Study Protocol Number:	COMIRB # 19-2727
Study Protocol Title:	Phase II Trial to Evaluate Safety and Efficacy of GM-CSF/Sargramostim in Alzheimer's Disease (SESAD)
Sponsor(s):	Huntington Potter, PhD Department of Neurology University of Colorado School of Medicine
Safety Monitor	Vijaya Knight, MD, PhD
Lead Medical Sub-Investigator	Gemlyn George, MD
Investigational Product Name:	Sargramostim
Indication:	Alzheimer's Disease
Phase:	2
Version Date:	17JUL2025
IND Number	152023
Confidentiality Statement:	This document, and the information contained within is confidential. It contains proprietary information of the sponsor. Any disclosure of such information that is not authorized in writing by the sponsor is prohibited. The information contained in this protocol may be only used for the purpose of reviewing and/or conduct of this study.

TABLE OF CONTENTS

1 Protocol Synopsis

Compound Name:	Sargramostim
Active Ingredient:	Recombinant human Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)
Study Protocol Title:	Phase II Trial to Evaluate Safety and Efficacy of GM-CSF/Sargramostim in Alzheimer's Disease (SESAD)
Principal Investigator:	Huntington Potter, PhD
Site:	Anschutz Medical Campus Department of Neurology University of Colorado School of Medicine
Study Period:	For an individual participant, the total study period is up to 12 weeks of screening, 24 weeks of double-blind placebo controlled treatment, and ~6 weeks (45 days) of follow-up, totaling approximately 38 weeks.
Objectives	
Primary Objective:	• To assess long-term tolerability and safety of sargramostim in individuals with mild-to-moderate Alzheimer's Disease (AD).
Secondary Objective:	• To test the hypothesis that sargramostim treatment, as compared to placebo, slows, or halts cognitive decline, or improves cognitive function, in individuals with mild-to-moderate AD.
Exploratory Objectives	• To assess the effect of sargramostim treatment, as compared to placebo, on clinical progression in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on metabolic activity in brain regions known to be affected by AD, in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on brain amyloid deposition in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on AD related changes in peripheral blood and CSF biomarkers in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on AD related changes in blood-brain barrier permeability in individuals with mild-to-moderate AD.

1 Protocol Synopsis (Continued)

	• To assess the effect that Apolipoprotein E genotype may have upon predicting sargramostim treatment outcomes in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on inhibiting, reducing or slowing medial temporal atrophy (MTA) in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on changes in microstructural white matter measurements, in regions known to be affected by AD, in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on changes in sleep-related measures in individuals with mild-to-moderate AD. • To assess the effect of sargramostim treatment, as compared to placebo, on changes in speech measures in individuals with mild-to-moderate AD.
Study Design:	The study is double-blind, placebo-controlled in the Alzheimer's Disease population, and will include individuals with mild AD and moderate AD, consistent with Montreal Cognitive Assessment (MoCA) score range of 4-24 at time of screening. Individuals who meet inclusion/exclusion criteria will be randomized in a double-blind manner, to receive either sargramostim (178.57 $\mu g/m^2$/day subcutaneously 7days per week for 24 weeks) or placebo (7 days per week) in an approximate 2:1 randomization ratio. The study population will be recruited from the Memory Disorders Clinic at the University of Colorado Hospital (UCH) as well as from the community at large. The estimated maximum duration of participation for each individual participant is approximately 10.5 months (12 week screening phase, 24 week post-randomization double blind treatment phase, and ~6 week (45 day) post-treatment follow-up phase.
Number of Study Participants	Approximately 150 individuals and their study partners will be screened, ~47 participants randomized, with an estimated 42 individuals completing the treatment and follow-up for full analysis (~28 individuals to the sargramostim 178.57 $\mu g/m^2$/day subcutaneously arm of the study (1250 $\mu g/m^2$ per week) and ~ 14 individuals to the placebo control arm of the study.)

Protocol Synopsis (Continued)

Key Inclusion Criteria	Individuals between age 60 and 85 years, inclusive, at time of consent who meet criteria for probable AD dementia according to the National Institute of Aging – Alzheimer's Association (NIA-AA) research framework criteria (2018), and have a diagnosis of mild or moderate AD, or a study MD provisional diagnosis consistent with mild-to-moderate AD, with a screening MoCA score between 4-24, inclusive, have (optional per PI judgment of necessity) positive plasma biomarker for Alzheimer's disease, and have positive biomarker for brain amyloid pathology by CSF assay, obtained via screening lumbar puncture, **or** a positive amyloid PET scan.
Key Exclusion Criteria	Any individuals with a first degree relative diagnosed with AD before 55 years of age, have contraindication to lumbar dural puncture, PET or MRI, have other neurological or psychiatric condition (other than AD) that can impact cognition or procedure adherence, a BMI ≥35, active cancer or malignant neoplasm within 5 years of screening other than non-melanoma skin cancers, have had prior treatment with an investigational anti-amyloid or anti-tauopathy therapy, or AD vaccine, a history of latex or yeast allergy or has a history of, or treatment for, Rheumatoid Arthritis. Full Inclusion / Exclusion Criteria is presented later in this protocol.
Study Treatment	Sargramostim is commercially available from the manufacturer, Partner Therapeutics, and will be obtained by the sponsor. Seven doses of sargramostim (178.57 $\mu g/m^2$or placebo doses that look identical, will be prepared weekly by an unblinded pharmacist into small syringes for injection at home by the participant / caregiver. Doses are to be administered at approximately the same time every scheduled administration day. First dose will be administered at the Baseline visit overseen by trained staff at the CTRC, with training provided for at home dosing to the participant and study partner.

1 Protocol Synopsis (Continued)

Data Safety Monitoring Board	An independent Data Safety Monitoring Board (DSMB) will be chartered for this trial to review safety and other relevant data. The DSMB will convene at regular, predetermined time points to monitor the overall safety of the study, and to make recommendations, when necessary, related to the safety of the study, and the ability of the study to continue unchanged or with modifications, to the Sponsor. The study will proceed with regular order during DSMB safety reviews.
Assessments	
Efficacy Assessments	The Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Alzheimer's Disease Assessment Scale – Cognitive (ADAS-Cog), Clinical Dementia Rating (CDR), Alzheimer's Disease Cooperative Study – Activities of Daily Living (ADCS-ADL), Trail Making Test –A (TMT-A), lexical and semantic fluency, speech and verbal performance measures, and the Neuropsychiatric Inventory (NPI) are well validated and reliable instruments for measurement of cognitive performance. Selected cognitive assessments will be performed during Screening for study inclusion, and at Baseline, week 12, End of Treatment (EOT), and at the follow-up visit.
Blood and CSF Biomarker Assessments	Per PI judgement, plasma may be collected via venipuncture during screening to assess for AD biomarkers (such as Aβ(42)/ Aβ(40) ratio or pTau 217). If drawn, the findings will be confirmed via Cerebrospinal Fluid (CSF) collected via lumbar puncture (LP) later in screening, and CSF will be assayed for Aβ(1-42) and tau:Aβ(1-42) ratios. (Note: Participants could opt for Amyloid PET in lieu of LP for screening confirmation). If the LP option is chosen, a venipuncture blood collection will occur at the same visit as the LP procedure and assayed in conjunction with CSF serum and CSF albumin levels to determine an albumin index for assessing AD-related changes in blood brain barrier permeability. If opting for LP, randomized participants will also have CSF collected via LP, and a venipuncture blood collection, after the final dose of treatment article, post visit week 24. The LP and venipuncture procedures will occur on the same day, which will be at least 2 days after and up to 10 days following the last day of treatment article injection. Exploratory biomarker analyses will assess individual changes in measurements between each participant's EOT blood samples compared to their baseline samples and between each participant's EOT CSF samples compared to their screening samples, as well as determining group differences from sargramostim treatment, as compared to placebo treatment. Changes in the albumin index will be used as

1 Protocol Synopsis (Continued)

Blood and CSF Biomarker Assessments (Continued)	an indication for treatment-related effects on blood brain barrier permeability and as a correlate with MRI analyses for any incidence of vasogenic edema or microhemorrhage. CSF and plasma will be assayed for Aβ(1-42), Aβ(1-40), t-tau, p-tau, tau:Aβ(1-42) ratios, NfL, GFAP, UCHL1, and panels of cytokines, chemokines, and other circulating factors to determine any treatment-related effects that might correlate with cognitive and activities of daily living changes. Serum Anti-Drug Antibody (ADA) Testing for Anti-GM-CSF Antibody will be assayed from serum collected by venipuncture blood draws at baseline, week 3, week 12, week 18, EOT, and follow-up visits. Apolipoprotein E (ApoE) Genetic Status will be determined via blood sample collected at Baseline visit.
Imaging Assessments	Fluorodeoxyglucose F18 (FDG) PET scan will be completed as part of baseline and EOT visit. MRIs of the brain will be conducted throughout the study period, including during screening period, week 6 visit, week 12 visit, week 18 visit, EOT visit, and at the follow-up visit. Amyloid PET utilizing an approved radiotracer (such as Vizamyl (flutemetamol F^{18})) will be done as confirmation of elevated amyloid, or as an optional sub-study if LP is completed as confirmation. If participant consents, the PET will occur during Screening and at the follow-up visit.
Safety Assessments	Blood work including CBCs with differential and comprehensive metabolic panels (CMPs) will be collected at baseline visit, for the remainder of the treatment period, CBCs with differential twice a week and CMP once a week. At the End of Treatment Visit and follow-up visits, CBC with differential only. CBC will be repeated approximately 2 days and 9 days after last dose. Physical exams by study physician or qualified designee will be conducted at screening visit, Baseline, week 12, EOT, and follow-up visits. Electrocardiograms (ECGs) will be conducted at screening visit, week 12, and EOT visits. MRIs of the brain will be conducted throughout the study period, including during screening (Tier 2), week 6 visit, week 12 visit, week 18 visit, EOT visit, and at the follow-up visit. Vital signs and adverse events (AEs) will be monitored weekly. C-SSRS assessment of suicidality will be conducted at Screening, Baseline week 12 and EOT visits.

2 Schedule of Events

Schedule of Events - Screening Period	
Visit:	**Screening Visit**
End of week relative to study randomization day[1]:	**Week -12 to Week -1**
Tolerance Interval for Visit (in days)[1]	**84**
Screening Visit Procedures (By Tier)	
Tier 0.5	
Screening Procedures	
Mini Informed Consent	**X**
Demographics	**X**
Cognitive Health Review Battery[13]	**X**
Optional blood draw for biomarker AD evaluation (such as Aβ(42)/Aβ(40) ratio or pTau 217[14]	**X**
Tier 1	
Screening Procedures	
Full Informed consent (participant and study partner)[2]	**X**
Demographics	**X**
Medical History	**X**
Previous / Concomitant Meds	**X**
Physical / Neurological Exam	**X**
Diagnostic assessment for mild-to-moderate AD (as necessary)[12]	X
Screening Entry Diagnostics	
MoCA	**X**
Vital signs (i.e. BP, HR, Temperature, Respirations)[3]	**X**
Height and Weight	**X**
ECG	**X**
GDS[4]	**X**
GAD-7[5]	**X**
C-SSRS[6]	**X**
Screening Laboratory Diagnostics	
Urinalysis	**X**
Prohibited drugs	**X**
Pregnancy (if applicable)	**X**
CBC+ Diff, CMP, PT/INR	**X**
Blood draw for counter-indicated conditions[7]	**X**
Tier 2	
Screening Imaging Diagnostics	
MRI Scan[8]	**X**
Tier 3	
Screening Laboratory Diagnostics	
Lumbar Puncture for Aβ CSF Assay (if selected for entry criteria)[9]	**X**
Amyloid PET[10] (if selected for entry criteria)	**X**
Blood draw for serum/CSF Albumin index, CSF and Serum Glucose, CSF Protein & Total cell count (if LP conducted)	**X**
Vital signs prior to Lumbar Puncture (i.e. BP, HR, Temperature, Respirations) if LP conducted)[3]	**X**

Review of NIA-AA Criteria	**X**
Review of Inclusion/Exclusion Criteria[11]	**X**
Tier 4 (Optional)	
Screening Laboratory and Imaging Diagnostics	
Amyloid PET[10] (if selected for optional substudy)	**X**
Lumbar Puncture for Aβ CSF Assay (if selected for optional substudy)[9]	**X**

[1]The interval between the screening visit and the baseline visit is a maximum of 84 days to allow for completion of all screening procedures, assessments and evaluations. The visit is not considered complete until all procedure to determine eligibility (Tiers 0.5-3) are completed and reviewed. Screening visit can take place over multiple days, with expectation that each separate Tier will be completed within a 1 day visit, with an expected 3 mandatory site visits and 1 optional visit.
[2]The participant and the study partner must have a completed, signed and dated informed consent before any data is collected.
[3]Blood pressure and pulse should be measured in the sitting position only after sitting for 5 minutes. Refer to section 8.4.5.3 Data Monitoring Plan for acceptable BP parameters.
[4]The Geriatric Depression Scale (GDS) will be administered at screening. Participants who score 20 or higher at screening, indicating severe depression, will be excluded from the study.
[5]The Generalized Anxiety Disorder (GAD-7) will be administered at screening. Participants who score 15 or higher at screening, indicating severe anxiety, will be excluded from the study.
[6]The baseline version of the C-SSRS will be administered at screening. Participants at higher imminent risk ('yes' to question 4 or 5 on the C-SSRS) at screening, will be excluded from the study.
[7]The blood draw will be examined for hepatitis B, hepatitis C, HIV, and/or spirochetal (syphilis) infections. Positive results of any of these tests will be reported to the state or local public health agency according to CDC or other governmental guidelines.
[8]A screening MRI will be performed as part of the screening visit eligibility criteria. The MRI and/or MRI report will be reviewed by the Principal Investigator or qualified sub-Investigator designee for inclusion.
[9]If selected for entry criteria or as optional substudy, the lumbar puncture will collect a sample of CSF for assay to determine the presence of elevated amyloid burden in the brain. The assay will examine Aβ(1-42) and tau:Aβ (1-42) ratios (ADmark® Phospho-Tau/Total-Tau/ABeta42; Athena Diagnostics). The participant should meet all other eligibility criteria before the LP is conducted. Platelet and INR results to be reviewed prior to procedures, and must be within preceding 30 days of LP.
[10]An Amyloid PET scan will be conducted within the screening period if the participant consents to the optional sub-study. If chosen as inclusion criteria for amyloid burden,, participant must meet all other inclusion criteria before the Amyloid PET will be conducted. The Amyloid PET will be reviewed by the Principal Investigator or qualified designee.
[11]A final review of all inclusion and exclusion criteria will be conducted and given to the Principal Investigator or qualified sub-Investigator for approval before the participant can move to the baseline visit for randomization.
[12] A diagnostic assessment may be conducted by a study doctor, as necessary, to confirm probable mild or moderate AD in a participant who otherwise meets criteria to begin screening, but may not have a neurologist verified Dx of mild-to-moderate AD. It will consist of a clinical interview and measures of functioning such as the DSRS and/or FAQ, and consultation and consensus with one additional study doctor.
[13] The Cognitive Health Review Information System Battery is designed around standardized diagnostic criteria and practice guidelines to identify common mimics of Alzheimer's disease, some of which can co-exist with Alzheimer's disease pathology. Questionnaires include Everyday Assessment of Cognition (ECog), Neuropsychiatric Symptoms Questionnaire (NPI-Q), Dementia Cognitive Fluctuation Scale, Early screen from the Columbia Suicidality Scale, Epworth Sleepiness Scale, Lewy Body Composite Risk Score, Functional Activities Questionnaire (FAQ), a Dementia Severity Rating Scale (DSRS), an assessment of activities of daily living, and the Revised Self-Monitoring Scale – Expressive Score (RSMS-Ex). Depending on answers given, additional questionnaires may include the RSMS, Behavior Inhibition Scale, Interpersonal Reactivity Index, and Unified Parkinson's Disease Rating Scale–II (UPDRS-II). Other questions were created specifically for the purpose of more specific diagnosis or ensuring best management according to recommended clinical practice guidelines. Answers are provided on a secure Qualtrics system,and provided by caregivers. The questionnaires take approximately one hour to fill out.
[14]Any assay such as the plasma assay for Aβ(42)/ Aβ(40) ratio or pTau 217 will be a clinically validated plasma assay to determine levels of circulating Aβ(42) and Aβ(40) or pTau, which has been normed against CSF derived levels.

STUDY SCHEDULE OF EVENTS																										
	DOUBLE BLIND RANDOMIZATION PERIOD																									
	WEEK of Visit																									
Visits	**Baseline**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**EOT[l]**	**F/U**
Tolerance Interval for Visit (days)	0	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+2-10	+/-7
Concomitant Med Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical / Neuro Exam	X[b]											X[b]													X[b]	X[b]
Contact pharmacy to dispense study drug[c]	X[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Study Drug Injection Training	X[d]																									
Study Medication Administration	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]		
Assessment of compliance		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Cognitive Assessments[f]																										
MMSE[f]	X											X													X	X
MoCA[f]	-X											X													X	X
ADAS-Cog$_{13}$[f]	X											X													X	X
TMT A[f]	X											X													X	X
ADCS-ADL[f]	X											X													X	X
CDR[f]	X											X													X	X
NPI[f]	X											X													X	X
Verbal Fluency[f]	X											X													X	X
Sleep Assessments[g]	X											X													X	X
Speech and Verbal Performance Measures[g]	X											X													X	X
Safety Assessments																										
C-SSRS, GDS, GAD-7[h]	X											X													X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Injection site review[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital Signs[j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Anti-Drug Antibody (ADA)	X					X						X						X							X	X
ECG												X													X	

	WEEK of Visit																								
Visits	**Baseline**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**EOT[l]**	**F/U**
Tolerance Interval for Visit (days)	**0**	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+2-10	+/-7
Safety Assessments (Continued)																										
MRI						**X**						**X**						**X**							**X**	**X** [n]
Safety blood draws[k]	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**[p]	**X**
PT/INR																								**X**		
Enhanced safety blood draw-cytokine panel[o]	**X**					**X**						**X**						**X**							**X**	
Enhanced safety blood draw-t-cell[o]	**X**											**X**													**X**	
Additional Assessments																										
LP and blood draw for Aβ CSF assay, albumin index, total cell count and biomarkers (if opted in)[O]																									**X**[l]	
Blood Draw (biomarkers)	**X**																								**X**[l]	**X**[m]
FDG PET	**X**																								**X**[m]	
Amyloid PET (if opted in)																										**X**
ApoE Genotyping	**X**																									

[a]Final review to confirm participant has met all screening eligibility criteria and can be randomized
[b]Neuro and physical examinations are to be conducted by the Principal Investigator or a qualified designee.
[c]The unblinded pharmacist will used pre-approved method of assigning participants into either the study drug or placebo controlled arm, and continue to dispense the study drug/placebo throughout the study treatment period.
[d]Training on how to inject the study drug will be provided to the participant and study partner, or other designated caregiver if not the study partner, for administering the daily injections. Training will include methods of administration as well as how to fill out the injection site diary. Training will be refreshed on an as needed basis.
[e]Clinic injections to be completed once weekly, for the first 3 weeks, by study partner, LAR, or caregiver with observation by study nurse or qualified designee for appropriate injection technique with additional observation at in person visits (Wk 6, Wk 12, Wk 18) and ongoing education as needed. If they are unable to administer the once weekly dose at the in-person visit with study staff, a study nurse may administer. Home injection administration to be completed by study partner and logged in injection diary.
[f]At Visits where the cognitive and functional assessments will be conducted, they should be administered before any medical procedures that could be stressful for the participant. All attempts should be made to administer the assessments at the same time of day, and with the same individual, designated by the site, as the administrator.
[g]The Insomnia Severity Index (ISI), and Pittsburgh Sleep Quality Index (PSQI) sleep scales will be administered and the Speech and verbal performance measures will be performed and recorded for the Baseline, Week 12, EOT and Follow-up visits.
[h]The "Since last visit" where assessed version of the C-SSRS will be utilized for the Baseline, Week 12, EOT and Follow-up visits. Standard forms for GDS and GAD-7 will be used for the Baseline, Week 12, EOT and Follow-up visits.
[i]Study nurse will assess the injection site(s) at each in-person visit for injection site reaction. If necessary, and in consultation with the Principal Investigator or appropriate sub-investigator or designee, a topical agent can be prescribed to address any swelling or redness at the injection site.
[j]Temperature, respirations, and seated blood-pressure and pulse after sitting for 5 minutes. Refer to section 8.4.5.3 Data Monitoring Plan for acceptable BP parameters.
[k]Safety blood draws will include CMP (once per week) and CBC + diff (twice per week). EOT and FU visits will not have a CMP, only one CBC draw will be collected for these visits.

[l]The LP (if opted in) and blood draw for end of treatment should be conducted at least 2 days after and within 10 days following the final treatment injection, and both performed on the same day with the LP procedure performed first. PT/INR and platelets must have been performed within 3 months of LP procedure, refer to manual of procedures for LP parameters.
[m]The blood draw for follow-up visit, FDG PET, and Amyloid PET (if opted in) should be conducted within ± 7 days of anticipated visit.
[n]The MRI for follow-up visit is only scheduled for participants who participate in the optional Amyloid PET procedure. If the LP is done as a substudy, not for confirmation of the plasma results Aβ(42)/ Aβ(40) ratio, it will not be tested at outside lab as inclusion LP is for confirmatory results.
[O]The Enhanced safety blood draws will consist of a cytokines panel (IL 1 beta, IL 2, IL 6, TNF alpha such as from CHCO Lab 10055) recommended by study immunologist and safety monitor at baseline, week 6, 12, 18 and EOT, and lab (such as CHCO LAB9496) testing characterization of activated, senescent, and exhausted T cells done at visits (Baseline, Week 12 and EOT visits)
[p]The CBC w/ diff will be repeated approximately 2 days and 9 days after last participant dose at end of treatment or withdrawal from study.

