varied across time point and treatment group. The placebo treatment group showed greater improvement in ADCS-ADL-Sev total score at Weeks 5 and 9 than either bryostatin treatment group in Stratum 1 or 2, and also at Week 13 in Stratum 2. At Week 13 in Stratum 1, the 20 µg bryostatin treatment group showed the best response compared to placebo or the 40 µg bryostatin treatment group.

There was a significant difference in the FAS between the placebo treatment group and the 20 µg bryostatin treatment group (P=0.031), and between the placebo treatment group and the pooled bryostatin treatment group (P=0.041) in an MMRM analysis of change from baseline in SIB total score at the 30-day follow up visit.

Additional post-hoc analyses were performed on the TEAE by MMSE-2 baseline strata, SIB by BSA-adjusted Dose, ADCS-ADL-Sev by BSA-adjusted Dose, TEAE by BSA-adjusted Dose, SIB by memantine use, ADCS-ADL-Sev by memantine use, the NPI 10-item total score by memantine use, and administration site-specific TEAE. The SIB and ADCS-ADL-Sev scores also appeared to be affected by BSA-adjusted dose, as the lowest tertile of bryostatin dose in general had a better response to treatment than higher tertiles or placebo (these data were also not analyzed statistically). Participants treated with bryostatin who did not take memantine at baseline had significantly better improvement in SIB score compared to the placebo group in an MMRM analyses of SIB total score in both the CAS and FAS. Both the pooled bryostatin and 20 µg bryostatin treatment groups had significantly better scores than the placebo treatment group at Weeks 5, 13, and at 30-day Follow-up. Similar results (which were not statistically analyzed) were seen in the ADCS-ADL-Sev test and in the NPI 10-item score.

Pharmacokinetics

Mean plasma bryostatin concentrations increased approximately proportional to dose for an increase in dose between 20 µg and 40 µg, infused IV over 45 minutes. The median time-to-peak measure (Tmax) occurred at the end of the 45-min infusion. Mean peak drug concentration (Cmax) ranged from 0.563 ng/mL to 0.653 ng/mL after 20 µg and from 0.792 ng/mL to 1.34 ng/mL after 40 µg. Mean T1/2 ranged from 1.33 h to 5.37 h. Mean CL was similar for both dose levels and ranged from 24.97 L/h to 28.42 L/h for Weeks 0 and 3. The mean CL on Weeks 7 and 11 was slightly lower, ranging from 13.38 L/h to 23.91 L/h. Within a given dose level, the ranges of values for Cmax, AUClast, and AUCinf were consistent across weeks, and there was no indication of systemic accumulation of bryostatin during administration of bryostatin once every 2 weeks.

Summary

Bryostatin treatment was, in general, safe to use and well tolerated.

The 20 µg bryostatin treatment group had similar numbers of TEAEs to the placebo treatment group except in injection site reactions and diarrhea. The 40 µg treatment group had more TEAEs and SAEs than the 20 µg or placebo treatment groups across many SOCs.

The 40 µg dose of bryostatin was associated with more TEAEs. With a decrease in systemic CL especially noted with 40 µg bryostatin dosing, the higher drug exposure may further explain the increased number of TEAEs. The most common treatment related TEAEs were diarrhea, headache, fatigue, and myalgia, and all were reported in greater frequency with the 40 µg dose of bryostatin. Participants given the 40 µg dose of bryostatin also reported more TEAEs of seizure and fall.

The primary efficacy endpoint at Week 13 for the SIB and secondary efficacy endpoint for the ADCS-ADL- Sev was not met in the FAS; however post hoc analyses showed statistically significantly differences from placebo at Weeks 5 and 13 in the CAS for the ADCS-ADL-Sev with the 20 µg dose. The 40 µg dose was not statistically significant from the placebo

SIB improvement was nearly significant for the Week 5 FAS measurement ($p < .056$). No other primary or secondary endpoints were met in the FAS.

Multiple exploratory analyses in the FAS for covariates in the primary and secondary endpoints revealed additional significant differences, but these occurred predominantly at the Week 5 time point.

3.2.4 Study NTRP101-201

This was a randomized, double-blind, Placebo-controlled safety study of a single dose of bryostatin in participants with mild to moderate AD (MMSE: 14-26). Participants were randomized 2:1 to receive bryostatin 25 $\mu g/m^2$ or placebo. The primary objective was to evaluate the safety and tolerability of bryostatin by the incidence of AEs and SAEs. Secondary safety endpoints included assessment of PE, hematology including CBC and platelet count, coagulation parameters, serum chemistries, ECG, urinalysis and vital signs.

The primary efficacy endpoint was a composite end point of change from baseline in the Hopkins Verbal Learning Test-Revised (HVLT-R) delayed recall and Repeatable Battery of Assessments for Neuropsychological Status (RBANS) figure recall at 48 hours post study drug infusion.

The study included single dose PK, and measurement of PKCε in peripheral blood mononuclear cells (PBMCs) as a potential biomarker.

Demographics

The study enrolled nine participants, 4 male and 5 female, with a mean age of 71.8 ±7.4 years (range, 62 to 82). The mean MMSE-2 at baseline was 22.5 for the three placebo participants (range, 19-24) and 22 (range, 16-26) for the six bryostatin-treated participants.

Safety

Bryostatin treatment was safe and well tolerated. There were no deaths or SAEs reported. No participants had an AE leading to withdrawal during the study. There were five treatment emergent adverse events occurring in three participants: headache, dizziness, and papular rash. There were no reported episodes of myalgia, a known side effect of bryostatin. The only adverse event in the bryostatin treated group was headache, which was not considered related to study drug. All adverse events were mild and resolved without treatment. All laboratory assessments, including hematology, chemistry, coagulation, renal function and liver function as well as cardiac assessments were unremarkable after treatment and there was no clinically significant change in any vital sign.

Efficacy

The study was not powered to detect an effect. As such, there was no difference in HVLT-R delayed recall or the RBANS delayed figure recall at 48 hours. Additional time points of assessment for these measures (day 2, day 4, and day 15) did not indicate any difference between groups. Additional endpoints included the change from baseline in Digit Symbol Coding (DSC), and MMSE-2 at various time points. There was no difference in mean values between groups for these endpoints at any time point. Both the treatment and the placebo group showed an improvement in the MMSE-2 score most likely due to practice effects since the MMSE-2 was administered five times in 2 weeks.

In summary, there was no clinically significant difference between the mean or mean change from baseline between bryostatin and placebo in the HVLT-R or MMSE-2 assessments or any other efficacy assessments to suggest a treatment effect after a single dose of 25 $\mu g/m^2$ bryostatin.

Pharmacokinetics

The pharmacokinetics of bryostatin were assessed in 6 participants following 25 $\mu g/m^2$ bryostatin administered as a single 1-hour IV infusion. Individual bryostatin plasma concentrations were observed to increase and approach steady-state within the 1-hour infusion periods, and then rapidly decrease following the end of the infusion. The bryostatin maximum plasma concentrations occurred at the end of the IV

infusions, and had a mean (±standard deviation (SD)) value of 1.09±0.25 ng/ml. A terminal elimination rate could not be calculated for most participants due to sampling frequency, the rapid drug elimination, and the sensitivity limitations of the assay. Based on the observed individual bryostatin plasma concentration profiles, the elimination $t_{1/2}$ associated with the observed drug exposure was estimated to be less than 30 minutes. Total drug CL was observed to be high (~ 40 L/h), consistent across the individual administered doses, and did not appear to be related to body weight, BSA, or sex. The bryostatin pharmacokinetic parameters were observed to have low to moderate intersubject variability.

Pharmacodynamics/ PKCε

Preliminary assessment of PKCε concentration in PBMCs suggests there is an increase in the total amount of PKCε (cytosol plus membrane bound concentration) following treatment with bryostatin. Additional analysis is underway to further characterize the increase and pharmacodynamics. Time course of total PKCε measured in PBMC samples from six Phase IIa subjects treated with bryostatin 1. A) Expanded scale (0–7 h). B) Full scale (0–340 h). There was an increase in total PKCε in the bryostatin group but not in the placebo at 1 h after the start of infusion (bryostatin $F(7.013, 21.039) = 3.026$, $p = 0.023$; placebo $F(10,20) = 0.75$, $p = 0.67$, repeated measures ANOVA; $p = 0.0185$ at 1 h, two-tailed matched pair t-test). This statistically significant increase in PKCε in patients who had received a single i.v. infusion of a single-dose (25 mcg/m2) closely tracked a statistically significant increase of bryostatin measured in the patients' blood samples.[29]

Summary

This was the first controlled, double-blind assessment of the safety of bryostatin treatment in participants with AD. The study met it primary endpoint of safety and tolerability. Bryostatin appears safe and well tolerated in this cohort of participants with mild to moderate AD. No improvement was detected in cognitive function on the efficacy measurements though the study was not powered to detect cognitive benefit. There was no evidence of an adverse effect on cognition following treatment with bryostatin.

4. STUDY OBJECTIVES

4.1 Primary Objective

To evaluate the safety and tolerability of bryostatin for the treatment of MS.

4.2 Exploratory Objective

To evaluate the changes in clinical, imaging, and cognitive measures during and following treatment as signals of efficacy.

5. INVESTIGATIONAL PLAN

5.1 Overview of Study Design

NTRP103-301 is a single-site, single-arm, open-label, Phase 1 study assessing the safety and tolerability of bryostatin in participants with MS. Exploratory outcome measures will be assessed to capture signals of efficacy. The study is 42 weeks in duration. Eligible participants will be treated with bryostatin for 26 weeks. Study drug is administered IV by continuous infusion. At weeks 0, 1, 15, and 16, the dose of study drug will be a loading dose 20% higher (i.e., 24 µg) than the assigned fixed dose and will be administered one week apart. For all other weeks of treatment, the assigned fixed dose will be 20 µg and will be administered every other week. Participants are scheduled to receive a total of 14 doses over 26 weeks. Safety and exploratory assessments will be done as indicated on the Schedule of Activities (Table 1).

