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## STUDY PROTOCOL

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

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Protocol Number:	NTRP-101-203
Date:	9 May 2019
Study Drug:	Bryostatin 1; matching Placebo
Investigational new Drug Application (IND) Number:	71,276
Version:	3.0
Planned FPI to LPLV	Jun 2018 to Jul 2019
Study Sponsor:	Neurotrope, Inc.
	NCT03560245

### 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

**SPONSOR'S SIGNATURE PAGE****Title:**

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

**Protocol**                    NTRP-101-203  
**Number:**                    Version 3.0, 9 May 2019

**Sponsor's Authorized Representatives:**

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

**Protocol Number:** NTRP-101-203

**Protocol Version and Date:** Version 3.0 9 May 2019

**Investigational Medicinal Product:** Bryostatin 1

## PI'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: \_\_\_\_\_

\_\_\_\_\_  
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## PROTOCOL SYNOPSIS

<b>Name of Company:</b> Neurotrope, Inc.	<b>Name of Study Medication:</b> Bryostatin 1	<b>Name of Active Ingredient:</b> Bryostatin 1
<b>Title of Study:</b>  A Randomized, Double-Blind, Placebo-Controlled, Confirmatory Phase 2 Study Assessing the Safety, Tolerability and Efficacy of Bryostatin in the Treatment of Moderately Severe to Severe Alzheimer’s Disease Subjects Not Receiving Memantine Treatment		
<b>Study center(s):</b> Approximately 30 sites in United States (US)		
<b>Protocol Number:</b> NTRP-101-203		
<b>Study Duration:</b> Approximately 12 months	<b>Study Phase:</b> 2	
<b>Objective:</b>  <b>Primary objective:</b> To evaluate the safety, tolerability, and efficacy of bryostatin 1 (hereafter referred to as bryostatin) for the treatment of moderately severe to severe Alzheimer’s disease (AD).		
<b>Study Drug:</b> Bryostatin 1 or matching Placebo		
<b>Route of Administration:</b> Intravenous by continuous infusion over 45±5 minutes		
<b>Number of Subjects:</b> 100 randomized subjects (approximately 145 subjects screened)		
<b>Study Design</b>  This is a randomized double-blind Placebo-controlled, Phase 2 study comparing bryostatin to placebo for the treatment of moderately severe to severe Alzheimer’s disease in subjects not receiving memantine treatment. The study is 15 weeks in duration, including a safety and efficacy evaluation 30 days after the last dose of study drug. Subjects will receive 7 doses of study drug during the study. The primary efficacy endpoint is defined as the change from baseline to Week 13 in the Severe Impairment Battery (SIB) total score.  <b>Randomization and Treatment</b>  Eligible subjects will be stratified based on Mini Mental State Exam (MMSE-2) scores 4-9 vs. 10-15 and will be randomized 1:1 to one of two treatment arms: 20µg bryostatin or placebo for twelve weeks. The first two doses of study drug will be a loading dose 20% higher (i.e., 24µg) than the assigned dose and will be administered one week apart.		

<b>Name of Company:</b> Neurotrope, Inc.	<b>Name of Study Medication:</b> Bryostatin 1	<b>Name of Active Ingredient:</b> Bryostatin 1
<p>Thereafter, the assigned dose of 20µg will commence with the third dose and be administered every other week. Drug is administered IV by continuous infusion over 45(±5) minutes. Subjects are scheduled to receive seven doses over 12 weeks.</p>		
<p><b>Evaluation Methods</b></p>		
<p>Safety and tolerability will be determined through evaluations of adverse events (AE), serious adverse events (SAE), physical examination (PE), vital signs, 12-lead electrocardiogram (ECG), the Columbia Suicide Severity Rating Scale (C-SSRS), and clinical laboratory assessments.</p>		
<p>Cognitive improvements will be evaluated primarily with the Severe Impairment Battery (SIB) and secondarily with functional improvements in the Alzheimer’s Disease Cooperative Study – Activities of Daily Living - Severe Impairment Version (ADCL-ADL-Sev), Clinical Global Impression of Improvement (CGI-I), and Neuropsychiatric Inventory (NPI) metrics.</p>		
<p><b>Safety Assessments</b></p>		
<ul style="list-style-type: none"> <li>• Treatment emergent AEs and SAEs</li> <li>• Vital signs, hematology, blood chemistry, physical examination including body weight</li> <li>• ECG parameters</li> <li>• C-SSRS</li> </ul>		
<p><b>Primary Efficacy Endpoint</b></p>		
<p>The primary efficacy endpoint is defined as the change from baseline to Week 13 in the SIB total score.</p>		
<p><b>Secondary Efficacy Endpoints</b></p>		
<ul style="list-style-type: none"> <li>• The changes from baseline at Weeks 5, 9 and 15 in the SIB total score</li> <li>• The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 4-9 stratification group</li> <li>• The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 10-15 stratification group</li> <li>• Individual patient’s slope over time in SIB total score evaluated via Weeks 0, 5, 9, and 13</li> </ul>		
<p><b>Exploratory Efficacy Endpoints</b></p>		

<b>Name of Company:</b> Neurotrope, Inc.	<b>Name of Study Medication:</b> Bryostatin 1	<b>Name of Active Ingredient:</b> Bryostatin 1
<ul style="list-style-type: none"> <li>• Change from baseline in Alzheimer’s Disease Cooperative Study - Activities of Daily Living – Severe Impairment Version (ADCS-ADL-Sev) total score</li> <li>• Change from baseline in Mini Mental State Examination, 2<sup>nd</sup> edition (MMSE-2) total score</li> <li>• Change from baseline in 10-point Neuropsychiatric Inventory (NPI) total score</li> <li>• Clinical Global Impression of Improvement Scale (CGI-I) score</li> </ul>		
<p><b>Statistical Considerations:</b></p> <p>The primary objective for efficacy is to evaluate whether bryostatin is better than the control with respect to the primary and secondary SIB endpoints.</p> <p>Efficacy analyses will be conducted for the full analysis set (FAS), consistent with the modified intention-to-treat principle, defined as all randomized subjects who received at least one dose of randomized study medication and who had at least one post-baseline assessment. The completer analysis set (CAS) will be defined as all randomized subjects who completed 12 weeks of treatment, and who have a week 13SIB assessment.</p> <p>Adverse event and other safety data will be analyzed descriptively in all subjects who received any dose of study drug (including partial infusions). These data will be summarized by treatment group and by time in study.</p>		

**Eligibility Criteria:****Inclusion**

1. Written informed consent from caregiver and subject (if possible) or legally acceptable representative if different from caregiver
2. Male and female subjects 55-85 years of age inclusive
3. Cognitive deficit present for at least 2 years that meet the diagnostic criteria for probable Alzheimer's dementia.<sup>1</sup> The diagnosis must be confirmed at the time of the screening visit
4. MMSE-2 score of 4-15 inclusive (applies to Screening Visit only)
5. Patients must be able to perform at least one item on the SIB and may not have a SIB score >93 at screening
6. Neuroimaging computerized tomography (CT) or Magnetic Resonance Imaging (MRI) within the last 24 months consistent with a diagnosis of probable AD without any other clinically significant co-morbid pathologies. If there has been a significant change in the subject's clinical status since the last imaging study that is not consistent with progression of the subject's AD, an imaging study should be performed to confirm eligibility
7. Reliable caregiver(s) or informant(s) who attends the subject at least an average of 3 hours or more per day for 3 or more days per week and who will agree to accompany the subject to the clinic visits and reliably complete the caregiver questions
8. Adequate vision and motor function to comply with testing
9. If taking an approved cholinesterase inhibitor for treatment of Alzheimer's disease, must be on a stable dose for at least 3 months prior to entry into study and the dose must not change during the study unless a change is required due to an adverse effect of the prescribed medication or a clinically significant change in the patient's status
10. Subjects who are memantine naïve or have been off memantine for at least 30 days prior to initial treatment with study drug
11. Subjects on neuroleptic medications must be on a stable dose for  $\geq 4$  weeks at screening (dose adjustments will be permitted if medically necessary at the discretion of the PI)
12. 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 ( $\beta$ -hCG) test for pregnancy at screening

13. 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
14. In the opinion of the PI subjects should be in reasonably good health over the last 6 months and any chronic disease should be stable

#### **Exclusion**

1. Dementia due to any condition other than AD, including vascular dementia (Rosen-Modified Hachinski Ischemic score  $\geq 5$ )
2. Evidence of significant central nervous system (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
3. Clinically significant neurologic disease or condition other than AD, 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
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 subject 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. Creatinine clearance (CL) of  $<45$ ml/min
6. Poorly controlled diabetes, at the discretion of the Principal Investigator
7. Concomitant treatment with NMDA receptor antagonists such as but not limited to memantine or drug combinations containing memantine, dextromethorphan (a cough suppressant), ketamine, phencyclidine (PCP), methoxetamine (MXE), nitrous oxide ( $N_2O$ ) and the following synthetic opioids: penthidine, levorphanol, methadone, dextropropoxyphene, tramadol, and ketobemidone.
8. Use of vitamin E  $> 400$  International Units (IU) per day within 14 days prior to screening
9. Use of valproic acid within 14 days prior to screening
10. Use of an active Alzheimer's vaccine within 2 years prior to screening
11. Use of a monoclonal antibody for treatment of AD within 1 year prior to screening
12. Any medical or psychiatric condition that is likely to require initiation of additional medication or surgical intervention during the course of the study
13. Any screening laboratory values outside the reference ranges that are deemed

clinically significant by the PI

14. Use of an investigational drug within 30 days prior to screening
15. Suicidality defined as active suicidal thoughts during the 6 months prior to screening or at Baseline [Type 4 or 5 on C-SSRS], or history of suicide attempt in previous 2 years, or at serious suicide risk in PI's judgment
16. Major psychiatric illness such as current major depression according to Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> Edition<sup>2</sup>, 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
17. Diagnosis of alcohol or drug abuse within the last 2 years
18. Abnormal laboratory tests that suggest an alternate etiology for dementia. If the patient has prior history of serum B12 abnormality, anemia with hemoglobin  $\leq 10$ g/dl, thyroid function abnormality, electrolyte abnormality, or positive syphilis serology the patient should be reevaluated to determine if these potential causes of dementia have been addressed. Only if these causes have been ruled out as the cause of the dementia can the patient be enrolled.
19. History of prolonged QT or prolonged QT on screening ECG (QTcB or QTcF  $>499$  per central reader)
20. 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]<sup>3</sup>
21. Known to be seropositive for human immunodeficiency virus (HIV)
22. Known to be seropositive for Hepatitis B or C, unless successful curative treatment for Hepatitis C (e.g., Harvoni) has been received and there is documentation that there is no Hep B/C virus detected 3 months after completion of treatment
23. AST or ALT  $>3$ x upper limit of normal (ULN) and total bilirubin  $>2$ x ULN or International Normalized Ratio (INR)  $>1.5$
24. Prior exposure to bryostatin, or known sensitivity to bryostatin or any ingredient in the study drug
25. Any other concurrent medical condition, which in the opinion of the PI makes the subject unsuitable for the clinical study

<sup>1</sup> McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Phelps CH. 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, 7:263–9.

<sup>1</sup> American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: DSM-5 – May 27, 2013. American Psychiatric Association. Washington, D.C.

<sup>1</sup> [http://www.heart.org/HEARTORG/Conditions/HeartFailure/AboutHeartFailure/Classes-of-Heart-Failure\\_UCM\\_306328\\_Article.jsp](http://www.heart.org/HEARTORG/Conditions/HeartFailure/AboutHeartFailure/Classes-of-Heart-Failure_UCM_306328_Article.jsp)List of References

## Table of Contents

<b>SPONSOR'S SIGNATURE PAGE .....</b>	<b>2</b>
<b>STUDY CONTACT INFORMATION .....</b>	<b>4</b>
<b>STUDY ADMINISTRATIVE STRUCTURE .....</b>	<b>5</b>
<b>PROTOCOL SYNOPSIS .....</b>	<b>6</b>
<b>1 INTRODUCTION.....</b>	<b>19</b>
1.0 Alzheimer's disease .....	19
1.1 Rationale for the use of bryostatin in the treatment of AD.....	20
1.2 Pharmacokinetics, Toxicology and Drug Metabolism in Animals .....	20
1.3 Genotoxicity.....	21
<b>2 CLINICAL TRIAL DATA.....</b>	<b>21</b>
2.0 Oncology Data .....	21
2.1 Alzheimer's Disease Data.....	22
2.1.1 Study NTRP101-202.....	22
2.1.2 Methodology .....	22
2.1.3 Demographics .....	22
2.1.4 Safety .....	23
2.1.5 Efficacy .....	24
2.1.6 Pharmacokinetics .....	26
2.1.7 Summary .....	26
2.2 Study NTRP101-201.....	26
2.2.1 Results.....	27
<b>3 STUDY OBJECTIVES AND HYPOTHESIS.....</b>	<b>28</b>
3.0 Primary Objective .....	28
3.1 Hypothesis.....	28
<b>4 INVESTIGATIONAL PLAN .....</b>	<b>28</b>
4.0 Overview of Study Design.....	28
4.1 Dose Rationale.....	29
4.2 Risk/Benefit .....	29
4.3 Study Endpoints.....	30
4.3.1 Safety Assessments.....	30
4.3.2 Primary Efficacy Endpoint .....	30
4.3.3 Secondary Efficacy Endpoints.....	30
4.3.4 Exploratory Endpoints.....	30
4.4 Study Population.....	31
4.4.1 Inclusion Criteria .....	31
4.4.2 Exclusion Criteria .....	32
<b>5 PRODUCTS USED IN THIS STUDY .....</b>	<b>33</b>
5.0 Bryostatin.....	33
5.1 Placebo.....	34
5.2 Packaging and Labeling of Study Drug Kits .....	34
5.3 Storage and Preparation of Study Drug .....	35
5.4 Study Drug Accountability and Disposal .....	36