LP/PET sequences based on which is used for study inclusion:

If Choose Lumbar Puncture for Study Inclusion (with OPTIONAL AMYLOID PET SUBSTUDY)

	Screening Tier 3 Visit	Screening Tier 4 Visit	Baseline Visit	End of Treatment Visit	Follow-up Visit
Lumbar Puncture (Mandatory)	X			X	
FDG PET (Mandatory)			X	X	
Amyloid PET (Optional)		X			X

If Choose Amyloid PET for Study Inclusion (with OPTIONAL LUMBAR PUNCTURE SUBSTUDY)

	Screening Tier 3 Visit	Screening Tier 4 Visit	Baseline Visit	End of Treatment Visit	Follow-up Visit
Amyloid PET (Mandatory)	X				X
FDG PET (Mandatory)			X	X	
Lumbar Puncture (Optional)		X		X	

3 List of Abbreviations

Abbreviation	Term
Aβ	amyloid beta
Aβ(1-x)	amyloid beta (1-x) (i.e., the amyloid beta monomer from amino acid 1 to x [e.g. 1-42])
ADCC	Alzheimer's Disease Core Center
ADRC	Alzheimer's Disease Research Center
ADCS-ADL	Alzheimer's Disease Cooperative Study – Activities of Daily Living
AChEI	acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADA	Anti-Drug Antibodies
ADAS-Cog	Alzheimer's Disease Assessment Scale - cognitive subscale
AE	adverse event
AI	albumin index
anti-HCV	Hepatitis C antibodies
anti-HIV	Human Immunodeficiency Virus antibodies
ApoE	Apolipoprotein E
APP	amyloid precursor protein
BP	blood pressure
BIC	Brain Imaging Center
CBC	complete blood count
CDR	Clinical Dementia Rating
CDR-SB	Clinical Dementia Rating -Sum Of Boxes
CIT	Cognitive Interference Task
CMP	Comprehensive Metabolic Panel
CNS	central nervous system
COMIRB	Colorado Multiple Institutional Review Board
CRF	case report form
CSF	cerebrospinal fluid
C-SSRS	Columbia Suicide Severity Rating Scale
CUACC	University of Colorado Alzheimer's and Cognition Center
CU-AMC	University of Colorado – Anschutz Medical Campus
CU-RIC	University of Colorado Research Imaging Center
DSMB	data safety monitoring board
DTI	diffusion tensor imaging
ECG	Electrocardiogram
eCRF	electronic case report form
ED	early discontinuation
EDTA	Ethylenediaminetetraacetic acid
EOT	End of Treatment
FA	fractional anisotropy
FDA	Food and Drug Administration
FDG PET	Fluorodeoxyglucose (^{18}F-FDG) positron emission tomography
GAD	Generalized Anxiety Disorder
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
GDS	Geriatric Depression Scale
GFAP	glial fibrillary acidic protein
GFR	glomerular filtration rate
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor

HBsAg	Hepatitis B surface antigen
HIMSR	Human Immune Monitoring Shared Resource
HIPPA	Health Insurance Portability and Accountability Act of 1996
HR	heart rate
ICF	Informed Consent Form
IND	Investigational New Drug
IP	investigational product
IRB	Institutional Review Board
ISI	Insomnia Severity Index
LP	lumbar puncture
MD	mean diffusivity
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
MTA	medial temporal atrophy
NACC	National Alzheimer's Coordinating Center
NDI	neurite density index
NIA-AA	National Institute of Aging-Alzheimer's Association
NfL	neurofilament light
NFTs	neurofibrillary tangles
NODDI	neurite orientation and dispersion density imaging
NREM	orientation dispersion index
ODI	non-rapid eye movement
PD	pharmacodynamic(s)
PET	positron emission tomography
PK	pharmacokinetic(s)
PRN	pro re nata
PSQI	Pittsburgh Sleep Quality Index
p-Tau	phosphorylated-Tau
QTc	corrected QT interval
QTcF	QTc interval calculated using Fridericia's formula
REDCap	Research Electronic Data Capture
SAE	Serious adverse event
SAP	Statistical analysis plan
SOP	standard operating procedure
SST	serum separation tube
SUSAR	suspected unexpected serious adverse reaction
SWA	slow wave activity
TEAE	treatment-emergent adverse event
TEMAV	treatment-emergent markedly abnormal laboratory values
TIA	transient ischemic attacks
t-Tau	total Tau
UCH	UCHealth
UCHL1	Ubiquitin C-terminal hydrolase L1
vMRI	volumetric magnetic resonance imaging
WBC	white blood cell (count)
WM	white matter

4 Introduction

4.1 Introductory Statement and General Investigational Plan

This trial protocol is designed to evaluate primarily whether the long-term use of sargramostim (recombinant human GM-CSF), administered five days per week for six consecutive months (24 weeks), will be tolerated by and safe for use in participants with mild-to-moderate AD, secondarily whether sargramostim can slow, halt, or reverse cognitive decline, and exploratory whether sargramostim can slow, halt, or reverse decline in activities of daily living, reverse or improve several biomarkers associated with AD, as evaluated by multimodal neuroimaging techniques and blood and cerebrospinal fluid analyses. This trial extends the safety results from recently completed Phase 2 double-blind, placebo-controlled clinical trial in mild-to-moderate AD participants (NCT01409915, COMIRB#12-1273), using sargramostim that was administered five days per week for three consecutive weeks and in which there were no incidence of drug-related serious adverse events (SAEs). This concluded in December 2019, and although the trial only treated a small number of participants for a very short treatment phase and was not powered to adequately evaluate efficacy measures, an interim analysis has hinted at potential improvements in some secondary and exploratory efficacy measures. Thus, this 6-month trial protocol aims to evaluate sargramostim treatment in participants with mild-to-moderate AD over an extended period of time and determine whether sargramostim can indeed safely ameliorate cognitive decline and reverse pathological biomarkers of AD, in a similar fashion to our preclinical studies showing GM-CSF's effects in transgenic animal models of AD ([1], attached as Appendix B).

4.2 Background and Research Rationale

As the sixth leading cause of death and a major cause of disability in the U.S., AD affects around 12% of those over age 65 and around 40–50% of those over age 85, for a total of about 5.8 million people in the U.S. and more than 35 million people worldwide. Without significant medical breakthroughs, the number of people age 65+ with AD in the U.S. is projected to reach about 14 million by 2050. Beyond its toll on affected individuals, AD is also extremely costly to society, with an estimated annual cost of $290 billion in the U.S. alone, which is expected to quadruple by 2050. Although treatments slowing the clinical course of the disease, such as cholinesterase inhibitors or a glutamate receptor antagonist are available, their benefits are minor and they do not attack the origin of the AD pathogenic pathway. It is therefore imperative that new pharmacological interventions be developed that can prevent or reverse the disease and the associated cognitive decline.

Most AD treatments that are currently under investigation are designed to attack the formation of the Aβ peptide or its polymerization into neurotoxic oligomers/fibrils and subsequent amyloid plaque deposition. Anti-amyloid immunotherapy strategies have provided encouraging results in transgenic animal models of AD. However, the human trials have thus far not been very successful and have been plagued with serious adverse events (SAEs) defined as amyloid-related imaging abnormalities (ARIAs), including cerebral microhemorrhage and vasogenic edema [2]. Evidently, a completely new therapeutic approach to AD may be necessary [3]. In particular, the essential role of neuroinflammation in AD has remained relatively unexplored as a potential therapeutic target.

Amyloid deposition and cognitive decline in transgenic animals is fundamentally different from that which leads to AD in humans, perhaps because it develops over a relatively short period of time and in the absence of clear neuronal cell death, and this concern always overshadows the use of animal models for developing AD therapies. We therefore sought a different approach to identify a new mechanism that would provide protection against AD. Our investigation is based on epidemiological evidence that rheumatoid arthritis (RA) patients have about an 8-fold reduced risk of developing AD [4], motivating us to determine the underlying basis for their AD resistance.

RA is an autoimmune disease in which inflamed synovial tissue and highly vascularized pannus form and irreparably damage the cartilage and bone. In this inflammatory pannus, leukocyte populations are greatly expanded, perhaps as an endogenous, but ineffective attempt to remove the autoantibody-mediated inflammatory insult. As a result, many other proinflammatory factors are produced that work together in feed-forward mechanisms to further increase leukocytosis, cytokine/chemokine release, osteoclastogenesis, angiogenesis, and continued autoantibody production (rheumatoid factors and anti-citrullinated protein antibodies) [5, 6]. Additionally, the adaptive immune system presents a Th17 phenotype within $CD4^+$ lymphocytes, with ultimate production of interleukin 17 (IL-17), which is then responsible for inducing much of the pro-inflammatory effects [7, 8], concurrently with the reduction in the number of and function of T regulatory lymphocytes [9-11]. Further enhancements of leukocyte populations come from increased expression of structurally unrelated hematopoietic colony-stimulating factors (CSFs), namely M-CSF (macrophage colony-stimulating factor), G-CSF (granulocyte colony-stimulating factor), and GM-CSF (granulocyte-macrophage colony-stimulating factor) [12-15].

While it has been commonly assumed that RA patients' usage of non-steroidal anti-inflammatory drugs (NSAIDs) was protective against the onset and progression of AD [4], clinical trials with NSAIDs have proven unsuccessful in AD patients, and, in some cases, such as the trial with mild cognitive impairment (MCI) subjects, the NSAIDs accelerated the progression to AD [16]. We took a reverse approach and developed and investigated the new hypothesis that intrinsic factors within RA pathogenesis itself may underlie the protective effect(s) of RA. After researching the literature regarding the potential interplay between the innate immune system and AD [17-20], we focused on the hematopoietic colony-stimulating factors that stimulate production of their respective innate immune system leukocytes, which we postulated might traffic to the brain to prevent AD onset or to reverse AD pathogenesis.

4.3 Preliminary Preclinical Results

Intrahippocampal injections of colony-stimulating factors: In our first experiments, we performed bolus injections of either M-CSF, G-CSF, or GM-CSF into the hippocampus of one (ipsilateral) brain hemisphere, and with vehicle (i.e., artificial cerebrospinal fluid), injected into the contralateral hippocampus as a control, into cohorts of aged cognitively-impaired transgenic AD (PS/APP) mice [1]. The mice were then sacrificed one week later and analyzed. Whereas M-CSF injection resulted in significant hyperplasia to the treatment hemisphere and no effect on amyloid deposition, G-CSF showed a modest reduction in amyloid deposition in the injected side. This outcome was later confirmed by colleagues using daily peripheral G-CSF injections, resulting in reduced amyloidosis and cognitive deficits in the AD mice [21] and leading to a small clinical trial in mild-to-moderate AD participants (NCT01617577), which found Filgrastim to be safe and well tolerated in people with AD but efficaciously inconclusive [22]. In contrast to the mild effect observed with G-CSF in AD mice, our intrahippocampal GM-CSF injections resulted in pronounced effects of over 40% reductions in amyloid deposition, relative to the negative control hemispheres [1].

Daily subcutaneous injection of GM-CSF: Based on the remarkably positive results of GM-CSF at only 7 days post-injection, we next investigated the effects of a daily subcutaneous (SC) GM-CSF injection on AD pathology and cognitive function. Both APPswe transgenic (Tg) mice and non-transgenic control (NT) mice were used in these experiments. After confirming cognitive impairment of Tg mice using a widely-used radial arm water maze (RAWM) task of working memory, Tg and NT mice were each sub-divided into two cognitively balanced groups. The mice then received 20 days of a daily SC injection of either GM-CSF or saline control, and ending behavioral testing consisting of RAWM testing during injection days 10-14 and a double RAWM task (i.e. cognitive interference task (CIT)) performed during injection days 16-20, after which the animals were sacrificed and tissues analyzed for AD pathology. Surprisingly, the GM-CSF-treated Tg mice performed as well as or better than saline-treated NT mice at the end of only 14 days, which was confirmed by the CIT results at 20 days. Interestingly, some blocks of testing also showed that GM-CSF-treated NT mice exhibited improved cognitive performance as compared to saline-treated NT mice. After sacrifice, histochemical analyses of GM-CSF-treated Tg mice showed over 50% less amyloid

plaque deposition as compared to saline-treated Tg mice, which correlated with increased neuronal synaptic area and microglial densities in the GM-CSF-treated Tg mice [1].

After these findings that only 14 days of daily subcutaneous GM-CSF injections completely reversed the cognitive impairment of the transgenic AD mice and that 20 days of GM-CSF reduced over half of their cerebral amyloid deposition, we researched the literature for GM-CSF's effects in other neurological diseases. There is an accumulating body of research showing that GM-CSF has beneficial effects for stroke [23-32], spinal cord injury [33-42], traumatic brain injury [43, 44], retinal degeneration [45-47], and Parkinson's disease (PD) [48-51], including in a recent PD clinical trial using sargramostim for 56 consecutive days and that was successful in achieving its primary endpoint [52]. GM-CSF is also anti-apoptotic, neuroprotective, and a growth factor for neural stems cells [53-58], plays a major role in neuronal plasticity critical for learning and memory [59], and has also recently been shown to improve the function of mesenchymal stem cells [60, 61]. GM-CSF is being investigated as a treatment for other inflammatory conditions [62-65], as well as recent studies that support or have replicated our work for GM-CSF to treat AD [66-68] or to improve cognition in general [69].

4.4 Preliminary Clinical Results

4.4.1 Sargramostim effects in chemotherapy-induced cognitive impairment

Together with Dr. Heather Jim of the Moffitt Cancer Center, Tampa, FL, we performed a retrospective analysis of the data that were gathered from a study assessing cognition in bone marrow transplant patients, who acquired cognitive deficits from the chemotherapy or irradiation procedures they underwent ([70], attached as Appendix C; [71]). Their cognitive function was measured before treatments and then six months and 12 months after hematopoietic cell transplantation (HCT) and associated treatment with a combination of GM-CSF plus G-CSF or treatment with G-CSF alone.

The neuropsychological measures used included some of the cognitive measures that are used routinely to assess AD patients. Total neuropsychological performance z scores (TNP) were calculated by summarizing the cognitive domains of memory, executive functioning (i.e., complex cognition), and attention. Scores indicate change in TNP from pre-transplant baseline. Kruskal-Wallis one-way analyses of variance were conducted at six months and 12 months after baseline assessment and HCT, using all available data to compare between-group changes in TNP by receipt of GM-CSF. Wilcoxon signed rank tests were conducted using all available data to examine within-group changes in TNP by receipt of GM-CSF. Despite having a high level of education (average of 13.89 years), the subjects displayed a statistically significant cognitive deficit at baseline. Our preliminary results show that patients who received GM-CSF plus G-CSF improved their cognitive functions (neuro minus motor features) between baseline and six months, whereas subjects receiving only G-CSF did not improve. This result validates in humans that GM-CSF can improve cognition in an at-risk population using the standard recommended, FDA-approved dosage that we also propose to use in this new protocol. This human study also showed that a small number of subjects (less than 20) who received GM-CSF plus G-CSF was sufficient to reveal that GM-CSF led to improved cognition at six months after treatment, compared to the much larger group who received G-CSF alone.

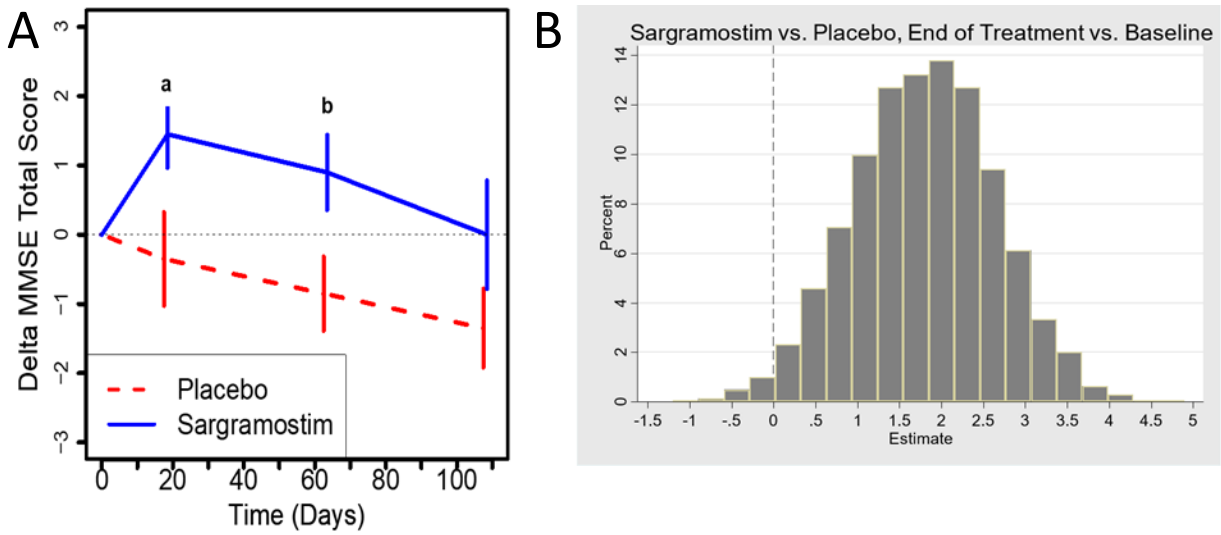
4.4.2 Safety and Efficacy Trial at CU-Anschutz and USF (COMIRB# 12-1273):

After GM-CSF's preclinical results described above in models of AD and other neurodegenerative diseases and the association of GM-CSF with improved cognition in bone marrow transplant patients, the pilot safety and efficacy trial was initiated at the University of South Florida (USF), in which sargramostim/Leukine® was administered to mild-to-moderate AD participants for 15 days over a 19-day period (NCT01409915). At USF, the first 14 participants completed the study given placebo or sargramostim at a dosage of 125 $\mu g/m^2$/day SC, which is half of the FDA-recommended dose. The Data Safety Monitoring Board (DSMB) met and determined that sargramostim was safe, and approved subsequent participants to receive treatment dosage of sargramostim at the full FDA-recommended dose of 250 $\mu g/m^2$/day SC. Then with the relocation of Drs. H. Potter and T. Boyd to Colorado, the trial was halted and amended to increase new enrollment to

40 participants (i.e. 20 sargramostim-treatment, 20 placebo treatment). All participants (3 at USF and 37 at CU-AMC) completed the trial either with placebo or at the full FDA-recommended dose of 250 μg/m^2/day SC. The only AEs reported have been mild expected reactions to sargramostim, including injection site reactions, such as redness, swelling, itching, and bruising. No drug-related SAEs were reported, including no incidence of amyloid-related imaging abnormalities (ARIAs), indicating vasogenic edema (ARIA-E) and/or microhemorrhage (ARIA-H), which have been observed in all other trials testing anti-amyloid immunotherapy drugs in AD participants.

Final analysis of 40 participants showed a statistically significant treatment effect of sargramostim as assessed by MMSE, but none for the other assessments (ADAS-cog, CDR-SB, ADCS-ADL, TRAILS A).

4.5 Specific Objectives



Improved MMSE change scores in rHuGM-CSF/sargramostim-treated participants compared to placebo-treated participants. Mixed model analysis of MMSE data of the completed study of 20 sargramostim-treated participants and 20 placebo-treated participants shows a statistically significant improvement in the sargramostim-treated group at the EOT visit (**a**) compared to baseline (p=0.074) and to the placebo-treated group (p=0.037), and at the first follow-up visit (45 days post-EOT) compared to the placebo-treated group (p=0.0272) (**b**). (**B**) **Distribution of delta MMSE scores at the EOT for random groups of 10 sargramostim-treated participants and 10 placebo-treated participants.** The 20 sargramostim-treated participants and 20 placebo-treated participants were divided into 10,000 random groups of 10 participants each, and the mean Mini-Mental State Exam (MMSE) scores were calculated and graphed as a delta distribution. The mean and median delta was 1.8, as was the delta of the entire 20 sargramostim-treated participants and 20 placebo-treated participants analyzed in (**A**) showing that the statistically significant difference between the sargramostim-treated participants and the placebo-treated participants at the EOT reflected all parts of the trial.

The specific objectives address two hypotheses: The primary hypothesis is that long-term administration of sargramostim (recombinant human GM-CSF) will be tolerated by and safe for use in participants with mild-to-moderate AD; and the secondary and exploratory hypotheses that sargramostim treatment in participants with mild-to-moderate AD will slow or halt cognitive decline and/or activities of daily living, or improve cognitive functioning and/or activities of daily living,, reduce amyloid load, inhibit progression of medial temporal atrophy (MTA), improve AD-related microstructural white matter changes, improve brain metabolic activity, improve sleep-related measures, improve indices of semantic verbal fluency, and induce changes in peripheral blood and cerebral spinal fluid (CSF) biomarkers toward levels measured in

individuals without dementia. These aims are expressed through primary, secondary and exploratory objectives with related endpoints.

Primary Objective	Primary Endpoint
To assess long-term tolerability and safety of sargramostim in individuals with mild-to-moderate Alzheimer's Disease (AD).	Effects of sargramostim on white blood cell mobilization will be determined by CBCs with differential. Comprehensive metabolic panels (CMPs), electrocardiograms (ECGs), serology testing for anti-drug antibodies (ADAs), physical exams, and high-resolution MRI scans to identify amyloid-related imaging abnormalities (ARIAs) will be performed, and vital signs and adverse events (AEs) will be monitored.
Secondary Objective:	**Secondary Endpoint**
To test the hypothesis that sargramostim treatment, as compared to placebo, slows or halts cognitive decline, or improves cognitive functioning in individuals with mild-to-moderate AD.	Change in cognition from Baseline to End of Treatment (EOT) visit as measured by: • The Mini-Mental State Examination(MMSE)
Exploratory Objectives	**Exploratory Endpoints**
To assess the effect of sargramostim treatment, as compared to placebo, on clinical progression in individuals with mild-to-moderate AD.	Change in cognition from Baseline to EOT visit as measured by: • the change in the MoCA (Montreal Cognitive Assessment) score • the change in the ADAS-cog$_{13}$ (Alzheimer's Disease Assessment Scale-cognitive subscale) score • the change in the Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) score • the change in the Trail Making Test A (TMT A) score • the change in the ADCS-ADL (Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory) score • the change in the Neuropsychiatric Inventory Score (NPI) • the change in tests of Verbal Fluency (Semantic and Lexical) scores
To assess the effect of sargramostim treatment, as compared to placebo, on metabolic activity in different brain regions in individuals with mild-to-moderate AD.	Change from Baseline to EOT visit in FDG-PET assessment of metabolic activity, overall, as well in different brain regions, particularly in the medial temporal lobe in potential correlation with changes in MTA
To assess the effect of sargramostim treatment, as compared to placebo, on brain amyloid deposition in individuals with mild-to-moderate AD.	CSF assay will be performed examining Aβ(1-42) and tau:Aβ(1-42) ratios to test the hypothesis that sargramostim treatment of mild-to-moderate AD participants reduces cerebral amyloid load. If participant opts in, the CSF assay will be conducted as part of screening and at the end of treatment. Additionally, there is an option utilizing Amyloid PET scans, performed using Vizamyl, produced by the Pharmalogic cyclotron laboratory located adjacent to the CU Research Imaging Center (CU-RIC), which allows for amyloid-beta

	neuritic plaque density imaging. If the participant opts in, the PET scans will be performed during screening and at the follow-up visit, or within a week thereof depending upon scheduling availability, to assess for treatment-related changes in neuritic plaque density, as well as to correlate with any changes in tau:Aβ(1-42) ratios.
To assess the effect of sargramostim treatment, as compared to placebo, on AD related changes in peripheral blood and CSF biomarkers in individuals with mild-to-moderate AD.	Biomarkers from peripheral blood and CSF collection will include various forms of the hallmark proteins that form amyloid plaques in AD (i.e., amyloid-beta [Aβ] peptides), as well as other proteins whose levels are known to be altered in AD (e.g., phosphorylated Tau (p-Tau), Total Tau (t-Tau), and various AD- and inflammation-associated cytokines, chemokines, and other factors, for example, NfL, UCHL1, GFAP, IL-6, BDNF, etc.). Blood for these biomarker purposes will be collected at baseline, and at the EOT visit. CSF will be collected during screening, and at the EOT visit.
To assess the effect of sargramostim treatment, as compared to placebo, on AD related changes in blood-brain barrier permeability in individuals with mild-to-moderate AD.	CSF will be collected from participants choosing CSF as confirmation for inclusion, or as an optional substudy, during screening, and at the EOT visit. A venipuncture blood draw will be performed at the same visit of each LP procedures to obtain serum. Albumin levels will be quantitated within CSF (in mg/dL) and serum (in g/dL) to calculate an Albumin Index (AI). Because albumin is neither synthesized nor metabolized intrathecally, albumin that is found within CSF that is free of blood contamination indicates that the albumin must have come from the plasma through the blood-brain barrier (BBB). An AI value < 9 indicates an intact BBB, values 9-14 as slight impairment, values 14-30 as moderate impairment, values 30-100 as severe impairment, and values > 100 as complete breakdown of the BBB [72]. AI values will be calculated at screening and at EOT visit to assess any treatment-related changes in BBB permeability and to correlate values with any potential trial emergent ARIA events.
To assess the effect that ApoE genotype may have upon predicting sargramostim treatment outcomes in individuals with mild-to-moderate AD.	ApoE genotyping will be performed using blood samples collected at Baseline visit of this trial for each participant. ApoE genotype will be determined by UC Health lab using established methods. Outcomes in the context of ApoE genotype will be compared. The genetic testing will be carried out at the end of the trial from stored blood, and participants will not be notified of any genetic testing results.
To assess the effect of sargramostim treatment, as compared to placebo, on inhibiting MTA in individuals with mild-to-moderate AD.	Atrophy in the entorhinal cortex (ERC), parahippocampal gyrus, and hippocampus (HPC), measured on high-resolution MRI scans, predict future cognitive decline and conversion to AD among individuals with mild cognitive impairment (MCI). Severity of MTA, assessed with high-resolution MRI scans, is strongly associated with severity of medial temporal lobe degenerative pathology, especially the severity of neurofibrillary pathology at autopsy. High-resolution MRI scans will be performed at screening, EOT visit, (and among participants in the optional amyloid PET procedure, at follow-up (45 days post-treatment)).