5.2 Dose Rationale/Justification

Understood to be the mechanism of action is bryostatin's ability to modulate Protein Kinase C (PKC).

Factors that drastically alter the overall effects of bryostatin on PKC modulation include the length of exposure to bryostatin and the concentration of bryostatin used. In the NCI trials, the goal was to maximize the antiproliferative effects of bryostatin by causing a profound reduction of total cellular PKC, known as down regulation. This was accomplished by exposure to high concentrations and prolonged exposures of cells to the drug. In trials designed to treat neurodegenerative (*e.g.*, AD, MS) and neurodevelopmental (*e.g.*, FXS) diseases with bryostatin, the goal is to prolong the activation or the upregulation of PKC by exposure to low concentrations and short exposure times.

The primary objective of treatment for MS with bryostatin-1 lies in its known safety profile, anti-inflammatory, neuroprotective, re-myelination, and neurogenesis stimulatory properties (synaptogenesis, anti-apoptosis), and purported ability to cross the blood-brain barrier.

Safety Profile

The NCI previously held an IND for bryostatin in oncology (IND 42780). Fifty-seven clinical trials were conducted in patients with various solid and liquid tumors under that IND. Approximately 1430 oncology patients have been dosed across a wide range of doses and regimens (5 μg/m^2 to 225 μg/m^2 administered as infusions ranging from 1 to 72 hours in duration). Those studies established bryostatin's maximum tolerated dose range (25-50 μg/m^2) or 45-90 μg for a standard 70 kg man. The predominant dose-limiting toxicity observed was myalgia, which is dose dependent and cumulative, thus variable in severity. Symptoms generally occurred within 24-48 hours after the second or third dose but resolved within a week. In the only study in pediatric oncology where 22 children with refractory solid tumors were treated with rising doses of bryostatin, 3/6 patients at dose-level 57μg/m^2 had a Grade 3 dose-limiting myalgia. Grade 1 or 2 myalgia was observed at all lower dose levels except the lowest dose of 20 μg/m^2.

In the Synaptogenix NTRP101- 201 AD study, there were no reports of myalgia after a single 25 μg/m^2 infused dose of bryostatin, administered to 6/9 patients with mild AD. The only AE reported in the bryostatin treatment group was headache. In NTRP101-202 in which AD patients with moderately severe to severe AD received 7 fixed doses of infused study drug (either 20 μg or 40 μg), bryostatin treatment was generally safe and well-tolerated. The 20 μg bryostatin treatment group had similar numbers of TEAEs to the placebo group, except in injection site reactions and diarrhea. There were no clear differences in SAEs between the 20 μg bryostatin treatment group and the placebo treatment group. The 40 μg treatment group had more TEAEs and SAEs that the 20μg or placebo treatment groups across many SOC. The most common treatment related TEAEs were diarrhea, headache, fatigue and myalgia – all reported in greater frequency with the 40 μg dose of bryostatin. There were no dose-limiting toxicities. In its NTRP101-203 and NTRP101-204 trials, similar safety findings were observed at the 20 μg fixed infused dose compared to placebo in patients with moderately-severe to severe AD. Treatment with bryostatin at the loading dose of 24 μg and the assigned dose of 20 μg was well tolerated by all subjects. One death in NTRP101-203 was reported during the study in the placebo group where the cause was unknown. No other doses were evaluated in those two studies.

Efficacy

NTRP101-201 was a safety study that evaluated a single dose of bryostatin in nine eligible subjects with mild to moderate AD. The study was not powered to detect an effect; consequently, the 25 μg/m^2 dose did not show a difference in cognitive improvement when comparing bryostatin treated patients (6/9) to placebo-treated patients (3/9).

NTRP101-202 examined the efficacy of 2 doses of bryostatin in subjects with moderate to severe AD. The primary efficacy endpoint was the change from baseline on the SIB at Week 13, and the secondary efficacy endpoints were the change from baseline on the ADCS-ADL-SIV, NPI, and MMSE-2 tests at Week 13. The primary efficacy endpoint and secondary efficacy endpoint for the ADCS-ADL-SIV

was met in the CAS with the 20 μg fixed dose, which was statistically significantly different from placebo at these time points. The 40 μg dose was not statistically significant from the placebo. No primary or secondary endpoints were met in the FAS. Multiple exploratory analyses in the FAS for covariates in the primary and secondary endpoints revealed additional significant differences that occurred predominantly at the Week 5 time point.

In NTRP101-203 and NTRP101-204, as described above in Section 3.2.2 and 3.2.1, respectively, the primary efficacy endpoints were not met. Exploratory, post hoc analyses of a subgroup of subjects with moderately severe AD suggest that those patients may derive some improvement on an SIB score with a fixed dose of 20 μg bryostatin. No other dose was evaluated.

Bryostatin, delivered intraperitoneally (IP) at a dose of 30 μg/kg x 3 days/week, provided a noticeable benefit in mice with experimental autoimmune encephalomyelitis (EAE).[21] A drug administered IP, is absorbed more slowly than intravenous (IV) absorption, as the primary route of IP absorption is into the mesenteric vessels which drain into the portal vein and pass through the liver where it undergoes first pass hepatic metabolism before reaching the systemic circulation. When 40 μg/kg bryostatin was administered IV and IP to mice, the Cmax was shown to be 92.94 ng/ml and 13.52 ng/ml, respectively, albeit at different times.[43] This suggests that only 14.5 % of the administered IP dose of bryostatin reached the systemic circulation. Thus, in the EAE mouse model, one may surmise that only 4.35 μg/kg/30μg/kg administered reached the systemic circulation. That being the case, the estimated human equivalent dose (HED) is determined to be 0.35μg/kg.[44] For a 70-kg man, the fixed dose is 24.7 μg.

Based on the above, a fixed dose of 20 μg bryostatin will be administered to eligible MS patients with a primary focus on safety; however, exploratory outcome measures will be captured that represent signals of efficacy, such as evidence of improved cognition or remyelination.

The introduction of a rest between doses at Week 13 stems from concern about tachyphylaxis, or a reduced response to bryostatin after successive doses favoring down-regulation of PKC. A drug holiday followed by loading doses of bryostatin should refresh the response to bryostatin administration and enhance possible effects on exploratory endpoints.

5.3 Risk/Benefit

It is anticipated that the risk to subjects enrolled in NTRP103-301 will be minimal, based on prior use of bryostatin studied in over 1500 cancer and AD patients.

Participants in this study with MS will have been stable on their current DMT for at least 1 year.

Participants will also be identified with mild to moderate cognitive deficits as defined by a pre-study PST[26] with a z-score falling between -1.0 and -2.5 of the normal population.

As reproductive risks with bryostatin are unknown, females participating in the study must be surgically sterilized for at least 6 months, post-menopausal, or 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. 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.

Sperm and egg donation are not permitted during the study and for at least 30 days after the last dose of study treatment.

The potential anti-inflammatory, neuroprotective, re-myelinating, and neurogenesis stimulatory (synaptogenic, anti-apoptotic) properties, and the positive safety profile observed in adult patients with AD suggest that the potential clinical benefits of treatment with bryostatin at the proposed fixed dose of 20 µg in the protocol outweigh the risks.

5.4 Study Endpoints

Safety Assessments

Safety and Tolerability Primary Outcomes

- Frequency of Adverse Events [Treatment Emergent AEs (TEAEs), Serious Adverse Events (SAEs), and Suspected Unexpected Serious Adverse Reactions (SUSARS)]
- Frequency of study medication discontinuation and reason thereof
- Potential CNS inflammatory effects as captured by clinical monitoring and MRI

Exploratory Outcomes

Changes from baseline to Week 11, Week 28, and Week 40 in the following:

- MRI biomarkers
  - lesion volume and brain parenchymal fraction
  - default mode network node connectivity
  - diffusion MRI measures of tissue integrity
  - anatomical connectivity of transcallosal motor pathway
  - myelin water fraction
  - measures of myelination derived from magnetization transfer ratio, quantitative T2* and quantitative susceptibility mapping
  - grey matter atrophy, including grey matter fraction, white matter fraction, and cortical atrophy as measured by CLADA
- Expanded Disability Status Scale (EDSS)
- Montreal Cognitive Assessment (MoCA) test
- Controlled Oral Word Association Test (COWAT)
- MS Performance Test Domains
  - lower extremity function
  - upper extremity function
  - cognition
- Quality of Life in Neurological Disorders (Neuro-QoL)

Change from baseline to Week 28 and Week 40 in the following:

- California Verbal Learning Test- 3rd Edition (CVLT 3)
- Brief Visuospatial Memory Test - Revised (BVMT-R)
- Judgement of Line Orientation (JOLO)
- Boston Naming Test (BNT)
- Delis–Kaplan Executive Function System (D-KEFS) Sorting Test

The time-course of changes will be assessed for those outcomes with more frequent assessments (i.e. COWAT, MoCA, and MSPT)

5.5 Study Population – Eligibility Requirements

Participants with MS are eligible to enroll. Participant 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

1. Written informed consent signed by participant
2. English-speaking
3. Hospital Anxiety and Depression Scale <11
4. Male and female participants, 18-65 years of age inclusive
5. Established diagnosis of MS, as defined by the 2017 revision of McDonald Diagnostic Criteria (any form of MS).[14] 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[27]
7. EDSS between 0.0 and 7.0, inclusive
8. Adequate vision and motor function to participate in assessment procedures
9. Participants must be off of a DMT or 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

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[43]
8. Use of valproic acid and/or lithium within 14 days prior to screening
9. Use of carbamazepine within 7 days prior to screening
10. Use of teriflunomide within 90 days prior to screening

11. Use of dalfampridine within 7 days of screening.
12. Current use of acetaminophen, ciprofloxacin, and/or trimethoprim/sulfamethoxazole
13. Use of any potent or moderate, inhibitor or inducer, of CYP3A4, CYP2C8, or CYP2C9. Concomitant medicines will be examined on a case by case basis against the Flockhart Table by Sponsor-Investigator, and if needed, the Medical Monitor, to determine allowability
14. Current use of St. John's Wort, within 2 weeks prior to screening
15. Consumption of grapefruit juice from screening until end of study
16. 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
17. Any screening laboratory values outside the laboratory reference ranges that are deemed clinically significant by the PI
18. Use of an investigational drug within 30 days prior to screening
19. 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 PI's judgment
20. Major psychiatric illness such as currently uncontrolled major depression according to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition [45], 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
21. Diagnosis of alcohol or drug abuse within the last 2 years
22. 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][46]
23. 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]
24. 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
25. Known to be seropositive for human immunodeficiency virus (HIV)
26. Pregnancy or breastfeeding during the study. A β-hCG serum pregnancy test will be performed at Screening for female patients of child-bearing potential.
27. 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
28. History of significant bleeding disorders
29. Moderate baseline thrombocytopenia (platelets <100K/uL)
30. Elevated INR (>2.0)
31. Prior exposure to bryostatin, or known sensitivity to bryostatin or any ingredient in the study drug
32. Any other concurrent medical condition, which in the opinion of the PI makes the participant unsuitable for the clinical study

6.0. PRODUCTS USED IN THIS STUDY

Active study drug, bryostatin, will be provided as described below.