5.5 Randomization .....36

5.6 Blinding.....37

5.7 Study Drug Administration.....37

**6 STUDY PROCEDURES AND ASSESSMENTS .....37**

6.0 Table 1 - Schedule of Activities.....38

6.1 Assessments .....39

6.1.1 Safety .....39

6.1.2 Efficacy / Psychometric Assessments.....41

6.2 Visit Procedures .....42

6.2.1 Screening and Randomization (Days -28 to -2).....42

6.2.2 Week 0 (Day 0 Dose 1).....43

6.2.3 Week 1 (Day 7 ±2 days Dose 2) .....44

6.2.4 Week 2 (Day 14 ±2 days/ No dose).....44

6.2.5 Week3 (Day 21 ±2 days/ Dose 3) .....44

6.2.6 Week 5 (Day 35 ±2 days/ Dose 4).....44

6.2.7 Week 7 (Day 49 ±2 days/ Dose 5).....45

6.2.8 Week 9 (Day 63 ±2 days/ Dose 6).....45

6.2.9 Week 11 (Day 77±2 days/ Dose 7).....45

6.2.10 Week 13 (Day 91 ±2 days) .....46

6.2.11 Week 15 or End of Treatment Follow-up Visit .....46

6.3 Concomitant Medications .....47

6.3.1 Medications for AD .....47

6.3.2 Concomitant Medications for Management of Myalgia .....47

6.3.3 Prohibited Medications .....47

**7 ADVERSE EVENTS AND OTHER SAFETY EVALUATIONS .....48**

7.0 Definition of Adverse Events.....48

7.1 Adverse Event of Special Interest - Myalgia .....48

7.2 Definition of Serious Adverse Event .....48

7.3 Assessment of Intensity .....49

7.4 Relationship to Study Drug.....49

7.5 Table 2 Relationship of AE to Study Drug or Trial-Related Procedures .....50

7.5.1 Unexpected Adverse Event.....50

7.6 Reporting Adverse Events .....50

7.6.1 SAE reports.....51

7.6.2 Reporting to Regulatory Authorities.....52

7.7 Criteria for Withdrawal of Subjects.....52

7.8 Criteria for Permanent Discontinuation of Study Drug .....52

7.9 Study Discontinuation.....53

**8 SAFETY MONITORING .....53**

**9 DATA ANALYSIS / STATISTICAL METHODS.....53**

9.0 Sample Size Determination.....54

9.1 Statistical Methods.....55

9.2 Exploratory Analysis .....55

9.3 Safety Analysis .....56

9.4 Analysis of Primary Efficacy Endpoint .....56

9.5 Secondary Efficacy Endpoints.....56

9.5.1 Changes from baseline at Weeks 5, 9 and 15 in the SIB total score.....56

9.5.2 The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 4-9 stratification group.....57

9.5.3 The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 10-15 stratification group.....57

9.5.4 Individual patient’s slope over time in SIB total score evaluated via Weeks 0, 5, 9 and 13 57

9.6 Analysis of Exploratory Endpoints.....57

9.7 Safety Assessments.....57

9.8 Analysis of Safety Data .....58

9.8.1 Adverse Events .....58

9.8.2 Clinical Laboratory Evaluations .....58

9.8.3 Physical Examination.....59

9.8.4 Vital Signs.....59

9.8.5 ECG.....60

9.8.6 Columbia Suicide Severity Rating Scale (C-SSRS) .....60

**10 DATA MONITORING.....60**

10.0 Source Documentation.....60

10.1 Study Documentation and Record Retention.....60

10.2 Site Monitoring.....61

10.3 Quality Assurance and Quality Control.....62

10.4 Audits and Inspections.....62

**11 ETHICS .....62**

11.0 Institutional Review Board .....62

11.1 Ethical Conduct of the Study.....63

11.2 Written Informed Consent .....63

**12 STUDY MANAGEMENT.....63**

12.0 Data Collection and Management.....63

12.1 Data Quality Control.....63

12.2 Data Management and Data Storage.....64

12.3 Inspection of Records .....64

12.4 Retention of Records.....64

12.5 Confidentiality .....65

12.6 Protocol Amendments.....65

12.7 Protocol Deviations.....65

12.8 Data Corrections .....65

12.9 Insurance.....65

**13 PUBLICATION AND DISCLOSURE POLICY .....66**

**14 APPENDIX 1 - RESTRICTED CONCOMITANT MEDICATIONS .....67**

**15 APPENDIX 2 LIST OF REFERENCES .....72**

**16 APPENDIX 3 SUMMARY OF CHANGES 73**

## LIST OF ABBREVIATIONS

<b>Abbreviation</b>	<b>Definition</b>
A $\beta$	Beta-amyloid
AD	Alzheimer's Disease
ADCS-ADL-Sev	Alzheimer's Disease Cooperative Study – Activities of Daily Living - Severe Impairment Version
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Amino Transferase
APP	Amyloid Precursor Protein
AST	Aspartate Amino Transferase
A $\beta$ 1-42	Beta-Amyloid 1-42
ARIA	Amyloid Related Imaging Abnormalities
$\beta$ APP	Beta-amyloid Precursor Protein
$\beta$ -hCG	Human chorionic gonadotropin
BDNF	Brain-derived Neurotrophic Factor
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
C-CASA	Columbia-Classification Algorithm of Suicide Assessment
CAS	Completer Analysis Set
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression – Improvement
CL	Clearance
CNS	Central Nervous System
CPK	Creatine phosphokinase
CRC	Cancer Research Campaign
CSF	Cerebrospinal Fluid
CT	Computerized tomography
C-SSRS	Columbia Suicide Severity Rating Scale
DSMB	Data Safety Monitoring Board
DSC	Digit Symbol Coding
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMG	Electromyography

<b>Abbreviation</b>	<b>Definition</b>
ERB	Ethical Review Board
FAS	Full Analysis Set
FDA	Food and Drug Administration
FLAIR	Fluid Attenuated Inversion Recovery
GCP	Good Clinical Practice
GGT	Gamma Glutamyl Transferase
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GRE	Gradient Refocused Echo
IAP	Interim Analysis Plan
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
IRT	Interactive Response Technology
ITT	Intention-To-Treat
IU	International Unit
IV	Intravenous
LDH	Lactate Dehydrogenase
LSM	Least-Squares Means
MAP	Mitogen Activated Protein
MMRM	Mixed Model for Repeated Measures
MMSE-2	Mini Mental State Examination
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NYHA	New York Heart Association
NMDA	N-Methyl-D-Aspartate
NOAEL	No Adverse Effect Level
NPI	Neuropsychiatric Inventory
PAP	Pharmacokinetic Analysis Plan
PBMCs	Peripheral Blood Mononuclear Cells

<b>Abbreviation</b>	<b>Definition</b>
PET	Tween 80 (polysorbate 80)
PI	Principal Investigator
PK	Pharmacokinetics
PKC	Protein Kinase C
PKCε	Protein Kinase C Epsilon
PP	Per Protocol Analysis Set
PT,	Prothrombin Time
PTT,	Partial Prothrombin Time
PVC	Polyvinylchloride
QTcB	Corrected QTc – Bazett’s formula
QTcF	Corrected QTc – Fridericia’s formula
RBANS	Repeatable Battery of Assessments for Neuropsychological Status
SA	Safety Analysis Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SIB	Severe Impairment Battery
SUSAR	Suspected Unexpected Serious Adverse Reactions
ULN	Upper limit of normal
USP	United States Pharmacopeia
US	United States
WHO-DRL	World Health Organization Drug Reference List

# 1 INTRODUCTION

## 1.0 Alzheimer's disease

Alzheimer's disease is the most common cause of dementia, affecting approximately 5.3 million people in the United States (US) and 30 million people worldwide. There is a disproportionate representation of women with nearly 2/3 of the affected individuals being female. Of the 5.3 million affected Americans, 5.1 million are older than 65 (Alzheimer's Association website [www.alz.org](http://www.alz.org), 2015). Since aging is the single most important risk factor for development of dementia and medical advances are prolonging survival, the incidence of AD will increase. The US Census Bureau data suggest that the number of individuals living until age 100 between the years 2000 and 2020 will increase by more than 200% and the number of individuals living until age 90-95 will double.<sup>4</sup> By 2050 the number of individuals with Alzheimer's disease will almost triple to a projected 13.8 million. In 2013, 15.5 million family and friends provided 17.7 billion hours of unpaid care to those with Alzheimer's and other dementias – care valued at \$220.2 billion (Alzheimer's Association website [www.alz.org](http://www.alz.org), 2015).

The currently approved treatments for AD provide modest symptomatic benefit and do not alter the disease progression. These therapies primarily consist of cholinesterase inhibitors [tacrine (Cognex), donepezil (Aricept), rivastigmine (Exelon), and galantamine (galanthamine, Reminyl)] and the N-methyl-D-aspartate (NMDA) antagonist, memantine (Namenda). The development of a medication that produces a significant improvement in clinical symptoms and/or slows the progression of the disease would be a significant advance in the therapeutics of AD.

The protein kinase C (PKC) signaling pathways have been shown to play an integral role in learning and memory. Several animal models have demonstrated that PKC is involved in the learning process.<sup>5,6</sup> In addition, PKC activity regulates phosphorylation of Tau and cleavage of A $\beta$  amyloid through its effects on alpha secretase and GSK3 $\beta$ .<sup>13</sup> The single most important risk factor for the sporadic form of AD, increased age, has also been linked to impaired PKC-mediated  $\alpha$ -secretase activation.<sup>7</sup> Aged animal models, for example, have shown age-specific changes of PKC isozyme distribution in the brain,<sup>8,9</sup> impaired PKC translocation, reduced levels of the PKC anchoring protein, RACK1,<sup>10</sup> alterations in mitogen activated protein (MAP) kinase Erk1/2,<sup>11</sup> and reduced levels of the  $\alpha$ -secretase cleaved Amyloid Precursor Protein (APP), soluble amyloid precursor protein alpha (sAPP $\alpha$ ), in the cerebrospinal fluid (CSF).<sup>12</sup> Aging of normal human fibroblasts also reduced secretion of sAPP $\alpha$ .

The demonstration of reduced levels of PKC in the brain of Alzheimer's subjects suggests a potential target to improve cognition in AD is the activation of PKC which in turn activates a series of downstream pathways that enhance synaptic function and promote synaptogenesis. Bryostatin is a potent stimulator of PKC $\epsilon$  and is non-tumorigenic. Hongpaisan et.al<sup>13</sup> have recently demonstrated the cognitive benefits and the effect on synaptogenesis in two strains of transgenic AD mice. Their work was recently reproduced by Schrott, et.al.<sup>14</sup> Activation of PKC $\epsilon$  by bryostatin was associated with reduced levels of  $\beta$  amyloid, increased levels of the Brain Derived Neurotrophic Factor (BDNF), prevention of synapse loss, reduced plaque formation and restored memory and learning even in the presence of plaques.

## 1.1 Rationale for the use of bryostatin in the treatment of AD

The pharmacological basis for the use of bryostatin for the treatment of AD is based on the hypothesis that low dose, intermittent administration of bryostatin will activate PKC isozymes  $\alpha$  and  $\epsilon$ . Activation of PKC is associated with:

- Enhanced associative learning and recent memory via PKC-mediated phosphorylation of downstream substrates
- Increased synthesis of proteins such as MAP kinase Erk1/2, and synaptogenic proteins such as BDNF required for long-term memory
- Activation of  $\alpha$ -secretases, thereby increasing non-toxic fragments of the beta-amyloid precursor protein ( $\beta$ APP) and reducing the neurotoxic fragment of  $\beta$ APP,  $A\beta_{1-42}$ , and the associated neuropathology
- Activation of  $A\beta_{1-42}$  – degrading enzymes
- Phosphorylation of GSK-3 beta, inhibiting production of Tau and the associated neuropathology
- Improved memory and learning in 3 different strains of transgenic mice with single or multiple Alzheimer's gene mutations even in the presence of amyloid plaque<sup>14, 13, 15</sup>

Thus, activation of PKC can enhance existing synaptic function, reduce the toxic effects of amyloid and promote synaptogenesis, all potential targets to improve cognitive function in AD.