To assess the effect of sargramostim treatment, as compared to placebo, on AD-related changes in microstructural white matter measures in individuals with mild-to-moderate AD.	Numerous neuroimaging studies have shown that white matter (WM) tracts are associated with cognitive function, and that microstructural abnormalities are found in AD and other neurodegenerative diseases, as determined from high-resolution MRI diffusion tensor imaging (DTI) techniques [73-77]. DTI metrics have been proposed as sensitive biomarkers for disease progression over short periods of time [78], which makes it useful for assessing any WM changes in response to treatments, and specific DTI patterns have been shown to help distinguish between AD and other comorbid neurodegenerative diseases [79, 80], to correlate with AD-related verbal fluency impairments [81], and with using neurite orientation dispersion and density imaging (NODDI) sequences of DTI, changes in neurite density can be approximated [82, 83]. High-resolution MRI with DTI and NODDI sequences will be performed at screening, weeks 6, 12, 18, and EOT, (and among participants in the optional amyloid PET procedure, at follow-up (45 days post-treatment)) and metrics of DTI (e.g. FA, MD, NDI, ODI, etc.) will be monitored for any treatment-related changes.
To assess the effect of sargramostim treatment, as compared to placebo, on changes in sleep-related measures in individuals with mild-to-moderate AD.	Changes in quality of sleep have been long reported in onset and progression of AD [84-86], and decreases in non-rapid eye movement (NREM) slow wave activity (SWA) is known to be associated with Aβ deposition [87]. Our preclinical work and others have revealed that GM-CSF quickly removes amyloid in AD models [1, 68], of which it is not known whether quality of sleep improves with reductions in amyloid. For assessing any changes in qualitative measures of sleep in participants, the participant and the study partner/caregiver interview will be given the ISI and PSQI assessments at baseline, weeks 12, EOT, and at follow-up (45 days post-treatment) visits to assess any changes in participant sleep-related measures. Mechanistically, melatonin and its signaling have been shown to be decreased in AD progression [88-92], and GM-CSF has also been reported to increase melatonin secretion in preclinical studies [93]. Melatonin is made and secreted by the pineal gland, and calcification of the pineal gland increases with AD progression [94-97]. High-resolution MRI scans, using susceptibility weighted imaging (SWI) sequence, will be performed at screening, weeks 3, 12, 18, EOT, (and among participants in the optional amyloid PET procedure, at follow-up (45 days post-treatment)) to assess changes in pineal calcification measurements.
To assess the effect of sargramostim treatment, as compared to placebo, on changes in speech measures in individuals with mild-to-moderate AD.	AD is known to adversely impact temporal characteristics of spontaneous speech, such as speech tempo, number of pauses in speech, and their length, with speech alterations often noticed years before other cognitive deficits become apparent [98, 99]. Among the neuropsychological measures frequently used to assess semantic memory impairment in AD are verbal fluency tests, or speeded word-list generation, which consists of two types: phonemic (letter) fluency tests and category fluency tests. Fluency performance involves multiple brain processes and regions that are adversely impacted by AD pathology, and both phonemic and category fluency performances reveal declines in AD [100, 101]. Word retrieval in connected speech is also impaired in AD, and by using picture naming and picture

	descriptions, the total word output, percentages of content words, percentages of nouns, and percentages of pronouns out of all words, type-token ratio of all words and type-token ratio of nouns alone, mean frequency of all words and mean frequency of nouns alone, and mean word length can be analyzed [102]. A study participant interview will be given at baseline, week 12, EOT, and at follow-up (45 days post-treatment) visits to assess changes in verbal fluency and word retrieval measures.

4.6 Benefits and Risks Assessment

4.6.1 Benefits

There is a tremendous unmet medical need for treatments to slow, halt, or reverse AD pathogenesis and cognitive decline. Animal models, as shown above, suggest that GM-CSF-treated Tg mice performed equally well or better than NT control mice during individual blocks and overall. The reversal of cognitive dysfunction and reduced cortical amyloidosis of GM-CSF-treated Tg mice were paralleled by increased synaptic area and microglial density as compared to saline-treated Tg mice, as well as increasing cognitive function in NT control mice [1], and of which effects have subsequently been replicated by others [68, 69].

In human preliminary clinical results, a retrospective analysis on data assessing cognition among bone marrow transplant patients, found that patients receiving GM-CSF plus G-CSF had improved cognitive function between baseline and at six and twelve months, as well as being significantly improved over G-CSF alone at 6 months [70].

Further, recombinant human GM-CSF (Leukine®/sargramostim) has been FDA-approved and safely used worldwide since 1991 for leukopenia, to shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections. Specifically, recombinant human GM-CSF (sargramostim) is a leukocyte growth factor indicated for use following induction chemotherapy in AML, for use in mobilization and following transplantation of autologous peripheral blood progenitor cells, for use in myeloid reconstitution after autologous bone marrow transplantation, for use in myeloid reconstitution after allogeneic bone marrow transplantation, and for use in bone marrow transplantation failure or engraftment delay. Any adverse events (AEs) associated with sargramostim are usually rare mild-to-moderate pyrogenic effects that subside upon reducing the dosage of sargramostim by half or by halting administration. Sargramostim has not been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness. In fact, a briefing package composed by Genzyme Corporation in May 2013 for an FDA Advisory Committee Meeting states that "*a large body of safety data exists with Leukine®, in both approved and investigational therapeutic settings, representing over 21 years of post-marketing experience*," that "*approximately 470,000 patients have received Leukine® treatment in the post-marketing setting from the time of product launch in March 1991 through December 2012*," and that "*the safety profile observed to date has been consistent and predictable across multiple indications and across special patient populations (healthy volunteers, pediatric and geriatric subjects)*" ([103], attached as Appendix D). As such, sargramostim has an excellent safety profile for use among many human populations, and current results from our ongoing clinical trial within mild-to-moderate AD participants (NCT01409915) have revealed no drug-related SAEs, including no incidence of ARIAs that have plagued all other anti-amyloid immunotherapy trials.

4.6.2 Risks

Potential risks for GM-CSF (sargramostim) include reported side effects including “first-dose effect," wherein the first dose of sargramostim may cause a person to experience low blood pressure, fast heart rate, flushing, lightheadedness or feeling faint, and tends not to happen with future doses. As well, some individuals have reported diarrhea, local reactions at the injection site (i.e. swelling, redness and/or tenderness), and feelings of weakness and fatigue. Less common potential side effects include mild flu-like syndrome (i.e. fever, headache, generalized aches and pains, weakness and fatigue) and swelling in hands and feet. A rare (<1%) side effect is a problem with blood clots. There is a potential risk related to immunosuppression (neutropenia or leukopenia) that may occur in select individuals taking sargramostim, which may increase risk of infection. There is also a risk of infection independent of low neutrophil count which could range from mild to life-threatening. This does not appear to have been previously reported in other use of sargramostim.

In addition to the potential drug-related risks, there are study procedure-related risks. All procedures have varying degrees of risk, ranging from very low risk (ECG) to higher risk (lumbar puncture), and are all standard clinical assessments. All procedures have limited associated risks for the participant and are described in the respective parts of this clinical study protocol (e.g. associated risk from low to higher - ECG, blood draw, MRI, and PET, lumbar puncture). Please see section 8.2 for more discussion on specific procedural risks.

An independent DSMB will be chartered for this study to review safety and other relevant data on an ongoing basis.

A placebo arm is necessary to allow for an accurate assessment of safety and tolerability, as well as the potential beneficial effect on cognition and function. Because there is currently limited treatments available for participants at risk for Alzheimer’s disease dementia, the use of a placebo is warranted. Initiation of approved symptomatic treatment is possible during the course of the study, if that is deemed necessary by the participant and the respective treating physician.

5 Study Design and Methods

5.1 Overall Design

The study is a Phase 2, double-blind, placebo-controlled study of sargramostim in the Alzheimer's disease population. It will include individuals diagnosed with mild AD or moderate AD (or meeting criteria consistent with a provisional research diagnosis), consistent with MoCA score range of 4-24 (137, 138,139)at time of screening and NIA-AA (2018) research criteria. The study includes a screening period of up to 12-weeks, a treatment period of 24-weeks, and an approximately 6-week follow-up. Individuals who meet inclusion/exclusion criteria will be randomized in a double-blind manner, in an approximate 2:1 randomization ratio to one of the following treatments:

- Sargramostim 178.57 μg/m^2/day subcutaneously (7 days per week) (This is adjustment from original 250 μg/m^2/day subcutaneously (5 days per week). Any participant on the original dosing schedule will be changed to the new dosing schedule the first refill after amendment is approved)
- Placebo equivalent volume subcutaneously (7 days per week).

The estimated maximum main study visits for each individual participant is approximately 55, including 4 screening visits (Tier .5-3), 48 treatment period visits (i.e. 4 in-person clinic visits, 20 at home visits, and 24 weekly blood draw at central laboratory for 2nd safety CBC w/diff), 1 End of Treatment visit, 1 FDG PET visit, and 1 visit in the post-treatment follow-up phase. Participants who opt to participate in the optional PET substudy or optional LP substudy would have 2 additional visits, 1 during screening, and 1 during either EOT (LP) or follow-up (PET). All participants will have the option of visits to happen at the main study site when the home nursing visit option is available.

5.1.1 Investigational Product:

Recombinant human GM-CSF is commercially available from Partner Therapeutics as Leukine®/sargramostim, which is FDA-approved for use in elderly patients (> 55 years old) and has a long and well documented safety record. The investigational product will be obtained by the study Sponsor for use in this clinical trial, and prepared by an unblinded pharmacist for administration to the randomized participant.

5.1.2 Justification for Dose

Recombinant human GM-CSF (Leukine®/sargramostim) is currently marketed in the U.S. by Partner Therapeutics. Leukine® has been FDA-approved since 1991 and safely used worldwide for leukopenia, in multiple stem cell transplantation settings, and following induction chemotherapy in patients 55 years and older with acute myelogenous leukemia (AML) to shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections. Specifically, sargramostim is a leukocyte growth factor indicated for use following induction chemotherapy in AML, for use in mobilization and following transplantation of autologous peripheral blood progenitor cells, for use in myeloid reconstitution after autologous bone marrow transplantation, for use in myeloid reconstitution after allogeneic bone marrow transplantation, and for use in bone marrow transplantation failure or engraftment delay ([104], attached as Appendix E). The FDA-approved recommended dosage is 250 μg/m^2/day SC for up to 42 days or until absolute neutrophil count (ANC) reaches 1,500 cells/mm^3 for three consecutive days. Although it is not possible to directly translate the dosage used in mice to that to be used in humans for cognitive enhancement, the dosage of GM-CSF administered to transgenic AD mice that resulted in cognitive improvement and amyloid plaque reduction was about twice the recommended dosage of sargramostim (~500 μg/m^2/day SC or ~167 μg/kg/day) for a total of 20 injections (cumulative amount per mouse = 100 μg). However, several clinical trials with sargramostim have shown no serious AEs at dosages of ≥500 μg/m^2/day [105-107], and the maximum amount of sargramostim that can safely be administered by single or multiple dose has yet to be determined. Doses up to 100 μg/kg/day (4,000 μg/m^2/day or 16 times the

recommended dose) were administered to four patients in a Phase I uncontrolled study by continuous intravenous infusion for 7 to 18 days and with WBC reaching up to 200,000 cells/mm^3. All AEs (dyspnea, malaise, nausea, fever, rash, sinus tachycardia, headache and chills) were reversible upon discontinuation of the drug, as discussed above ([104], attached as Appendix E). These and other results, from the approximately 470,000 patients that have received sargramostim during its post-marketing setting ([103]; attached as Appendix D), indicate that sargramostim has an extremely good safety profile.

Notably, the Moffitt Cancer Center study, described in the Preliminary Clinical Results section above, used the recommended FDA-approved dosage (250 μg/m^2/day SC) and found highly significant ($p < 0.01$) cognitive enhancement in patients treated with GM-CSF plus G-CSF compared to those treated with G-CSF alone ([70], attached as Appendix C). And, most importantly, in the recently completed pilot safety and efficacy trial (described above, NCT01409915) in which sargramostim (250 μg/m^2/day SC) was administered to mild-to-moderate AD participants for 15 days over a 19-day period, the randomized participants have completed the trial with no drug-related serious AEs, including no evidence of ARIAs. Therefore, randomized participants will be started at the full FDA-approved recommended sargramostim dose.

During the course of this clinical investigation, the study team has determined that full dosing at 250 μg/m^2/day SC for the full 6 month treatment period of the study may have unknown risks related to immune functioning of participants, and the decision was made to change the dosing schedule from all 24 weeks at 250 μg/m^2/day SC five days a week, to 178.57 μg/m^2/day SC seven days a week. This maintains total weekly dosing while reducing total daily dosing.

5.2 Recruitment of Participants

This phase 2 clinical trial of sargramostim in AD will recruit participants with mild-to-moderate AD. At CU-AMC, Individuals who are patients in the Memory Disorders Clinic at the University of Colorado Hospital (UCH), will be recruited by study personnel. Recruitment of participants will also take place within the larger community surrounding CU-AMC. To recruit from the community, study flyers will be posted on the CU-AMC campus and social media may be utilized. Additionally, Clinicaltrials.gov, the CU-AMC Clinical Trials Website, and sites such as TrialMatch.alz.org will be utilized. The recruitment communication will emphasize the voluntary nature of the research study, and the fact that declining participation will not alter clinical care. To help defray the costs of participation, participants will be informed that, if randomized, they will be receiving a $75.00 gift card at the end of the 24-week treatment period, even if they cannot or choose not to complete the study. They will also receive a $25.00 gift card after the follow-up visit (45-days post-treatment), for a subtotal of $100.00. Additionally, the participants will receive a $75.00 gift card after receiving each LP procedure, for a subtotal of $150.00, and a $125.00 gift card after undergoing each Amyloid PET procedure, for a subtotal of $250.00, which all add up to an overall trial total maximum of $500.00.

5.3 Screening Period (Screening Visit)

At the first contact for the Screening Visit, the study will be explained to the participant and the study partner and legally authorized representative (LAR) if applicable and a different individual than the study partner, by the study Principal Investigator or delegated sub-investigator. Informed consent must be obtained before any study procedures are conducted. The Screening Visit consists of four Tiers (0.5-3) of events (with an additional Tier for participant in optional amyloid PET substudy or LP substudy) that must be completed within 84 days (day -84 to day 0). The Screening study events and assessments, and their related Tiers, are shown in in the Schedule of Events section (Section 2). Informed consent discussion for Tier 0.5 procedures may occur remotely or in person, with the remote taking place via HIPAA-compliant platform, in which case e-consent will be obtained using 21 CFR Part 11 compliant version of eSignature system. If e-consent is not possible, potential participants may scan or fax the signed consent form to the study team, or send them a picture, and will be asked to bring the original signed version to the first in-

person study visit, should one occur. Decisional capacity will be assessed by a medically qualified and delegated investigator at all relevant time points (ie Tier .5 and Tier 1 of screening) using a standardized checklist.

5.3.1 Screening Procedures

Screening procedures are listed below and shown in Schedule of Events section (Section 2). Assessments and procedures used throughout the study are described in more detail in Section 8. If any results of the screening procedures warrant concern for the participant's well-being and/or additional care, the participant will either be referred to their appropriate medical provider or if urgent, escorted or transferred to the Emergency Department for follow-up care.

All mandatory Tier 0.5 screening procedures and assessments must be completed and evaluated by the PI/sub-I before any Tier 1 procedures and assessments are initiated. All Tier 1 screening procedures and assessments must be completed and evaluated by the PI/sub-I before any Tier 2 procedures and assessments are initiated. Similarly, all Tier 2 assessments and procedures (Screening MRI) must be completed and evaluated by the PI/sub-I before any Tier 3 procedures (Screening LP or Screening Amyloid PET). The optional amyloid PET or optional LP substudy procedures (Tier 4) will be only be conducted after results of the Tier 3 entry criteria are evaluated and the participant meets inclusion/exclusion criteria.

- **Tier 0.5**
  - Mini-Informed Consent
  - Demographics
  - Cognitive Health Review Battery*
    - *At study physician discretion, the cognitive health review battery can be minimized to included only the FAQ and DSRS based upon available medical history.
  - Optional (per PI/sub-I discretion for clarity on eligibility for screening) Blood draw for screening biomarker for Alzheimer's disease, such as plasma Aβ(42)/ Aβ(40) ratio or pTau 217 may be conducted

- **Tier 1**
  - Full Informed Consent
  - Physical and neurological exam
  - Montreal Cognitive Assessment (MoCA): After informed consent is obtained, the cognitive entry criteria, the MoCA, will be one of the first assessments or procedures, performed by a trained cognitive rater. Participants who do not meet the MoCA screening criteria are not to have any further screening procedures performed, and participants who qualify based on the MoCA entry criteria will then proceed to the remaining screening criteria.
  - Medical history review
  - Previous and concomitant medications
  - Vital signs (i.e. BP, HR, Temperature, Respirations) Blood pressure and pulse should be measured in the sitting position only. Refer to section 8.4.5.3 Data Monitoring Plan for acceptable BP parameters.
  - Height and weight
  - ECG
  - **Diagnostic assessment for mild-to-moderate Alzheimer's disease:** In the case of a potential participant who otherwise meets criteria for inclusion in the study, has a confirmed diagnosis of mild cognitive impairment, but does not have a confirmed diagnosis of mild or moderate AD, the PI or sub-I may conduct an evaluation as part of screening utilizing clinical interview and validated measures of functioning related to disease severity (such as the Functional Ability Questionnaire (FAQ) and/or the Dementia Severity Rating Scale (DSRS) to create a research provisional diagnosis of mild or moderate AD. NOTE: The PI or sub-I who conducts the

diagnostic assessment will meet with at least one additional study doctor or qualified sub-Investigator to arrive at consensus of the research provisional diagnosis.

- Safety evaluations (C-SSRS, GDS, GAD-7)
  - **Columbia Suicide Severity Rating Scale (C-SSRS):** The C-SSRS will be administered at the Screening Visit to assess psychological health. It was designed to capture the occurrence, severity and frequency of suicide related thoughts and behaviors during the defined assessment period. At screening, the C-SSRS version to be administered will be the "Baseline" version, with individuals who score at high risk for suicidality (positive response to items 4 and/or 5) will be excluded. If any results of the C-SSRS warrant concern for the participant's well-being, the participant will either be referred to their appropriate medical provider or if urgent, escorted or transferred to the UCH Emergency Department for follow-up care.
  - **Geriatric Depression Scale (GDS):** The GDS is a simple 30-item measure used to identify depression in elderly individuals. The simplicity of the GDS (questions are answered only by "yes" or "no") enables the scale to be used with ill or moderately cognitively impaired individuals. One point is assigned to each answer and the cumulative score is rated on a scoring grid. The grid sets a range of 0 to 9 as 'normal', 10 to 19 as 'mildly depressed', and 20 to 30 as 'severely depressed'.
  - **Generalized Anxiety Disorder (GAD)-7:** The GAD-7 is a simple seven item questionnaire used to identify anxiety levels in individuals. The GAD-7 is calculated by assigning scores of 0, 1, 2, and 3, to the response categories of "*not at all*," "*several days*," "*more than half the days*," and "*nearly every day*," respectively. GAD-7 total score for the seven items ranges from 0 to 21. Scores of 5, 10, and 15 represent cut-points for mild, moderate, and severe anxiety, respectively. Though designed primarily as a screening and severity measure for generalized anxiety disorder, the GAD-7 also has moderately good operating characteristics for three other common anxiety disorders: panic disorder, social anxiety disorder, and post-traumatic stress disorder.
- Screening Laboratory Assessments
  - Approximately 16 ml of blood will be drawn for a CBC with differential and CMP, PT INR, and for serology evaluation of treponema, HBsAg and anti-HCV, anti-HIV1, and anti-HIV2 antibodies.
  - The creatinine level from the CMP will be used to calculate the estimated GFR to assess kidney function if not provided by the central laboratory.
  - Urine for exclusionary substances.
  - For women who cannot confirm they are post-menopausal per clinician satisfaction, a urine pregnancy test will be performed.

- **Tier 2**
  - Screening MRI: A screening MRI will be performed as part of eligibility to assess for evidence of prior or existing neurological disease (e.g. stroke, microhemorrhage, cancer, etc.), to exclude other potential causes of dementia, and to assess for MTA. Aside from the Tier 3 procedure, the participant should have met all other screening inclusion criteria before the MRI is performed. The MRI scan will be reviewed by a Radiologist and then results and/or the scan will be reviewed by the Principal Investigator to determine eligibility. The PI will share any medically relevant information that might indicate a health risk with the participant and LAR that is in the MRI report.

- **Tier 3**
  -
  - Participant may choose either as entry criteria to confirm plasma Aβ(42)/ Aβ(40) ratio from Tier .5

- *Screening Lumbar Puncture (LP) for CSF Assay:* A lumbar puncture (LP) will be performed to obtain cerebrospinal fluid (CSF) and each participant's amyloid status will be evaluated at a central screening laboratory via CSF Aβ1-42 and p-tau:Aβ(1-42) ratios using validated assay methods demonstrating high sensitivity and specificity (e.g. Athena Diagnostics; ADmark® Phospho-Tau/Total-Tau/A Beta42). These assays will be performed at a central laboratory (i.e. Athena Diagnostics) with the p-tau:Aβ(1-42) ratio results being reported to the investigator for eligibility determination. The accepted reference values in non-AD individuals is Aβ(1-42) >1026 pg/mL and p-tau/Aβ(1-42) ratio ≤0.023. For inclusion criteria consistent with the presence of pathological changes associated with AD, the values for Aβ(1-42) should be <1026 pg/mL and the values for p-tau/Aβ(1-42) ratio ≥0.023. CSF will also be assessed for glucose and protein levels to ensure safety.
- Additionally at this Tier 3 visit, approximately 4ml of blood will be drawn at the same visit to be used in conjunction with the CSF to quantitate both serum and CSF albumin levels and calculate an albumin index (AI) for assessment of AD-related permeability changes in the blood brain barrier [108].
    - CSF total protein
    - CSF Total cell count
    - CSF and serum glucose
    - Extra CSF sample that is not used for these assays will be aliquoted and stored for biomarker assays (e.g. 5.3.1.1 below), which are dependent on the participant meeting all inclusion criteria and being enrolled into the trial. If the participant does not enroll into the trial, the extra CSF samples will be removed from storage, discarded, and not used for any biomarker assays, unless the participant consents for these samples to be banked and used in further research studies.
    - It is anticipated that results of the CSF assay will be available within the 84 day screening period. However, it will not be considered a protocol violation should the assay results not be available until after the 84 day screening period has concluded. It will be allowable for the screening period to be extended if the CSF assay results are not available within the 84 day screening window.
    - Vital signs including BP, pulse, respirations, and temperature will be collected prior to the LP procedure, if performed.