6.1 Bryostatin

The investigational drug product, bryostatin for Infusion, is a sterile, pyrogen-free, lyophilized powder intended for IV infusion upon reconstitution and dilution. Bryostatin will be supplied in a 10 mL vial containing 0.025 mg bryostatin, 2.5 mg povidone lyophilized from 40% t-butanol. Accompanying each vial of bryostatin will be a 10 mL vial containing 2 mL of sterile PET diluent [60% v/v polyethylene glycol 400, 30% v/v dehydrated ethyl alcohol, and 10% v/v Tween-80 (polysorbate 80)] for reconstitution of the lyophilized bryostatin for Infusion.

6.2 Packaging and Labeling of Study Drug

Vials of bryostatin for Infusion and vials of PET diluent will be batch shipped to the site.

Study drug vials will be labeled in English according to US FDA's current Good Manufacturing Practice (GMP) and local regulations.

Bryostatin for Injection vial label will contain the following information:

1. Bryostatin for Infusion
2. Lot # XXXXX
3. Store refrigerated at 2-8°C
4. Caution New Drug - - Limited by Federal Law (US) to Investigational use.
5. Each vial contains 0.025 mg bryostatin and 2.5 mg povidone C-17. Contains no antibacterial preservatives
6. Reconstitute with 1 mL PET Diluent. Swirl to dissolve.
7. Further dilute with 9 mL sodium chloride for injection
8. Manufactured for Synaptogenix by Lyophilization Technology, Inc.

PET Diluent vial label will contain the following information:

1. PET Diluent for Reconstitution of bryostatin for Infusion
2. Lot #: XXXX
3. Volume: 2 mL
4. Caution New Drug - - Limited by Federal Law (US) to Investigational use
5. Contains Polyethylene Glycol 400 (60%), dehydrated Ethyl Alcohol (30%) and Polysorbate 80 (10%).
6. For Single Use Only
7. Manufactured for Synaptogenix by Lyophilization Technology, Inc.

6.3 Storage and Preparation of Study Drug

The study drug will be stored under refrigeration (2-8°C), in a refrigerator that is securely locked and continuously temperature monitored. Access to the stored study drug will be restricted to the investigational site pharmacy or designated staff member.

Study drug will be prepared and dispensed for administration by a qualified member of site pharmacy staff. This individual will be responsible for reconstitution, dilution, and preparation of study drug. The investigational drug is to be administered only according to the conditions of this protocol.

Study drug will be administered by the study PI or his/her designees. Study drug should be allowed to come to room temperature prior to administration. The study drug contains no antibacterial preservatives and must be used within 8 hours of reconstitution. Study drug should be reconstituted with 1 mL of PET diluent. After swirling the 10 mL vial to completely dissolve the contents, the resulting solution must be diluted immediately with 9 mL of 0.9% sodium chloride (NS) injection, United States Pharmacopeia (USP). The necessary volume of this solution to achieve the assigned dose (a 9.6 mL loading dose for the first two doses and an 8.0 mL maintenance dose for the remaining 5 doses) should then be added to an IV infusion bag containing 50 mL of NS. Once the infusion bag is filled with the assigned dose, the 45±5 minute continuous infusion must be completed within 3 hours of the IV infusion bag fill.

The infusion system (IV infusion bag and tubing) should be made of a polyolefin plastic, such as polypropylene or polyethylene, or a combination of both. The use of polyvinylchloride (PVC) plastic bags and tubing is NOT to be used as plasticizer from the PVC is leached, and there is absorption of bryostatin to

PVC plastics.

The prepared infusion bag containing study drug should be labeled with the following information:

1. Protocol number
2. Participant number Date and time prepared
3. Instructions for dosing (e.g., entire contents of IV infusion bag infused within 45 ±5 minutes)

6.4 Study Drug Accountability and Disposal

Neither the Investigational Pharmacy, designated drug preparer, the PI, nor any of his/her designees may provide drug to any person not enrolled in this study. Adequate records of study drug receipt and use must be maintained in order to comply with governmental regulations and with the protocol in addition to preventing unauthorized distribution.

Study drug orders, records of receipts, dispensing records, and inventory forms will be examined and reconciled throughout the study. All study drug that is used during the course of the study must be accounted for on a drug accountability form.

Unless otherwise directed at the end of the study all unused study medication must be destroyed onsite following the site's specific standard operating procedures (SOPs) after drug accountability has been verified by the monitor. If destruction on site is not possible, study medication should be retained and returned to the drug vendor at the end of the study. A copy of all completed drug accountability forms will be collected by the monitor or appropriate designee upon completion of the study.

Note: The medications should not be disposed of prior to monitoring and approval.

6.5 Blinding

The study is open-label.

6.6 Study Drug Administration

Study drug is scheduled to be administered by continuous intravenous infusion only, via a pump, at the same rate over 45 minutes (±5 min) once a week for 2 weeks and then every 14 days ±2 days thereafter. The entire volume of study drug in the infusion bag is to be administered. Infusion times should not be extended or shortened. If a participant misses a dose, the dose should be administered as soon as possible. The participant should then continue on the original dosing schedule.

Bryostatin absorbs PVC plastics. Infusion bags and sets (*e.g.*, tubing) used in the delivery of the study drug must be made of a polyolefin plastic, such as polyethylene or polypropylene or a combination of both.

7. STUDY PROCEDURES AND ASSESSMENTS

All participants will be treated with the study drug bryostatin 1. 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).

7.1 Assessments

Every effort should be made to ensure that protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances outside the control of the PI that may make it unfeasible to perform a test. In these cases, the PI must take all steps necessary to ensure the safety and well-being of the participant. When a protocol required test cannot be performed, the PI will document the reason for the missed test and any corrective and preventative actions taken to ensure that the required processes are adhered to as soon as possible. The study team must be informed of these incidents in a timely manner.

Safety

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.

Laboratory

Blood samples will be obtained for routine laboratory tests, if indicated as outlined in the Schedule of Activities (Table 1). Laboratory tests will be run at the Cleveland Clinic Laboratories and processed routinely. Lab normal will be listed with results at the time of the report.

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

Clinical chemistry tests will include comprehensive metabolic panel (sodium, potassium, chloride, blood urea nitrogen (BUN), creatinine, estimated glomerular filtration rate (eGFR), glucose, calcium, CO2, total protein, albumin, ALT, and AST).

Screening blood tests will include hematology, clinical chemistry, βhCG serum pregnancy for females of child-bearing potential, TSH, B12, creatinine phosphokinase (CK), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), uric acid, and bilirubin.

Routine laboratory tests after screening will include Hematology, Clinical Chemistry, GGT, LDH, uric acid, and bilirubin. Additionally, serum samples will be collected at screening, Week 11, and Week 28 for future biomarker analysis.

Urine pregnancy tests will be performed prior to study drug administration at Day 0, Day 21, Day 49, Day 105, Day 126, Day 154, and Day 182. If pregnancy test is positive, study drug will not be administered.

In order to monitor for weight loss potentially related to study drug, weight will be monitored at screening and on Day 35, Day 77, Day 140, and Day 196. If at any time a clinically significant weight loss is observed, study drug will be permanently discontinued. A clinically significant weight loss is defined as a loss of more than 5% of usual body weight.

In the event of any reported myalgia, additional serum CK and B12 tests will be performed.

Some non-clinical data described in the Investigator's Brochure suggest a potential risk of bleeding associated with bryostatin. Therefore, at study visits, participants will be evaluated for clinical signs and symptoms of bleeding. If evidence of increased and/or unexpected bleeding is noted, the Principal Investigator will be contacted for further evaluation of the participant and possible study discontinuation.

Physical Examination

All PEs must be performed by the PI or qualified designee (physician, physician's assistant, or nurse practitioner). The complete PE conducted during the screening period and at designated subsequent time points should include, but is not limited to the following:

1. General appearance
2. Weight
3. Height (screening only)

4. Ears
5. Eyes
6. Nose
7. Throat
8. Neck
9. Respiratory system
10. Cardiovascular system
11. Abdomen
12. Musculoskeletal /Neurologic
13. Extremities
14. Skin
15. Lymph nodes

Examination of other systems as needed to explore reports of AEs should be conducted as needed by the PI, or designee. Any clinically significant physical findings that were not present on the initial PE will be considered AEs and documented on the AE electronic case report form (eCRF), as well as in the participant's source documentation and on the physical examination eCRFs.

Vital Sign Measurements
Single supine blood pressure and pulse rate will be measured at Screening and at other visits as specified in the Schedule of Activities (Table 1). Vital signs will include: blood pressure, heartrate, and temperature.

Electrocardiogram
A 12-lead ECG will be collected at screening and at time points specified in the Schedule of Activities.