## 1.2 Pharmacokinetics, Toxicology and Drug Metabolism in Animals

Single dose toxicity of bryostatin has been characterized in four non-GLP studies conducted under National Cancer Institute's (NCI) IND, including three IV toxicity studies in mice and one IV toxicity study in rats. Bryostatin was evaluated in a single GLP-repeated dose 21-day IV toxicity study in rats. The single dose toxicity studies were conducted using two different dosing formulations: bryostatin in ethanol/saline or bryostatin in polyethylene glycol, ethanol, and Tween 80 (PET) diluent. LD<sub>50</sub> ranged from a low of 38 $\mu$ g/kg to 75 $\mu$ g/kg. The 21-day repeat-dose toxic study performed in rats at doses of 0, 10, 15 and 25 $\mu$ g/m<sup>2</sup> with the ethanol/saline formulation resulted in no relevant toxicology findings. The maximum tolerated dose (MTD) and no adverse effect level (NOAEL) were noted to be 25 $\mu$ g/m<sup>2</sup> the highest dose studied. However, since there were no toxicologically relevant findings, the report suggested that the MTD and NOAEL are greater than 25 $\mu$ g/m<sup>2</sup>.

Two preliminary dose range finding IV toxicology studies were performed in the rat and dog to characterize the toxicity of bryostatin and to estimate the maximum tolerable dose (MTD) when administered by bolus injection and continuous infusion. In the Sprague-Dawley rat bryostatin was tolerated up to 10 $\mu$ g/kg (infusion) and 15 $\mu$ g/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 $\mu$ g/kg (infusion and bolus). There were no significant differences in toxicity between the two routes of administration. A more detailed results summary can be found in the Investigator's Brochure.

Limited pharmacokinetics (PK) data are available in animals. The pharmacokinetics of bryostatin was analyzed in female CD1/F2 mice by using [C26-3H]-labeled bryostatin following IV and IP administration (Zhang et al 1996). Following IV administration of 40µg/kg (120mg/m<sup>2</sup>), the plasma disappearance curve for bryostatin could be described by a 2-compartment model, with a distribution t<sub>1/2</sub> of 1.05hours and an elimination t<sub>1/2</sub> 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 t<sub>1/2</sub> of 0.81hours and an elimination t<sub>1/2</sub> of 28.76hours.

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 lung, liver, gastrointestinal (GI) tract, and fatty tissue.

### 1.3 Genotoxicity

Bryostatin 1 was evaluated using the bacterial reverse mutation assay (i.e., the Ames test) with no positive responses observed. Bryostatin 1 was evaluated as negative (nonclastogenic) in the micronucleus assay; it was also evaluated as negative (non-DNA damaging) in the Comet Assay. Overall, based on the results of the Ames test and the combined micronucleus/comet assay, bryostatin 1 is not considered to be genotoxic.

## 2 CLINICAL TRIAL DATA

### 2.0 Oncology Data

Safety data are available from published clinical studies of bryostatin for the treatment of cancer. Altogether, over 1400 oncology subjects received bryostatin, mainly under NCI's IND # 42,780, with exposures to bryostatin in both single and combination agent studies. About 584 subjects received bryostatin as monotherapy, with dose levels ranging from 5µg/m<sup>2</sup> to >180µg/m<sup>2</sup>. Most subjects in both the monotherapy and combination therapy studies received bryostatin at doses >25µg/m<sup>2</sup>, 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 subjects received treatment for several weeks (See Investigators Brochure).

Adverse events occurring in the single agent studies that resulted in discontinuation from the studies were myalgia (28 subjects), acute transient reaction (dyspnea, flushing, hypotension, and bradycardia; 4 subjects each), phlebitis (attributed to ethanol in the formulations, 6 subjects), fatigue (3 subjects), and 1 subject 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 reported in the single agent clinical studies in cancer subjects included: alkaline phosphatase (ALP) (elevated; subject 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.

## 2.1 Alzheimer's Disease Data

### 2.1.1 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 Alzheimer's disease subjects. Exploratory objectives characterize the pharmacokinetics (PK) and pharmacodynamics of bryostatin in this subject population. Seven doses of study drug were administered by a 45-minute continuous intravenous (IV) infusion over 12 weeks.

### 2.1.2 Methodology

Eligible subjects with moderately severe to severe AD were stratified based on Mini Mental State Exam (MMSE-2) scores 4-9 vs. 10-15 and randomized 1:1:1 to one of three treatment arms: 20µg, 40µg, or placebo for twelve weeks. Trial drug was administered IV by continuous infusion. The first two doses (24µg, 48µg) of each of the two active treatment arms were a loading dose, 20% higher than the assigned dose (20µg, 40µg), and were administered one week apart. Thereafter, the assigned dose commenced with the third dose and was administered every other week. Subjects were scheduled to receive seven 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.

### 2.1.3 Demographics

A total of 264 subjects were screened in the trial. Of these, 147 subjects were randomized on a 1:1:1 basis to a treatment group. There were 50 subjects randomized to the placebo treatment group, 49 randomized to the 20µg bryostatin treatment group, and 48 randomized to the 40µg bryostatin treatment group. A total of 141 of the randomized subjects were treated with investigational product (IP) and were included in the Safety Analysis Set (SAS). A total of 135

subjects provided a post-baseline efficacy assessment and were analyzed as the Full Analysis Set (FAS). A total of 113 subjects 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 subjects in the SAS completed the trial (75.2%). A total of 35 subjects in the SAS and 29 subjects in the FAS withdrew early from the trial. The most common reason for early withdrawal was withdrawal of consent (18 subjects), followed by AE (11 subjects).

#### 2.1.4 Safety

Overall, the 20 $\mu$ g bryostatin treatment group showed minimal differences from the placebo treatment group in safety assessments. The 40 $\mu$ g bryostatin treatment group had significantly greater AEs than the other treatment groups. There was also no clear difference in Serious Adverse events (SAEs) between the 20 $\mu$ g bryostatin group and placebo treatment groups, and again the 40 $\mu$ g bryostatin treatment group had significantly greater SAEs than the other treatment group. Similarly, the placebo and 20 $\mu$ g group had similar numbers of TEAEs (28 events vs 30 events, respectively). The TEAEs observed more often in the 20 $\mu$ g 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 $\mu$ g treatment group was associated with more TEAEs than either the 20 $\mu$ g 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 subjects; 4 of those subjects 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 $\mu$ g 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%) subjects in all treatment groups reported 287 separate TEAEs. Of these, 49 (34.8%) subjects reported 107 separate treatment-related TEAEs. There were 8 (16.7%) subjects with 20 treatment-related TEAEs in the placebo treatment group, 17 (37.0%) subjects with 30 events in the 20 $\mu$ g bryostatin treatment group, and 24 (51.1%) subjects with 57 events in the 40 $\mu$ g bryostatin treatment group. Six subjects had a TEAE that lead to trial drug discontinuation, 2 in the placebo treatment group, 1 in the 20 $\mu$ g bryostatin treatment group, and 3 in the 40 $\mu$ g bryostatin treatment group. Overall, 43 (30.5%) subjects had a TEAE that was mild in intensity, 46 (32.6%) subjects had a TEAE that was moderate in intensity, and 8 (5.7%) subjects had a TEAE that was severe in intensity.

There was 1 death in the trial, a subject in the 40 $\mu$ g bryostatin treatment group who suffered a severe TEAE of worsening of AD that was unrelated to the IP.

There were 12 (8.5%) subjects who had 14 treatment emergent non-fatal SAEs during the trial; 4 subjects with 4 events in the placebo treatment group, 2 subjects with 2 events in the 20 $\mu$ g bryostatin treatment group, and 6 subjects with 8 SAEs in the 40 $\mu$ g bryostatin treatment group. A total of 4 subjects in the 40 $\mu$ g 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, PEs, and ECG. However, there was a decline in weight in the bryostatin groups, which was more prominent in the higher dose group: 20µg bryostatin treatment group (mean loss of  $-1.65 \pm 2.77$ kg) and the 40µg bryostatin treatment group ( $-2.98 \pm 2.10$ kg), while there was a slight weight gain in the placebo treatment group ( $0.442 \pm 2.52$ kg). Further, 5 subjects in the 40µg bryostatin treatment group had 5 TEAEs of weight decreased, 3 of which were judged to be related to the IP. No weight related TEAEs were observed in the 20µg bryostatin dose group. There were no differences between treatment groups on the C-SSRS as most subjects did not have suicidal thoughts during the trial, and there were no attempts at suicide by any subject during the trial.

### 2.1.5 Efficacy

Consistent with the initial hypothesis for bryostatin effects on PKC $\epsilon$  and cognition, treatment with 20µg of bryostatin is effective in the treatment of Alzheimer's disease in a FAS and CAS. The overall weight of data shows that subjects treated with 20µg bryostatin displayed significant improvement on the SIB, ADCS-ADL-Sev, and other secondary and exploratory endpoints (one-sided at  $\alpha=0.10$ ), as outlined below.

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 20µg 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 and either the 20µg bryostatin treatment group or 40µg 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. Baseline scores were similar across all treatment groups. There was a statistically significant difference, however, between the placebo treatment group and the 20µg bryostatin treatment group at Week 5 ( $P=0.016$ ), and the treatment difference at Week 13 was also statistically significant ( $P=0.070$ ). Further, when both bryostatin treatment groups were pooled, there was a statistically significant difference between the placebo treatment group and pooled group at Week 5 ( $P=0.039$ ) and a statistically significant treatment difference at Week 13 ( $P=0.094$ ). There were no statistically significant differences between the placebo treatment group and the 40µg 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 20µg 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, NPI, or 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 an MMRM analysis of change from baseline in SIB total score, excluding all subjects from sites that recruited 2 or fewer subjects during the trial, there was a significant difference between the placebo treatment group and the 20µg bryostatin treatment group at Week 5 ( $P=0.068$ ). There was a significant difference between the placebo treatment group and the 20µg

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 ANCOVA was performed to assess site effect on SIB, there was a significant difference between the placebo treatment group and 20 $\mu$ g 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 AChEI or memantine at baseline as an additional covariate, at Week 5 the 20 $\mu$ g 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 $\mu$ g 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 subjects with > median baseline ADCS-ADL-Sev total score. There was a statistically significant difference between the placebo and 40 $\mu$ g bryostatin treatment groups at Week 5 ( $P=0.099$ ) in an ANCOVA of change from baseline in NPI Total score to assess site effects. When performing an ANCOVA of change from baseline in NPI total score for subjects with > median baseline NPI total score, there were statistically significant differences between the placebo treatment group and the 40 $\mu$ g bryostatin treatment group at Week 9 ( $P=0.054$ ) and Week 13 ( $P=0.096$ ).

No clear differences were apparent between treatment groups for Caregiver 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 $\mu$ g bryostatin treatment group had a more positive response than any treatment group at Week 5 and Week 9 and the 40 $\mu$ g 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 $\mu$ g bryostatin treatment group showed the best response compared to placebo or the 40 $\mu$ g bryostatin treatment group.

There was a significant difference in the FAS between the placebo treatment group and the 20 $\mu$ g 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). Subjects 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 $\mu$ g 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.

### 2.1.6 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 T<sub>max</sub> occurred at the end of the 45-min infusion.

Mean C<sub>max</sub> 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 T<sub>1/2</sub> 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 C<sub>max</sub>, AUC<sub>last</sub>, and AUC<sub>inf</sub> were consistent across weeks and there was no indication of systemic accumulation of bryostatin during administration of bryostatin once every 2 weeks.

### 2.1.7 Summary

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

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

The 40µg dose of bryostatin was associated with more TEAEs. With a decrease in systemic CL especially noted with 40µg 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 40µg dose of bryostatin. Subjects given the 40µg dose of bryostatin also reported more TEAEs of seizure and fall.

The primary efficacy endpoint for the SIB and secondary efficacy endpoint for the ADCS-ADL-Sev was met in the CAS with the 20µg dose, which was statistically significantly different from placebo at Weeks 5 and 13. The 40µg dose was not statistically significant from the placebo. SIB improvement was 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.

## 2.2 Study NTRP101-201

This was a randomized, double-blind, Placebo-controlled safety study of a single dose of bryostatin in subjects with mild to moderate AD (MMSE: 14-26). Subjects were randomized 2:1 to receive bryostatin 25µg/m<sup>2</sup> 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 physical examination, hematology including complete blood count (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 (HVLTR) 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.

## 2.2.1 Results

### 2.2.1.1 Demographics

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

### 2.2.1.2 Safety

Bryostatin was well tolerated. There were no deaths or SAEs reported. No subjects had an AE leading to withdrawal during the study. There were five treatment emergent adverse events occurring in three subjects: 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 signs.

### 2.2.1.3 Efficacy

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  $255 \mu\text{g}/\text{m}^2$  bryostatin.

### 2.2.1.4 Pharmacokinetics

The pharmacokinetics of bryostatin were assessed in 6 subjects following  $25 \mu\text{g}/\text{m}^2$  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 ( $\pm$ standard deviation (SD)) value of  $1.09 \pm 0.25$  ng/mL. A terminal elimination rate could not be calculated for most subjects 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.

#### 2.2.1.5 Pharmacodynamics/ PKC $\epsilon$

Preliminary assessment of PKC $\epsilon$  concentration in PBMCs suggests there is an increase in the total amount of PKC $\epsilon$  (cytosol plus membrane bound concentration) following treatment with bryostatin. Additional analysis is underway to further characterize the increase and pharmacodynamics.

#### 2.2.1.6 Conclusions

This was the first double-blind assessment of the safety of bryostatin treatment in subjects with Alzheimer's disease. The study met its primary endpoint of safety and tolerability. Bryostatin appears safe and well tolerated in this cohort of subjects with mild to moderate Alzheimer's disease. 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.