- *Amyloid PET scan*: An Amyloid PET scan will be performed using an FDA approved ligand Vizamyl, produced at Pharmalogic, which allows for amyloid-beta neuritic plaque density imaging, and will be used to explore the hypothesis that sargramostim treatment of individuals with mild-to-moderate AD reduces cerebral amyloid load. The Amyloid PET scans will be performed during the screening period and at the follow-up visit, or within a week thereof depending upon scheduling availability. The radiologists who read the MRI and PET imaging scans will remain blinded to the participant's identity and test article treatment group. Vizamyl is FDA approved for research and clinical use.

- **Tier 4 (Optional)**
    - After either the screening Amyloid PET scan or the LP results have been received, participants have an option to participate in an optional substudy for either LP or amyloid PET scan.
        - If receiving an LP for entry, and consenting to an optional Amyloid PET scan it will be conducted using Vizamyl, Pharmalogic, which allows for amyloid-beta neuritic plaque density imaging, and will be used to explore the hypothesis that sargramostim

treatment of individuals with mild-to-moderate AD reduces cerebral amyloid load and will follow the same schedule of events as listed for using the Amyloid PET used for entry criteria.

- If receiving an amyloid PET scan for entry, and consenting to an optional LP, it will follow the same schedule of events as listed for using the LP procedure used for entry criteria.

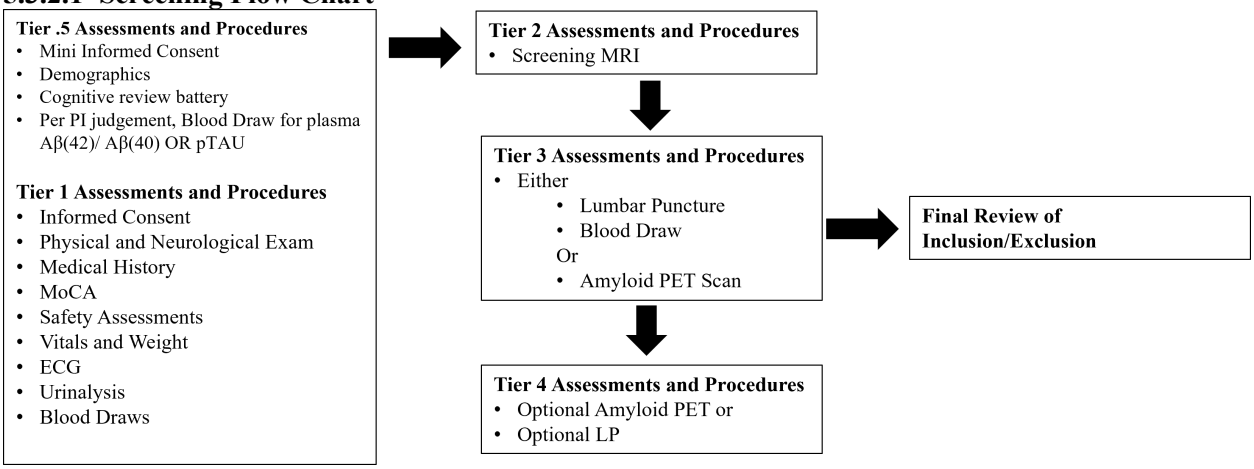
- Participation in, or result of, either the optional Amyloid PET or optional LP are **not** required for entry to the study.

5.3.2 Additional Screening Procedures and Considerations

Participants will also have to meet the additional inclusion/exclusion criteria (such as, but not limited to, stability on medications per appendix H). As well, in regards to hematologic values from screening visit labs, if screening ANC>70% or 7.7X10^3/mm3 or WBC>11,500/mm^3 and if this is sustained for > two weeks on recheck, a medical evaluation and clearance for underlying conditions, as well as return to normal prior limits, must be met prior to consideration for further enrollment procedures.)

To determine whether inclusion/exclusion criteria are met, a review committee consisting of the principal investigator or delegated sub-Investigator and the trial coordinator will review all screening data points. If all inclusion/exclusion criteria are met, with results reviewed by the principal investigator or qualified sub-investigator, the principal investigator or delegated sub-investigator will provide documented confirmation that the participant is eligible to be randomized. After that confirmation, the participant may complete their optional Tier 4 Screening Visit or Baseline Visit. More detailed information of this process can be found in a separate document, the Manual of Procedures.

5.3.2.1 Screening Flow Chart



5.4 Double Blind Treatment Period (Baseline Visit through Visit 24)

The treatment period is double-blinded, beginning at the Baseline Visit. At the Baseline Visit, appointments for all remaining visits should be made and scheduled as close as possible to the targeted date, based on the Baseline Visit. Visits done outside the allowable window will be considered a protocol deviation. Participants who meet the entry criteria for the clinical trial will be enrolled, randomized, and receive 24 weeks of treatment with either sargramostim or placebo.

The participant will follow the schedule of 7 days (Sunday through Saturday) of subcutaneous injection (178.57 μg/m^2/day sargramostim or placebo) over the course of 24 weeks, for a total maximum of 168

treatment days. The first injection will be administered at the time of baseline (randomization) visit in the site's designated facility. The participant and study partner will receive training and a schedule on how to administer additional doses at home. For the first 3 weeks, the participant will receive a weekly dose administered by their caregiver with observation by a study nurse for correct injection technique. After the first 3 weeks, the injection will be observed by a study nurse at in-person visits at the site (Wk 6, Wk 12, Wk 18). Ongoing injection technique education will be provided as needed. If the caregiver is unable to administer the in-person visit injection, the injection will instead be administered by a trained medical professional. The investigational product will be distributed weekly to the participant either at their in-person clinic visit (Baseline, week 6, week 12, week 18) or at-home visit for subsequent subcutaneous injection at home, along with a new injection diary. An empty sharps container will be provided at the baseline visit and be replaced as needed for participants/caregivers to place used syringes into at home.

At the weekly double-blind treatment period site visits (Visit 2- through Visit 24), delegated study personnel will review and record changes to conmeds, adverse events, compliance and vital signs. As well, qualified staff will review inject sites to assess for possible adverse reactions.

5.4.1 Double-Blind Treatment Procedures

All treatment visit administrative procedures, cognitive assessment, safety assessments and laboratory assessments are to be done before administration of the investigational product.

5.4.1.1 Baseline Visit Procedures

- Randomization
- Concomitant medication and AE review
- Physical/neurological exam
- Vital sign / weight
- Cognitive assessments and other measures
  Participant
    - Mini-Mental State Examination (MMSE)
    - Alzheimer's Disease Assessment Scale – cognitive subscales (ADAS-Cog_{13})
    - Trail Making Test-A (TMT-A)
    - Verbal Fluency (Lexical and Semantic Fluency)
    - MoCA
    - Clinical Dementia Rating Scale (CDR)
    - GDS
    - GAD-7
    - C-SSRS

  Study Partner
    - CDR
    - Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL)
    - Neuropsychiatric Inventory (NPI)
    - Sleep assessments (ISI, PSIQ)
- Laboratory assessments
- FDG PET
- IP administration training
- Administration of first dose
- Dispensing of IP

Note: It is acceptable to perform the required procedures of the baseline visit over more than one visit to accommodate the participants and study site scheduling.

At the Baseline Visit, the pharmacy will be contacted to perform participant randomization (this may be

done in advance of the visit to allow for study drug / placebo preparation by the pharmacy), a physical exam is performed, vital signs are recorded, approximately 52 ml of blood will be drawn for a CBC with differential, a CMP, ADA evaluation (anti-sargramostim antibodies), and for biomarker assays (e.g. NfL, UCHL1, GFAP, IL-6, BDNF, etc.). As well, a sample will be obtained and stored for later ApoE genotyping.

Baseline assessments of cognition will be conducted by trained personnel, including the, MMSE, MoCA, ADAS-cog$_{13}$, ADCS-ADL, Trail Making Test-A, CDR, NPI, and the verbal fluency measures (lexical and semantic). (Note that the qualified clinical rater who administers the CDR and ADCS-ADL will not be the same individual who administers the remainder of the cognitive assessment, thereby ensuring that CDR raters are blind to other clinical data.) Additionally, safety assessments, (i.e. C-SSRS, GDS and GAD-7) will be administered. More information about these testing assessment procedures can be found in a separate document, the Manual of Assessments.

The investigational product will be prepared by the unblinded pharmacist for acceptance by study personnel, with the initial dose administered by the study partner or designated caregiver after injection technique education by a study nurse with study nurse oversight, or by a study nurse if needed. Investigational product administration training will be given, with injection diary, to the participant and study partner. The remaining injections will be administered by or under the supervision of the study partner or other designated caregivers in the home setting until the next in-person visit. More detailed information can be found in a separate document, the Manual of Procedures.

5.4.2 Additional Double Blind Treatment Study Procedures

In addition to the regularly scheduled collection of vital signs, conmed and AE review, labs, cognitive assessments, imaging and a physical/neurological exam will be completed at visits 12 and EOT. More detailed information can be found in a separate document, the Manual of Procedures.

Pharmacy orders will be submitted using the most recent weight documented for each participant, and coordinators will ensure the weight is within 3-weeks of the order submission. If a new weight is documented after order submission and by noon the day before the pharmacy dispenses, the coordinator will verify if the weight has changed by more than 3%. If the change is greater than this amount, a new pharmacy order will be submitted with the updated weight. If the change is less than this amount, the order will proceed with the original submission.

5.4.2.1 Double-Blind Treatment Labs – Visit 2 through 24

Prior to investigational product administration at weekly visits (Visit 2 through Visit 24), either at-home visits or research site visits, qualified medical staff will draw blood for safety labs and metabolic functioning (approximately 8 ml for CBC with differential and CMP) with two CBC with differential and one CMP per study week. Additionally, prior to administration of investigational product, on specific visits (Visit 6, 12, 18 and 24), there will be an additional blood draw for ADA testing (approximately 9 ml). If there are outstanding clinically significant lab values after CMP during week 24, additional CMP lab draw(s) may take place to resolve these clinically significant values, or the participant referred to their health care provider for clinical care follow-up, based upon the judgement of the PI.

An additional, weekly, safety blood draw (i.e. CBC with differential) will be completed. Depending on the participant's preference, the participant can either visit the CU -AMC site for the blood draw or the participant can travel to a local contracted lab site and have the safety draw (i.e. central contracted laboratory with phlebotomy services, as specified in Manual of Procedures). Lab results will be forwarded to study staff for review by the study physician. The EOT and FU Visits will comprise of a safety blood draw CBC w/differential and other specified labs per the schedule of events).

Additionally, a PT INR will also be drawn at Visit 24 in preparation for review prior to the EOT.

5.4.2.2 Double-Blind Treatment Cognitive Assessment – Visits 12 and End of Treatment (EOT)

At Visit 12 and EOT Visit, Cognitive assessments will be conducted by trained personnel, including the MMSE, MoCA, ADAS-cog$_{13}$, ADCS-ADL, Trail Making Test-A, CDR, NPI, verbal fluency measures (lexical and semantic), , and sleep-related measures (i.e. ISI and PSQI). As mentioned above, there will be blinding between the cognitive and the clinical rater. As well, all attempts are to be made to schedule the assessments at approximately the same time of day, with the same rater to reduce variability.

Additionally, safety C-SSRS, GDS and GAD-7 assessments will be administered by qualified personnel at Visit 12 and EOT Visit.

5.4.2.3 Double-Blind Treatment Imaging

High-resolution MRI scans will be completed at Visit 6, Visit 12, Visit 18 and EOT Visit.

In addition to the baseline FDG PET Scan, an End of Treatment FDG PET Scan will be completed at EOT Visit.

5.4.2.4 EOT / Double-Blind Treatment blood draw and Lumbar Puncture (if applicable)

Occurring within 10 days, and at least 2 days following the final treatment day of week 24, a lumbar puncture will be conducted. Vital signs and weight will be collected at lumbar puncture visits. CSF for assays including Aβ(1-42) and tau:Aβ(1-42) ratios, total cell count, and for biomarker assays (e.g. NfL, UCHL1, GFAP, IL-6, BDNF, etc.) will be collected. At the same visit as the LP procedure has obtained CSF, a blood draw of approximately 44 ml will be obtained for biomarkers assays (e.g. NfL, UCHL1, GFAP, IL-6, BDNF, etc.). Additionally, both blood and CSF samples will be analyzed by UC Health to determine a serum/CSF albumin index.

5.5 Follow-up Visit

After the 24-week double-blind treatment period, all participants will be monitored during a 45-day follow-up period. At this visit, a physical/neurological exam will be performed, vital signs recorded, draw approximately 52 ml of blood for a CBC with differential, ADA analysis, and biomarker assays, and administer cognitive tests, including the MoCA, MMSE, ADAS-cog$_{13}$, the ADCS-ADL scale, Trail Making Test A, CDR, NPI, verbal fluency measures (lexical and semantic), and sleep-related measures (i.e. ISI, PSQI).

If the participant opted to engage in the Amyloid PET scan (either as entry criteria or optional), the PET will be performed using Vizamyl, and an MRI will be performed.

5.6 End of Treatment / End of Study Definitions

End of Treatment is the date of the last Visit or scheduled procedure for a participant, as listed in the Schedule of Events (Section 2)

End of Study is the date of the last Visit or scheduled procedure for the last participant randomized, as listed in the Schedule of Events (Section 2)

Every attempt should be made to follow participants through their final visit of the double-blind treatment phase and follow-up visit. If a participant discontinues the study prematurely for any reason, all reasonable attempts will be made to complete an Early Termination Visit, which will consist of the events listed for the End of Treatment Visit.

5.7 At-Home Visits

As noted above, when the option becomes available to participants, nurses, contracted through an accredited traveling or in-home nursing organization, will be utilized to provide at-home visits during the treatment period of the clinical trial. The nurse(s) will visit the participant's home one day per week (Monday through Friday, as their selected day as designated by study staff) during weeks 2–24 (excepting weeks with in-clinic visits, week 6, week 12, and week 18, as described above) to verify delivery/presence of the weekly doses of the study drug, record vital signs, perform injection sites review, record conmed changes, record adverse events, and draw blood. Participants will also be given the option to come to the study site for their weekly visits, rather than have home visits. Except under extenuating circumstances, participants will be discouraged from switching the location of the weekly visits.

5.7.1 Narrative Summary of Home Visits:

Each participant's **first injection** will be administered at the time of baseline (randomization) visit in the Clinical and Translational Research Center (CTRC) at the CU-AMC campus. As noted above, injections for the first 3 weeks, at in-person visits either at home, or in-clinic (select visits) will be administered by the study partner under observation by a trained (blinded) study nurse after training by study staff, and under observation at Wk 6, Wk 12, and Wk 18 on site study visits, with refresher training as necessary. At the Home visits (weeks 2-5, 7-11, 13-17, 19-24), the nurse will verify delivery of the study drug (by a contracted delivery service), collect vital signs, perform an injection site review, and record adverse events. As well, the nurse will perform a blood draw of approximately 4 mL for CBC plus differential on the day of each home visit for safety labs, and deliver to the contracted central laboratory.

5.7.2 Summary of Traveling Nurse Duties During at Home Visits:

- Verification of delivery study drug to home of participant
- Perform and record vital signs
- Injection sites review
- Record and monitor Adverse Events.
- Record conmed changes, if any
- Blood draw (CBC + Diff,) at each home visit;,; deliver blood samples to contracted laboratory drop-off site within two hours of collection.
- Oversee administration, by study partner, of study drug injection until Week 3 and as needed thereafter.
- Provide injection training refresher, as necessary.
- Return injection diary and all other study visit related paperwork to study staff.

5.7.3 Logistics Associated with Traveling Nurses:

Day before/day of visit: The study drug will be collected from the CU Outpatient Research Pharmacy by for delivery to the participants residence. The study drug syringes will be placed in hard cases, in an insulated bag with a cold pack for transport. Refrigeration required for drug: drug to be placed in the refrigerator at the participant's residence, with a recording thermometer (supplied by the study).

Day of Visit: Nurse will travel to participant's residence to perform the required duties (verify delivery of drug, injection site review, check vital signs, monitor adverse events, oversee injection of study drug by study partner as needed, draw blood, complete source documentation, injection site diary, drop off blood samples to lab). Submit data updates daily/ every-other-day (unless urgent) to study team.

At Home Visit Nurse requirements:

- Current nursing license with the State of Colorado (LPN or higher)
- Current driver's license with no outstanding citations.
- Reliable vehicle
- Coordinated back up from employer

5.7.4 Drug Handling Details (when performed by courier service for at home visits):

Study drug will be provided with a temperature monitoring device, which will record the temperature and record an excursion if the temperature is outside of the established range for the study drug when stored in the participant's home refrigerator. On the designated day for the home visit (or day before if able to be appropriately stored), the study drug will be picked up by an authorized staff member at CUAMC Research Pharmacy, and either deliver it to the participant's home, or delivery will pick up from AMC staff and deliver it to the participants home,and the study partner will placed in the refrigerator. During transit from CUAMC to the participant's home, the pre-filled syringes will be maintained in a temperature-controlled container in a hard case designed to prevent any inadvertent changes to the syringes. As well, a temperature-monitoring device will be placed in the container with the drug to ensure proper temperature exposure and provide a read-out of temperature reading if an excursion is noted. The temperature-monitoring device will be left in the participant refrigerator with the study drug and reviewed at in-person visits by the study team.

6 Study Population

6.1 Inclusion and Exclusion Criteria

6.1.1 Inclusion Criteria

All participants must meet the following criteria to be included into the study:

Inclusion Criteria	
	Participant Characteristics
1	Males or females between age 60 and 85 years, inclusive, at time of consent.
2	Have a dedicated partner/caregiver informant who is in the company of the participant at least 12 hours a week, who can accompany them to scheduled visits, and who is able to provide accurate reporting upon the behavioral, cognitive and functional abilities of the participant. Additionally, the study partner (or LAR or caregiver, if different) must be willing and able to administer the treatment injections. If a study partner must withdrawal from study participation, a replacement study partner may be allowed, at the investigators discretion. The replacement study partner, would need to agree to and sign a separate informed consent on the first visit that he/she accompanies the participant to participate.
3	Be physically able to participate by medical history, clinical exam, and other testing, with adequate visual acuity and auditory discrimination.
4	Be willing / able to provide written informed consent or assent. If assent is provided, consent must be provided by a legally authorized representative (LAR) or proxy decision maker, who may or may not be the dedicated study partner / caregiver. Documentation of LAR or proxy decision maker status will follow local laws and regulations. Note: Individuals utilizing a proxy decision maker will not be able to participate in optional sub-studies as a proxy decision maker cannot provide consent for optional non-therapeutic interventions.
5	Must reside within a proximity of the study site that will not preclude their regularly-scheduled participation in the trial, as well as a catchment area for local lab blood draws (i.e. central contracted laboratory).
	Disease Characteristics
6	Meet criteria for probable AD dementia according to the National Institute of Aging – Alzheimer's Association (NIA-AA) 2018 core research criteria[109], and have the following at screening: • A diagnosis of mild AD or moderate AD, or • A provisional research diagnosis consistent with probable mild AD or moderate AD, and • MoCA score of 4-24 inclusive.
7	Have positive biomarker for brain amyloid pathology as shown by:

	• (Optional, per PI judgment for clarification on appropriateness for screening) Positive plasma assay for AD biomarkers (such as Aβ(42)/ Aβ(40) ratio or pTau217) AND/OR • Either positive CSF assay for AD assessment (Aβ(1-42) is <1026 pg/mL, p-tau:Aβ(1-42) ratio is ≥0.023) or positive amyloid PET, per PI read.
8	If receiving anti-dementia treatment (i.e., cholinesterase inhibitor and/or Memantine), be on stable treatment for at least 60 days before initial screening visit and up to the baseline visit. Be stable on all other medications for at least 30 days prior to initial screening visit.
9	Have had a dental exam within 6 months of date of screening.

6.1.2 Exclusion Criteria

Individuals who meet any of the following criteria will be excluded from this study:

Exclusion Criteria	
	Participant Characteristics Exclusions
1	Individuals with a first degree relative diagnosed with AD before 55 years of age.
2	BMI ≥35.
3	Is unable to read/write at an appropriate level to reliably participate in clinical trial psychometric assessments.
4	Is a prisoner.
	Medical Condition Exclusions
5	Other neurological or psychiatric condition (other than AD) that can impact cognition, as well as atypical presentations of AD and AD related dementias, including logopenic primary progressive aphasia (PPA), or posterior cortical atrophy (PCA); or, CT/MRI evidence of potentially significant intracranial abnormalities not related to AD (e.g., evidence of major stroke or lacune in an area critical to cognition, infections, cancer, hydrocephalus, multiple sclerosis, etc.); or abnormal CSF not consistent with AD.
6	Presence of current, serious mood or anxiety disorder, and/or a psychotic disorder, and/or a substance-related disorder according to Diagnostic and Statistical Manual of Psychiatric Disorders, Edition IV, text revision (DSM-IV-TR) or DSM-V that, in the opinion of the Principal Investigator, might impact cognitive assessment, affect participants ability to complete the study, or confound interpretation of the study drug effect; or is considered suicidal or shows suicidal ideation as assessed by the study physician, including, but not limited to: • Endorsing positive answer to C-SSRS suicidal ideation (items 4 or 5) or is positive for any suicidal behaviors within 6 months of screening, or has been hospitalized or treated for suicidal behaviors within past 5 years before screening.

- Severe depression as indicated by a GDS score of 20 or higher.
- Severe anxiety as indicated by a GAD-7 score of 15 or higher.
- Recent history (within 2 years of Screening) of alcohol or substance abuse or dependence, as determined by DSM-V criteria and in the judgement of the investigator.
- Positive urine drug panel (due to non-prescription drug) at Screening, or use of cannabinoids (prescription or recreational).

7 History of unprovoked deep vein thrombosis, pulmonary embolism, familial predisposition for deep vein thrombosis, or pulmonary embolism, per study doctor judgement.

8 Active cancer / malignant neoplasm within 5 years of screening other than non-melanoma skin cancers (e.g. Basal cell or squamous cell). Previous diagnosis of Leukemia, despite remission state or length of time, is considered exclusionary.

9 History of a latex or yeast allergy.

10 Presence/history of drug hypersensitivity; or known hypersensitivity to sargramostim, yeast-derived products, any other component of the product, or benzyl alcohol (present in bacteriostatic water or saline for injection).

11 History of asplenia, hyposplenia, or splenectomy (for any indication).

12 History of, or treatment for, an autoimmune disease (e.g. Rheumatoid Arthritis, Multiple Sclerosis, Myasthenia Gravis, etc.).

13 Untreated or unstable medical condition that could interfere with the study assessments in the opinion of the study physician, or may require use of excluded medications or treatments as specified in Appendix H.

14 History of seizures (except infant febrile seizures).

15 Pregnant or breastfeeding female, or female of childbearing potential and not protected by highly effective contraceptive method of birth control (i.e., oral or depot contraceptives or intrauterine device (IUD) or participant was surgically sterilized) and/or unwilling or unable to be tested for pregnancy; Male refusing to use condoms, if partner can get pregnant.

Lumbar Puncture, MRI, PET, Vital Signs, ECG, Laboratory Tests and Physical Examination Exclusions

16 MRI evidence of >4 micro-hemorrhages; participants who may be prone to spontaneous ARIA-H and/or may be more susceptible to adverse effects of the ARIA-H.

17 Laboratory results that are, in the judgement of the investigator, indicative of an untreated medical or hematologic condition that could increase risk or interfere with study assessments, including untreated hypo- or hyperthyroidism, vitamin B12

deficiency, hyperleukocytic syndrome (including, but not restricted to, chronic myelogenous leukemia, Hodgkin and non-Hodgkin lymphoma), monoclonal gammopathy, and/or thrombocythemia. (Regarding hematologic values, if screening ANC>70% or $7.7X10^3$/mm3 or WBC>11,500/mm^3 and if this is sustained for > two weeks on recheck, a medical evaluation and clearance for underlying conditions, as well as return to normal prior limits, must be met prior to consideration for further enrollment procedures.)