Suicide Behaviors Questionnaire Revised (SBQ-R)
The 4-item SBQ-R is a measure of past suicidal thoughts and attempts, which have proved to be significant predictors of future suicidality.[47] The items ask if the respondent has ever thought about or attempted suicide, how frequent was suicidal thoughts in the past year, have they told someone about such thoughts, and what is the likelihood of attempting suicide in the future.

Suicide Behaviors Questionnaire-Revised (SBQ-R) scale will be obtained and reviewed at each study visit and for any participant with SBQ-R score ≥7, the study neurologist will be notified to immediately assess and manage suicidality.

Magnetic Resonance Imaging (MRI)
Potential pro-inflammatory effects will be evaluated through the presence of abnormal post-Gd T1 enhancement. A clinical read of all scans will be performed by a board-certified neuroradiologist and results recorded.

Exploratory Efficacy Assessments

Magnetic Resonance Imaging (MRI)
Participants will be scanned on a 7T scanner (Terra; SIEMENS) as follows:

- MRI will include the following:
    - Prescans (localizer, etc.)
    - T1-weighted (MP2RAGE)
    - Fluid-Attenuated Inversion Recovery (FLAIR)
    - Resting State-Functional MRI (rs-fMRI)

  - Diffusion Tensor Imaging (DTI)
  - Visualization of Short Transverse (ViSTa)
  - Magnetization Transfer Ratio (MTR)
  - Multi-echo gradient recalled echo (mGRE)
  - T1-weighted post-gadolinium (MP2RAGE)
- T1-weighted and FLAIR imaging will be used for volumetric measures and lesion measures.
- rs-fMRI will be used to assess inter-node functional connectivity within the default mode network including a reverse phase encode scan to correct for image distortion.
- DTI will be used to assess tissue integrity.[48] This scan is combined with rs-fMRI to derive the Structural and Functional Connectivity Index (SFCI).[49]
- ViSTa is a novel approach to measuring myelin water fraction that can be performed across the entire brain quickly and without the numerical instability associated with alternative methods like multi-echo imaging or multicomponent driven equilibrium single pulse observation of T1 and T2 (mcDESPOT).[50] ViSTa will provide measures of myelin water fraction in normal-appearing white matter and in white matter lesions.
- MTR will provide measures of myelination in normal-appearing white matter and in white matter lesions and has been adapted for 7T imaging.[51]
- Multi-echo gradient recalled echo (mGRE) is a single imaging acquisition that can be analyzed to generate maps of quantitative T2* (qT2*) and quantitative susceptibility maps (QSM). qT2* has been found correlate with myelination.[52] QSM has been found to correlate with myelination and with iron.[53] The sensitivity of the mGRE sequence to iron makes it a candidate for identifying paramagnetic rim lesions.[54] The mGRE acquisition can also be used to generate susceptibility-weighted imaging (SWI) for detection of paramagnetic rim lesions.[55]
- Grey matter atrophy assesses the specific impact of treatment on grey matter structures. In this study, this will include grey matter atrophy, including grey matter fraction, white matter fraction, and cortical atrophy as measured by CLADA

Expanded Disability Status Scale (EDSS): The EDSS was developed to quantify disability based on the neurological exam, ambulatory capacity, and ability to carry out activities of daily living.[56] The scale takes into account a wide range of neurological functions relevant to MS. The EDSS has several advantages. It is accepted by regulators for disability measurement. It is familiar to MS clinicians and allows comparison of participants on a 0-10 scale. However, several limitations to the EDSS have been widely discussed.[57] The scale is an ordinal measure, where changes between scores at different points are not necessarily of the same clinical meaning. Despite these limitations, the EDSS remains a widely used measure in clinical trials, MS registries, and clinical practice.

Quality of Life in Neurological Disorders (Neuro-QoL): The Neuro-QoL assessment is a survey consisting of a series of "life item banks" related to different domains which affect an individual's quality of life.[58] Answers given to series of questions within each domain determine a numerical score indicating the level of functioning for that individual at that time. A computer adapted version was developed and assessed in an MS population and was shown to provide similar results as the full battery.[59]

Short Cognitive Battery and Performance Test:

- Montreal Cognitive Assessment (MoCA) – MoCA is a brief, 30-question assessment of cognitive impairments for detecting early AD, measuring executive functions, and multiple cognitive domains which are important components not measured by the MMSE.[60]
- Controlled Oral Word Association Test (COWAT) – The COWAT consists of three word

conditions. The participants' task is to produce as many words as he can that begin with the given letter (C, F, or L;P, R, or W) within a 1-min time period. The COWAT and other measures of verbal fluency have proven to be sensitive indicators of frontal lobe dysfunction.[61]

- Multiple Sclerosis Performance Test (MSPT) – The MSPT was developed as an iPAD based version of the Multiple Sclerosis Functional Composite (MSFC),[62] which allows administration to take place independently, without the use of a study coordinator. The MSFC is an alternative approach to address some of the shortcomings of the EDSS.[63] The MSFC is based on quantitative neuroperformance tasks that were selected after extensive analysis of a collection of clinical datasets. The original MSFC was manually administered by trained personnel and contained the following three domains: lower extremity function [timed 25-foot walk (T25FW)], upper extremity function [9-hole Peg Test (9HPT)], and cognition [Paced Auditory Serial Addition Test (PASAT)]. Psychometrically, the measurement is favorable as each domain yields a single continuous score of function relevant to MS. Although practice effects can occur, these can be mitigated with pre-baseline run-in testing. The MSPT adapted the key elements of the MSFC into an electronic format. In the current version of the MSPT, the Walking Speed Test (WST) replaces the T25FW, the Manual Dexterity Test (MDT) replaces the 9-HPT, the Processing Speed Test (PST) replaces the PASAT. A vision measure – the Low-contrast Letter Acuity (LCLA) test – has been added, to form a 4-domain measure of neurologic function relevant to MS, but will not be utilized in this trial. The MSPT has been validated for use in the MS population.[64]

Extensive Cognitive Battery:

- California Verbal Learning Test- 3rd Edition (CVLT 3) – is a comprehensive, detailed assessment of verbal learning and memory deficits.[65]
- Brief Visuospatial Memory Test - Revised (BVMT-R)- The BVMT-R assesses visuospatial learning and memory abilities in research and clinical settings.[66]
- Judgement of Line Orientation (JOLO) – JOLO is a widely used measure of visuospatial judgment that measures accuracy of angular orientation based on judgments about a pair of angled lines that visually match an identical pair immersed within a semicircular array of 11 lines.[67]
- Boston Naming Test (BNT)– BNT is a test of word retrieval used to evaluate aphasia. Drawings of objects are presented, and the participant provides the name of each object.[68]
- Delis–Kaplan Executive Function System (D-KEFS) Sorting Test– The *Sorting Test* explores executive abilities by measuring concept-formation skills, modality-specific problem-solving skills (verbal/nonverbal), and the ability to explain sorting concepts abstractly.[69]

7.2 Visit Procedures

Screening (Days -28 to -2)

In order to avoid unnecessary screen failures, a prescreening telephone call to rule out clearly exclusionary conditions is advised prior to scheduling a screening visit. Informed Consent must be obtained before any study related procedures are performed. Screening procedures will take place within an approximate 4-week period prior to first dose administration (Days -28 to -2).

Participants who are screen-failed (e.g., due to clinically significant laboratory abnormality or an active medical condition) may be re-screened if the laboratory abnormality or medical condition stabilizes or improves as assessed by the PI. The Study Administration should be consulted prior to re-screening. Participants who are Screen Failures may be rescreened only once.

The following procedures should be performed first to avoid unnecessary procedures for ineligible participants:

Study Nurse:

- Informed Consent
- Collection of participant demographic data
- Review of concomitant medications
- Review of medical history
- Lab- Blood samples for initial Safety Monitoring
- Biobank samples
- Vital signs
- Weight

Study Coordinator:

- ECG
- SBQ-R
- Short Cognitive Battery and Performance Test
- Neuro-QoL
- Schedule MRI
- Hospital Anxiety and Disability Scale

Study Neurologist:

- EDSS
- Complete PE

Neuropsychologist:

- Extensive Cognitive Battery

If changes in the participant's health or mental status occur during the screening period, including changes in medication affecting the mental status, the Study Administration should be notified. The Study Administration will advise the PI regarding any assessments that should be repeated to ensure eligibility requirements are met.

At weeks 0, 1, 15, and 16, the dose of study drug will be a loading dose 20% higher (i.e., 24 $\mu g/m^2$) than the assigned dose and will be administered one week apart. For all other weeks of treatment, except for Week 13 when no dose is administered, the assigned dose will be 20 $\mu g/m^2$ and will be administered every other week.

Week 0 (Day 0/Dose 1) (Bryostatin loading Dose, 24 µg)

A final assessment of the inclusion/exclusion criteria, including any changes in medications or health status will be done before dosing to confirm the participant's eligibility.