### 3 STUDY OBJECTIVES AND HYPOTHESIS

#### 3.0 Primary Objective

To evaluate the safety, tolerability and efficacy of bryostatin for the treatment of moderately severe to severe Alzheimer's disease in subjects not receiving concurrent memantine treatment.

#### 3.1 Hypothesis

Patients treated with bryostatin will experience a greater improvement in cognitive function as measured by the SIB from baseline to the primary efficacy endpoint after 12 weeks of treatment, as compared to patients on placebo during the same time period. The null hypothesis will be rejected at a significance level of a two-sided  $\alpha = 0.05$ . The test of the null hypothesis will be a superiority test based on the two-sample t-test, and only an improvement in the SIB score from baseline is of clinical significance.

### 4 INVESTIGATIONAL PLAN

#### 4.0 Overview of Study Design

This is a randomized double-blind placebo-controlled exploratory study assessing bryostatin for the treatment of moderately severe to severe Alzheimer's disease in subjects not receiving memantine treatment. The study is 15 weeks in duration, including safety and efficacy evaluation 30 days after the last treatment. Eligible subjects will be stratified based on MMSE-2 scores 4-9 vs. 10-15 and will be randomized 1:1 to treatment with 20 $\mu$ g bryostatin or placebo for 12 weeks of

treatment. Study drug is administered IV by continuous infusion. The first two doses of study drug will be a loading dose 20% higher (i.e., 24 $\mu$ g) than the assigned dose and will be administered one week apart. Thereafter, the assigned dose of 20 $\mu$ g will commence with the third dose and be administered every other week. Subjects are scheduled to receive seven doses over 12 weeks. Safety and efficacy assessments will be done as indicated on the Schedule of Activities (Table 1).

#### 4.1 Dose Rationale

The primary objective of treatment for cognitive disorders with bryostatin is activation of PKC $\epsilon$ , not downregulation as was attempted for the oncology indications. Bryostatin activation of PKC $\epsilon$  is dose dependent, where lower doses activate PKC $\epsilon$  and appear to increase de-novo synthesis of the enzyme, while higher doses of bryostatin downregulate PKC $\epsilon$ .

The dosing regimen is based on the recently completed phase 2 study, NTRP101-202, in which the safety and efficacy analyses indicated a clear advantage of the 20 $\mu$ g dose over the 40 $\mu$ g dose.

The oncology data suggest that the dose-limiting side effect of myalgia is dose dependent and cumulative and likely related to the down regulation of PKC $\epsilon$ . This was not seen in the NTRP101-201 study, in which there were no reports of myalgia after a single 25 $\mu$ g/m<sup>2</sup> dose of bryostatin was administered, nor in the recently completed NTRP101-202 study, in which myalgia was considered an adverse event of special interest. In NTRP101-202, in which subjects received 7 doses of study drug, myalgia was seen in 5 subjects; 4 of those subjects were given the higher 40  $\mu$ g dose of bryostatin. Myalgia observed was mostly mild and managed with analgesics.

Dosing in this study will begin with two initial loading doses at one-week intervals that are 20% higher than the maintenance dose to assure enzyme activation. Dosing will then continue every other week.

#### 4.2 Risk/Benefit

Bryostatin treatment in study NTRP101-202 was shown to be generally safe and well tolerated. The 20 $\mu$ g bryostatin treatment group had similar numbers of TEAEs to the placebo treatment group except in injection site reactions and diarrhea. *Post hoc* analysis in NTRP101-202 clearly showed that bryostatin-treated subjects 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 (20  $\mu$ g and 40  $\mu$ g) and 20 $\mu$ g bryostatin treatment groups had significantly better scores than the placebo treatment group at Weeks 5, 13, and at 30-day Follow-up. There were no statistical differences in SIB score between treatment groups in subjects given memantine at baseline. NTRP101-203 will prospectively seek to replicate these findings and demonstrate a clear benefit both in terms of safety and efficacy in this patient population when comparing the 20 $\mu$ g bryostatin dose to placebo.

Therapeutic antibodies targeting  $\beta$ -amyloid have been associated with the occurrence of amyloid related imaging abnormalities (ARIA), which is similar in appearance to cerebral vasogenic edema and microhemorrhage. A minority of cases have been associated with symptoms of headache, visual disturbance, loss of coordination, or disorientation. Asymptomatic ARIA was reported in a clinical study of the small molecule gamma-secretase inhibitor BMS-708163. Bryostatin has a different mechanism of action than antibodies directed at amyloid, but in the transgenic mouse

model of AD treated with bryostatin a decrease of A $\beta$  deposition was observed. Given the limited bryostatin experience in AD, the potential for ARIA and associated clinical events cannot be excluded. In NTRP101-202, however, there was no clinical evidence suggestive of vasogenic edema or microhemorrhage that required an MRI scan.

Subjects in this study, with moderately severe to severe dementia, are those who have had a progressive dementia to the degree that their activities of daily living and their ability to care for themselves may be compromised. Subjects with MMSE-2 scores of 15 or less display significant cognitive impairments that are disabling. Most if not all of these subjects will have progressed despite treatment and have no alternative treatments available. Based on the available nonclinical and clinical data, and the low risk of ARIA, the potential benefits of treatment with bryostatin at the proposed dose in this protocol outweigh the risks.

### 4.3 Study Endpoints

#### 4.3.1 Safety Assessments

- Treatment emergent AEs and SAEs
- Vital signs, hematology, blood chemistry, and physical examination including body weight
- ECG parameters
- C-SSRS

#### 4.3.2 Primary Efficacy Endpoint

The primary efficacy endpoint is defined as the change from baseline to Week 13 in the SIB score.

#### 4.3.3 Secondary Efficacy Endpoints

- The changes from baseline at Weeks 5, 9 and 15 in the SIB total score
- The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 4-9 stratification group

The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 10-15 stratification group

- Individual patient's slope over time in SIB evaluated via Weeks 0, 5, 9, and 13.

#### 4.3.4 Exploratory Endpoints

- Change from baseline in Alzheimer's Disease Cooperative Study - Activities of Daily Living – Severe Impairment Version (ADCS-ADL-Sev) total score
- Change from baseline in Mini Mental State Examination, 2<sup>nd</sup> edition (MMSE-2) total score
- Change from baseline in 10-point Neuropsychiatric Inventory (NPI) total score
- Clinical Global Impression of Improvement Scale (CGI-I) score

## 4.4 Study Population

Subjects with moderately severe to severe Alzheimer's disease defined as a MMSE-2 score of 4-15 inclusive are eligible to enroll. Subjects will be permitted to continue present FDA-approved treatments for Alzheimer's disease but no new treatments can be initiated. OTC medications taken for cognitive improvement such as Ginkgo Biloba or other empiric medications are permitted while participating in the study but should not be initiated or dose modified after screening. Subjects who are no longer on medications for Alzheimer's disease can be enrolled.

### 4.4.1 Inclusion Criteria

1. Written informed consent from caregiver and subject (if possible) or legally acceptable representative if different from caregiver
2. Male and female subjects 55-85 years of age inclusive
3. Cognitive deficit present for at least 2 years that meet the diagnostic criteria for probable Alzheimer's dementia.<sup>16</sup> The diagnosis must be confirmed at the time of the screening visit
4. MMSE-2 score of 4-15 inclusive (applies to Screening Visit only)
5. Patients must be able to perform at least one item on the SIB and may not have a SIB score >93 at screening
6. Neuroimaging computerized tomography (CT) or Magnetic Resonance Imaging (MRI) within the last 24 months consistent with a diagnosis of probable AD without any other clinically significant co-morbid pathologies. If there has been a significant change in the subject's clinical status since the last imaging study that is not consistent with progression of the subject's AD, an imaging study should be performed to confirm eligibility
7. Reliable caregiver(s) or informant(s) who attends the subject at least an average of 3 hours or more per day for 3 or more days per week and who will agree to accompany the subject to the clinic visits and reliably complete the caregiver questions
8. Adequate vision and motor function to comply with testing
9. If taking an approved cholinesterase inhibitor for treatment of Alzheimer's disease, must be on a stable dose for at least 3 months prior to entry into study and the dose must not change during the study unless a change is required due to an adverse effect of the prescribed medication or a clinically significant change in the patient's status
10. Subjects who are memantine naïve or have been off memantine for at least 30 days prior to initial treatment with study drug
11. Subjects on neuroleptic medications must be on a stable dose for  $\geq 4$  weeks (dose adjustments will be permitted)
12. 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 ( $\beta$ -hCG) test for pregnancy at screening
13. 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
14. In the opinion of the PI subjects should be in reasonably good health over the last 6 months and any chronic disease should be stable

#### 4.4.2 Exclusion Criteria

1. Dementia due to any condition other than AD, including vascular dementia (Rosen-Modified Hachinski Ischemic score  $\geq 5$ )
2. Evidence of significant central nervous system (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
3. Clinically significant neurologic disease or condition other than AD, 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
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 subject 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. Creatinine clearance (CL) of  $<45$ ml/min
6. Poorly controlled diabetes, at the discretion of the Principal Investigator
7. Concomitant treatment with NMDA receptor antagonists such as but not limited to memantine or drug combinations containing memantine, dextromethorphan (a cough suppressant), ketamine, phencyclidine (PCP), methoxetamine (MXE), nitrous oxide ( $N_2O$ ) and the following synthetic opioids: pentidine, levorphanol, methadone, dextropropoxyphene, tramadol, and ketobemidone.
8. Use of vitamin E  $> 400$  International Units (IU) per day within 14 days prior to screening
9. Use of valproic acid within 14 days prior to screening
10. Use of an active Alzheimer's vaccine within 2 years prior to screening
11. Use of a monoclonal antibody for treatment of AD within 1 year prior to screening
12. Any medical or psychiatric condition that is likely to require initiation of additional medication or surgical intervention during the course of the study

13. Any screening laboratory values outside the reference ranges that are deemed clinically significant by the PI
14. Use of an investigational drug within 30 days prior to screening
15. Suicidality defined as active suicidal thoughts during the 6 months prior to screening or at Baseline [Type 4 or 5 on C-SSRS], or history of suicide attempt in previous 2 years, or at serious suicide risk in PI's judgment
16. Major psychiatric illness such as current major depression according to Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> Edition<sup>17</sup>, 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
17. Diagnosis of alcohol or drug abuse within the last 2 years
18. Abnormal laboratory tests that suggest an alternate etiology for dementia. If the patient has prior history of serum B12 abnormality, anemia with hemoglobin  $\leq 10$ g /dl, thyroid function abnormality, electrolyte abnormality, or positive syphilis serology the patient should be reevaluated to determine if these potential causes of dementia have been addressed. Only if these causes have been ruled out as the cause of the dementia can the patient be enrolled.
19. History of prolonged QT or prolonged QT on screening ECG (QTcB or QTcF  $>499$  per central reader)
20. 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]<sup>18</sup>
21. Known to be seropositive for human immunodeficiency virus (HIV)
22. Known to be seropositive for Hepatitis B or C, unless successful curative treatment for Hepatitis C (e.g., Harvoni) has been received and there is documentation that there is no Hep B/C virus detected 3 months after completion of treatment
23. AST or ALT  $>3$ x upper limit of normal (ULN) and total bilirubin  $>2$ x ULN or International Normalized Ratio (INR)  $>1.5$
24. Prior exposure to bryostatin, or known sensitivity to bryostatin or any ingredient in the study drug
25. Any other concurrent medical condition, which in the opinion of the PI makes the subject unsuitable for the clinical study

## 5 PRODUCTS USED IN THIS STUDY

Active study drug, bryostatin, and a matching placebo will be provided as described below.

### 5.0 Bryostatin

The investigational drug product, bryostatin, 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 0.05mg bryostatin, 2.5mg povidone lyophilized from 40% t-butanol. Accompanying

each vial of Bryostatin will be a 10mL vial containing 2mL of sterile PET diluent [60% v/v polyethylene glycol 400, 30% v/v dehydrated ethyl alcohol, and 10% v/v Tween-80 (polysorbate 80)].

## 5.1 Placebo

The placebo is a sterile, pyrogen-free lyophilized powder intended for IV infusion upon reconstitution and dilution. It will be supplied in a 10 mL vial containing 0.0mg bryostatin, 2.5mg povidone lyophilized from 40% t-butanol. Accompanying each vial of placebo will be a 10 mL vial containing 2mL of the same sterile PET diluent as described above for bryostatin. The placebo is identical to bryostatin in appearance, including color, consistency, volume, and odor.

## 5.2 Packaging and Labeling of Study Drug Kits

Study drug kits will contain 7 vials of Bryostatin for Infusion or 7 vials of Placebo for Infusion and 7 vials of PET diluent. Each kit will be identified by kit number only and assigned to a subject via the Interactive Response Technology (IRT), according to the established randomization scheme.

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

The kit label will contain the following information:

Bryostatin or Placebo for Infusion Kit

Kit # XXXXX

Store refrigerated at 2-8°C.

Caution New Drug - Limited by Federal Law (US) to Investigational use.

Each kit contains 7 vials Bryostatin for Infusion, 0.05 mg bryostatin/vial or 7 vials Placebo for Infusion, 0.0 mg bryostatin per vial and 7 vials PET Diluent

Manufactured for Neurotrope by Lyophilization Technology, Inc.