18 Evidence of:

- Clinically significant pre-existing fluid retention (clinical or radiological);
- respiratory symptoms (e.g., dyspnea), moderate-to–severe lung disease (e.g. COPD, pulmonary infiltrates);
- cardiovascular symptoms or electrocardiographic evidence of cardiac disease that warrant therapeutic intervention (e.g., congestive heart failure, supraventricular arrhythmia, heart block, uncontrolled atrial fibrillation, etc.)
- a resting pulse less than 50, as reviewed by the study physician;
- prolonged QTc interval >470 ms in females, 450 ms in males).
- screening blood pressure measurement of greater than 160 systolic and/or 95 diastolic. If initial screening blood pressure is outside these parameters, BP will be taken in triplicate over the course of the visit with average BP to be evaluated by the delegated clinical sub-I.

19 Known renal dysfunction or serum creatinine >150 μmol/L, or Glomerular Filtration Rate (GFR) less than 55 ml/min.

20 Known hepatic dysfunction (apart from Gilbert's syndrome) or serum ALT ≥3 times the upper limit of normal (ULN).

21 Positive serology for hepatitis B surface antigen (HBs Ag), anti-hepatitis C virus (anti-HCV), anti-human immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti-HIV2 Ab) or spirochetal infection (e.g. syphilis).

23 Contraindication or inability to complete magnetic resonance imaging (e.g., cardiac pacemaker/defibrillator, ferromagnetic metal implants) or PET scan.

24 Sensitivity to fluorodeoxyglucose F 18

25 Having past or planned exposure to ionizing radiation that would, together with the radiation resulting from the administrations of the PET tracer(s) used in this study, exceed applicable institutional, local, or national recommendations for annual or lifetime exposure.

26 Poor venous access.

27 History of chronic or recurrent bacterial infections, at the discretion of the PI or delegated sub-I.

Prior / Concomitant Therapy Exclusions

28	Is taking any prohibited medication or therapy (see 7.7 Concomitant Therapy section and Appendix H).
	Prior / Concurrent Clinical Trial Experience
29	Be the recipient of an investigational drug within 60 days of screening, or within 5 times the elimination half-life of that drug, whichever is the longest.
30	Prior treatment with an investigational anti-amyloid or anti-tauopathy therapy, or AD vaccine, unless it can be documented that they were on placebo.
31	Participation in the treatment phase of an investigational sargramostim clinical trial within 6-months of screening.
32	Have previously withdrawn from this study (This exclusion does not apply to participants who are eligible to rescreen before randomization in this study)
	Other Exclusions
33	Any interested participant who: a. Is in the judgement of the Principal Investigator likely to be non-compliant with study protocol, including, but not limited to, leaving the area of the study for any extended period; or separate from the designated caregiver/informant, without acceptable replacement, for any of the scheduled assessment visits during the study. b. Is unable to cooperate because of a language problem or because of a developmental disability. c. Oversees or implements any aspect of the study, or is employed by Partner Therapeutics or its affiliates or subsidiaries, or is an employee of the University of Colorado Alzheimer's and Cognition Center and is engaged in the conduct of the study, or first degree relative of such.
34	Any technical/administrative reason that makes it impossible to randomize the participant in the study (e.g. kyphosis or other physical condition that prevents neuroimaging acquisition or other examinations, conflicts in participant schedules, etc.).

NOTE: Investigators will ensure that all study enrollment criteria have been met at screening. If a participant's status changes (including laboratory results or receipt of additional medical records) after screening but before the first dose of study drug is given such that he or she no longer meets all eligibility criteria, then the participant will be excluded from participation in the study.

6.2 Lifestyle Restrictions

1. Participants should refrain from donating blood or blood products from the time of the screening visit until 6 months following last dose of the study drug.
2. Participants should avoid excessive consumption of alcohol from screening visit until after follow-up visit is completed. Excessive consumption for this study is defined as more than 1 drink per day for female participants, or more than 2 drinks a day for male participants, on average.
3. Avoidance of products containing tetrahydrocannabinol (THC) and other recreational drugs.

6.3 Screen Failures

Screen failures are defined as participants who sign an informed consent to participate, but fail to meet eligibility criteria. A minimal set of data from screen fails is required to be maintained, including basic demographics, reason for screen failure, and any serious adverse events that occurred between the signing of the consent and time of screen failure.

Individuals who do not meet criteria for study participation (screen failure) may not be rescreened if the screen failure is due to non-eligibility due to plasma Aβ(42)/ Aβ(40) ratio assay, pTau217 assay, confirmatory CSF assay, or confirmatory amyloid PET scan. If the screen failure is due to not meeting the cognitive inclusion criteria (MoCA out of range), one rescreen may be allowed, at the investigators discretion, after 6 months if the MoCA score is above the cut-off, or if previous score is within range of amended MoCA range. If the screen failure is due to prohibited treatments (see Appendix H), or changes in medication, the individual may be rescreened if they subsequently discontinue use of the prohibited drug(s) and are then stable on all other medications for at least 30 days prior to rescreening, and previously collected data such as LP and/or MRI scans may be used for rescreening if the visit is within two months of said procedure. Other participants who fail screening procedures may be rescreened at the discretion of the investigator.

6.4 Withdrawal from Study

A withdrawal from the study shall be defined as withdrawing any time after being randomized into the double-blind treatment period, and before completing the events of the End of Treatment Visit. Participants who permanently discontinue use of the investigational product will be considered to be withdrawn from the study intervention, but may, if willing, complete other timepoint visits (such as EOT or F/U visits .

Participants may withdraw from the study at any time and for any reason. The investigator (or qualified designee) must document the reason for withdrawal in the participant file. If the participant withdraws consent for the continued use of data, biological or genetic samples, or imaging, the request must be received by the Sponsor, in writing, from the participant or legally authorized representative.

6.4.1 Withdrawal Procedures

If a participant prematurely discontinues treatment with the investigational product, the investigator must make every reasonable effort to perform the evaluations scheduled for the End of Treatment Visit as an Early Termination Visit. In the case where the participant permanently discontinues study medication between scheduled clinic visits, he/she should be requested to visit the clinic as soon as possible for the Early Termination Visit.

If a participant is lost to follow-up, every reasonable effort must be made by study personnel to contact the participant and study partner to inquire about the reason for discontinuation/withdrawal, and follow up with any unresolved AEs/SAEs. A minimum of 3 attempts at contact should be made, with all measures taken to contact the participant and information received during those attempts documented.

7 Treatments

7.1 Treatment Administered

The investigational product (IP) in this study is defined as recombinant human GM-CSF (sargramostim) administered at a dosage of 178.57 μg/m^2/day by subcutaneous injection and its matching placebo. Sargramostim is commercially available from Partner Therapeutics, and will be obtained by the study Sponsor for this clinical trial. The sargramostim injections and placebo injections will be prepared by an unblinded UCH pharmacist or other qualified pharmacy personnel delivered to the blinded staff at location of the clinical visit. The IP and placebo will indistinguishable from each other.

The investigational product for the double-blind treatment period will be supplied as individual syringes sufficient for weekly dosing until their next scheduled dosing visit. In-clinic and at-home visit injections will be administered by the study partner with observation by a blinded study nurse or qualified designee for appropriate injection technique for the first 3 weeks, and at on site visits (Wk 6, Wk 12, Wk 18), with ongoing education as needed. If the study partner is unable to administer the once weekly in-person dose, qualified personnel may administer. Home injection administration to be completed by trained study partner with take home syringes for home administration. The IP will be placed into a hard-case for travel and storage and then within a travel container that will be monitored and that is designed to ensure temperature compliance. In conjunction with the take home syringes, an empty sharps container will be provided for the take home syringes to be placed into after they have been administered each treatment day. If sharps containers are filled they may be returned to the site and exchanged for empty containers as needed, otherwise the sharps container should be retuned at the end of the treatment. This cycle will be repeated every week for the 24 week double-blind treatment period. Additional details of these procedures are found in the Manual of Procedures.

The investigational product is to be stored in the home refrigerator of the participant. A temperature monitor will be provided, which will be reviewed every week to check for extreme temperature excursions. At the end of each dosing period (the projected number of dosing days between deliveries), if there are unused doses due to IP being held or form not being administered for any other reason, the participant and study partner will be instructed to dispose of the syringes in the sharps container, even if they are not expired, and to continue dosing only with the newly delivered doses.

The investigator or qualified designee is responsible for the following:

- explaining and training of the correct storage and use of the investigational product to the participant and her/his legal representative and her/his study partner (if different from LAR).
- verifying that the instructions are being properly followed
- maintaining accurate records of all investigational product dispensing and compliance.

For dosing modifications or titration, please refer to Section 7.4.

7.2 Method of Treatment Assignment

Participants who meet all criteria for enrollment will be randomized into sargramostim or placebo groups at Baseline Visit, at a 2:1 ratio. This enrollment ratio was determined using the sample size power analysis, as well as to maximize recruitment and minimize attrition. The randomization schedule will be enacted and maintained at the CU Research Pharmacy (CURP), which has policies and procedures in place that describe randomization and blinding. Briefly, the pharmacy has built and implemented an Excel spreadsheet that produces random number sets that may be used for simple, weighted, block (two-, four-, or six-sized), and stratified randomization. For blinding, the pharmacy staff includes the pharmacist manager and technician. The pharmacy staff has been involved in several clinical trials that require double blinding, and double dummy. The pharmacy is an independent entity, and has the ability to ensure and maintain blinding

in any regard so that the staff at the clinical site will not know which study participants are receiving the study drug or the placebo.

7.3 Blinding

As this is a double-blind study, to preserve the blind, a minimal number of study administration personnel will have access to the randomization table and treatment assignments before the study is deemed complete. These individuals will be designated by the Sponsor, Huntington Potter, PhD, and kept in a log. The Data Safety Monitoring Board (DSMB) may be unblinded for safety evaluations or dose reduction decisions related to safety.

Additionally, preselected key personnel may have access to unblinded data to conduct pre-planned interim analysis for safety or for pre-planning activities for future clinical trials. Related, an external, independent Steering Committee will be established by the Sponsor to review data from the interim analysis to provide guidance on future trial planning.

Emergency unblinding for AEs may be performed through communication with the Investigational Pharmacy. This option can ONLY be used if the participant's well-being requires knowledge of the treatment assignment. The principal investigator holds the sole responsibility for determination of unblinding is warranted for medical management of the event. In such cases, the safety of the participant must always be the primary consideration for such determinations.

If an emergency unblinding occurs, the investigator must promptly document the decision and rationale, and notify the Sponsor and DSMB at earliest opportunity.

Decision to either discontinue the participant or continue with the study will rest with the Sponsor after consultation with the investigator and/or with the DSMB.

A list of all those who are unblinded to study treatment as the study progresses will be maintained and stored with the final documentation of the study.

7.4 Dosage Modification

Note: As the safety of the participant is paramount, the participants and their study partners will be told at the time of the informed consent that the study team will be monitoring their health and well-being through review of adverse events, laboratory results, and examinations performed during the trial. To help minimize the possibility of any unblinding of participants or study partners within any of these scenarios listed below, all participants will be informed at their time of consent that they may be asked to do one of three things if they are having adverse effects or abnormal laboratory results including: stopping treatment; receiving new, replacement syringes; or continuing treatment with the same syringes. Additionally, they will be told that the study team will continuously monitor their lab results, but they may not be told their specific results. Additionally, they will be informed that the trial physician or clinician designee will take the opportunity to examine them for clinical condition as a general safety measure if they are ever instructed to return to the CU-AMC site for an unscheduled visit.

The greatest safety concern noted with the investigational product is excessive leukocytosis. As advised by the product safety instructions, a CBC with differential, including examination for the presence of blast cells, will be competed twice weekly during the 24-week-long treatment phase. Additionally the product insert states that "***If the ANC exceeds 20,000 cells/mm^3 or if the platelet count exceeds 500,000/mm^3, LEUKINE administration should be interrupted or the dose reduced by half. The decision to reduce the dose or interrupt treatment should be based on the clinical condition of the patient***." If a participant has CBC with differential results which indicate that their ANC has exceeded 20,000 cells/mm^3, their white blood cell count has exceeded 50,000 cells/mm^3, or their platelet count has exceeded 500,000/mm^3, the participant and the study partner will be immediately contacted, asked whether the participant is experiencing any adverse health effects, AND instructed NOT to administer any additional study product

injections. Whether the participant is experiencing any adverse health effects or not, treatments will be interrupted until the following steps are completed and the Principal Investigator or qualified medical designee has determined whether to resume treatments at full dosage (178.57 μg/m^2/day) or 50% reduced dosage (89.29 μg/m^2/day):

- If there <u>are</u> adverse health effects occurring, the participant and study partner will be instructed to immediately hang up and dial 911 if deemed an emergency or, if not deemed an emergency, to as quickly as possible return to the CU-AMC site to be examined by the trial physician or qualified clinician designee AND to bring all unused study product/syringes with them. (Note: At the time of initial contact with the participant and/or study partner, the trial personnel have the capability to immediately contact 911 emergency services if they believe the participant needs immediate attention.)

  OR

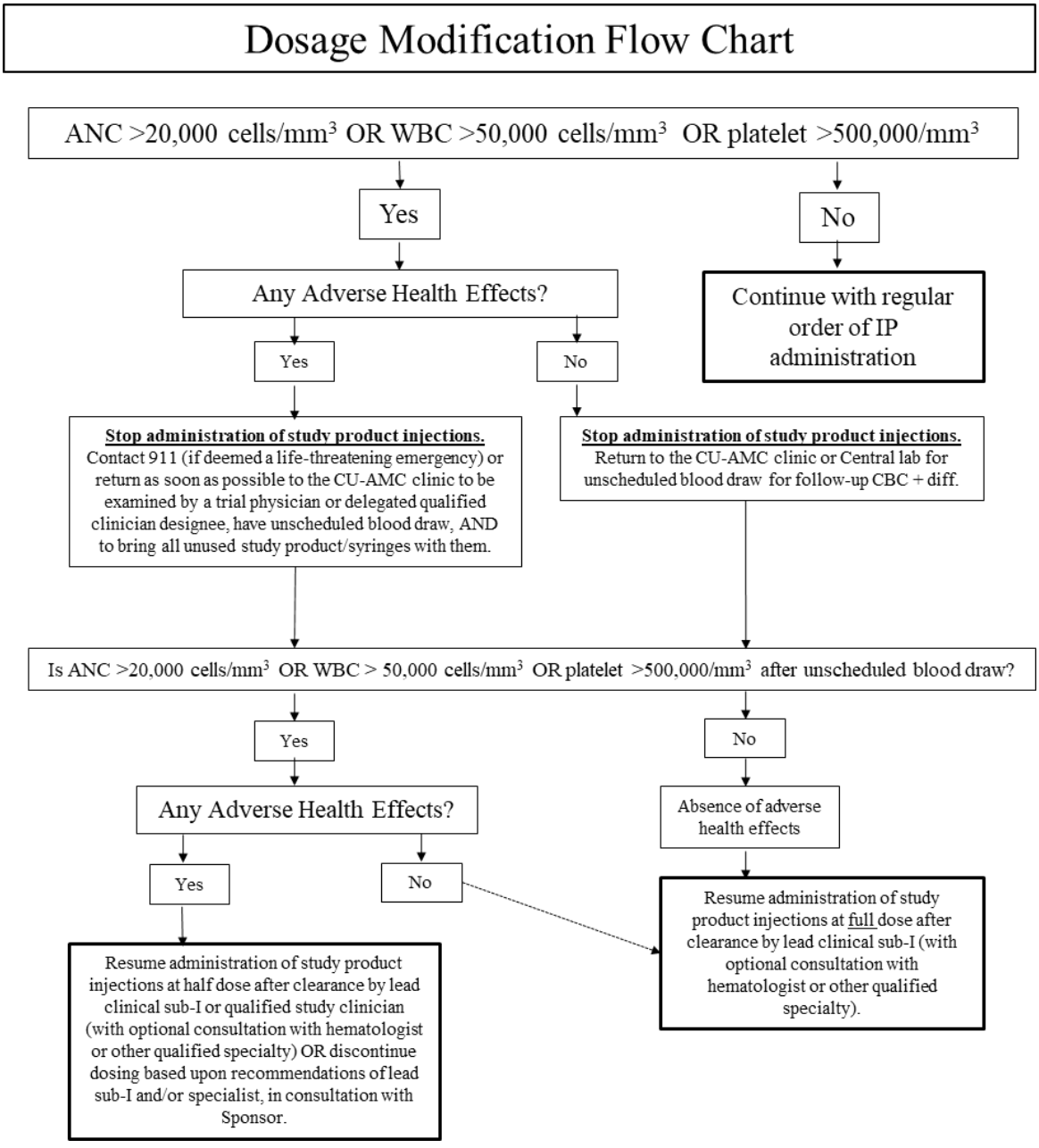
- If there <u>are not</u> any adverse effects occurring (other than the blood value), the participant and study partner will be instructed to, as soon as possible, either return to the CU-AMC clinic or to a central contracted laboratory with phlebotomy services, whichever is quickest, to have an unscheduled blood draw performed "*due to potentially abnormal or inconclusive lab results that need to be confirmed* (exact phrasing may vary as deemed necessary by study personnel in their best attempts to prevent any un-blinding to the participant and study partner)." At this unscheduled visit to the CU-AMC site or central contracted laboratory, a follow-up CBC with differential will be performed and results obtained as quickly as possible.
- The ensuing steps will be followed pending results of this follow-up CBC with differential:

    - If the results indicate that the ANC levels are now less than 20,000 cells/mm^3, white blood cell count is less than 50,000 cells/mm^3, and the platelet count is less than 500,000/mm^3, and if determined appropriate by the trial's lead clinical sub-Investigator or delegated study clinician, the participant and study partner will be contacted and instructed to resume any additional study product injections and actions according to their regular daily scheduled times, **NOT** to administer any of the syringes that were missed during the days in which the unscheduled CBC with differential was performed and analyzed, if out of date, and to return any used AND unused syringes as normally done for each treatment week when they have their next study visit.

      OR

    - Although rarely expected based upon the experiences of the completed Leukine-AD trial (NCT01409915), the dosage may be reduced by half (i.e. 89.29 μg/m^2/day subcutaneous injection) if the ANC exceeds 20,000 cells/mm^3, the white blood cell count exceeds 50,000 cells/mm^3, or the platelet count exceeds 500,000/mm^3 in the most recent CBC, and if the trial's delegated sub-Investigator has determined, perhaps in consultation with a hematologist, oncologist or other qualified designee of the Sponsor, that the clinical condition of the participant warrants changing the dosage or continued interruption of treatment. If the results from the unscheduled follow-up CBC with differential has indicated that the ANC levels are still greater than 20,000 cells/mm^3, and/or the white blood cell count is greater than 50,000 cells/mm^3, and/or the platelet count is more than 500,000/mm^3, the participant and the study partner will be immediately contacted again and instructed not to administer any additional study product injections AND to bring all unused and used study product/syringes to the clinic as soon as possible, so that they can

be assessed for clinical condition by a trial physician or qualified clinician designee AND to then potentially receive new syringes of the study product, which will be provided by the Research Pharmacy (prescribed by the lead clinical sub-Investigator) using procedures designed specifically for these types of unusual circumstances. In contacting and interacting with the study partner and participant for such a scenario, extreme care will be taken to not inadvertently unblind the participant, study partner, or any trial personnel. If unblinding is suspected, provisions described in prior section 7.3 will be followed and a decision will be made by the Sponsor regarding whether to discontinue the participant or to continue with the study.



In addition to these key blood values above, the responsible clinician will review the absolute eosinophil value, with a critical value, determined by the hematology specialist, set at ≥1.5x10E3/uL as a potential adverse event/clinically significant lab value. Management of this value will be as ongoing AE until the value returns to < 1.5x10E3/uL and is evaluated by the responsible clinician as not clinically significant. Once the AE is resolved, a subsequent occurrence of an absolute eosinophil ≥1.5x10E3/uL will be classified as a new AE. For each new adverse event due to absolute eosinophil values, a symptom check will be conducted using guidelines developed by the hematology specialist. If they are free of symptoms, injections will continue on the regular schedule. If the participant is positive for any symptoms, they will be instructed to hold the injections pending further follow-up by the responsible sub-I. Within each ongoing AE, symptom checks will occur each time the participant's absolute eosinophil count is above the previous high value for that AE.

7.5 Interruption of Treatment

Temporary interruption of treatment is allowed if a short-term treatment with an excluded medication is necessary, secondary to hospitalization, personal circumstances, or to evaluate the study drug impact on an uncertain AE, including but not limited to abnormal CBC with differential results (see Section 7.4), Grade 2 or higher AEs, and Grade 2 or higher abnormal laboratory values. Interruption of treatment should only be limited to significant events. If temporary interruption is due to an AE, it will be reported to the DSMB as part of the regularly scheduled aggregate reports. In such cases as above, reinitiation of treatment is at the trial physician(s) or qualified delegated clinician(s) judgement.

If a participant has to interrupt treatment for an extended period of time due to a significant medical condition or life event (such as greater than 7 dosing days off drug), it is possible to re-initiate treatment, and restarting the treatment will be at the trial physician's discretion, though it is suggested that if deemed necessary, consultation with the Sponsor may be done. Treatment re-initiation should occur as soon as possible and has to be confirmed by trial physician. Re-initiation of treatment will include a general safety examination by qualified medical personnel and safety labs. Restarting treatment after an interruption period that is greater than 10% of the trial length (i.e. 18 consecutive days) will be discussed between sponsor and trial physician before a final decision is made to re-initiate treatment at the discretion of the Sponsor. All information relating to interruption of treatment and re-initiation will be documented. Missed doses from the above category will not be considered in determining non-compliance.

7.5.1 Interruption of Treatment not related to short term treatment with excluded medication, hospitalization, personal circumstance or AE.

At the discretion of Principal Investigator, participants may request an interruption of treatment due to commitments such as travel obligations, for one block of time, up to 4 weeks in duration (28 dosage days). The study recording of interruption of treatment will allow for the addition of the time taken off to the study treatment period, up to the predetermined 28 dosage days. This interruption will not be factored into non-compliance, and a safety examination by study clinician is not required prior to resuming treatment.

7.6 Treatment Compliance

Compliance will be assessed at study visits via participant diary when the materials are returned at each weekly study visit at the clinic or by the traveling nurse if the weekly visit occurs at home. When possible, clinic injections will be performed by caregivers with delegated study staff observation for confirmation of correct injection technique. Ongoing injection technique education will be provided as needed. Delegated study staff will perform clinic injections as needed at visits if caregivers are unable to administer the investigational product or placebo. Non-compliance is generally defined as a willful disregard for following the treatment plan by the participant or study partner).

Only participants who use 90% (151 out of 168 administration days) of the prescribed daily dosage will be considered compliant (missed doses due to circumstances described in Sections 7.5 and 7.5.1 are not factored into treatment compliance). Participants deemed non-compliant may be, at the discretion of the

investigator, in consultation with the Sponsor, discontinued from the study. If a participant is deemed non-compliant within the first 3 weeks of the trial, a discussion at the next in-person clinical visit or via phone prior to or during the next in-person visit with the PI or sub-I or other delegated study staff and retraining on treatment administration and compliance will occur to attempt to increase compliance.

As stated above, reduction in dosage (i.e. reduction from 178.57 $\mu g/m^2$/day to 89.29 $\mu g/m^2$/day) OR doses withheld due to AEs or other factors relevant, as determined and approved by the PI, and Sponsor, will not be factored into non-compliance of a participant.