The following procedures will be done on Day 0:

Study Nurse:

- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Dosing by IV infusion will be done according to the procedures outlined in Section 5.7.
- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- AEs

- Concomitant medications

Week 1 (Day 7 ±3 days/Dose 2) (Bryostatin loading Dose, 24 µg)
One week after the first dose of study drug, participants will return for a second infusion. The following procedures will be performed:

Study Nurse:

- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Dosing by IV infusion
- AEs
- Concomitant medications

Week 3 (Day 21 ±3 days/ Dose 3) (Bryostatin Dose, 20 µg)

Study Nurse:

- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Vital signs prior to infusion, then at 30 (±5), 60 (±5), and 90 (±5) minutes from start of the infusion
- Routine Labs
- Dosing by IV infusion
- AEs
- Concomitant medications

Study Coordinator:

- SBQ-R
- Short Cognitive Battery and Performance Test

Week 5 (Day 35 ±3 days/ Dose 4) (Bryostatin Dose, 20 µg)

Study Nurse:

- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Dosing by IV infusion
- Weight
- AEs
- Concomitant medications

Study Neurologist:

- PE

Week 7 (Day 49 ±3 days/ Dose 5) (Bryostatin Dose, 20 µg)

Study Nurse:

- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Vital signs prior to infusion, then at 30 (±5), 60 (±5), and 90 (±5) minutes from start of the infusion
- Dosing by IV infusion
- AEs
- Concomitant medications

Study Coordinator:
- SBQ-R
- Short Cognitive Battery and Performance Test

Week 9 (Day 63 ±3 days/ Dose 6) (Bryostatin Dose, 20 µg)

Study Nurse:
- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Dosing by IV infusion
- AEs
- Concomitant medications

Week 11 (Day 77±2 days/ Dose 7) (Bryostatin Dose, 20 µg)

Prior to dosing:

Study Nurse:
- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Routine Labs
- Weight
- Biobank sample
- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion

Study Coordinator:
- ECG

After dosing:

Study Nurse:
- AEs
- Concomitant medications

Study Neurologist:
- PE
- EDSS

Study Coordinator:
- SBQ-R
- Short Cognitive Battery and Performance Test
- Neuro-QoL
- Schedule MRI

Week 13 (Day 91) No clinical visit

Week 15 (Day 105 ±3 days/ Dose 8) (Bryostatin loading Dose, 24 µg)

Study Nurse:
- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion

- Dosing by IV infusion
- AEs
- Concomitant medications

Week 16 (Day 112 ±3 days/Dose 9) (Bryostatin loading Dose, 24 µg)

Study Nurse:

- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Dosing by IV infusion
- AEs
- Concomitant medications

Study Coordinator:

- SBQ-R
- Short Cognitive Battery and Performance Test

Week 18 (Day 126 ±3 days/Dose 10) (Bryostatin Dose, 20 µg)

Study Nurse:

- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Routine Labs
- Dosing by IV infusion
- AEs
- Concomitant medications

Week 20 (Day 140 ±3 days/Dose 11) (Bryostatin Dose, 20 µg)

Study Nurse:

- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Weight
- Dosing by IV infusion
- AEs
- Concomitant medications

Study Coordinator:

- SBQ-R
- Short Cognitive Battery and Performance Test

Study Neurologist:

- PE

Week 22 (Day 154 ±3 days/Dose 12) (Bryostatin Dose, 20 µg)

Study Nurse

- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Vital signs prior to infusion, then at 30 (±5), 60 (±5), and 90 (±5) minutes from start of the infusion

- Dosing by IV infusion
- AEs
- Concomitant medications

Week 24 (Day 168 ±3 days/Dose 13) (Bryostatin Dose, 20 µg)

Study Nurse

- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- Dosing by IV infusion
- AEs
- Concomitant medications

Study Coordinator

- SBQ-R
- Short Cognitive Battery and Performance Test

Week 26 (Day 182 ±3 days/Dose 14) (Bryostatin Dose, 20 µg)

Study Nurse

- Urine pregnancy test for women of childbearing potential, prior to study drug administration
- Routine Labs
- Vital signs prior to infusion, then at 30(±5), 60(±5), and 90(±5) minutes from start of the infusion
- AEs
- Concomitant medications

Week 28 (Day 196 ±3 days)

Study Nurse

- Routine Labs
- Biobank sample
- Vital signs
- Weight
- AEs
- Concomitant medications

Study Coordinator

- SBQ-R
- Short Cognitive Battery and Performance Test
- Neuro-QoL
- Schedule MRI
- ECG

Study Neurologist

- EDSS
- PE

Neuropsychologist

- Extensive Cognitive Battery

Week 32 (Day 224 ±3 days; 30-day follow up)

Study Nurse

- AEs
- Concomitant medications

Week 40 (Day 280 ±3 days; 3-month follow up)

Study Nurse

- Concomitant medications

Study Coordinator

- Short Cognitive Battery and Performance Test
- Neuro-QoL
- Schedule MRI

Study Neurologist

- EDSS

Neuropsychologist

- Extensive Cognitive Battery

Early Termination

Study Nurse

- Routine Labs
- Vital signs
- AEs
- Concomitant medications

Study Coordinator

- Short Cognitive Battery and Performance Test
- SBQ-R
- ECG
- Neuro-QoL
- Schedule MRI

Study Neurologist

- EDSS
- PE

Neuropsychologist

- Extensive Cognitive Battery

7.3 Concomitant Medications

During the screening visit, the participant will provide a list of currently used medications. All concomitant medications used should be recorded on the source documents and also in the eCRF using the generic name for the drug. Assessment of concomitant medications will take place at each study visit. Any changes to chronic medications should be noted as well as new and discontinued medications.

Participants taking allowed antidepressant medications may be enrolled in the study (see Appendix 1). The dose and dose regimen for these medications should be stabilized for at least 30 days prior to enrollment in the study. Every effort should be made to keep the dose and dose regimen of antidepressant medications stable throughout the study.

Medications for MS

Participants either off MS DMT or currently taking Food and Drug Administration (FDA) approved DMT (as well as rituximab) for the treatment of MS may be enrolled in the study. For those taking disease modifying therapy, the participant must be on a stable dose for at least 1 year prior to entry into the study (see 4.5 Inclusion Criteria). Participants should not be anticipated to change MS DMT over the course of the study.

Administration of MS DMTs, whether by injection or infusion, must occur on days that the participant is not receiving a bryostatin administration. To decrease IFN-β1 site reactions, it is recommended that bryostatin infusions occur in a different limb than was used for the most recent IFN-β1 injection, if possible.

Concomitant Medications for Management of Myalgia

Non-steroidal anti-inflammatory drugs such as ibuprofen and naproxen sodium are permitted. Use of acetaminophen as an analgesic is not allowed as it has the ability to inhibit PKC activation.

Prohibited Medications

High dose Vitamin E (> 400 IU / day), Valproic Acid, lithium, and divalproex sodium are prohibited.

If the PI determines that initiation of any of the prohibited medications is required to ensure the participant's safety, the medication may be initiated with the notification of the Medical Monitor and proper documentation of a protocol deviation.

A full list of restricted concomitant medications is shown in Appendix 1.

8. ADVERSE EVENTS AND OTHER SAFETY EVALUATIONS

8.1 Definition of Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (e.g., clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality.

The PI is responsible for obtaining information about all medical emergencies during the clinical study. The PI's contact information will be located in the body of the informed consent form (ICF) and participants will be encouraged to contact the PI or clinical site personnel during any clinical study-related emergency.

All AEs spontaneously reported by the participant in response to an open question or revealed by observation will be recorded during the study regardless of relationship to the study drug. All AEs will be monitored and recorded for the progress of the event until it resolves or reaches a clinically stable outcome. Adverse events that are not resolved at the time of database lock will be recorded as ongoing.

The AE and SAE reporting period starts from the time of consent to the last study visit.

8.2 Adverse Events of Special Interest (AESI)

8.2.1 Myalgia

Myalgia has been reported as the predominant dose limiting toxicity across oncology studies. The incidence of myalgia appears to be dose dependent and cumulative. In the AD studies, subjects who received a fixed dose of 20 μg, the proposed dose in this study (NTRP103-301), did not experience myalgia.

However, it has been reported in participants receiving doses as low as 5 μg/m^2.

Myalgia has been investigated in some studies but not all. No increase in muscle enzymes were found in the cases investigated. Electromyography (EMG) was abnormal in one participant who received 65 μg/m^2 and suggested a patchy myositis. An MRI done in another participant was normal.

For all cases of myalgia (regardless of severity), a narrative will be created documenting onset, severity, treatment, and outcome. CK and B12 tests will be collected for all cases of myalgia. Additional investigations are at the discretion of the investigator.

To reduce the effects of myalgia, an analgesic such as ibuprofen may be administered. Acetaminophen as an analgesic is not allowed as it has the ability to inhibit PKC activation.

Participants taking interferon-β1 will be counseled to receive bryostatin infusions on a different day than interferon-β1 injection to decrease the likelihood of myalgia side effects.

8.2.2 Systemic Infusion Related Reactions

Bryostatin 1 is administered through IV infusion. Infusion-site reactions, including cellulitis, and generalized infusion reactions occurred in previous human trials. As a result, a guide for preventing, recognizing, and treating these issues is outlined. All necessary supplies to treat infusion-related complications should be on-site during all infusions.

Complication	Signs/Symptoms	Management	Prevention
Systemic infusion reaction	• Dyspnea • Flushing • Bradycardia/tachycardia • Chest pain • Fever • Chills • Generalized itching/hives • Flushing • Rash • Dizziness or lightheadedness • Abdominal cramps	• Slow infusion rate or temporarily hold infusion • H1 blocker (i.e. diphenhydramine) • H2 blocker (i.e. ranitidine or famotidine) • IV fluids • Methylprednisolone	(none)
Anaplylaxis/ anaphylaxoid	The same as for systemic infusion reaction, plus: • Laryngeal edema • Bronchospasm • Diaphoresis • Hypoxia • Hypotension • Angioedema • Cyanosis • Loss of consciousness, seizure • Vomiting/diarrhea	Stop infusion immediately Medications as for systemic infusion reaction, plus: • Oxygen • Epinephrine • Trendelenberg positioning • Assess airway, breathing, circulation, vital signs, mental status • Consider transfer to ER	Do not infuse if patient has prior allergic reaction to bryostatin or similar agents

8.2.3 Infusion Site Reactions

Injection site reactions are seen with IFN-β1, glatiramer acetate, and ofatumumab medications. These reactions are typically mild and self-limiting but can occasionally be associated with serious skin infection or damage.