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

Bryostatin for Infusion or Placebo for Infusion vial label will contain the following information:

Bryostatin for Infusion, 0.05mg bryostatin or Placebo for Infusion 0.0mg bryostatin

Kit # XXXXX

Store refrigerated at 2-8°C

Caution New Drug - - Limited by Federal Law (US) to Investigational use.

Each vial contains 0.05 mg bryostatin or 0.0 mg bryostatin and 2.5 mg povidone C-17.  
Contains no antibacterial preservatives

Reconstitute with 1 mL PET Diluent. Swirl to dissolve

Further dilute with 9 mL sodium chloride for injection

Manufactured for Neurotrope by Lyophilization Technology, Inc.

PET Diluent vial label will contain the following information:

PET Diluent for Reconstitution of Bryostatin for Infusion or Placebo for Infusion

Lot #: XXXX

Volume: 2 mL

Caution New Drug - - Limited by Federal Law (US) to Investigational use

Each mL contains Polyethylene Glycol 400 (60%), dehydrated Ethyl Alcohol (30%) and Polysorbate 80 (10%).

For Single Use Only

Manufactured for Neurotrope by Lyophilization Technology, Inc.

### 5.3 Storage and Preparation of Study Drug

The study drug kits will be stored under refrigeration conditions (2-8°C) in a refrigerator, refrigerated cabinet or other refrigerated enclosure, which is securely locked and temperature continuously monitored. Access to the stored study drug kits 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 the study staff (e.g., pharmacist, pharmacist-designee, nurse or physician trained in aseptic handling techniques). This individual will be responsible for reconstitution, dilution and preparation of study drug (active and placebo) according to the randomization assignment for each subject. 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 eight hours of reconstitution. Study drug should be reconstituted with 1mL of PET diluent. After swirling the 10 mL vial to completely dissolve the contents, the resulting solution must be diluted immediately with 9mL of 0.9% sodium chloride (NS) injection, USP. The necessary volume of this solution to achieve the assigned dose (a 4.8 mL loading dose for the first two doses and a 4.0 mL maintenance dose for the remaining 5 doses) should then be added to an IV infusion bag containing 50 mL of normal saline. 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:

Protocol number

Kit/Subject number

Date and time prepared

Instructions for dosing (*e.g.*, entire contents of IV infusion bag infused within 45 ±5 minutes)

#### 5.4 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 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 Singota Solutions 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.

#### 5.5 Randomization

Once all eligibility criteria for the study have been met, and the site has received approval by the Medical Monitor (MM) and Clinical Assessment Technologies (CAT) group, via the EDC system, the subject can be randomized via Interactive Response Technology (IRT) system. A randomization number will be assigned, and drug for that randomization number will be shipped to the site for twelve weeks of treatment. Randomization and scheduling of the first study drug infusion should be timed to allow for receipt of study drug prior to the scheduled study treatment. The drug kits will be shipped to the individual who will be responsible for kit storage and drug preparation and may not be handled by any other study staff member. The study drug kit will not identify the vial containing study drug as either placebo or bryostatin.

## 5.6 Blinding

All subjects, PIs, and investigational clinical site personnel will be blinded to dose assignment.

Since there is no known antidote to bryostatin, the blind should only be broken in exceptional circumstances and is at the discretion of the PI. The Medical Monitor should be contacted as soon as possible to discuss the situation, but this should not delay any treatment.

In a non-emergency situation, when unblinding is requested, the site should discuss the clinical circumstances with the Medical Monitor to determine if breaking the blind will alter the subject's treatment. The decision to break the blind is ultimately the decision of the PI. If the blind is broken for a subject, the PI will record the date and reason for breaking the blind in the electronic case report form (eCRF) and study drug treatment will be discontinued. However, the subject will continue to be monitored per protocol for safety and efficacy.

## 5.7 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 ( $\pm 5$  min) once a week for 2 weeks and then every 14 days  $\pm 2$  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 subject misses a dose, the dose should be administered as soon as possible. The subject should then continue on the original dosing schedule. Provided all efficacy assessments have been completed, if deemed necessary by the PI, a short acting sedative may be administered to reduce a subject's anxiety or agitation during the infusion. All medication is to be recorded on the Concomitant Medication eCRF.

# 6 STUDY PROCEDURES AND ASSESSMENTS

Subjects will be randomized to study drug, either bryostatin or placebo treatment. A follow-up visit will take place 30 days after the last dose of study drug for all subjects, including subjects that have discontinued treatment before completion of the study. Assessments will be performed according to the Schedule of Activities (Table 1).

## 6.0 Table 1 - Schedule of Activities

		NTRP101-203										
Week	Screening	0	1	2	3	5	7	9	11	13	15/ET	
Day (±2 days)	(Days -28 to -2) <sup>d</sup>	0	7	14	21	35	49	63	77	91	105	
Dose	Rand	1	2		3	4	5	6	7			
Informed Consent	X											
Medical history	X											
Demographics	X											
Rosen-modified Hachinski Scale	X											
SIB	X <sup>a</sup>					X*		X*		X	X	
MMSE-2	X					X*		X*		X	X	
ADCS-ADL-Sev <sup>b</sup>	X					X*		X*		X	X	
NPI <sup>b</sup>	X					X*		X*		X		
CGI-I						X*		X*		X		
CSSRS	X			X			X*			X		
Labs <sup>^^</sup>	X			X			X*			X		
ECG	X (x3)			X			X			X		
PE	X						X* <sup>+</sup>			X		
Vitals	X	X <sup>c</sup>	X <sup>c</sup>	X	X <sup>c</sup>	X <sup>c</sup>	X <sup>c</sup>	X <sup>c</sup>	X <sup>c</sup>	X <sup>c</sup>	X	X
Randomization		X <sup>a</sup>										
Confirm Eligibility	X <sup>◊</sup>	X										
Study Drug Dosing		X	X		X	X	X	X	X			
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	
Con meds	X	X	X	X	X	X	X	X	X	X	X	

<sup>a</sup>Baseline SIB administered during screening must be done within 3 weeks of the first dose of study drug. If the screening period exceeds 3 weeks, the SIB should be repeated on the day of first dose, prior to dosing; \* before dose; ^^Labs: CBC including differential, coagulation, clinical chemistry, TSH at screening (B12, T-3 and T-4 if TSH abnormal), CPK at screening and event of myalgia, βhCG if indicated, HbA1C if clinically indicated; <sup>◊</sup> CT scan if necessary per inclusion criterion #6; <sup>+</sup> abbreviated physical examination; <sup>a</sup> Randomization after initial screening procedures indicate eligibility; <sup>b</sup> The ADCS-ADL-Sev and NPI may be administered via telephone at the discretion of the investigator, within the allowed time window for the scheduled visit; <sup>c</sup> Vital signs prior to infusion, then at 30, 60 and 90 minutes from start of the infusion (+/- 5min). <sup>d</sup>The visit window of ±2 Days does not apply to the Screening and Randomization period which is a maximum of 28 days.

## 6.1 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 subject. When a protocol required test cannot be performed, the PI will document the reason for the missed test and any corrective and preventative actions which were 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.

### 6.1.1 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 C-SSRS, clinical chemistry, hematology, and coagulation lab tests.

#### 6.1.1.1 Laboratory

Blood samples will be obtained for routine laboratory tests, including hematology, chemistry, coagulation and  $\beta$ hCG if indicated as outlined in the Schedule of Activities (Table 2).

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

Clinical chemistry tests will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, estimated creatinine CL, glucose, calcium, CO<sub>2</sub>, total protein, albumin, ALP, ALT, AST, gamma glutamyl transferase (GGT) lactate dehydrogenase (LDH), uric acid and bilirubin. A serum creatine phosphokinase (CPK) will be done at screening and in the event of myalgia. A TSH will be done at screening, and B12, T-3 and T-4 will be done if TSH result is abnormal.

Samples will be sent to a central lab for testing. Refer to the Laboratory Manual for detailed instructions.

#### 6.1.1.2 Physical Examination

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

- General appearance
- Weight
- Height (screening only)
- Ears

- Eyes
- Nose
- Throat
- Neck
- Respiratory system
- Cardiovascular system
- Abdomen
- Musculoskeletal /Neurologic
- Extremities
- Skin
- Lymph nodes

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

- General appearance
- Weight
- Respiratory system
- Cardiovascular system
- Abdomen

Examination of other systems as needed to explore reports of AEs should be conducted as needed by the PI. Any clinically significant physical findings that were not present on the initial physical examination will be considered AEs and documented on the AE eCRF as well as in the subject's source documentation and on the physical examination eCRFs.

### 6.1.1.3 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.

### 6.1.1.4 Electrocardiogram

An average of triplicate 12-lead ECGs will be collected at screening. The triplicate ECG measurements should be obtained approximately 2-4 minutes apart. Single 12-lead ECGs will be collected at time points specified in the Schedule of Activities.

### 6.1.1.5 Columbia Suicide Severity Rating Scale

The C-SSRS is an interview-based rating scale to systematically assess suicidal ideation and suicidal behavior. The scale should be administered by an individual with appropriate clinical training, who has also taken the specific rater training for the scale, which will be provided by an agent of the Sponsor prior to the study start. If at any visit from baseline on, there are "YES" answers on items 4, 5 or on any behavioral question of the C-SSRS, a risk assessment should be done by a qualified clinician to determine whether it is safe for the subject to continue to participate in the study. A suicidality narrative should be constructed for subjects who have

undergone any post-baseline risk assessment, using information from the C-SSRS, and available information from the NPI, prior screening and baseline information, the clinician assessment and the narrative guide. Subjects who answer “YES” on items 4, 5 or on any behavioral question of the C-SSRS on more than one occasion during a study should be discontinued from the study. Suicidality AEs or other clinical observations may, based on the judgment of the PI, also trigger a risk assessment and a narrative. When there is a positive response to any question on the C-SSRS, the PI should determine whether an AE has occurred.

### **6.1.2 Efficacy / Psychometric Assessments**

All raters for the efficacy assessments need to be trained and qualified on the administration of the scales. A qualified rater is required for the administration of the SIB, NPI and MMSE-2.

The same qualified rater should perform a given Efficacy/Psychometric Assessment at approximately the same time of day for a given subject throughout the study. All changes in raters for a given assessments must be noted in the subject’s source documents. The caregiver designated to provide assessments (ADCS-ADL-Sev and NPI) at the start of the study should be the same individual throughout the study. Should a change be necessary during the course of the study, the reason for the change and the first corresponding visit must be noted in the subject’s source document.

All psychometric tests performed on study subjects on the same day should be administered in the following order: SIB followed by MMSE-2, except at the screening visit as described in Section 6.2.1. If the MMSE-2 score obtained at screening does not fall within the 4-15 range for inclusion, no further screening tests should be performed. Efficacy tests at subsequent visits should be administered prior to dosing.

#### **6.1.2.1 Rosen-Modified Hachinski Scale**

The Rosen-Modified Hachinski Scale will be evaluated at screening to differentiate Alzheimer’s type dementia from multi-infarct dementia. The 8-item scale results in a score of 0-12; and a score of 5 or above is exclusionary for the study.

#### **6.1.2.2 Severe Impairment Battery**

The SIB is used to assess cognition in subjects with moderate and severe AD. It is divided into nine subscales that include attention, language, orientation, memory, praxis, visuospatial ability, construction, social skills, orienting head to name. Non-verbal responses are allowed, thus decreasing the need for language output. Forty questions are included with a point score range of 0-100. Lower scores indicate greater cognitive impairment.

#### **6.1.2.3 Mini Mental State Examination, 2<sup>nd</sup> Edition**

The Standard version of the MMSE-2, with two alternate forms, will be used. This version has the structure and scoring of the original 30-item MMSE-2 and scores are comparable. The MMSE-2 is a brief, widely used test for assessing overall cognitive state. The MMSE-2 measures selected aspects of cognition such as memory, orientation, attention, language, and praxis on a scale of 0-30. Lower scores indicate greater cognitive impairment.

#### 6.1.2.4 Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory – Severe Impairment Version

The ADCS-ADL-Sev is a 19-item functional assessment of the performance of activities of daily living for subjects with moderate to severe Alzheimer's disease. Informants are queried via a structured interview format as to whether subjects attempted each item in the inventory during the previous 4 weeks, as well as their level of performance. Each item is rated from the highest level of independent performance to complete loss. Total score ranges from 0-54 with lower scores indicating greater functional impairment. The ADCS-ADL-Sev may be administered via telephone at the discretion of the PI, within the allowed time window for the scheduled visit.

#### 6.1.2.5 Neuropsychiatric Inventory

The NPI is a caregiver interview-based rating scale assessing 10 behavioral disturbances occurring in dementia subjects. The NPI may be administered via telephone at the discretion of the investigator, within the allowed time window for the scheduled visit. Items are scored for both frequency and severity. Total scores range from 0-120 with higher scores indicating greater behavioral disturbances. For each item, the associated caregiver distress is also assessed.

#### 6.1.2.6 Clinical Global Impression of Improvement Scale

The CGI-I is used to assess global change in the subject's condition compared to baseline before treatment. The same Clinician must complete the CGI-I scale at each scheduled visit. This is a seven-point scale ranging from (1) very much improved to (7) very much worse.

### 6.2 Visit Procedures

#### 6.2.1 Screening and Randomization (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 and randomization will take place within an approximate 4-week period prior to first dose administration (Days -28 to -2).