7.7 Concomitant Therapy

All concomitant medications taken during the study are to be recorded on the concomitant therapy log maintained by the study personnel. The participant and study partner will be asked at each weekly visit if there have changes to the concomitant medications, and if changes occur, they will be recorded and reviewed by the investigator.

Participants and/or their study partner will be instructed to consult the investigator or other qualified study personnel before initiation of new medications or changes to existing medications or supplements.

Use of approved standard of care medications for AD is permitted during the study with PI/Sponsor approval, as long as the duration meets the inclusion criteria, and there is no unapproved change through the double-blind treatment period. Participants and/or study partners are instructed to notify the study team as soon as they are aware of any medication changes. If unplanned initiation, change or stopping of standard of care medication occurs prior to the EOT Visit without express approval for continued participation okayed by the PI prior to medication changes, the Sponsor should be consulted to determine if the participant should continue in the study.

See Appendix H for list of prohibited concomitant therapies.

8 Study Assessments and Procedures

The Schedule of Events (Section 2) and Section 5 outline the study procedures and timing.

8.0 Screening Assessments (from Tier .5)

The Cognitive Health Review Information System Batter is Battery is designed around standardized diagnostic criteria and practice guidelines to identify common mimics of Alzheimer's disease, some of which can co-exist with Alzheimer's disease pathology. Questionnaires may include:

FAQ (Functional Activities Questionnaire)- Will be included
The FAQ is a well validated instrument used in both clinical and research settings to measure instrumental activities of daily living (iADL), providing useful information in determination of level of ADLs to help qualified professionals stage impairment related to dementia. It has been widely used as part of the National Alzheimer's Coordinating Center (NACC) longitudinal research initiatives.

DSRS (Dementia Severity Rating Scale) – Will be included
The DSRS is a functional evaluation tool that is a commonly used instrument in staging dementia, and utilizes a semi-structured interview format to collect information about an individual's ability to function in various domains, allowing a trained clinician stage the current severity of impairment displayed by an individual.

ECog (Everyday Assessment of Cognition) – At study physician discretion
The ECog is an carefully validated assessment of cognition filled out by a care partner that reflects basic mental functioning, and is widely used within clinical practice.

NPI Q (Neuropsychiatric Symptoms Questionnaire) - At study physician discretion
The NPI-Q is a study partner/caregiver self administered questionnaire to assess any changes in neuropsychiatric status in such domains as hallucinations, delusions, agitation, depression, anxiety, disinhibition, apathy and aberrant motor behaviors.

DCFS (Dementia Cognitive Fluctuation Scale) - At study physician discretion
The DCFS is a scale developed to distinguish levels of cognitive fluctuation among patients with dementia, and help provide distinguishing characteristics between various types of dementia. It is widely used in clinical trials and clinical practice.

Early screen from the Columbia Suicidality Scale - At study physician discretion
The early screen from the Columbia Suicidality scale is a study partner answered questionnaire about past and current suicidal ideation and behaviors. It is a validated and reliable scale that is widely used across clinical practice and clinical research trials.

ESS (Epworth Sleepiness Scale) - At study physician discretion
The ESS is a wall validated and widely used instrument across clinical practice and clinical research to identify issues with sleep and daytime sleepiness that often is apparent within dementia populations.

Lewy Body Composite Risk Score - At study physician discretion
The Lewy Body Composite Risk score is a 10 item questionnaire designed to capture signs and symptoms associated with Lewy Body pathology, and help distinguish from other forms of dementia. It is widely used across clinical practice and clinical trials.

RSMS-Ex (Revised Self-Monitoring Scale – Expressive Score) - At study physician discretion

The RSMS-Ex is a scale developed to detect socioemotional deficits in functioning, and effectively distinguish neurodegenerative diseases from psychiatric expressions that might be similar. It is widely used in clinical practice to aid in the diagnosis of such diseases.

Depending on study physician discretion, and potential answers given, additional questionnaires may include the full RSMS, Behavior Inhibition Scale, Interpersonal Reactivity Index, and Unified Parkinson's Disease Rating Scale–II (UPDRS-II) for diagnostic clarification, all instruments used within both clinical practice and clinical trials to gain more information regarding cognitive and functional abilities. The questionnaires are provided on a secure Qualtrics system, or RedCap (with the option for paper and pen if potential participant does not have access to a computer or internet access, and answers provided by caregivers. The questionnaires take, at maximum, approximately one hour to fill out.

8.1 Efficacy Assessments

Cognitive and clinical testing for each participant should be performed at approximately the same time on each assessment day to reduce potential variability. As previously noted, the CDR and the ADCS-ADL should be administered by a different rater than the cognitive scales (i.e. MoCA, MMSE, ADAS-Cog, TMT A, tests of verbal fluency), and all attempts made to maintain these two raters administering the same scales with the same participant throughout the study. The principal investigator holds the responsibilities for selection of the raters who will administer the scales, and verifying that they have met all training requirements.

On visits when administered, the cognitive and clinical assessments should be completed before medical procedures that may be stressful to the participant (for example blood draws, ECG), and that some procedures (such as MRI) can be conducted on other days within the visit window, if necessary.

MMSE (Mini-Mental State Exam)

The Folstein Mini-Mental State Exam is a brief psychometric instrument developed to assess cognitive function in elderly populations. It is a standard assessment used by all NIH Alzheimer's Disease Centers (ADCCs and ADRCs) to identify and monitor individuals with AD. The range for scores in the MMSE is from 0 to 30, with lower scores indicating greater impairment.

MoCA (Montreal Cognitive Assessment)

The MoCA is a brief, assessment developed for detection and tracking of cognitive impairment and is sensitive for detecting Alzheimer's disease. Measuring multiple domains, it is commonly used in both clinical and research settings, and is well validated, with a range of scores from 0-30.

ADAS-Cog$_{13}$ (Alzheimer's Disease Assessment Scale-cognitive subscale)

ADAS was designed to measure the severity of the most important symptoms of AD. Its subscale ADAS-Cog$_{13}$ is the most popular cognitive testing instrument used in clinical trials of nootropics (drugs or agents that improve cognitive function). It consists of 13 tasks measuring the disturbances of memory, language, praxis, attention and other cognitive abilities, which are often referred to as the core symptoms of AD.

ADCS-ADL (Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory)

ADCS-ADL is a caregiver/study partner rated questionnaire of 23 items, with possible scores over a range of 0–78, where 78 implies full functioning with no impairment. The ADCS-ADL assesses functional capacity across a wide spectrum of severity and will be the primary tool for collecting ADL data.

TRAIL MAKING TEST-A

Psychomotor speed will be assessed by the Trail Making Test-A**,** a timed test in which participants must connect a series of numbers randomly placed on a page.

CDR (Clinical Dementia Rating)
The CDR is a study partner/caregiver and participant based interview to assess changes in domains such as memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each domain is rated as 0 (no dementia), 0.5 (uncertain dementia), 1 (mild dementia), 2 (moderate dementia), or 3 (severe dementia). Further, the Sum of Boxes score (CDR-SB) shall be tallied for each administration of the instrument All raters in this trial will have completed the Washington University Alzheimer's Disease Research Center CDR Training.

Tests of Verbal Fluency
Tests of verbal fluency test are assessments where the participant is asked to produce, as fast as they can, all the words that can think of in 60 seconds. The two areas are Semantic fluency (producing words that belong in a category, such as animals, also known as category fluency) and Lexical fluency (producing words that start with a specific letter of the alphabet, also known as letter fluency). The more correct words that a participant produces, the higher the score.

Neuropsychiatric Inventory (NPI)
The NPI is a study partner/caregiver interview to assess any changes in neuropsychiatric status in such domains as hallucinations, delusions, agitation, depression, anxiety, disinhibition, apathy and aberrant motor behaviors.

Sleep Quality Assessments
Changes in quality of sleep have been well documented in onset and progression of AD [84-86]. The Pittsburgh Sleep Quality Index (PSQI) and Insomnia Severity Index (ISI), will be administered to both the participants and their partner/caregiver at Baseline, Week 12, EOT, and Follow-up (45 days post-treatment) visits to assess measures of sleep quality and any changes that may occur throughout the duration of the trial.

8.1.1 Functional Ability Assessments (if needed by study clinician for diagnostic clarity)

Functional Activities Questionnaire (FAQ)
The FAQ is a well validated instrument used in both clinical and research settings to measure instrumental activities of daily living (iADL), providing useful information in determination of level of ADLs to help qualified professionals stage impairment related to dementia. It has been widely used as part of the National Alzheimer's Coordinating Center (NACC) longitudinal research initiatives.

Dementia Severity Rating Scale (DSRS)
The DSRS is a functional evaluation tool that is a commonly used instrument in staging dementia, and utilizes a semi-structured interview format to collect information about an individual's ability to function in various domains, allowing a trained clinician stage the current severity of impairment displayed by an individual.

8.2 LABORATORY ASSESSMENTS:

During the Screening Visit, and at in-person (clinic or at home) research weekly visits (Baseline through Visit 24), phlebotomy will be performed by trained personnel of the UCH CTRC or other qualified study personnel, such as a contracted nurse. These qualified personnel may perform additional blood draws, if necessary, at unscheduled visits.

During the double-blind treatment period of the study, a second weekly blood draw / phlebotomy will be performed at a local central contracted laboratory site with phlebotomy services by trained staff. If preferred by the participant, the second weekly blood draw can be scheduled for the main study site (i.e. CU-AMC).

Drawing blood may cause mild pain at the site of needle insertion and occasionally local ecchymosis formation. With phlebotomy, rarely people may experience vasovagal reactions that are typically limited and resolve spontaneously. The total amount of blood (cumulative amount for research and clinical procedures) drawn will not exceed five percent of estimated total blood volume over a 24-hour time period.

Complete blood count (CBC) with differential and complete metabolic panel (CMP):
These labs will be processed by the central contracted laboratory (see Manual of Procedures). Blood samples will be delivered to a local contracted central laboratory drop –off location by designated staff personnel within an acceptable time frame to maintain viability of sample. The CBC and CMP results will be reported by the contracted central laboratory to the Study Coordinator or qualified designee and presented to qualified medical designee to evaluation.

Lumbar puncture
A lumbar puncture (LP or "spinal tap"), in which 25 to 30 ml (approximately 2 tablespoons) of spinal fluid is removed, will be performed for either entry criteria or as an optional sub-study. The LP procedure will use an atraumatic needle that is designed to reduce the risk of post-dural-puncture headache. The CSF will be used to measure a number of biomarkers, including amyloid and tau protein, which are markers for Alzheimer's disease (AD) progression, as well as for measuring albumin levels in correlation with blood serum albumin levels, in order to assess AD-associated changes in blood brain barrier permeability. The procedure will involve the insertion of a very thin needle into the participant's lower back in order to reach the spinal canal. The procedure will be done by a licensed medical provider and under sterile conditions using local anesthetic in the area in which the needle is inserted. The participant will rest for at least one half \ hour after this procedure. Each participant will be screened by history, and if there is suspicion of increased risk associated with the LP, additional assessment will be done before the procedure. Individuals on blood thinners including but not limited to: warfarin (Coumadin), rivaroxaban (Xarelto), apixaban (Eliquis), and dabigatran (Pradaxa) will be excluded from this procedure, and excluded from study entry. Those taking PRN regimens of ibuprofen, or other NSAIDs will be considered if they agree to stop said regiment for 7 days prior to the procedure. For those taking regimens of daily 81 mg aspirin, the dose should be held for 24 hours prior to the procedure. Additionally, individuals with history of spinal fusions (most often in the lumbar region) or other major back procedures will be excluded from the procedure unless imaging can be provided demonstrating that their anatomy will not increase the chances of an unsuccessful LP.

The following risks exist related to lumbar puncture: pain, bleeding, infection and headache. To limit discomfort, the skin, surrounding tissue and underlying muscle will be numbed with lidocaine. During the LP, participants may experience brief pain lasting seconds in the lower back during insertion of the needle, especially if the needle contacts bone. This occurs about 1 in 5 times. Less than 1 in 10 patients experience a brief pain shooting down the leg if the LP needle touches a nerve root. Less than 1 in 100 patients are allergic to the iodine solution used to clean their back and will develop rash. After LP, 5 to 15 out of 100 individuals develop a headache that is worse when standing and disappears when lying down. It typically begins 6-48 hours after the LP, and may last 1 to 6 days. The research team will instruct the participants to lie flat and take caffeine (via coffee consumption or via prescription caffeine sodium benzoate, according to PI or qualified designee discretion) if by chance this does occur. LP induced headaches may also be treated with oral hydration, analgesics such as acetaminophen, or ibuprofen and caffeine.

If a participant's headache persists for more than 7 days, an epidural blood patch may be advised for relief. Post-lumbar puncture headache is thought to be due to continued leakage of spinal fluid out of a hole made by the spinal needle in the membrane sac that holds the spinal fluid. An epidural blood patch involves injection of some of the patient's own blood in the area of the spinal puncture, with the local blood clot then 'plugging' this hole. This procedure is effective in eliminating the headache in about 90

percent of people with post spinal tap headache. The cost of this procedure would not be covered by the study.

Sharing of optional LP Information with Participant and/or Providers:
The study team does not plan to release (optional) LP results to study participants, as they are used for study analysis, not diagnostic or entry criteria.

8.3 Biomarkers

Whole blood and CSF samples will be received in the CU-AMC Neurology laboratory under the administration of the Sponsor, Huntington Potter, PhD, and processed as quickly as possible to maintain viability of the samples using SOPs that were developed to conform to NACC guidelines for processing Alzheimer's disease biospecimens. CSF samples will be centrifuged, any leukocyte pellets will be cryopreserved, and the CSF will be aliquoted into storage tubes. The whole blood samples that are collected in EDTA vacutainer tubes will be processed for storage aliquots of plasma, cryopreserved buffy coat, and erythrocytes, whereas whole blood samples collected in serum separator tubes (SSTs) will be processed only for aliquots of serum. All sample aliquots will be immediately placed for storage within -80ºC freezers or in liquid nitrogen storage within the University of Colorado Alzheimer's and Cognition Center (CUACC)'s human biorepository until samples are needed for testing. Testing of these biomarkers will occur using core facilities at CU-AMC (i.e. HIMSR and/or the Neurology Translational Core), and biomarker results will be analyzed by the trial's biostatistician, who has no direct contact with trial participants, and who will receive the treatment code from the UCH pharmacist. However, biomarker evaluation will only occur after completion of the trial or at predetermined interim analysis time-points, of which results would only be disclosed for select individuals, for example in pursuit of grants or other funding opportunities.

8.3.1 Serum Anti-Drug Antibody (ADA) Testing: Anti-GM-CSF Antibody

Serum samples collected from blood draws in visits at baseline, weeks 6, 12, 18, EOT, and follow-up will be screened using a bridging electrochemiluminescence assay for anti-sargramostim antibodies by a dedicated research laboratory associated with Partner Therapeutics. Each participant's baseline ADA values will be determined from serum that was obtained at their baseline time point blood draw. Any treatment-related ADA response (positive, negative, or inconclusive) will be assessed by comparing ADA values from the treatment phase visits and the follow-up visit to baseline values. All individual data, including rechecked values, will be recorded. Serum samples will be shipped in batches to the designated laboratory after a participant has completed all study visits.

On visit days that the participants receive their test article injection, the participants will be monitored by the study physician(s), or other clinicians or qualified study personnel after the test article injection for any symptoms of acute anaphylaxis reaction. Each participant's study partner will be trained for symptoms of anaphylaxis and for immediate emergency procedures that they must perform if anaphylaxis occurs, for the days in which the study partners are administering the test article to the participants at home. However, anaphylaxis reactions or any other SAEs due to ADA responses are not expected. In a recent clinical trial within participants having Parkinson's disease, full FDA-approved dosage of sargramostim was administered to participants for 56 consecutive days ([52]; attached in Appendix G) The authors stated that "*Serum anti-sargramostim antibodies were detected in the drug group by week 4 of treatment (visit 5), but diminished by week 8 (visit 7). Antibody levels were marginal at 4 weeks after drug cessation*," and no participants had to discontinue the trial due to ADA response.

8.3.2 MRI

Atrophy in the entorhinal cortex (ERC), parahippocampal gyrus, and hippocampus (HPC), measured on MRI scans, predicts future cognitive decline and conversion among individuals with mild cognitive impairment (MCI) to AD [110, 111]. Severity of MTA, assessed with MRI scans, is strongly associated with

severity of medial temporal lobe degenerative pathology, especially the severity of neurofibrillary pathology, at autopsy [112]. Because of the high prevalence of AD in the elderly, 85–90% of all degenerative pathology in the medial temporal lobe, either alone or in combination with other diseases, is AD pathology [113].

An automated rating system to grade the severity of atrophy in the entire medial temporal region (MTA) will be employed using the FreeSurfer software automated rating system of individual medial temporal structures, i.e., the HPC, ERC, parahippocampal gyrus and perirhinal cortex (PRC), which are specific regions of interest (ROIs) in AD pathogenesis. It has been shown that these individual and summed MTA scores distinguish AD and acute MCI individuals from normal elderly control individuals and predict progression from acute MCI to dementia/AD [114, 115].

Numerous neuroimaging studies have shown that white matter (WM) tracts are associated with cognitive function, and that microstructural abnormalities are found in AD and other neurodegenerative diseases, as determined from high-resolution MRI diffusion tensor imaging (DTI) techniques [73-77]. DTI metrics have been proposed as sensitive biomarkers for disease progression over short periods of time [78], which makes it useful for assessing any WM changes in response to treatments, and specific DTI patterns have been shown to help distinguish between AD and other comorbid neurodegenerative diseases [79, 80], to correlate with AD-related verbal fluency impairments [81], and with using neurite orientation dispersion and density imaging (NODDI) sequences of DTI, changes in neurite density can be approximated [82, 83]. Because GM-CSF has been shown to be a growth factor for oligodendrocytes [116], induce oligodendrocyte progenitor cells concurrent with amelioration of experimental spinal cord injury [34, 117], and increase synaptic area concurrent with reversing cognitive impairment and pathology in transgenic AD models [1, 68], high-resolution MRI with DTI and NODDI sequences will be performed at screening, weeks 6, 12, 18, EOT, and among participants of the optional amyloid PET procedure, at follow-up (45 days post-treatment) and metrics of DTI (e.g. FA, MD, NDI, ODI, etc.) will be monitored for any treatment-related changes.

Melatonin and its signaling have been shown to be decreased in AD progression [88-92], and GM-CSF has also been reported to increase melatonin secretion in preclinical studies [93]. Melatonin is made and secreted by the pineal gland, and calcification of the pineal gland increases with AD progression [94-97]. High-resolution MRI scans, using susceptibility weighted imaging (SWI) sequence, will be performed at screening and EOT to assess changes in pineal calcification measurements.

Brain MRI scans will be obtained on a 3.0-tesla MRI machine using proprietary three-dimensional magnetization-prepared rapid-acquisition gradient echo or the three-dimensional spoiled gradient recalled echo sequences; MRI scans will be acquired with isotropic resolution, and contiguous slices with thickness of 1.5 mm or less will be reconstructed. Multiple MRI scans without contrast are safe as they do not involve exposure to radiation. Some individuals may have difficulty lying motionless during the scan or may experience anxiety while in the scanner. These issues can be mitigated by administering a sedative, prescribed by the study physician(s), prior to scanning. If necessary for the procedure, prescription/use of the prescription (e.g. lorazepam) will be documented by the study team, and the study will reimburse the participant for the cost of the prescription. If such a prescribed drug is used in the conduct of an MRI, the participant will need a driver (friend or family) to provide transportation for the visit. As well, any risks associated with the administration or use will be described to the study participant.

Brain MRI scans will be performed in Building 400 in the BIC or in Biosciences 3 on the CU-AMC campus, which is part of the AMC's comprehensive CU-Research Imaging Center. A separate Radiologist, blinded to participant identity and test article group, will examine each MRI scan for any evidence of ARIAs (i.e. vasogenic edema and microhemorrhage), and results, including any incidental findings, will be reported to the Study Coordinator and Study Physician. Another Radiologist or designate, also blinded to participant identity and test article group, will perform the FreeSurfer analyses.

Brain MRIs will be conducted at the Screening Visit, and Visit 6, 12, 18, EOT and, for individuals participating in the optional amyloid PET, at Follow-up.

8.3.3 PET

Fluorodeoxyglucose F18 (FDG)

The FDG PET procedure will utilize the CU-RIC research PET scanner for human imaging studies. The Philips Gemini 64TF PET/CT imaging system is located in Biosciences 3 on the Anschutz Medical Campus at the University of Colorado. An additional GE Signa PET/MR imaging system is also located within the CU-RIC for use as necessary.

Fluorodeoxyglucose F18 (FDG) is an FDA approved radiotracer that has been used extensively to evaluate glucose metabolism within many organs within the body, and in relation to the brain, FDG PET has been at the forefront of functional neuroimaging over the past 3 decades, used to measure local cerebral metabolic rate of glucose that is indicative of synaptic activity in the brain, and contributing to the understanding of cognitive functions in healthy humans and how these functional patterns change with cognitive alterations. FDG has also been approved by the FDA for the identification of regions in human brain of abnormal glucose metabolism associated with foci of epileptic seizures, as well as being used as a useful biomarker of the regional reductions in glucose metabolism associated with the different forms of dementia. Evaluation of regional brain metabolism with FDG, might also prove useful or necessary to make a diagnosis, particularly in difficult or atypical cases. Perhaps FDG's best use may be in combination with other neuroimaging modalities, such as with structural MRI (sMRI) analyses and in relation to AD, to help predict conversion of mild cognitive impairment (MCI) to AD. The FDG PET scans will be performed at baseline and at EOT Visit.

FDG PET Imaging Procedure

Prior to the visit, participants will be asked to abstain from food or drinks for 4 hours before the procedure. Each study visit will take approximately 2 hours. A 5-10 mCi intravenous injection of fluorodeoxyglucose F18 will be administered. After the fluorodeoxyglucose F18 dose is injected, a 3-5 minute head CT scan will be performed to aid in alignment and calibration, and adjust for attenuation effects. Dynamic images will be acquired for approximately 20-30 minutes. The IV catheter will be removed at the conclusion of the scan and materials will be disposed of in biohazard containers or decay chambers until they can be safely disposed.

Indications and Usage

FDG is a PET radiopharmaceutical that was first administered to human normal volunteers in 1976, and that has since been used in oncology imaging since the 1980s, in order to determine abnormal glucose metabolism and to assist in evaluating malignancy in patients with abnormalities found by other testing modalities or in patients with an existing diagnosis of cancer. FDG is indicated in PET for diagnostic use in patients for the evaluation of pulmonary nodules to distinguish benign from malignant and the evaluation of non-small cell and small cell lung cancers for staging and restaging and for the evaluation of colorectal cancer for recurrence, restaging, and distant metastases. For lung cancer evaluation, certain thoracic area non-cancerous lesions may show FDG uptake including acute and chronic infections (such as abscesses, tuberculosis and histoplasmosis), and inflammatory / granulomatous conditions (such as sarcoidosis, bronchiectasis, or post radiotherapy sites) that could mimic tumor accumulation. Absent or less intense relative uptake of FDG may be observed in specific lesions including bronchoalveolar, mucinous and lobular carcinoma as well as carcinoid and fibroadenoma sites. For colorectal cancer evaluation, certain abdominal / pelvic area non-cancerous lesions may show FDG uptake including sites of post radiation or post-surgical inflammatory response, lesion site flare following chemotherapy, colonic adenomas and bladder diverticula that could mimic tumor accumulation. Absent or less intense relative uptake of FDG may be observed in specific lesions including mucinous carcinoma. Lesion size may also affect detectability

based on relative FDG accumulation and PET imaging system resolution, as it has been shown that FDG PET imaging may have a lower sensitivity in evaluating lesion sizes of less than 1 cm.