Complication	Signs/Symptoms	Management	Prevention
Phlebitis	• Redness • Pain • Tenderness • Swelling	• Re-site canula • Elevate limb • Topical anti-inflammatory gel or cream • Anti-inflammatory analgesics • Consider infection as cause and treat as cellulitis	• Universal infection control • Use smaller cannula • Avoid insertion near bony prominence or venous valve • Slow infusion rate
Extravasation	• Pain • Redness • Burning • Skin pallor • Edema • Decreased IV flow or flush • Skin blistering • Necrosis	• Stop infusion • Remove cannula • Elevate limb • If severe, consider irrigation with 0.9% saline • Localized supportive treatment	• Careful insertion of cannula • Careful monitoring for extravasation
Hematoma	• Skin discoloration bruising • Swelling • pain/discomfort, tenderness to touch	• Apply ice with light pressure • Elevate limb	• Careful insertion of cannula • Consistent pressure after removing cannula
Cellulitis	4 cardinal signs • Localized • pain/tenderness • Swelling • Erythema, warmth Additionally: • Borders are typically not elevated • Regional lymphaden-opathy • Systemic signs including malaise, chills, fever	Needs immediate medical evaluation and management, including consideration of antibiotics	• Universal precautions • Diligent skin preparation and post-infusion skin care • Infusion staff wash hands frequently

8.3 Definition of Serious Adverse Event

An SAE is any untoward medical occurrence that fulfills any of the following criteria:

1. Results in death (fatal)
2. Is life threatening (an event is considered "life threatening" if, in the view of either the investigator or sponsor-investigator, its occurrence placed the participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death).
3. Requires in-patient hospitalization or prolongation of existing hospitalization
4. Results in a persistent or significant disability/incapacity
5. Is a congenital anomaly/birth defect
6. Is an important medical event that may not result in death, may be immediately life threatening or require hospitalization, may be considered serious when based upon appropriate medical judgment, may jeopardize the participant, or may require medical or surgical intervention to prevent any of the outcomes listed above. Examples include allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.
7. The following situations may not, by themselves, constitute sufficient grounds to be considered as an SAE:
    a. Hospitalization solely for a diagnostic purpose, even if related to an AE,
    b. Elective hospitalization for an intervention planned before the participant enrollment in the study
    c. Admission to a day care facility or sleep laboratory

8.4 Assessment of Intensity

Severity is a clinical determination of the intensity of an AE and will be determined by the PI based on the following classification criteria for all AEs occurring during the clinical study:

Mild - Awareness of signs or symptom, but easily tolerated, may require additional therapy
Moderate - Discomfort, enough to cause interference with usual activity and to require intervention or additional therapies.
Severe - Incapacitating with inability to work or perform usual activity

Note: It should be noted that a severe AE need not be serious in nature and that an SAE is not, by definition, severe. Regardless of intensity, all SAEs and significant events must be reported.

8.5 Relationship to Study Drug

The causal relationship between the investigational drug and each AE will be determined by the PI based on his/her medical judgment in consideration of all relevant factors, including pattern of reaction, temporal relationship, concomitant medication, co-existing diseases, and relevant medical history. The PI will classify every AE according to its relationship to study drug or trial-related procedures. The categories are listed in the following Table 2.

8.6 Table 2 Relationship of AE to Study Drug or Trial-Related Procedures

Rating	Classification	Definition
1	Probable	An AE that: • Occurs at a reasonable time interval after administration of the study drug; • Follows a known response pattern to the study drug and; • Cannot be reasonably explained by the known characteristics of the participant's clinical state or by other therapies.
2	Possible	An AE that: • Occurs at a reasonable time interval after administration of the study drug; • Follows a known response pattern to the study drug, but; • Could have been produced by the participant's clinical state or by other therapies.
3	Unlikely*	An AE for which: • sufficient information exists to indicate that the etiology is unrelated to the study drug; • Another etiology is specified.

*If the AE is classified as unlikely, the PI should provide a likely cause, other illness, concomitant medication, or other.

Unexpected Adverse Event

An AE is considered "unexpected" if it is not listed in the Investigator's Brochure (IB) or is not listed at the specificity or severity that has been observed. Unexpected refers to AEs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation. However, an event that is more specific or more severe than described in the IB will be considered unexpected.

8.7 Reporting Adverse Events

The PI should instruct all participants on the procedure for reporting AEs/SAEs to the appropriate clinical site personnel. For each AE reported, clinical site personnel should obtain all required information to complete the source document and eCRF.

The PI or designee should document all AEs/SAEs in participant's source documentation, on the AE Source Document and in the eCRF.

In addition to standardized reporting procedures, worsening or exacerbation of concurrent conditions in participants will also be reported as AEs, and will follow the designated reporting format.

The following should be recorded with each SAE/AE:

1. The nature of the AE, with a diagnosis wherever possible
2. Date of event
3. Assessment of intensity
4. Whether the event is an SAE

5. Relationship to study drug or trial-related procedures
6. Action taken regarding study drug treatment
7. Outcome

If the intensity of an already reported AE increases, then a new AE eCRF must be completed for that AE. The date of change would be included as the end date for the originally reported AE, and the start date for the new AE of greater intensity.

The clinical research associate is responsible for source document verification of all safety events.

If the clinical site becomes aware of an SAE, regardless of causality, within 30 days following the last administration of investigational product, the SAE should be recorded and reported immediately to the sponsor-investigator. An SAE that occurs after the Week 40 (3-month follow up) visit will NOT be collected unless the PI considers that the event is related to the investigational product.

The sponsor should be informed if the PI becomes aware of any unusual safety information or any potential drug-related safety information, even after a participant completes the study.

SAE reports

All SAEs, whether or not considered associated with study treatment or study-related procedures, must be reported on the eCRF immediately and no later than 24 hours after the site becomes aware of the event. Follow-up information must be provided promptly as requested.

The PI is obligated to provide as much information about the event as possible on the eCRF provided and as requested by the Medical Monitor.

In general, this will include a description of the SAE in sufficient detail to allow for a complete medical assessment of the case. The Medical Monitor or designee will review the SAE including the SAE criteria, the relationship to study medication, the expected or unexpected assessment, and inform the sponsor-investigator by phone and e-mail immediately.

The following AEs should also be reported to the sponsor-investigator's designated Medical Monitor immediately:

1. severe injection/infusion site reactions (ulceration or necrosis that is severe; operative intervention indicated)
2. systemic hypersensitivity reactions

All additional follow-up evaluations must be reported by the site to the medical monitor, or designee, immediately after notification of the additional information.

An SAE will be followed until it resolves or reaches a clinically stable outcome. AEs/SAEs that have not resolved by study closure will be considered ongoing.

An SAE that occurs after the Week 40 (3-month follow up) visit will NOT be collected unless the PI determines that the event is related to the investigational drug product.

Reporting to Regulatory Authorities

The PI, or designee, is responsible for informing the Institutional Review Board (IRB) of any unexpected SAEs, as well as any additional SAEs according to the IRB's policy.

Any SAE that is serious, suspected to be related to investigational study drug, and unexpected (Suspected Unexpected Serious Adverse Reaction; SUSAR) will be promptly reported to regulatory authorities by the sponsor-investigator according to expedited reporting requirements. Subsequent relevant information after the initial submission of the IND Safety Report to the regulatory authorities will be submitted in a follow-up IND Safety Report to the regulatory authorities in the expedited period by the sponsor-investigator.

8.8 Criteria for Withdrawal of Participants

Participants may be withdrawn from the clinical study for the following reasons:

1. The PI believes withdrawal to be medically necessary or in the best interest of the participant
2. Noncompliance with the protocol as judged by the sponsor-investigator/PI
3. Participants who are enrolled in violation of inclusion and/or exclusion criteria
4. An AE that presents an unacceptable consequence or risk to the participant as judged by the PI, sponsor-investigator, sponsor, or Medical Monitor
5. Lost to follow up
6. Withdrawal of consent

Participants may voluntarily discontinue their participation in the study at any time without prejudice to further treatment.

8.9 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

8.10 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.

Stopping Rules:

Results will be examined for potential early stopping at every DSMB meeting. DSMB meetings will be held as outlined in the DSMP. Additional DSMB meetings may be convened any time the Medical Safety Monitor or Study PI requests a DSMB meeting due to safety concerns.. Additionally, discontinuation of the trial will be considered by the DSMB if 2 participants within the first 6 of 20 planned experience significant AEs classified as a SUSAR (Suspected Unexpected Serious Adverse Reaction) or if 3 participants experience significant AEs classified as a SUSAR (Suspected Unexpected Serious Adverse Reaction) at any time.

9. CLINICAL DATA AND SAFETY MONITORING

9.1 Clinical Monitor

A Clinical Monitor will assess the implementation and progress of study, NTRP103-301, and will review accumulating trial data to monitor the safety of bryostatin administered to participants and to assure that the data collected and recorded is complete and accurate. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse event monitoring
- Source data verification
- Response assessment

Clinical monitoring activities will be performed by a CRO to be contracted specifically for this purpose, so that independence is maintained.

9.2 Medical Safety Monitor

A Medical Safety Monitor will be appointed who will review safety data on an ongoing basis and report any concerns to the Treating Neurologist, Principal Investigator, or DSMB as appropriate. This Medical Safety Monitor will be independent from the Institution running the study and will not be located at the clinical site.

The Medical Safety Monitor (MSM) serves two major roles in the evaluation of AEs and SAEs for the trial:

1. They perform ongoing, real-time reviews of all individual SAE reports
2. They perform quarterly reviews of cumulative AE (serious and non-serious) data to judge whether there are concerning trends in the occurrence of events, and the possible relationship of those trends to the trial.

AEs for which the significance for potential stopping of the trial is ambiguous will be preliminarily reviewed by the Medical Monitor and confirmed by the DSMB.

The Medical Safety Monitor evaluates each SAE and completes a review within three (3) business days of receipt. The Medical Safety Monitor will use the detail, when needed, to clarify the context or assumptions used in the evaluation.

9.3 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be established to serve as the primary data and safety monitoring group for the trial. The monitoring of subject safety and data quality will follow

the Guidelines for Data and Safety Monitoring in Clinical Trials. A DSMB will be appointed by the sponsor-investigator to review study data provided by the study statistician. This committee will monitor rates of AEs and safety endpoints in the trial. The DSMB will review interim safety data, evaluate whether the study should be stopped or amended for safety or other reasons, and make such recommendations to the Steering Committee. In addition, the DSMB will provide input to the Steering Committee concerning the study protocol. The membership, responsibilities, and procedures of the DSMB are outlined in detail in the DSMB Operating Guidelines.