Subjects who are screen-failed (e.g. due to clinically significant laboratory abnormality or an active medical condition) may be re-screened if their medical condition stabilizes or improves as assessed by the PI. Subject who are screen-failed due to MMSE score may be rescreened in 3 months.

The Medical Monitor should be consulted prior to re-screening. Subjects who are Screen Failures may be rescreened only once.

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

- Collection of subject demographic data
- Review of concomitant medications

- Review of medical history
- Assessment of neuroimaging (CT or MRI performed within 2 years of screening)
- Evaluate Rosen-Modified Hachinski Scale
- Evaluate MMSE-2

**After confirming that these parameters satisfy eligibility criteria, the remaining screening procedures should be performed;**

- SIB. The SIB should be done within 3 weeks before the first study drug dose. If the screening period exceeds 3 weeks, the SIB should be repeated on the day of the first dose, prior to dosing. The repeat SIB score obtained closest to the day of dosing should be recorded in the eCRF as an unscheduled visit.
- NPI
- ADSC-ADL-SIV
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Blood samples for routine safety including hematology, PT, PTT, INR and serum-chemistry, CPK, TSH (B12, T-3 and T-4 if indicated), and  $\beta$ -hCG, if indicated
- 12-lead ECG (x3)
- Complete physical examination
- Vital signs
- AEs and concomitant medications

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

Sites will not be able to randomize subjects before confirmation that eligibility criteria have been met per the review of the Rosen-Modified Hachinski Scale, MMSE-2, CSSRS, and other eligibility criteria by the Worldwide Clinical Trials' Clinical Assessment Technologies (CAT) group and Medical Monitor (MM).

### 6.2.2 Week 0 (Day 0 Dose 1)

If the subject has had no intervening medical issues or changes in medications between the date of randomization and Week 0, dosing may proceed as scheduled.

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

The following procedures will be done on Day 0:

- *Dosing by IV infusion* will be done according to the procedures outlined in Section 5.7.

- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- AEs and concomitant medications

### 6.2.3 Week 1 (Day 7 $\pm$ 2 days Dose 2)

One week after the first dose of study drug, subjects will return for a second infusion. The following procedures will be performed:

- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- *Dosing by IV infusion*
- AEs and concomitant medications

### 6.2.4 Week 2 (Day 14 $\pm$ 2 days/ No dose)

- C-SSRS
- Blood samples for routine safety
- 12-lead ECG
- Vital signs
- Adverse events and concomitant medications

### 6.2.5 Week3 (Day 21 $\pm$ 2 days/ Dose 3)

- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- *Dosing by IV infusion*
- Adverse events and concomitant medications

### 6.2.6 Week 5 (Day 35 $\pm$ 2 days/ Dose 4)

- Prior to dosing:
  - SIB
  - MMSE-2
  - ADCS-ADL-Sev
  - NPI
  - CGI-I

- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- *Dosing by IV infusion*
- AEs and concomitant medications

#### 6.2.7 Week 7 (Day 49 $\pm$ 2 days/ Dose 5)

- Prior to dosing:
  - C-SSRS
  - Blood samples for routine safety
  - 12-lead ECG
  - Abbreviated Physical exam
- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- *Dosing by IV infusion*
- AEs and concomitant medications

#### 6.2.8 Week 9 (Day 63 $\pm$ 2 days/ Dose 6)

- Prior to dosing:
  - SIB
  - MMSE-2
  - ADCS-ADL-Sev
  - NPI
  - CGI-I
- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- *Dosing by IV infusion*
- AEs and concomitant medications

#### 6.2.9 Week 11 (Day 77 $\pm$ 2 days/ Dose 7)

- Vital signs prior to infusion, then at 30( $\pm$ 5), 60( $\pm$ 5) and 90( $\pm$ 5) minutes from start of the infusion
- *Dosing by IV infusion*

- Adverse events and concomitant medications

#### 6.2.10 Week 13 (Day 91 ±2 days)

At Week 13, subjects will undergo efficacy assessments as well as routine safety assessments. The following will be performed:

- SIB
- MMSE-2
- ADCS-ADL-Sev
- NPI
- CGI-I
- CSSRS
- Blood samples for routine safety
- 12-lead ECG
- PE
- Vital signs
- AEs
- Concomitant medications

#### 6.2.11 Week 15 or End of Treatment Follow-up Visit

For subjects who have completed the study, a follow-up visit will take place at Week 15, 30 days after the last dose of study drug. Subjects who have withdrawn from the study will have a follow-up visit 30 days after the last dose of study drug. The following will be performed:

- SIB
- MMSE-2
- ADCS-ADL-Sev
- Vitals
- AEs and concomitant medications

## 6.3 Concomitant Medications

During the screening visit, the Caregiver will provide a history of prior medication use during the past 6 months and provide a list of currently used medications. All concomitant medications used should be recorded on the eCRF and in the source documents 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.

Subjects 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.

### 6.3.1 Medications for AD

Subjects taking FDA approved medications for the treatment of AD, **with the exception of memantine or any drug containing memantine**, may be enrolled in the study. The subject must be on a stable dose of permitted medications for at least 3 months prior to entry into the study and dose must not change during the study. Subjects may not initiate additional drugs for treatment of AD during the study other than the study drug.

### 6.3.2 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 contraindicated as it has the ability to inhibit PKC activation.

### 6.3.3 Prohibited Medications

Memantine is prohibited. Subjects previously treated with memantine must stop treatment at least 30 days prior to initiation of study drug treatment. Treatment with other NMDA receptor antagonists such as but not limited to dextromethorphan (a cough suppressant), ketamine, phencyclidine (PCP), methoxetamine (MXE), nitrous oxide (N<sub>2</sub>O) and the following synthetic opioids: pentidine, levorphanol, methadone, dextropropoxyphene, tramadol, and ketobemidone is prohibited and a 30-day washout period is required prior to initiation of study drug treatment.

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

If the PI determines that initiation of any of the prohibited medications is required to ensure the subject's safety, the medication may be initiated with the notification of the Medical Monitor and proper documentation of a protocol deviation. Other restricted concomitant medications are listed in Appendix 1.

## 7 ADVERSE EVENTS AND OTHER SAFETY EVALUATIONS

### 7.0 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 (also referred to as an adverse experience) 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 subjects/caregivers will be encouraged to contact the PI or clinical site personnel during any clinical study-related emergency.

All adverse events spontaneously reported by the subject and/or caregiver 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.

### 7.1 Adverse Event of Special Interest - Myalgia

Myalgia has been reported as the dose limiting toxicity across the oncology studies. The incidence of myalgia appears to be dose dependent and cumulative. However, it has been reported in subjects receiving doses as low as  $5\mu\text{g}/\text{m}^2$ . The myalgia has been investigated in some studies but not all. No increase in muscle enzymes were found in the cases investigated. EMG was abnormal in one subject who received  $65\mu\text{g}/\text{m}^2$  and suggested a patchy myositis. An MRI done in another subject was normal.

For all cases of myalgia despite the severity, a narrative will be created documenting onset, severity, treatment and outcome. Muscle enzymes, CPK, will be collected for all cases and compared to baseline values. Additional investigations are at the discretion of the investigator.

### 7.2 Definition of Serious Adverse Event

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

- Results in death (fatal)
- Is life threatening (an event is considered "life threatening" if, in the view of either the investigator or sponsor, its occurrence placed the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death).
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability/incapacity

- Is a congenital anomaly/birth defect  
or
- Is an important medical event (events that may not result in death, be immediately life threatening or require hospitalization; may be considered serious when based upon appropriate medical judgment, they jeopardize the subject and 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.

The following situations may not, by themselves, constitute sufficient grounds to be considered as an SAE:

- Hospitalization solely for a diagnostic purpose, even if related to an AE,
- Elective hospitalization for an intervention planned before the subject enrollment in the study
- Admission to a day care facility or sleeping laboratory

### 7.3 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.

### 7.4 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, positive, 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 according to World Health Organization guidelines are listed in the following Table

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

Rating	Classification	Definition
1	Probable	An AE that: <ul style="list-style-type: none"> <li>• Occurs at a reasonable time interval after administration of the study drug;</li> <li>• Follows a known response pattern to the study drug and;</li> <li>• Cannot be reasonably explained by the known characteristics of the subject's clinical state or by other therapies.</li> </ul>
2	Possible	An AE that: <ul style="list-style-type: none"> <li>• Occurs at a reasonable time interval after administration of the study drug;</li> <li>• Follows a known response pattern to the study drug, but;</li> <li>• Could have been produced by the subject's clinical state or by other therapies.</li> <li>•</li> </ul>
3	Unlikely*	<ul style="list-style-type: none"> <li>• An AE for which sufficient information exists to indicate that the etiology is unrelated to the study drug;</li> <li>• Another etiology is specified.</li> </ul>

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

### 7.5.1 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.

### 7.6 Reporting Adverse Events

The PI should instruct all subjects/caregivers on the procedure for reporting AEs/SAEs to the appropriate clinical site personnel. For each AE reported by parents/guardians, clinical site personnel should obtain all required information to complete the eCRF.

The PI or designee should document all AEs/SAEs in subjects’ source documentation and on the AE eCRF.

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

The following should be recorded with each SAE/AE:

- The nature of the AE, with a diagnosis wherever possible
- Date of event
- Assessment of intensity
- Is this an SAE?
- Relationship to study drug or trial-related procedures
- Action taken regarding study drug treatment
- 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. An SAE that occurs more than 30 days after the last dose 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 subject completes the study

### 7.6.1 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 Sponsor or 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 pharmacovigilance physician or designee will review the SAE including the SAE criteria, the relationship to study medication, the expected or unexpected assessment, and inform the Sponsor by phone and e-mail immediately.

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

- severe injection/infusion site reactions (ulceration or necrosis that is severe; operative intervention indicated)
- systemic hypersensitivity reactions
- myalgia

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 30-day safety visit will NOT be collected unless the PI determines that the event is related to the investigational drug product.

### 7.6.2 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 (SUSAR), will be promptly reported to regulatory authorities by the Sponsor 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.

### 7.7 Criteria for Withdrawal of Subjects

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

- The PI believes withdrawal to be medically necessary or in the best interest of the subject
- Noncompliance with the protocol as judged by the PI (requires discussion with the Sponsor)
- Subjects who are enrolled in violation of inclusion and/or exclusion criteria
- An AE that presents an unacceptable consequence or risk to the subject as judged by the PI, Sponsor or the Medical Monitor
- Lost to follow up
- Withdrawal of consent
- Subject is unblinded

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

### 7.8 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 PI believes that discontinuing treatment is in the best interest of the subject

## 7.9 Study Discontinuation

Neurotrope 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. Clinical study site discontinuation should only occur after mutual consultation between the PI and Neurotrope.

Should the clinical study be discontinued prematurely, all subjects should be brought in for early termination procedures as outlined for the 30-day Follow-up visit. All clinical study materials should be returned to Neurotrope or designee.

## 8 SAFETY MONITORING

An Independent Safety Monitor will provide consultation to Neurotrope to assess the implementation and progress of study NTRP101-203 and will review accumulating trial data to monitor the safety of bryostatin administered to patients with AD. Safety data will be reviewed when 30 subjects have received four doses of study drug, followed by additional safety analyses when 60 subjects have received a similar number of doses. Medical decisions relevant to this study will be made by the Sponsor in consultation with the Primary Investigator, Medical Monitor and Statistician.

Prior to each safety review, a cut-off date for the data to be provided will be established by the Sponsor in consultation with the WCT Supporting Biostatistician. The format and content of data listings will be approved by the Supporting Biostatistician. Data reports will be provided as partially blinded displays (e.g., data is grouped by unblinded treatment using "Group A, B" to indicate treatment). The identity of each group will be available to the safety reviewer via the Supporting Statistician.

Data review documents, safety data tables and listings, will be provided to the Independent Safety Monitor at least 7 business days prior to scheduled reviews. Serious Adverse Events (SAEs) reported during the 7 days before the scheduled review and therefore not included in the provided documentation will be provided by the WCT Drug Safety Manager as soon as possible before the review date. The reviews of the safety data will be summarized for the overall subject group only and will not be separated by treatment groups.

## 9 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 study unblinding and 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.

Descriptive summaries of safety and efficacy data including number of evaluable subjects, mean, median, SD, maximum and minimum for continuous variables will be provided by treatment group and 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 primary endpoint will be conducted on data collected through Week 13.

## 9.0 Sample Size Determination

Based on post hoc analyses of the NTRP 101-202 study for patients not treated with memantine, the SIB for the 20ug bryostatatin group demonstrated a mean (SD) increase of 4.5 (6.8) points from baseline by Week 13. In contrast, patients not treated with memantine in the placebo arm showed a decrease in SIB scores at the 13-week time-point from baseline, giving a mean (SD) SIB change of -0.9 (6.7) points. This resulted in group difference in means of the 13-week SIB measure from baseline SIB changes of 5.4 points.

Guided by these post hoc results, power analyses were performed based on 50 patients per treatment arm, and assuming differences of between 4.0 to 5.5 points in the treatment arm-specific means of the primary outcome (e.g. the treatment effect). Table 2 below gives the estimated power to detect various treatment effects, assuming a two-sided alpha of 0.05. Based on these analyses, 50 patients per group will give an approximate 83% power to detect a treatment effect of 4.0 points, and an estimated power of 98% to see a treatment effect of 5.5 points. Power calculation was based the two-sample t-test of the group difference in the mean 13-week SIB changes from baseline SIB.