Mechanism of Action
Fluorodeoxyglucose F 18 is a glucose analog that concentrates in cells that rely upon glucose as an energy source, or in cells whose dependence on glucose increases under pathophysiological conditions. Fluorodeoxyglucose F 18 is transported through the cell membrane by facilitative glucose transporter proteins and is phosphorylated within the cell to [18F] FDG-6-phosphate by the enzyme hexokinase. Once phosphorylated it cannot exit until it is dephosphorylated by glucose-6-phosphatase. Therefore, within a given tissue or pathophysiological process, the retention and clearance of Fluorodeoxyglucose F 18 reflect a balance involving glucose transporter, hexokinase and glucose-6-phosphatase activities. When allowance is made for the kinetic differences between glucose and Fluorodeoxyglucose F 18 transport and phosphorylation (expressed as the "lumped constant" ratio), Fluorodeoxyglucose F 18 is used to assess glucose metabolism. In comparison to background activity of the specific organ or tissue type, regions of decreased or absent uptake of Fluorodeoxyglucose F 18 reflect the decrease or absence of glucose metabolism. Regions of increased uptake of Fluorodeoxyglucose F 18 reflect greater than normal rates of glucose metabolism.

Radiation Exposure
Any exposure to radiation carries a very small risk of causing damage to tissues and the possibility of triggering a new cancer. However, the amount of radiation that each participant will be exposed to per (Fluorodeoxyglucose F18) PET scan is similar to the amount received from natural sources, such as the sun (background radiation) over the course of living in Denver for approximately 1.5 years. The FDG PET imaging will be performed at the Baseline visit and at EOT Visit. The administered dose of (Fluorodeoxyglucose F18) will be 5-10 mCi. For a (Fluorodeoxyglucose F18) dose of 10-15 mCi (185-370 MBq), the expected whole body effective dose is 6.3 mSv. In addition, the effective dose for a low dose head CT performed for localization and attenuation correction as part of the standard brain PET/CT protocol is 0.6 mSv. Thus, it is expected the trial participants will be exposed to about 7 mSv per fluorodeoxyglucose F18 scan, and when added to expected radiation exposure from flutemetamol F^{18} (Vizamyl, Optional), will remain well below the 50 mSv maximum annual dose of radiation exposure. On the pre-screening medical history form, participants and/or the study partner/caregiver will be asked to provide information whether the participant has been exposed to radiation during the past year (e.g. prior X-ray, CT, PET scans, etc.), so that the Study Physician and/or the Nuclear Medicine Imaging Professional can determine whether there will be a risk of the participant exceeding maximum allowed levels of radiation exposure.

Amyloid PET
The amyloid PET scan, for either entry criteria or as an optional sub-study, will use a common radiotracer (such as flutemetamol F^{18} (Vizamyl) which is a commonly used radiotracer that binds to β-amyloid plaques in the brain, as a biological marker of Alzheimer's disease. For evidence of β-amyloid neuritic plaques, if a participant opts to take part in the sub-study, they will receive a screening \ PET scan before the baseline visit, and a follow-up scan at the post-treatment follow-up visit, that will be assessed by a Nuclear Medicine Radiologist within the University of Colorado School of Medicine. The Nuclear Medicine Radiologist will remain blinded to participant identity and randomized test article group, and qualitative results, including any incidental findings, will reported to the Study Coordinator and Study Physician or qualified designee. If the PET scan is performed on the same day as the MRI scan, the PET scan will be performed after the MRI scan. The PET scans will be performed at an outpatient visit at the CU-RIC at CU-AMC. For quantification of the brain amyloid load, assessments will evaluate the screening PET scan before the baseline visit and another PET scan within +/- 7 days of the Follow-Up Visit approximately 45-days after the end of treatment with sargramostim or placebo. The change from the screening PET scan's SUVR to follow-up SUVR will be analyzed by a qualified nuclear medicine radiologist within the University of Colorado Hospital - Department of Radiology or by a contracted central reading facility (i.e., BioClinica, a

specialty clinical trial services provider) using the participant's MRI to reduce white matter contamination, thereby increasing the SUVR signal change by eliminating counts in the region that do not change (white matter). The amyloid PET scans and accompanying MRI scans will be stripped of any personal identifying information, containing only the trial's randomized ID number and date. The results of the PET quantitative analyses will not be used to make any treatment decisions.

Amyloid PET Imaging
The amyloid PET procedure will utilize the CU-RIC research PET scanner for human imaging studies. The Philips Gemini 64TF imaging system is located in Biosciences 3 building on the Anschutz Medical Campus at the University of Colorado.

Each study amyloid PET will take approximately 2 hours. A preliminary, 3-5 minute head CT scan will precede the administration of the radiotracer to aid in alignment and calibration, and adjust for attenuation effects. Immediately following, the participant will be comfortably positioned in a chair. An intravenous catheter will be inserted using standard aseptic technique. A 5 mCi (Vizamyl) intravenous injection will be administered. The participant will be placed on the scanning table and moved into the scanner. Dynamic images will be acquired for approximately 70 minutes. The IV catheter will be removed at the conclusion of the study and materials will be disposed of in biohazard containers or decay chambers until they can be safely disposed.

Indications and Usage
Vizamyl (flutemetamol F^{18}) is a radioactive imaging agent for Positron Emission Tomography (PET) imaging of the brain to estimate β-amyloid neuritic plaque density in adult patients with cognitive impairment who are being evaluated for Alzheimer's Disease (AD) and other causes of cognitive decline. A negative amyloid scan indicates sparse to no neuritic plaques, and is inconsistent with a neuropathological diagnosis of AD at the time of image acquisition; a negative scan result reduces the likelihood that a patient's cognitive impairment is due to AD. A positive amyloid scan indicates moderate to frequent amyloid neuritic plaques; neuropathological examination has shown this amount of amyloid neuritic plaque is present in patients with AD, but may also be present in patients with other types of neurologic conditions as well as older people with normal cognition. The use radiotracers such as Vizamyl has become a widely accepted amyloid imaging method in human subject research. Vizamyl received initial U.S. approval for use in research in 2013 and is in wide using in clinical trials and clinical practice.

Mechanism of Action
The radiotracer Vizamyl (flutemetamol F^{18}) is a F^{18} -labeled positron emission tomography (PET) imaging agent that binds with high affinity to the amyloid-β (Aβ) peptide fibrils that constitute amyloid plaques, and maps on to amyloid deposition in post-mortem studies [119]. Vizamyl contains flutemetamol F 18, a molecular imaging agent that binds to β-amyloid aggregates and is intended for use with PET imaging of the brain. Chemically, flutemetamol F 18, is described as 2-[3-[18F]fluoro-4-(methylamino) phenyl]-6-benzothiazolol. It has the molecular formula C H 18FN OS. .

Radiation Exposure
Any exposure to radiation carries a very small risk of causing damage to tissues and the possibility of triggering a new cancer. However, the amount of radiation that each participant will be exposed to per PET scan is similar to the amount received from natural sources, such as the sun (background radiation) over the course of living in Denver for approximately 1-2 years for Vizamyl. The optional amyloid PET imaging will occur at the Screening Visit and at the Follow-Up visit, or within a week thereof, depending upon scheduling availability. Administered dose of Vizamyl will be 5 mCi. For a Vizamyl dose of 5 mCi (185 MBq), the expected whole body effective dose is 5.92 mSv. In addition, the effective dose for a low dose head CT performed for localization and attenuation correction as part of the standard brain PET/CT protocol is 0.6 mSv. Thus, with two scans, it is expected the trial participants to be exposed to about 13 mSv

(Vizamyl) and when added to expected radiation exposure from FDG-PET, will remain well below the 50 mSv maximum annual dose of radiation exposure. On the pre-screening medical history form, participants and/or the participant's study partner/caregiver will be asked to provide information whether the participant has been exposed to radiation during the past year (e.g. prior X-ray, CT, PET scans, etc.), so that the Study Physician and/or the Nuclear Medicine Imaging Professional can determine whether there will be a risk of the participant exceeding maximum allowed levels of radiation exposure.

PET Safety Monitoring
All participants will be monitored carefully from injection until the conclusion of the imaging study. The PI and/or qualified designee will be available to consult if any questions arise during the PET scan. The research coordinator and Nuclear Medicine Technologist will be present through the entire duration of the PET scan, consistent with CU-RIC PET center guidelines. Each study participant will be contacted by phone approximately 2 (+/- 1) business days after they were injected with the specific PET ligand to confirm their well-being and query them about any new adverse events. If any AE's are present they will be reviewed by the PI.

Sharing of optional Amyloid PET Information with Participant and/or Providers:
The study team does not plan to release (optional) amyloid scan results to study participants, as they are used for study analysis, not diagnostic or entry criteria.

8.4 Safety Monitoring

8.4.1 Physical and Neurological Examinations

Physical and neurological examinations will be performed at the Screening Visit, Baseline Visit, Visit 12, EOT Visit, and the Follow-up Visit.

8.4.2 Electrocardiograms (ECG)

For each participant, an ECG will be conducted at the Screening Visit, Visit 12 and EOT Visit. The ECG will be conducted by qualified study personnel, and be interpreted by a qualified physician or other designated qualified medical personnel as soon as possible after the administration.

8.4.3 Vital Signs

Vital signs including blood pressure, heart rate, temperature and respirations will be measured at all at-home and in-clinic visits. Blood pressure and pulse should be measured with participant in sitting position for at least 5 minutes. Height will be measured at screening. Body weight will also be measured according to the schedule of events table in protocol section 2.

8.4.4 Additional Safety Assessments

C-SSRS
Consistent with FDA regulatory guidance, any occurrence of suicide related thoughts or behaviors will be assessed per the Schedule of Events (Section 2). The C-SSRS is an instrument that collects the occurrence, severity and frequency of suicide related thoughts or behaviors. The "Baseline" version will be used for the initial screening, and the "Since Last Visit" when assessed shall be used at following visits, when scheduled for administration.

Geriatric Depression Scale (GDS)
The GDS is a simple 30-item measure used to identify depression in elderly individuals. The simplicity of the GDS (questions are answered only by "yes" or "no") enables the scale to be used with ill or moderately cognitively impaired individuals. One point is assigned to each answer and the cumulative score is rated on a scoring grid. The grid sets a range of 0 to 9 as 'normal', 10 to 19 as 'mildly depressed', and 20 to 30 as 'severely depressed'.

Generalized Anxiety Disorder (GAD)-7
The GAD-7 is a simple seven item questionnaire used to identify anxiety levels in individuals. The GAD-7 is calculated by assigning scores of 0, 1, 2, and 3, to the response categories of "*not at all*," "*several days*," "*more than half the days*," and "*nearly every day*," respectively. GAD-7 total score for the seven items ranges from 0 to 21. Scores of 5, 10, and 15 represent cut-points for mild, moderate, and severe anxiety, respectively. Though designed primarily as a screening and severity measure for generalized anxiety disorder, the GAD-7 also has moderately good operating characteristics for three other common anxiety disorders: panic disorder, social anxiety disorder, and post-traumatic stress disorder.

8.4.5 Adverse Event(s), Serious Adverse Events and Monitoring

The principal investigator is responsible for monitoring the safety of participants in the trial, and, with medically qualified sub-Investigators, will oversee and document any event that seems unusual, and document the review of each laboratory safety report. The investigators are responsible for the follow-up for all AEs that are considered serious or otherwise medically important, considered related to the investigational product or procedures in the trial, or that caused the participant to discontinue the investigational product before the recognized end of study treatment. The frequency of follow-up evaluations is at the discretion of the investigator.

After the informed consent is signed, designated study staff will document and report the occurrence and nature of any new medical conditions or events since the informed consent signing as an adverse event. For each AE, the investigator will evaluate relatedness to study participation, severity, and expectedness, taking into account issues including AD pathology, concomitant treatments, and medical history.

A safety medical monitor will advise and consult with the Sponsor, PI, sub-Investigator(s) and study staff on the following matters: Review protocol halting rules; Advise protocol team on safety oversight; Evaluate adverse events/SAEs and reviews safety reports once quarterly or more often if deemed necessary. This individual will be independent of the study team, and properly credentialed.

As a note: If a participant receives two injections in one day, the study team will report this as a protocol deviation. If the team learns of this at the time of the injections, the participant will be instructed to skip the dose on the following day. As determined by Gemlyn George, M.D., hematologist and SESAD lead Medical Sub-I, in consideration of the phase 1 trial that administered sargramostim at 16 times the recommended dosage without causing harm, instances of two doses in one day for this trial does not constitute patient harm.

8.4.5.1 Serious Adverse Events

A serious adverse event, in relation to this clinical trial, is defined as something that results in:

- death.
- initial or prolonged inpatient hospitalization.
- a life-threatening event.
- persistent or significant disability.
- congenital birth defect.
- important medical events that might not be immediately life-threatening, or result in hospitalization, or death, but may require intervention to prevent such, including serious allergic reaction.

All AEs since the signing of the informed consent are to be assessed for seriousness, documented, and, if a serious adverse event, reported to the Sponsor within 24 hours of becoming known to the principal

investigator or qualified sub-Investigator. As well, the Data Safety Monitoring Board will monitor reported AEs and SAEs as a part of their role overseeing the protection of human participants.

8.4.5.2 Protection of Participants

Because of the extensive safety history of sargramostim in elderly patients, serious safety issues are not anticipated, however, vigilance in assessing safety is required. A review of published AEs for GM-CSF and the Leukine® Product Insert and the papers it references will be used to guide the safety monitoring protocol (attached as Appendix E). The greatest safety concern noted with sargramostim is excessive leukocytosis. Therefore, as advised by the product insert, a CBC with differential, including analysis for blast cells, will be conducted at two time-points every week during the 24- week-long treatment phase. Refer to section 7.4 for details on dosage modification guidelines. In addition, the DSMB (described below) will monitor laboratory results, the MRI studies, and the cognitive assessments for any indications of toxicity. Following are the abbreviated categories of the National Cancer Institute's (NCI) graded toxicity protocol used by Daud et al., (2008) for assessing safety of Leukine® [123], although the latest version of the NCI-CTCAE (Version 5.00: November 27, 2017) will also be referenced for all AEs (attached as Appendix F):

Anemia
Lymphopenia
Pain
Fever
Flu-like symptoms
Arthralgia/myalgia
Injection-site reaction
Fatigue
Abdominal cramps
GERD/indigestion
Insomnia
Cough
Sweating

Full description of the DSMB will be provided in the charter. The DSMB will be comprised of members of relevant disciplines to overseeing conduct of a clinical trial for AD. The DSMB will be charged with overseeing participant safety. An initial meeting of the DSMB will be held before enrollment occurs in order for the members to review the study protocol and the study/participant termination guidelines. The DSMB will be required to meet at a minimum of one time per year. Data will be reviewed according to the DSMB mandated timeframe if a treatment related serious AE (SAE) or serious unexpected AE (SUAE) is reported. As mentioned above, SAEs and SUAEs will be reported per DSMB timeframes to the DSMB to discuss the SAEs/SUAEs and to decide whether the study should continue. Of note, if SAEs of Grade 3 or 4 occur in 20% of the initial 14 participants following sargramostim (or placebo) administration, the study will be halted while the Sponsor consults with the DSMB for final determination of study status. On the other hand, if the trial data indeed demonstrate clear safety and positive efficacy results from administration of sargramostim into AD participants at the end of the trial analyses, the DSMB may ethically consider and provide recommendations for open label extension study for trial participants, in which case the Sponsor will seek guidance from COMIRB and the FDA regarding how to proceed with these recommendations. All DSMB meetings will be documented and submitted to SARC. Once chartered, the DSMB membership will be listed in Appendix I.

8.4.5.3 Data Monitoring Plan:

Data Recording:

These functions will be carried out by the study staff and transcribed into the case report forms (adverse events page). The principal investigator or qualified medical designee will review, edit, and sign off on the AE form, and the AE form filed by the PI or designated staff. Laboratory results and data from case report forms will be entered into a computerized data registry.

Data Verification: The PI or designated qualified sub-Investigator will discuss the AEs with the study coordinator(s), and will verify AE data entry.

Data to be monitored, assessed, and reported to/by the DSMB: (From Product insert)
Below is a list of possible data to be monitored, assessed and potentially reported to the DSMB. This list is not exhaustive, but comprised of information found in other studies of the investigational product.

Common but non-life threatening side effects include:

- Fatigue or asthenia
- Malaise
- Rash
- Itching (pruritus)
- Vomiting
- Diarrhea
- Abdominal pain
- Sore throat (pharyngitis)
- Chills
- Bone pains
- Joint pains
- Weight loss or gain
- Elevations in kidney or liver enzymes
- Fluid retention (peripheral edema)
- Injection site irritation
- Insomnia
- Headaches
- Transient Fevers

Less common but potentially serious side effects include:

- Bleeding into the eye
- Allergic reactions
- Excessive increase in white blood cell count
- Fluid retention in the lung lining (pleura) and in the heart lining (pericardium)
- Shortness of breath
- Occasional transient heart rhythm changes (supraventricular tachycardia)
- Sweating, tachycardia, claustrophobia, or anxiety induced by the MRI procedure

Potential side effects of unknown frequency:

- Leukopenia (low white blood cell count)
- Neutropenia (low neutrophil count)

Adverse event of special interest (AESI) include:
An AESI is an AE (serious or non-serious) of scientific and medical concern specific to the program, for

which ongoing monitoring and immediate notification to the DSMB is required. Such events may require further investigation in order to characterize and understand them. Adverse events of special interest may be added or removed during a study by protocol amendment.

- ADA
- Acute hypersensitivity/anaphylaxis
- Leukopenia (low white blood cell count)
- Neutropenia (low neutrophil count)

Report of Unexpected Problems to the DSMB: The study coordinator will notify the PI or qualified sub-Investigator of any unexpected problems. The PI will contact the chair of the **DSMB** when unexpected problems arise that require immediate action.

Criteria for Action:
Specific triggers that will dictate when a specified action (such as reporting or stopping) is required. Any SAE or unexpected problem that meets the COMIRB criteria of reporting will be reported per COMIRB guidelines. Any serious AEs meeting the FDA reporting criteria, the FDA will also be notified according to their reporting guidelines.

Stopping rules:
Study: If treatment related serious AEs (SAEs) of Grade 3 or 4 occur in 20% of participants following sargramostim administration, the study will be stopped (Appendix F).

Participant: For individual participants, the **PI or Sub-I** may discontinue any participant if they reach grade 3 or 4 in any category, if laboratory results indicate blast cell appearance, or if they worsen in any of the cognitive tests by more than two standard deviations than would be normally expected from someone at their baseline measurements, or if they have any values that have exceeded greater than two times upper limits of normal for any trial measures. Participants are, of course, free to withdraw from the study at any time for any or no reason.

Specific participant stopping criteria for Absolute Neutrophil (ANC) count values are as follows:

- If a participants Absolute Neutrophil Count (ANC) is less than 0.5x10E3/uL (500 cells per microliter) at any CBC with differential safety blood draw post randomization, the participant will be monitored for potential infections over the course of 1 week until another CBC with differential is completed. If the ANC value does not demonstrate improvement (returned to a value above 0.5x10E3/uL), administration of the study drug to the participant should be discontinued.

Participants will be followed-up according to the study procedures as specified in this protocol up to the scheduled date of study completion, or up to recovery or stabilization of any AE to be followed-up as specified in this protocol, whichever comes last. If possible, and after the permanent discontinuation of treatment, the participants will be assessed using the procedures normally planned for the EOT visit with the treatment/placebo, including, but not limited to, LP (if chosen), imaging assessments and cognitive assessments. All cases of permanent treatment discontinuation should be recorded after confirmation.

The PI may also determine that a trial participant should no longer remain in the trial if there is evidence of uncontrolled hypertension. For an individual who has consented to volunteer for the trial, meets inclusion criteria and is enrolled, then begins the treatment phase of the trial, but who subsequently is determined to exhibit uncontrolled hypertension for more than 2 weeks (14 treatment days), the DSMB will be notified, within accepted timeframe, adverse events reporting protocols will be followed, and the following criteria will be used to determine whether the participant needs to be removed from the trial:

Inclusion Screening Criteria for hypertension:

- The screening BP will be no greater than 160 systolic or 95 diastolic. If the initial BP reading is greater than 160 systolic or 95 diastolic, the BP will be measured in triplicate over the course of the visit, with the average BP calculated. (This limit will apply to all and any BP measurements during the screening process.)

Management Criteria during the treatment phase of the study:

- Should blood pressure rise above 165 systolic or 95 diastolic, indicating uncontrolled hypertension, the participant will have treatment interrupted and may be removed from the study. Determination of uncontrolled hypertension will be assessed through taking of BP in triplicate during the visit in which the average criteria of greater than 165 systolic or 95 diastolic is noted.
- If the participant displays symptoms related to, or meets clinical criteria of, hypertensive urgency or emergency, treatment will be interrupted and the participant referred to emergency services by the PI, clinical sub-Investigator or other qualified designee.
- The participant's hypertensive regimen may be adjusted by the primary care provider during the study as needed.
- If any participant, who has been interrupted from treatment for uncontrolled hypertension and depending upon the period of time taken for their BP to be controlled below 160 systolic or 95 diastolic, the trial physician, in consultation with the Sponsor, may decide for re-initiation of treatment (see Section 7.5 *Interruption of Treatment*).

Reporting:

Timeframe for reporting: Any Grade 2 AEs and abnormal Grade 2 laboratory results, including CBC with differential results, which require temporary interruption of treatment (Sections 7.4 - 7.5), will be reported to the **DSMB** for review within the standard DSMB aggregate report. Serious AEs (SAE) and unexpected SAEs related to the trial will be reported within five business days to the **DSMB** for review, and to COMIRB within their reporting guidelines according to their policies and procedures. The FDA will be notified by written report within 15 days of the Sponsor determining that the SAE or suspected adverse reaction meets FDA criteria for reporting.

Reporting mechanisms: AE forms will be completed and submitted on-line using the COMIRB AE reporting system. The report will include a description of the event, judgment as to whether or not it was related to study medication, and the outcome of the AE. All non-serious AEs, and those that are not unexpected or that are normal to the study medication, will be reported to the DSMB at the time in which the rest of data from an aggregate block of participants is submitted, or if the DSMB requests the information for their analysis in another trial-related matter.

Responsibility for preparing and submitting reports: The study coordinator will prepare the AE report and the PI or qualified sub-Investigator will review/edit as appropriate and the PI, study coordinator or other designated staff will submit the AE.

Confidentiality Procedures:

The meetings of the DSMB will be closed to respect the privacy of the research participants. Data will be kept in case report binders in a locked office when not in use. Data entered into the computerized data registry will only be accessible to those members of the research team.

9 Statistical, Data, and Other Considerations

9.1 Sample size considerations

As the primary goal of this clinical trial is the safety and tolerability of long-term use of sargramostim in participants with mild-to-moderate Alzheimer's disease, a sample size of 42 participants (28 sargramostim-treated [1250 mcg/m^2/ week subcutaneously], and 14 placebo equivalent) is considered sufficient to meet the endpoint of safety and is consistent with the lack of drug related SAEs in our recently-completed randomized, double blind, placebo-controlled trial of sargramostim in 40 (20 sargramostim-treated:20 placebo-treated) subjects with mild-to-moderate Alzheimer's disease.