10. DATA ANALYSIS / STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP). This separate document will be finalized prior to conduct of any statistical analyses. The SAP may modify and will take precedence over the plans outlined in the protocol; however, any major modifications or modification of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

Adverse event and other safety data will be analyzed descriptively in all participants who received any dose of study drug (including partial infusions). These data will be summarized by time in study.

Descriptive summaries of safety and exploratory data including number of evaluable participants, mean, median, SD, maximum, and minimum for continuous variables will be provided by scheduled visit. Categorical variables will be presented showing number evaluable, frequencies, and percentages. In addition, the same descriptive statistics will be provided for changes from baseline at each post-baseline visit.

The primary efficacy analyses of the safety and efficacy endpoints will be conducted on data collected through Week 40.

10.1 Sample Size Determination

This is an 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 performed.

10.2 Statistical Methods

The Safety Analysis Set (SAS) is defined as all participants who received one of more doses of study medication.

Demographics including age, sex, race, ethnicity, height, and body weight will be summarized using descriptive statistics.

Medical history, neuroimaging, prior and concomitant medications, existing disease, years since MS onset and diagnosis, baseline medical conditions, and baseline safety and neuropsychological assessments will be summarized.

Trajectories over time for exploratory efficacy assessments including neuroimaging outcomes, MRI biomarkers, EDSS, cognitive assessments, MS Performance tests, and PROs will be modeled using mixed-effects models. Random effects will include intercept and fixed effects will include time and additional growth terms, as appropriate. Change over time will be calculated and graphically presented along with measures of variability at each time point. Contrasts will estimate the change from baseline to follow-up time points, and sustainability between Week 28 and Week 40, for applicable endpoints.

Participant disposition will be summarized and will include numbers screened, dosed, and withdrawn with reason for withdrawal.

10.3 Safety and Tolerability Outcomes

AEs, safety laboratory, ECGs, physical exam, vital signs, and MRI data will be presented in tabular format and summarized descriptively. Incidence and severity of AEs will be summarized using System Organ Class (SOC). AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

Participant disposition will be summarized by numbers screened, dosed and withdrawn with reason for withdrawal.

10.4 Safety Assessments

Summaries and listings of safety data will be provided to the Data Safety Monitoring Board as described in Section 9.3. Analyses will be performed by an independent statistician.

10.5 Analysis of Safety Data

For continuous variables, data will be summarized by treatment using n, mean, SD, minimum, and maximum values. For categorical variables, data will be summarized by treatment using frequency and percentage.

Adverse Events

AEs will be coded using standard guidelines determined by the sponsor-investigator and are defined as events with an onset on or after the first treatment. TEAEs will be summarized by System Organ Class, and preferred term. The following TEAE summaries will be provided:

1. Overall (i.e., regardless of severity or relationship to treatment)
2. By severity grade (mild, moderate, severe, life threatening or death for SAEs)
3. By relationship to clinical trial treatment (definitely related, probably related, possibly related, unlikely related, unrelated)

Unless otherwise specified, at each level of participant summarization, a participant will be counted only once. If there is more than one occurrence of an event, the event of the worst severity or the worst-case relationship category will be summarized.

In addition, separate summaries of serious adverse events, and adverse events resulting in discontinuation of study treatment will be presented.

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.

Clinical Laboratory Evaluations

All available results of the clinical laboratory evaluations will be listed and summarized as follows:

Laboratory Values over Time

Summary statistics of raw data and change from baseline values for each laboratory parameter will be presented by time point. Data will be summarized as appropriate for the variable type.

For continuous data, summaries will include the number of observations, mean, SD, median, minimum, and maximum values.

For categorical data, frequency counts and percentages will be used.

For change from baseline summaries, participants with an undefined change from baseline, because of missing data, will be excluded.

Individual Participant Changes (Shift Tables)
Individual participant changes will be identified through shift tables. Shift tables will be presented for each laboratory parameter with counts and percentages of participants, by time point, for shift (change) from baseline, using the normal ranges from the laboratory.

Individual Clinically Significant Abnormalities
Clinically significant laboratory abnormalities (i.e., those laboratory abnormalities recorded as AEs) will be listed.

All results of laboratory evaluations will be presented as by- participant listings.

10.6 Physical Examination

All PE findings will be listed and/or summarized. Shift tables will also be presented to show any abnormality shifts from baseline to post-baseline visits.

10.7 Vital Signs

Tabulations of raw data and change from baseline values will be presented by time point for each vital sign parameter. Tabulations will include the number of observations, mean, standard deviation, median, and minimum and maximum values. For change from baseline summaries, participants with an undefined change from baseline, because of missing data, will be excluded.

10.8 ECG

All ECG findings will be listed and/or summarized. Shift tables will also be presented to show any abnormality shifts from baseline to post baseline visits.

10.9 The Suicide Behaviors Questionnaire-Revised (SBQ-R)

The 4-item SBQ-R is a measure of past suicidal thoughts and attempts which have proved to be significant predictors of future suicidality.[47] The items ask if the respondent has ever thought about or attempted suicide, how frequent was suicidal thoughts in the past year, have they told someone about such thoughts, and what is the likelihood of attempting suicide in the future.

10.10 Patient-Reported Outcomes (PROs)

PROs will be summarized using descriptive statistics by visit, including mean, SD, median, maximum, and minimum, as appropriate. A responder analysis will be conducted by summarizing meaningful change at the individual level.[70] 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.[71]

10.11 Magnetic Resonance Imaging (MRI)

All MRI findings will be listed and/or summarized.

10.12 Exploratory Outcomes

Descriptive statistics by visit will be provided for all exploratory data, where applicable. These descriptive statistics will include the mean, median, SD, maximum and minimum for continuous variables, and frequencies, percentage, and tabulations for categorical variables.

Durability of bryostatin activity in MS participants

Durability of a drug is its ability to delay progression of disease, in a safe and well tolerated manner. Here, the durability of bryostatin in improving cognitive function, neurologic function, and MRI biomarkers will be assessed. The durability of changes in Exploratory efficacy data (cognitive and MRI) will be

analyzed and summarized from baseline to Week 28 and Week 40 using mixed effects models, as described above.

Summary statistics will also be performed on both the participant demographics and participant clinical characteristics. Exploratory 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.

10.13 Analysis of Exploratory Endpoints

Exploratory endpoints for this study include the change from baseline to 28 and 40 weeks in the Extensive Cognitive Battery (MOCA, CVLT 3, BVMT-R, JOLO, BNT, COWAT, D-KEFS, and PST) scores, EDSS scores, MSPT, and MRI findings. Exploratory endpoints also include the change in the monthly assessment of MOCA, COWAT, and PST. All statistical tests for these efficacy signals will be two-sided tests, with α=0.05. Since all of the efficacy assessments are exploratory, there will be no adjustment for multiplicity.

Based on the review of participants treated with bryostatin in the following AD clinical trials, NTRP 101-202, NTRP101-203, and NTRP101-204, the expected number of participants who will drop out of the study prematurely will not be significant.

11. DATA MONITORING

11.1 Source Documentation

In accordance with ICH-GCP guidelines, source documents may include, but are not limited to the following:

1. Clinic, office, hospital charts
2. Copies of transcribed health care provider notes, which have been certified for accuracy after production
3. Recorded data from automated instruments such as x-rays and other imaging reports, sonograms, computed axial tomography scans, magnetic resonance images, radioactive images, electrocardiograms, electroencephalograms
4. Records of telephone contacts
5. Diaries, evaluation checklists, or questionnaires that are completed directly by participants and serve as their own source
6. Laboratory results and other laboratory test results, urine dip-stick results
7. Correspondence regarding a participants' treatment between physicians or memoranda sent to the IRB.
8. MSTP iPAD output data

11.2 Study Documentation and Record Retention

The PI or designees must enter all results collected during the clinical study into eCRFs. eCRF completion guidelines will be reviewed with clinical site personnel at the PI's Meeting and site initiation visits. PI or designees are responsible for approval of all entered or corrected data.

The medical records (source documents) upon which the eCRFs are based must be kept at the clinical site for at least a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and the FDA is notified. Synaptogenix must be informed if the records are passed on to any other person or institution during this period. Records related to nonclinical laboratory studies will be maintained by Synaptogenix or its designee for a minimum of 5 years per 21 Code of Federal Regulations (CFR) 58.195 (b) (2).

11.3 Synaptogenix Site Monitoring

Synaptogenix or its designee will be allowed to conduct site visits at the investigation facilities to monitor any aspect of the study. The PI will provide Synaptogenix, or its designee, with documentation of IRB approval of the Study Protocol and the Informed Consent prior to study initiation and IRB approval of any subsequent amendments to the protocol or revision to the Informed Consent. Before the study site can enter a participant into the study, the sponsor-investigator or a designee may review the study site to:

1. Determine the adequacy of the facilities
2. Discuss with the PI(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of Synaptogenix, or its representatives. This will be documented in a clinical study agreement between Synaptogenix and the sponsor-investigator.

During the study, a monitor from Synaptogenix, or representative may have regular contacts with the investigational site, for the following:

1. Provide information and support to the PI(s)
2. Confirm that facilities remain acceptable
3. Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that investigational product accountability checks are being performed
4. Perform source data verification. This includes a comparison of the data in the eCRFs with the participant's medical records at the hospital or practice, and other records relevant to the study.
5. Record and report any protocol deviations not previously sent to Synaptogenix
6. Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been reported and those SAEs that met criteria for reporting have been forwarded to the Ethics Committee (EC)/IRB/ Independent Ethics Committee (IEC).

The Synaptogenix monitor will be responsible for immediately reporting the site not adhering to the study protocol to the project manager and sponsor-investigator. Noncompliance may result in study suspension or closure.

The monitor will be available between visits to provide information or advice.