**Table 2:** Power to detect treatment effect sizes of between 4.0 to 5.5 points; 50 patients per group with an available primary efficacy endpoint.

<u>Diff. in Deltas</u>	<u>Power</u>	<u>N1</u>	<u>N2</u>	<u>Two-sided Alpha</u>
4.0	83%	50	50	0.05
4.5	91%	50	50	0.05
5.0	95%	50	50	0.05
5.5	98%	50	50	0.05

Power analyses were also preformed to determine detectable effect sizes that assume an estimated dropout rate of 25% (e.g. 50 patients per group at a baseline, dropping to 37 patients per group by Week 13). Table 3 gives the estimated powers for treatment effects of between 4.5 and 5.5 points, assuming a 25% dropout rate. Note that a minimum treatment effect of 4.5 points can be statistically detected with at least 80% power, given 37 patients per group with a primary efficacy endpoint.

**Table 3:** Power to detect treatment effect sizes of between 4.5 to 5.5 points; 37 patients per group with an available primary efficacy endpoint.

<u>Diff. in Deltas</u>	<u>Power</u>	<u>N1</u>	<u>N2</u>	<u>Two-sided Alpha</u>
4.5	80%	37	37	0.05
5.0	88%	37	37	0.05
5.5	93%	37	37	0.05

## 9.1 Statistical Methods

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

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

The completer analysis set (CAS) will be defined as all randomized subjects who completed 12 weeks of treatment, and who have a week 13 SIB assessment.

Demographics will include age, sex, race, ethnicity, height, and body weight and will be summarized by treatment group using descriptive statistics for both the SAS and FAS populations.

Medical history, neuroimaging, prior and concomitant medications, existing disease, years since AD onset and diagnosis, baseline medical conditions, and baseline safety and neuropsychological assessments will be summarized descriptively by treatment group for both the SAS and FAS populations.

Subject disposition will be summarized by treatment group and will include numbers screened, randomized, dosed and withdrawn with reason for withdrawal.

## 9.2 Exploratory Analysis

Descriptive statistics by visit and randomized treatment group will be provided for all efficacy data. These descriptive statistics will include the mean, median, SD, maximum and minimum for continuous variables, and frequencies, percentage, and tabulations for categorical variables. Summary statistics will also be performed on both the patient demographics and patient 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.

### 9.3 Safety Analysis

The SAS population will be used for all safety analyses with treatment group determined by the treatment actually received. The Safety Analysis Set (SAS) is defined as all randomized subjects who received any study medication (either partial or completed infusions of bryostatin or placebo). AEs, safety laboratory, ECGs, physical exam and vital signs data will be presented in tabular format and summarized descriptively by treatment group. No formal hypothesis testing will be carried out on these safety assessments. Incidence and severity of AEs will be summarized by treatment dose using System Organ Class (SOC). AEs will be coded according to standard MedDRA terms.

C-SSRS responses will be mapped onto the Columbia-Classification Algorithm of Suicide Assessment (C-CASA) scale, and the frequency distribution of the C-CASA scores will be tabulated by treatment group and study visit. No hypotheses associated with the C-SSRS or C-CASA scales will be tested.

Subject disposition will be summarized by treatment group and will include numbers screened, randomized, dosed and withdrawn with reason for withdrawal.

### 9.4 Analysis of Primary Efficacy Endpoint

The primary endpoint for this study is the change from baseline to Week 13 in the SIB total score. The primary analysis will be conducted on FAS population. The CAS population will be used as a supportive analysis. All statistical tests for efficacy will be two-sided tests, with  $\alpha=0.05$ .

Based on the review of patients not treated with memantine in the NTRP 101-202 clinical trial, the expected number of patients who will drop out of the study prematurely will not be significant. For efficacy evaluations, multiple imputation methods will be used to handle missing data. This imputation method is a robust method to impute missing measurements. The imputation will be carried out in SAS version 9.4 or later using PROC MI. Each imputation model will include the stratification factor (i.e., MMSE-2 score (4-9 vs. 10-15)) as a covariate in the model..

### 9.5 Secondary Efficacy Endpoints

#### 9.5.1 Changes from baseline at Weeks 5, 9 and 15 in the SIB total score

The change from baseline at Week 5, 9 and 15 in the SIB total score will be summarized descriptively and compared using ANCOVA adjusted for baseline SIB total score. If the normality assumption is not met, a non-parametric method or a rank-ANCOVA analysis will be used.

### **9.5.2 The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 4-9 stratification group**

Similar analysis methods as the primary endpoint will be used for analysis of this endpoint.

### **9.5.3 The changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects in the MMSE-2 10-15 stratification group**

Similar analysis methods as the primary endpoint will be used for analysis of this endpoint.

### **9.5.4 Individual patient's slope over time in SIB total score evaluated via Weeks 0, 5, 9 and 13**

Individual-specific SIB slopes will be estimated for all patients over each person's available SIB outcome measures. An indicator will be assigned to each patient depending on the sign of the SIB slope  $\beta$  (i.e.  $I=-1$  if  $\beta < 0$ ;  $1$  if  $\beta > 0$ ; no value otherwise). The proportions of patients with  $I=1$  and, respectively,  $-1$  will be compared using logistic regression to assess the overall trend between treatments. In addition, an overall population averaged slope will be estimated for each treatment arm, and two-sample t-test will be used to assess the difference between treatments.

## **9.6 Analysis of Exploratory Endpoints**

The following exploratory endpoints will be analyzed:

Change from baseline in ADCS-ADL-Sev total score

Change from baseline in MMSE-2 total score

Change from baseline in NPI total score

CGI-I score

Similar analysis methods as the primary endpoint will be used for analysis of the exploratory endpoints.

## **9.7 Safety Assessments**

Unblinded summaries and listings of safety data will be provided to the Independent Safety Monitor as described in Section 8. Analyses will be performed by an independent unblinded statistician so that study investigators and other sponsor personnel (and contractors) involved in study monitoring, data processing and other aspects of the study remain blinded.

## 9.8 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.

### 9.8.1 Adverse Events

Adverse events will be coded using the most recent version of Medical Dictionary for Regulatory Activities (MedDRA). Treatment Emergent AE's (TEAE) are defined as events with an onset on or after the first randomized treatment. TEAEs will be summarized by treatment group, System Organ Class, and preferred term. The following TEAE summaries will be provided:

- Overall (*i.e.*, regardless of severity or relationship to treatment)
- By severity grade (mild, moderate, severe, life threatening or death for SAEs)

By relationship to clinical trial treatment (definitely related, probably related, possibly related, unlikely related, unrelated)

Unless otherwise specified, at each level of subject summarization, a subject 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 Serious Adverse Events (SAEs) will also be summarized by treatment group and relationship.

### 9.8.2 Clinical Laboratory Evaluations

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

### **9.8.2.1 *Laboratory Values over Time***

Summary statistics of raw data and change from baseline values for each laboratory parameter will be presented by treatment group and 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, subjects with an undefined change from baseline, because of missing data, will be excluded.

### **9.8.2.2 *Individual Subject Changes (Shift Tables)***

Individual subject changes will be identified through shift tables. Shift tables will be presented for each laboratory parameter with counts and percentages of subjects, by treatment group and time point, for shift (change) from baseline, using the normal ranges from the laboratory.

### **9.8.2.3 *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-subject listings.

### **9.8.3 *Physical Examination***

All physical examination findings will be listed and/or summarized by treatment group. Shift tables will also be presented to show any abnormality shifts from baseline to post baseline visits.

### **9.8.4 *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, subjects with an undefined change from baseline, because of missing data, will be excluded.

### 9.8.5 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.

### 9.8.6 Columbia Suicide Severity Rating Scale (C-SSRS)

All data from C-SSRS will be listed. Descriptive summaries will be presented for each of the subscales (i.e. Suicidal Ideation and Suicidal Behavior).

## 10 DATA MONITORING

### 10.0 Source Documentation

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

- Clinic, office, hospital charts
- Copies of transcribed health care provider notes, which have been certified for accuracy after production
- 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
- Records of telephone contacts
- Diaries, evaluation checklists, or questionnaires that are completed directly by subjects or caregivers and serve as their own source
- Laboratory results and other laboratory test results, urine dip-stick results
- Correspondence regarding a subjects' treatment between physicians or memoranda sent to the IRB.

### 10.1 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. PIs are responsible for approval of all entered or corrected data. The PI, or

designees, must review and approve the data before database lock, or before any scheduled interim analyses, as required by the sponsor.

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. Neurotrope must be informed if the records are passed on to any other person or institution during this period. Records related to the study will be maintained by Neurotrope or its designee for a minimum of 5 years per 21 Code of Federal Regulations (CFR) 58.195 (b) 2.

## 10.2 Site Monitoring

The Sponsor 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 Neurotrope, 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 a study site can enter a subject into the study, the Sponsor or a designee will visit the study site to:

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

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

- Provide information and support to the PI(s)
- Confirm that facilities remain acceptable
- 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
- Perform source data verification. This includes a comparison of the data in the eCRFs with the subject's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each subject (e.g. clinic charts).
- Record and report any protocol deviations not previously sent to Neurotrope
- 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 EC/IRB/ Independent Ethics Committee (IEC).

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

The monitor will be available between visits if the PI(s) or other staff needs information or advice.

### 10.3 Quality Assurance and Quality Control

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

### 10.4 Audits and Inspections

Authorized representatives of Neurotrope, 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 International Conference on Harmonization, and any applicable regulatory requirements. The PI should contact Neurotrope immediately if contacted by a regulatory agency about an inspection.

## 11 ETHICS

### 11.0 Institutional Review Board

The PI 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. The PI must submit written approval to Neurotrope or its representative, before he or she can enroll any participant 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 subjects for the study. The protocol must be re-approved by the IRB upon receipt of amendments and annually, as local regulations require.

The PI is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from this or any other study conducted with the investigational product. Neurotrope, will provide this information to the PI.

In addition to SAEs that are suspected unexpected serious adverse reactions (SUSARs), the PI or designee will report any additional SAEs that may be required according to the IRB policy.

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

## 11.1 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 subjects 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/Neurotrope representatives.

## 11.2 Written Informed Consent

Written informed consent must be obtained from caregiver and subject (if possible) or legally acceptable representative if different from caregiver.

The PI should confirm to the extent possible that the subject has the necessary caregiver support and will 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 subjects. The written approval of the IRB together with the approved subject'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 subjects should be documented in the subjects' files.

# 12 STUDY MANAGEMENT

## 12.0 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 subject's final eCRF to signify that all of the information is correct and complete.

## 12.1 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, or designee. The eCRF data for each subject 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.

## 12.2 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, Medical Monitor, CRO, and Sponsor. 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 subject's participation in the study and should clearly document the dates and details of study procedures, AEs, and subject status. The eCRFs will identify study subjects 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 the World Health Organization Drug Reference List (WHO-DRL). Medical history, concurrent medical conditions, and AEs will be coded using MedDRA.

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 the CRO and the Sponsor.

## 12.3 Inspection of Records

Neurotrope, 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, subject charts and study source documents, and other records relative to study conduct.

## 12.4 Retention of Records

A searchable offline version of the eCRF will be forwarded to the Sponsor 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 Neurotrope or designee. No study documentation should be destroyed or moved to a new location without prior written approval by Neurotrope.

## 12.5 Confidentiality

All study findings and documents will be regarded as confidential. The PIs and members of their research teams must not disclose such information without prior written approval from Neurotrope or its representatives.

The anonymity of participating subjects must be maintained. A Protected Health Information statement will be provided to each subject either as a part of the Informed Consent document or as a separate form. Subjects will be identified on eCRFs and other documents by their initials, birth date, and subject number. Documents that identify the subject by name (eg, the signed Informed Consent Form) must be maintained in strict confidence by the PIs.

## 12.6 Protocol Amendments

Minimally, any change that significantly affects the safety of subjects, 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. Any changes that affect subject safety or welfare must be submitted to the relevant IRB and 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.

## 12.7 Protocol Deviations

Should a deviation from the protocol be deemed crucial for the safety and well-being of a particular subject, such a deviation will be instituted for that subject 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 protocol deviations for any Inclusion or Exclusion criterion will be permitted in this study.

## 12.8 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 subject's final eCRF to signify that all of the information is correct and complete.

## 12.9 Insurance

The Sponsor has taken out a liability insurance policy, which covers the liability of PIs. This policy is in accordance with local laws and requirements.

The Sponsor's insurance does not relieve the PI or the collaborators of any obligation to maintain their own liability insurance policy as required by the applicable law.

### **13 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/CRO and the institution/investigator. 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/investigator and the Sponsor/CRO. Additionally, the publication plan of the Sponsor, considering, among other items, proprietary patent issues and competitive strategic goals, must be complied with prior to the public disclosure of any aspect of this study by abstract, verbal presentation, invited lecture, journal article, or journal letter. Matters regarding authorship and the order of authorship on publications reporting the results of single study findings are covered in a separate agreement.

## 14 APPENDIX 1 - RESTRICTED CONCOMITANT MEDICATIONS

*Drugs Allowed (Y) and Not Allowed (N) as Concomitant medications in this study.*

Drug Class	Usage		Restrictions
	(p.r.n.)	Chronic Use	
Analgesics	(Y)	(Y)	<p>Only non-opioid containing analgesics can be administered chronically.</p> <p>Use of acetaminophen as an analgesic is contraindicated as it has the ability to inhibit PKC activation.</p> <p>Combination products containing codeine, hydrocodone or oxycodone may be used on a p.r.n. basis only (not to exceed 5 consecutive days) and not within 24-hours before a clinic visit.</p> <p>Opioid analgesics are contraindicated as they have the ability to act as an NMDA antagonist.</p>
Anesthetics			
• <i>General</i>	(N)	(N)	
• <i>Local</i>	(Y)	(N)	
Anorexics	(N)	(N)	
Antacids	(Y)	(Y)	
Antianginal agents	(Y)	(Y)	
Antiarrhythmics	(N)	(Y)	Dose must be stable for 1 month prior to Screening.
Antiasthma agents	(Y)	(Y)	
Antibiotics	(Y)	Call	Call Medical Monitor.
Anticholinergics	(N)	(N)	Includes Cogentin. Anticholinergics for bladder control are to be avoided if possible. When needed some, such as Detrol is allowed. Call Medical Monitor.

*Drugs Allowed (Y) and Not Allowed (N) as Concomitant medications in this study.*

Drug Class	Usage		Restrictions
	(p.r.n.)	Chronic Use	
Anticoagulants	(N)	(Y)	Heparin is not allowed
Anticonvulsants	(N)	(N)	Divalproex, Valproate, Topiramate are prohibited. Anticonvulsants with no known significant cognitive effects, such as Lamictal, Pregabalin, Levetiracetam are allowed. Call the medical monitor for other anticonvulsants. Patient must have been on a stable dose for 3 months prior to screening.
Antidepressants	(N)	(Y)	MAO inhibitors, antidepressants with anticholinergic effects (e.g. tricyclics), and chronic use of sedating antidepressants (e.g. mirtazipine) are not allowed. Sedating antidepressants can be used sparingly at low dose, as needed for sleep; avoid using 12 hrs prior to efficacy assessments. Dose and medication must be stable for 1 month prior to Screening.
Antidiarrheal preparations	(Y)	(N)	Kaolin, Imodium and Pepto-Bismol preparations are allowed. Call Medical Monitor for others.
Antiemetics	(Y)	(N)	Antiemetics with sedative properties (e.g. first generation antihistaminics) are not allowed. Antiemetics such as phosphoric acid preparations (Emetrol, Emecheck), Pepto-Bismol, and cola syrup are allowed.
Antifungal agents			
<ul style="list-style-type: none"> <li>• Systemic</li> <li>• Topical</li> </ul>	(N) (Y)	(N) (Y)	
Antihistamines	(Y)	Call	Non-sedating antihistaminics such as Allegra (fexofenadine), Zyrtec (cetirizine) etc are allowed. Sedating antihistaminics are not allowed. See cough and cold

*Drugs Allowed (Y) and Not Allowed (N) as Concomitant medications in this study.*

Drug Class	Usage		Restrictions
	(p.r.n.)	Chronic Use	
			preparations for combination products.
Antihypertensives	(N)	(Y)	Diupres (reserpine), Catapres (clonidine), Minipress (prazosin), Inderal (propranolol), Wytensin (guanabenz), Tenex (guanfacine) and Aldomet (methyldopa) are not allowed. All others - medication and dose must be stable for 1 months prior to Screening.
Anti-inflammatory drugs	(Y)	(Y)	Indomethacin and systemic corticosteroids are not allowed.
Anti-neoplastics	(N)	(Y)	Tamoxifen is allowed. Dose must be stable for 3 months prior to Screening.
Anti-obesity	(N)	(Y)	Xenical (orlistat) Cetilistat, Lorcaserin (Belviq) are allowed. Others such as Qsymia (a combination of phentermine and topiramate) are not allowed. Call Med Monitor. Dose must be stable for at least one month prior to Screening.
Anti-Parkinson's drugs	(N)	(N)	Includes dopaminergic agents, amantadine, selegiline, Cogentin, and MAO inhibitors.
Antipsychotics	(Y)	(Y)	Clozapine and antipsychotics with anticholinergic effects are not allowed. Limit use to low doses and only if absolutely needed. Call Medical Monitor. Doses must be stable for at least <i>one month</i> prior to Screening.
Antiviral agents	(Y)	(Call)	Zovirax, Valtrex, Famvir are allowed. For others, call Medical Monitor
Anxiolytics	(Y)	(N)	Limited use of short/medium acting benzodiazepines if needed is allowed. Do not use within 12 hrs before efficacy assessments.
BPH agents	(N)	(Y)	Alpha-1 blockers (Hytrin, Flomax, Cardura) and finasteride are

*Drugs Allowed (Y) and Not Allowed (N) as Concomitant medications in this study.*

Drug Class	Usage		Restrictions
	(p.r.n.)	Chronic Use	
			permitted. Drug and dose must be stable for 3 months prior to Screening.
Cholinesterase inhibitors	(N)	(Y)	Alzheimer's disease medications such as Aricept (donepezil) Exelon (rivastigmine), and Reminyl (galantamine) must be on a stable dose for at least 3 months prior to Screening. Usage must continue unchanged throughout the study.
Cough/Cold preparations	(Y)	Call	Decongestants containing dextromethorphan or narcotics are not permitted. Preparations containing pseudoephedrine or phenylpropanolamine are not permitted. <i>See Antihistamines.</i>
Diuretics	(N)	(Y)	Medication and dose must be stable for 1 months prior to Screening.
Ginko biloba	(N)	(Y)	Dose must be stable for at least one month prior to Screening.
H <sub>2</sub> Blockers	(Y)	(Y)	For patients on chronic therapy, medication and dose must be stable for 1 months prior to Screening.
Hormones	(N)	(Y)	Medication and dose must be stable for 3 months prior to Screening.
Hormone suppressants	(N)	(Y)	Proscar (finasteride) is allowed. Dose must be stable for 3 months prior to Screening.
Hypoglycemic agents	(N)	(Y)	Oral hypoglycemic agents and insulin are allowed. Dose must be stable for 3 months prior to Screening.
Hypolipidemics	(N)	(Y)	Statins are allowed. Dose must be stable for at least one month prior to Screening.
Insulin	(N)	(Y)	Patients must be well controlled and stable.
Laxatives	(Y)	(Y)	Fiber-based products and Colace

*Drugs Allowed (Y) and Not Allowed (N) as Concomitant medications in this study.*

Drug Class	Usage		Restrictions
	(p.r.n.)	Chronic Use	
			(docusate sodium) are allowed.
Muscle relaxants	(N)	(N)	
Psychotropic drugs not otherwise specified (including herbal products)	(N)	(N)	Call medical monitor
Sedatives/hypnotics	(Y)	(Y)	Zolpidem, zaleplon, trazodone, chloral hydrate, mirtazapine, and the occasional use of benzodiazepines for sleep is allowed. Where possible, these should not be used within 12 hrs prior to efficacy tests.
Steroids			
• <i>Systemic</i>	(N)	(N)	
• <i>Topical</i>	(Y)	(Y)	
• <i>Inhalant</i>	(Y)	(Y)	
Stimulants	(N)	(N)	Includes Ritalin, Concerta, any methylphenidate preparations, Cylert (pemoline), etc.
Tocopherol (Vitamin E)	(N)	(Y)	Dose more than 400 IU per day not allowed; Dose must be stable for at least one month prior to Screening.
Vaccines for AD	N	N	Patients treated with active vaccine against amyloid/tau within 2 years are not allowed.

## 15 APPENDIX 2 LIST OF REFERENCES

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- <sup>3</sup> [http://www.heart.org/HEARTORG/Conditions/HeartFailure/AboutHeartFailure/Classes-of-Heart-Failure\\_UCM\\_306328\\_Article.jsp](http://www.heart.org/HEARTORG/Conditions/HeartFailure/AboutHeartFailure/Classes-of-Heart-Failure_UCM_306328_Article.jsp)List of References
- <sup>4</sup> Nelson PT, Head F, Schmitt FA, et al. 2011. Alzheimer's disease is not "brain aging": neuropathological, genetic and epidemiological human studies. *Acta Neuropathol*. 121:571-87.
- <sup>5</sup> Hongpaisan J and Alkon DL. 2007. A structural basis for enhancement of long-term associative memory in single dendritic spines regulated by PKC. *PNAS*. 104 (49):19571-19576.
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- <sup>7</sup> Kern A, Roempp B, Prager K, Walter J, Behl C. 2006. Down-regulation of endogenous amyloid precursor protein processing due to cellular aging. *J Biol Chem* 281:2405-2413.
- <sup>8</sup> Battaini F, Elkabes S, Bergamaschi S, Ladisa V, Lucchi L, De Graan PN, Schuurman T, Wetsel WC, Trabucchi M, Govoni S. 1995. Protein kinase C activity, translocation, and conventional isoforms in aging rat brain. *Neurobiol Aging* 16:137-148.
- <sup>9</sup> Battaini F, Pascale A. 2005. Protein kinase C signal transduction regulation in physiological and pathological aging. *Ann N Y Acad Sci* 1057:177-192.
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- <sup>11</sup> Larbi A, Douziech N, Fortin C, Linteau A, Dupuis G, Fulop T, Jr. 2005. The role of the MAPK pathway alterations in GM-CSF modulated human neutrophil apoptosis with aging. *Immun Ageing* 2:6.
- <sup>12</sup> Anderson JJ, Holtz G, Baskin PP, Wang R, Mazzarelli L, Wagner SL, Menzaghi F. 1999. Reduced cerebrospinal fluid levels of alpha-secretase-cleaved amyloid precursor protein in aged rats: correlation with spatial memory deficits. *Neuroscience* 93:1409-1420.

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<sup>14</sup> Schroot LM, Jackson K, Yi P et al. 2015. Acute oral Bryostatins-1 administration improves learning deficits in the APP/PS1 transgenic mouse model of Alzheimer's disease. *Current Alzheimer Research* 12: 222-31.

<sup>15</sup> Etcheberrigaray R, Tan M, Dewachter I et al. 2004. Therapeutic effects of PKC activation in Alzheimer's disease transgenic mice. *PNAS*: 101 (30): 11141-11146.

## 16 APPENDIX 3 SUMMARY OF CHANGES

### 16.1 Version 2.0, 21 Aug 2018

Global changes:

- Neurotrope change of address and logo have been updated.
- Spelling correction: ADCS-ADL-Sev replaces ADCS-ADL-Siv in the list of abbreviations and wherever it occurs in the document
- IRT (Interactive Response Technology) replaces IwRS (Interactive Web Response System) in the list of abbreviations and wherever it occurs in the document.

Inclusion criterion #4 clarification: MMSE-2 inclusion range of 4-15 applies to screening criterion only and may deviate from this range during study participation.

Inclusion criterion #5: The upper limit SIB score for inclusion has been changed from 97 to 93 to optimize measurement of improvement.

Exclusion Criterion #4: Text has been added to describe parameters for eligibility with medical history of cancer: If there is a history of cancer the subject 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.

Section 5.3: Since all site personnel are blinded, there is no need to exclude individuals preparing study drug from participation in safety or efficacy assessments. The sentence "The individual designated to prepare the study drug may not participate in any study subject safety or efficacy evaluations." has been removed.

Section 5.5: Text has been added to describe randomization procedure details. The text now reads: "Once all eligibility criteria for the study have been met, and the site has received approval by the Medical Monitor (MM) and Clinical Assessment Technologies (CAT) group, via

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the EDC system, the subject can be randomized via Interactive Response Technology (IRT) system.”

Section 6.0, Table 1 Schedule of Activities: A footnote has been added to clarify time window for the screening window. The visit window of  $\pm 2$  Days for scheduled study visits does not apply to the screening and randomization period which is a maximum of 28 days.

Section 6.1.2: Text has been clarified to be consistent with Section 6.2.1. The MMSE-2 should be administered and assessed before administering the SIB at screening. For all study visits after randomization, the SIB should be administered first.

Section 6.2.1 Text has been added to describe subject re-screening: Subjects who are screen-failed (e.g. due to clinically significant laboratory abnormality or an active medical condition) may be re-screened if their medical condition stabilizes or improves as assessed by the PI. Subjects who are screen-failed due to MMSE score may be rescreened in 3 months. The Medical Monitor should be consulted prior to re-screening. Subjects who are Screen Failures may be rescreened only once.

Section 14, Appendix 1 Restricted Concomitant Medications: Memantine has been removed from the list of allowed AD medications.

### **16.2 Version 3.0, 18 April 2019**

No changes have been made to study procedures.

The primary efficacy endpoint was described in previous versions as the change in the Severe Impairment Battery (SIB) scale score after 12 weeks of treatment from baseline (taking the change in the average of SIB measures observed during the Week 13 to Week 15 time window from SIB at baseline).

The revised text is: The primary efficacy endpoint is defined as the change from baseline to Week 13 in the SIB total score. This change was made to assure consistency with the primary analysis endpoint as analyzed in study NTRP101-202, for which NTRP101-203 is considered a confirmatory study.

Secondary endpoints were added to analyze the changes from baseline at Weeks 5, 9, 13 and 15 in the SIB total score for subjects enrolled in the MMSE-2 4-9 stratification group and the MMSE-2 10-15 stratification group. Similar analysis methods as the primary endpoint will be used for analysis of these endpoints.