11.4 Quality Assurance and Quality Control

To ensure compliance with GCP and all applicable regulatory requirements, Synaptogenix, Inc. or its representative may conduct a quality assurance audit.

11.5 Audits and Inspection

Authorized representatives of Synaptogenix, a regulatory authority, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. The PI should contact Synaptogenix immediately if contacted by a regulatory agency about an inspection.

12. ETHICS

12.1 Institutional Review Board

The sponsor-investigator, who is also the PI in this study, must obtain IRB approval before initiating any study activities.

The final study protocol, including the final version of the Informed Consent Form, must be approved in writing by an IRB. Following IRB approval, the sponsor-investigator must submit written notice of approval to Synaptogenix or its representative, before receiving study drug from Synaptogenix and before any participant can be enrolled into the study.

The PI is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit participants for the study. The protocol must be re-approved by the IRB upon receipt of amendments and annually, as local regulations require.

Sponsor-investigator is responsible for providing the IRB and FDA with reports of all adverse drug reactions (ADRs) that are both serious and unexpected.

Progress reports will be provided to the IRB according to local regulations and guidelines.

12.2 Ethical Conduct of the Study

The conduct of this study will be consistent with ICH Guidance E6, GCP, and U.S. federal regulatory requirements, as applicable. This study will be conducted in accordance with applicable local law(s) and regulation(s) and the principles of protection of healthy human participants participating in clinical medical research that have their origin in the Declaration of Helsinki. The PI must agree to the direct access to source documents and inspection of clinical study-related records by the regulatory authority/ Synaptogenix representatives.

12.3 Written Informed Consent

Written informed consent must be witnessed, signed, dated, and obtained from the participant.

The PI should confirm to the extent possible that the participant has the means to be able to attend scheduled study visits for the duration of the study.

Before starting the clinical study, the PI must have the IRB's written approval or favorable opinion of the written ICF and any other written information to be provided to parents or guardians of participants. The written approval of the IRB together with the approved participant's information/ICF must be in the clinical study files. The process of obtaining informed consent must be in accordance with applicable regulatory requirements and must adhere to ICH E6 (R1) guidelines and the ethical principles in the Declaration of Helsinki. Written informed consent must be obtained and documented before any clinical study-specific procedure takes place.

Participation in the clinical study and dates of informed consent given by participants should be documented in the participants' files.

13. STUDY MANAGEMENT

13.1 Data Collection and Management

For all written documentation such as source documents, the data collected during the study must be legibly printed using a permanent ink pen. A single line should be drawn through any incorrect information. Opaque correction fluids or tapes are not permitted. All corrections or deletions to any of the source documents must be dated and initialed. All corrections or deletions to the eCRF will be documented via an electronic audit trail. The PI or designee will electronically sign each participant's final eCRF to signify that all of the information is correct and complete.

13.2 Data Quality Control

Periodic on-site review of communications between the PI and investigational site study monitors, and review of eCRF data and source documents are the responsibility of the sponsor-investigator, or designee. The eCRF data for each participant will be reviewed against source documents at the study sites by the investigational site study monitor.

The PI and investigational site will allow study related quality control monitoring and audits, EC/IRB/IEC review, and/or regulatory inspection and will cooperate in providing direct access to source data and documentation.

13.3 Data Management and Data Storage

Study procedures will be documented on source documents that will be retained at the site(s). An Electronic Data Capture (EDC) system will produce eCRFs that will be used to collect assessment data for this study. All study data entered into the eCRF will be compliant with regulatory requirements and 21CFR part 11. The system will allow differing levels of access and will accommodate roles for the PI and Study Staff. All data changes made within the system will be subjected to an audit trail. In compliance with GCP, source documentation supporting the eCRF data should indicate the participant's participation in the study and should clearly document the dates and details of study procedures, AEs, and participant status. The eCRFs will identify study participants with unique identifiers. Data are recorded from the source documents, directly onto the eCRF at the site.

Electronic CRF data items will undergo quality control standards of operation. Unresolved errors, omissions, or requests for clarification will trigger a query to the Investigational Site for resolution via electronic queries. The database will be corrected for completeness and accuracy. Prior and concomitant medications will be entered into the eCRF and coded using guidelines determined by the sponsor-investigator. Medical history, concurrent medical conditions, and AEs will be coded using guidelines determined by the sponsor-investigator.

A quality assurance audit will be conducted to verify the accuracy and completeness of the database and will be done prior to declaring database lock. The database will not be altered after lock, unless joint written agreement is obtained between Synaptogenix and the sponsor-investigator.

13.4 Inspection of Records

Synaptogenix, Inc., will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The PI agrees to allow the monitor to inspect the drug storage area, study drug stock, drug accountability records, participant charts and study source documents, and other records relative to study conduct.

13.5 Retention of Records

A searchable offline version of the eCRF will be forwarded to the sponsor-investigator for storage. A copy of each completed eCRF will remain in the PI's study file on a compact disk. All source documentation, eCRFs and administrative records will be retained by the PI for a minimum of 2 years following agency approval of the medication for the indication under study or following notification that the investigational application is closed for this indication. However, this may be adjusted based on the applicable local requirements. After this time, the documentation will either be destroyed or transferred to Synaptogenix or designee. No study documentation should be destroyed or moved to a new location without prior written approval by Synaptogenix.

13.6 Confidentiality

All study findings and documents will be regarded as confidential. The sponsor-investigator, co-investigators and members of their research teams must not disclose such information without prior written approval from Synaptogenix or its representatives.

The anonymity of participating participants must be maintained. A Protected Health Information statement will be provided to each participant either as a part of the Informed Consent document or as a separate form. Participant will be identified on eCRFs by their participant number and on source documents by their initials, and participant number. A separate document linking study number to identifiable information of the participants will be maintained separately and kept securely. Documents that identify the participant by name (e.g., the signed Informed Consent Form) must be maintained in strict confidence by the PIs.

13.7 Protocol Amendments

Minimally, any change that significantly affects the safety or welfare of participants, the scope of the

investigation, or the scientific quality of the study will be effected by means of a protocol amendment approved by the sponsor-investigator and submitted to the IND and the local IRB. Protocol amendments submitted to the relevant (local) IRB must be approved before implementation.

The PI will provide written agreement of the protocol amendment via the approval signature page. The PI will notify the IRB of the amendment and obtain approval prior to implementation. If the change is intended to eliminate an immediate hazard, the amendment will be implemented immediately, prior to IRB notification.

13.8 Protocol Deviations

Should a deviation from the protocol be deemed crucial for the safety and well-being of a particular participant, such a deviation will be instituted for that participant only. The PI or other attending physician should contact the Medical Monitor as soon as possible. In addition, the PI or designee should document in the source document the reasons for the protocol deviation and the ensuing events. No waivers for protocol deviations for any Inclusion or Exclusion criterion will be permitted in this study.

13.9 Data Corrections

For all written documentation such as source documents, the data collected during the study must be legibly printed using a permanent ink pen. A single line should be drawn through any incorrect information. Opaque correction fluids or tapes are not permitted. All corrections or deletions to any of the source documents must be dated and initialed. All corrections or deletions to the eCRF will be documented via an electronic audit trail. The PI will electronically sign each participant's final eCRF to signify that all of the information is correct and complete.

13.10 Insurance

The sponsor has taken out a liability insurance policy, which covers the liability of investigators. This policy is in accordance with local laws and requirements. The sponsor's insurance does not relieve the investigators of any obligation to maintain their own liability insurance policy as required by the applicable law.

14. PUBLICATION AND DISCLOSURE POLICY

Study findings are an integral part of the overall commercialization plan for this investigational compound. To this end, the contents of this protocol and any amendments and results obtained during the study shall be kept confidential by the investigator, the investigator's staff, and the IRB/IEC, and shall not be disclosed in whole or in part to others or used for any purpose other than reviewing or performing the study, without the review and prior written consent of the sponsor. These obligations of confidentiality and non-use shall in no way diminish such obligations as set forth in either the Confidentiality Agreement or Clinical Trial Agreement executed between the sponsor-investigator, PI (if different from sponsor-investigator), Cleveland Clinic, and Synaptogenix. All persons assisting in the performance of this study must be bound by the obligations of confidentiality and non-use set forth in either the Confidentiality Agreement or Clinical Trial Agreement executed between the Institution (Cleveland Clinic/sponsor-investigator) and Synaptogenix. Additionally, while proprietary patent issues and competitive strategic goals must be considered, Cleveland Clinic investigators may publish results of this study by abstract, verbal presentation, invited lecture, journal article, or journal letter. Authorship and the order of authorship on publications reporting the results of study findings are at the discretion of the sponsor-investigator or PI, if different.

15. APPENDIX 1 - RESTRICTED CONCOMITANT MEDICATIONS

Drugs Not Allowed (N) during the study.

- Acetaminophen
- Heparin
- Divalproex sodium, valproic acid, and topiramate
- Memantine
- Lithium carbonate
- Vitamin E > 400 International Units (IU) per day within 14 days prior to screening
- MAO inhibitors
- Cholinesterase inhibitors donepezil, rivastigmine, and galantamine are not restricted concomitant medications if at a stable dose for at least 3 months prior to screening and usage continued unchanged throughout the study.
- Carbamazepine
- Terifluonimide
- Ciprofloxacin,prohibited for 24 hours prior to each infusion and on the day of the infusion.
- Dalfampridine
- Bactrim (trimethoprim/sulfamethoxazole) prohibited for 24 hours prior to each infusion and on the day of the infusion.
- Use of any potent or moderate, inhibitor or inducer, of CYP3A4, CYP2C8, or CYP2C9. Concomitant medicines will be examined on a case by case basis against the Flockhart Table by Sponsor-Investigator, and if needed, the Medical Monitor, to determine allowability.
- Use of St John’s Wort is prohibited starting 2 weeks prior to screening and throughout the study.

Dietary Restrictions throughout the study:

- Grapefruit juice

16. APPENDIX 2 - LIST OF REFERENCES

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