Based on results for 40 participants at the EOT, 45-day follow-up, and 90-day follow-up in our recently completed sargramostim trial in participants with mild-to-moderate Alzheimer's disease, an effect size of around 2–4 was predicted by the MMSE. We believe that the 42 participants (28 sargramostim-treated [1250 µg/m^2/week (178.57 µg/m^2/day subcutaneously seven days a week], and 14 placebo equivalent) who will be recruited for this study will provide sufficient power to allow valid analyses of the secondary and exploratory endpoints. Therefore, at least 42 individuals with mild-to-moderate Alzheimer's disease, age 60–85, of either sex, will be randomized (28 sargramostim participants and 14 placebo participants) for this proposed placebo-controlled study. For effect sizes of 2, 4, 6, or 8 (the expected treatment group differences in change between baseline and follow-up for MMSE total score), **Table 1** shows the number of participants required to achieve 91–97% power. Recruitment and treatment initiation will be timed to allow for the interim assessments before further participants are treated, thus assuring maximum early knowledge of any safety concerns.

Mean Treatment Difference in Change from Baseline to End of Treatment (Day 18)	Total Number of Patients	Number of Sargramostim Patients	Number of Placebo Patients	Alpha	Power
2	102	68	34	0.05	0.91
4	27	18	9	0.05	0.92
6	12	8	4	0.05	0.92
8	9	6	3	0.05	0.97

Table 1. Model for MMSE Total Score, Assumes no Treatment Effect at Baseline (successful randomization).

The study will have two arms with participants assigned in permuted blocks examining placebo and full FDA-recommended sargramostim dose. As mentioned above, in the ongoing trial with participants who have thus far received sargramostim, there have been no serious AEs reported by participants receiving either the half dose or full dose sargramostim using the same methods proposed by this protocol. Therefore, participants will be started at the adjusted weekly dose (178.57 µg/m^2/day SC, seven days per week, 1250 µg/m^2/week equivalent.)

9.2 Data Analysis Plan

9.2.1 Primary Objective: To assess long-term tolerability and safety of sargramostim in individuals with mild-to-moderate Alzheimer's Disease

Generally, descriptive statistics will be utilized to examine safety of participants who received sargramostim injections compared to placebo injections. Safety will be assessed by measuring the toxicity grades of AEs. These toxicity grades have been standardized by the National Cancer Institute (NCI)

Common Toxicity Criteria for Adverse Events (CTCAE Version 5.00: November 27, 2017, attached as Appendix F). Differences in toxicity grades will be compared between treatments using a Wilcoxon test for ordered outcome. MRI scans will be assessed to identify ARIAs, including vasogenic edema and microhemorrhage. Additional safety assessments will include neurological and physical examinations, vital signs and suicidality evaluations. The current study will utilize a Data Safety Monitoring Board (DSMB) to review the clinical data and ensure participant safety within the parameters of the trial.

9.2.2 Secondary Objective: To test the hypothesis that sargramostim treatment, as compared to placebo, slows or halts cognitive decline or improves cognitive function in individuals with mild-to-moderate AD.

The foremost measure used for this secondary endpoint of assessing cognitive function will be the MMSE, as it has been reported to be most sensitive to cognitive changes over time due to treatments in people ranging from late mild cognitive impairment to AD in clinical trials up to 2 years in length [124]. The cognitive assessment will be based on the participants' MMSE total score changes from Baseline to End of Treatment.

9.2.3 Exploratory Objectives

To assess the effect of sargramostim treatment, as compared to placebo, on clinical progression of in individuals with mild-to-moderate AD.

Well validated neuropsychological assessments will be administered and scored using the standardized scoring methods associated with each of the neuropsychological tests. Analysis of the change in cognition from Baseline to EOT will be measured by the change in scores for the MoCA, ADAS-cog_{13} total scores, CDR-SB, TMT A, ADCS-ADL score, measures of verbal fluency (lexical and semantic) and the NPI score. Due to the multitude of both the neuropsychological tests and the different time points that these tests are administered, a correction method will be used to counteract the problem of multiple comparisons.

To assess the effect of sargramostim treatment, as compared to placebo, on metabolic activity in different brain regions in individuals with mild-to-moderate AD.

FDG-PET will be used to assess overall metabolic activity in different brain regions, particularly in the medial temporal lobe in potential correlation with changes in MTA, at baseline and at EOT. Standard protocols for 18F-FDG PET imaging studies in conjunction with amyloid PET imaging studies, will be used [125, 126].

To assess the effect of sargramostim treatment, as compared to placebo, on brain amyloid deposition in individuals with mild-to-moderate AD.

CSF assay may be performed examining Aβ(1-42) and t-tau:Aβ (1-42) ratio. The assay will be conducted during of screening and at the End of Treatment). Additionally, there will be an option utilizing Amyloid PET scans, performed using Vizamyl, produced at the CU-AMC / Pharmalogic cyclotron laboratory, which allows for amyloid-beta neuritic plaque density imaging. The Amyloid PET scans may be performed at Screening Visit and at the Follow-up Visit. Vizamyl is approved by the FDA for use in research and clinical application. For this protocol, the change from the screening scan's standardized uptake value ratio (SUVR) to follow-up SUVR will be similarly analyzed by a contracted central reading facility, using the participant's MRI to reduce white matter contamination, thereby increasing the SUVR signal change by eliminating counts in the region that do not change (i.e. white matter). The PET scans and accompanying MRI scans will be stripped of any personal identifying information, containing only the trial's randomized subject ID number and date. The results of the PET quantitative analyses will not be used to make any treatment decisions.

To assess the effect of sargramostim treatment, as compared to placebo, on AD related changes in peripheral blood and CSF biomarkers in individuals with mild-to-moderate AD.

Levels of AD-associated biomarkers in peripheral blood samples (collected at Baseline, 12, EOT and follow-up) and CSF samples (collected at Screening Visit and EOT) will be compared between placebo

controls and sargramostim treatment groups. The identification of circulating biomarkers in AD is a relatively new field, with inconsistent biomarker level results reported as increased, decreased, or unchanged between studies [127]. These mixed results mean that standardized levels for AD-associated circulating biomarkers have yet to be established. Therefore, we will compare observed biomarker levels from our project to other published levels to explore whether any similarities are observed. This comparison, as well as our own results, will add to the AD-associated blood and CSF biomarker fields of literature.

To assess the effect that ApoE genotype may have upon predicting sargramostim treatment outcomes in individuals with mild-to-moderate AD.
ApoE genotyping will be performed using blood samples collected on Day 1 of this trial for each participant. ApoE genotype will be determined either by Athena Diagnostics, or in the University of Colorado laboratory under the direction of Huntington Potter, PhD, using established methods. Outcomes in the context of ApoE genotype will be compared. The genetic testing will be carried out at the end of the trial, and participants will not be notified of any genetic testing results.

To assess the effect of sargramostim treatment, as compared to placebo, on inhibiting MTA in individuals with mild-to-moderate AD.
MTA will be assessed using an automated rating system to grade the severity of atrophy in the entire medial temporal region will be employed using the FreeSurfer software automated rating system of individual medial temporal structures, i.e., the hippocampus (HPC), entorhinal cortex (ERC) and perirhinal cortex (PRC), which are specific regions of interest (ROIs) in AD pathogenesis. FreeSurfer automatically computes cortical reconstruction and volumetric segmentation, including segmentation of the subcortical white matter and deep gray matter volumetric structures [128] and parcellation of the cortical surface [129] according to a previously published parcellation scheme [130]. This labels cortical sulci and gyri, and thickness values are calculated in the ROIs.

Group comparisons of changes in regional volumes and thicknesses will be analyzed using a series of one-way analyses of variance (ANOVAs). The Scheffé post hoc procedure will be used to examine differences between means, and Pearson product–moment correlation coefficients will be used to evaluate the strength of relationships between changes in regional volumes and thicknesses and cognitive measures. Receiver operator curve analyses will determine sensitivity and specificity of various MTA cut-off points for distinguishing between diagnostic groups. Hazard ratios (HRs) for specific predictors of MTA progression and regression will be calculated using a three-state Markov model in continuous time. The proportional intensity model will be used to analyze effects of covariates on HRs.

To assess the effect of sargramostim treatment, as compared to placebo, on AD related changes in blood brain barrier permeability in individuals with mild-to-moderate AD.
CSF may be collected during screening, and at the EOT visit (i.e. within 2-10 days following the final treatment article injection) for a subset of participants for entry into the study or as part of an optional substudy. A venipuncture blood draw will be performed at each LP procedure visit to obtain serum. Albumin levels will be quantitated within CSF (in mg/dL) and serum (in g/dL) to calculate an Albumin Index (AI). Because albumin is neither synthesized nor metabolized intrathecally, albumin that is found within CSF that is free of blood contamination indicates that the albumin must have come from the plasma through the blood brain barrier (BBB). An AI value < 9 indicates an intact BBB, values 9-14 as slight impairment, values 14-30 as moderate impairment, values 30-100 as severe impairment, and values > 100 as complete breakdown of the BBB [72]. The BBB is known to be impaired in AD [131-133], and cerebral amyloid angiopathy (CAA), characterized by Aβ deposition on vessel walls, has been found in over 90% of AD cases [134]. In immunotherapy trials targeting amyloid in the parenchyma and cerebral vascular, the removal of pre-existing vascular amyloid has been attributed to onset of ARIA events (i.e. vasogenic edema and microhemorrhage) [135], and while we and others have found GM-CSF to quickly reduce

amyloid in transgenic AD models [1, 68], no incidences of ARIAs have been observed in the current ongoing sargramostim-AD trial (NCT01409915). GM-CSF is known to induce angiogenesis and restore cerebral blood flow following experimental stroke [23, 24, 27, 28]. However, if sargramostim does indeed cause reductions in amyloid deposition over 24 weeks of administration in mild-to-moderate AD participants, evaluating treatment effects on BBB permeability in correlation with MRI safety measures is warranted. Thus, AI values will be calculated at screening and at EOT to assess any treatment-related changes in BBB permeability and to correlate values with any potential trial emergent ARIA events.

To assess the effect of sargramostim treatment, as compared to placebo, on AD-related changes in microstructural white matter measures in individuals with mild-to-moderate AD.
Numerous neuroimaging studies have shown that white matter (WM) tracts are associated with cognitive function, and that microstructural abnormalities are found in AD and other neurodegenerative diseases, as determined from high-resolution MRI diffusion tensor imaging (DTI) techniques [73-77]. DTI metrics have been proposed as sensitive biomarkers for disease progression over short periods of time [78], which makes it useful for assessing any WM changes in response to treatments, and specific DTI patterns have been shown to help distinguish between AD and other comorbid neurodegenerative diseases [79, 80], to correlate with AD-related verbal fluency impairments [81], and with using neurite orientation dispersion and density imaging (NODDI) sequences of DTI, changes in neurite density can be approximated [82, 83]. Because GM-CSF has been shown to be a growth factor for oligodendrocytes [116], induce oligodendrocyte progenitor cells concurrent with amelioration of experimental spinal cord injury [34, 117], and increase synaptic area concurrent with reversing cognitive impairment and pathology in transgenic AD models [1, 68], high-resolution MRI with DTI and NODDI sequences will be performed at screening, weeks 3, 12, 18, EOT and metrics of DTI (e.g. FA, MD, NDI, ODI, etc.) will be monitored for any treatment-related changes.

To assess the effect of sargramostim treatment, as compared to placebo, on changes in sleep-related measures in individuals with mild-to-moderate AD.
Changes in quality of sleep have been long reported in onset and progression of AD [84-86], and decreases in non-rapid eye movement (NREM) slow wave activity (SWA) is known to be associated with Aβ deposition [87]. Our preclinical work and others have revealed that GM-CSF quickly removes amyloid in AD models [1, 68], of which it is not known whether quality of sleep improves with reductions in amyloid. For assessing any changes in qualitative measures of sleep in the participants, two sleep assessments (i.e. ISI, and PSQI) will be administered to both them and their study partner/caregivers at baseline, week 12, EOT, and at follow-up (45 days post-treatment) visits to assess quality of sleep measures and any changes that may occur throughout the trial. Mechanistically, melatonin and its signaling have been shown to be decreased in AD progression [88-92], and GM-CSF has also been reported to increase melatonin secretion in preclinical studies [93]. Melatonin is made and secreted by the pineal gland, and calcification of the pineal gland increases with AD progression [94-97]. High-resolution MRI scans, using susceptibility weighted imaging (SWI) sequence, will be performed at screening, weeks 3, 12, 18, EOT, and at follow-up (45 days post-treatment) to assess changes in pineal calcification measurements.

To assess the effect of sargramostim treatment, as compared to placebo, on changes in speech measures in individuals with mild-to-moderate AD.
AD is known to adversely impact temporal characteristics of spontaneous speech, such as speech tempo, number of pauses in speech, and their length, with speech alterations often noticed years before other cognitive deficits become apparent [98, 99]. Among the neuropsychological measures frequently used to assess semantic memory impairment in AD are verbal fluency tests, or speeded word-list generation, which consists of two types: phonemic (letter) fluency tests and category fluency tests. Fluency performance involves multiple brain processes and regions that are adversely impacted by AD pathology, and both phonemic and category fluency performances reveal declines in AD [100, 101]. Word retrieval in connected speech is also impaired in AD, and by using picture naming and picture descriptions, the total word output, percentages of content words, percentages of nouns, and percentages of pronouns out of all

words, type-token ratio of all words and type-token ratio of nouns alone, mean frequency of all words and mean frequency of nouns alone, and mean word length can be analyzed [102]. A study participant interview will be given at baseline, week 12, EOT, and at follow-up (45 days post-treatment) visits to assess changes in speech fluency and word retrieval measures.

9.3 Additional Statistical Consideration

Additional statistical tests that may be utilized include: independent t-tests to examine differences between means; the Chi-square test to assess association between variables; logistical regression to calculate the ratio of the odds of an event occurring in one group compared to another group, and longitudinal regression to analyze the data over time.

9.3.1 Full Statistical Analysis Plan

A full statistical analysis plan will be developed and approved prior to final database lock of participant data and will be outlined in a separate Statistical Analysis Plan (SAP) document.

9.4 Interim Analyses

No interim analyses will be conducted as a part of this trial.

9.5 Data Storage:

All data collected at the study site will be stored in REDCap, a HIPPA compliant database. Data collected will include general participant information (e.g., age, phone, address, etc.), background health information, and research outcomes. The Principal Investigator is responsible for the individually identifiable private health information. Access to the REDCap database key will be restricted to the research team approved on the application in order to minimize risk of unintentional disclosure. All affiliated/local principal investigators, co-investigators, and research coordinators involved in consenting and carrying out the proposed study will complete Collaborative Institutional Training Initiative, an online basic Course in Human Subject Protections, and the HIPAA research course prior to submitting a protocol. Source data will be collected and stored in participant binders, OnCore CTMS, Epic EMR, and the CU_AMC computer system.

For the general manipulation of data, the clinical data will be transformed into a de-identified data set using the unique study IDs. Any paper record of the data will be kept under lock and key with the Principal Investigator.

9.6 Specimen Storage:

Samples will be stored for at least 10 years, or the entire course of this study. Excess samples may also be added to the University of Colorado Alzheimer's and Cognition Center (CUACC) Biobank for future internal or external studies, if given consent by the participant. If a participant withdraws from the study, the samples will be discarded at the written request of the participant or appropriate consenting party.

9.7 Control of Conflict of Interest:

The Sponsor and Principal Investigator, Huntington Potter, PhD, was one of the conceivers of the Arthritis/GM-CSF/G-CSF approach to AD therapy and is one of the inventors on several University of South Florida issued patents on the use of GM-CSF to improve cognition and/or to reduce amyloid deposition in AD and/or in chemobrain. These patents have never been licensed and are of no commercial value presently. To eliminate any real or even perceived conflict of interest, Dr. Potter has relinquished any and all personal rights to shared royalties from USF patents and has instructed the USF Technology Transfer Office to transfer any such income that might come in the future to the University of Colorado Foundation to support research by the Potter Alzheimer Dementia Fund. Furthermore, the following steps will be used to help further isolate Dr. Potter from potential influence related to the outcomes of the trial and to ensure that the trial is carried out with the highest level of scientific integrity:

1) All investigators who interact with participants directly and all investigators who collect and record data will have zero financial interest in the outcome of the study and will be blinded to the treatment group.
2) Endpoints are quantitative and objective (i.e., assessments of cognition using a battery of neuropsychological and clinical assessments used in the UCH clinic, brain imaging, and measures of blood biomarkers), and the investigators are blinded to the treatment group.
3) With regard to potential financial interests of any investigators with pending patent applications at CU Anschutz, no such pending patent applications have been licensed and they have no current commercial value.
4) An independent **DSMB** has been established to oversee participant safety.
5) Notification of any financial interests of Dr. Potter or of other investigators in the study that may arise in the future will be made to the CU Research Compliance Office and appropriately managed, to editors of journals publishing study-related results and to the public in the context of communication of any research results.

Appendix A

References Cited

1. Boyd TD, Bennett SP, Mori T, Governatori N, Runfeldt M, Norden M, Padmanabhan J, Neame P, Wefes I, Sanchez-Ramos J, Arendash GW, Potter H. GM-CSF upregulated in rheumatoid arthritis reverses cognitive impairment and amyloidosis in Alzheimer mice. Journal of Alzheimer's disease : JAD. 2010;21(2):507-18. doi: 10.3233/JAD-2010-091471. PubMed PMID: 20555144.
2. DiFrancesco JC, Longoni M, Piazza F. Anti-Abeta Autoantibodies in Amyloid Related Imaging Abnormalities (ARIA): Candidate Biomarker for Immunotherapy in Alzheimer's Disease and Cerebral Amyloid Angiopathy. Front Neurol. 2015;6:207. doi: 10.3389/fneur.2015.00207. PubMed PMID: 26441825; PMCID: PMC4585101.
3. Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem. 2009;110(4):1129-34. doi: 10.1111/j.1471-4159.2009.06181.x. PubMed PMID: 19457065.
4. McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology. 1996;47(2):425-32. Epub 1996/08/01. doi: 10.1212/wnl.47.2.425. PubMed PMID: 8757015.
5. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43(1):155-63. doi: 10.1002/1529-0131(200001)43:1<155::AID-ANR20>3.0.CO;2-3. PubMed PMID: 10643712.
6. Szekanecz Z, Koch AE. Macrophages and their products in rheumatoid arthritis. Curr Opin Rheumatol. 2007;19(3):289-95. doi: 10.1097/BOR.0b013e32805e87ae. PubMed PMID: 17414958.
7. Cox CA, Shi G, Yin H, Vistica BP, Wawrousek EF, Chan CC, Gery I. Both Th1 and Th17 are immunopathogenic but differ in other key biological activities. J Immunol. 2008;180(11):7414-22. PubMed PMID: 18490741; PMCID: PMC2753359.
8. Parsonage G, Filer A, Bik M, Hardie D, Lax S, Howlett K, Church LD, Raza K, Wong SH, Trebilcock E, Scheel-Toellner D, Salmon M, Lord JM, Buckley CD. Prolonged, granulocyte-macrophage colony-stimulating factor-dependent, neutrophil survival following rheumatoid synovial fibroblast activation by IL-17 and TNFalpha. Arthritis Res Ther. 2008;10(2):R47. doi: 10.1186/ar2406. PubMed PMID: 18433499; PMCID: PMC2453767.
9. Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. J Exp Med. 2004;200(3):277-85. Epub 2004/07/29. doi: 10.1084/jem.20040165. PubMed PMID: 15280421; PMCID: PMC2211983.
10. Niu Q, Cai B, Huang ZC, Shi YY, Wang LL. Disturbed Th17/Treg balance in patients with rheumatoid arthritis. Rheumatol Int. 2012;32(9):2731-6. Epub 2011/08/03. doi: 10.1007/s00296-011-1984-x. PubMed PMID: 21809006.
11. Wang W, Shao S, Jiao Z, Guo M, Xu H, Wang S. The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. Rheumatol Int. 2012;32(4):887-93. Epub 2011/01/12. doi: 10.1007/s00296-010-1710-0. PubMed PMID: 21221592.
12. Kawaji H, Yokomura K, Kikuchi K, Somoto Y, Shirai Y. [Macrophage colony-stimulating factor in patients with rheumatoid arthritis]. Nihon Ika Daigaku Zasshi. 1995;62(3):260-70. PubMed PMID: 7615698.
13. Nakamura H, Ueki Y, Sakito S, Matsumoto K, Yano M, Miyake S, Tominaga T, Tominaga M, Eguchi K. High serum and synovial fluid granulocyte colony stimulating factor (G-CSF) concentrations in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2000;18(6):713-8. PubMed PMID: 11138333.
14. Olszewski WL, Pazdur J, Kubasiewicz E, Zaleska M, Cooke CJ, Miller NE. Lymph draining from foot joints in rheumatoid arthritis provides insight into local cytokine and chemokine production and

transport to lymph nodes. Arthritis Rheum. 2001;44(3):541-9. doi: 10.1002/1529-0131(200103)44:3<541::AID-ANR102>3.0.CO;2-6. PubMed PMID: 11263768.
15. Xu WD, Firestein GS, Taetle R, Kaushansky K, Zvaifler NJ. Cytokines in chronic inflammatory arthritis. II. Granulocyte-macrophage colony-stimulating factor in rheumatoid synovial effusions. J Clin Invest. 1989;83(3):876-82. doi: 10.1172/JCI113971. PubMed PMID: 2646320; PMCID: PMC303761.
16. McGeer PL, Rogers J, McGeer EG. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. Journal of Alzheimer's disease : JAD. 2006;9(3 Suppl):271-6. PubMed PMID: 16914866.
17. Malm TM, Koistinaho M, Parepalo M, Vatanen T, Ooka A, Karlsson S, Koistinaho J. Bone-marrow-derived cells contribute to the recruitment of microglial cells in response to beta-amyloid deposition in APP/PS1 double transgenic Alzheimer mice. Neurobiol Dis. 2005;18(1):134-42. Epub 2005/01/15. doi: 10.1016/j.nbd.2004.09.009. PubMed PMID: 15649704.
18. Simard AR, Rivest S. Neuroprotective properties of the innate immune system and bone marrow stem cells in Alzheimer's disease. Mol Psychiatry. 2006;11(4):327-35. Epub 2006/02/24. doi: 10.1038/sj.mp.4001809. PubMed PMID: 16491130.
19. Simard AR, Soulet D, Gowing G, Julien JP, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron. 2006;49(4):489-502. Epub 2006/02/16. doi: 10.1016/j.neuron.2006.01.022. PubMed PMID: 16476660.
20. El Khoury J, Toft M, Hickman SE, Means TK, Terada K, Geula C, Luster AD. Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. Nat Med. 2007;13(4):432-8. Epub 2007/03/14. doi: 10.1038/nm1555. PubMed PMID: 17351623.
21. Sanchez-Ramos J, Song S, Sava V, Catlow B, Lin X, Mori T, Cao C, Arendash GW. Granulocyte colony stimulating factor decreases brain amyloid burden and reverses cognitive impairment in Alzheimer's mice. Neuroscience. 2009;163(1):55-72. doi: 10.1016/j.neuroscience.2009.05.071. PubMed PMID: 19500657.
22. Sanchez-Ramos J, Cimino C, Avila R, Rowe A, Chen R, Whelan G, Lin X, Cao C, Ashok R. Pilot study of granulocyte-colony stimulating factor for treatment of Alzheimer's disease. Journal of Alzheimer's disease : JAD. 2012;31(4):843-55. Epub 2012/07/04. doi: 10.3233/jad-2012-120196. PubMed PMID: 22751169.
23. Buschmann IR, Busch HJ, Mies G, Hossmann KA. Therapeutic induction of arteriogenesis in hypoperfused rat brain via granulocyte-macrophage colony-stimulating factor. Circulation. 2003;108(5):610-5. Epub 2003/07/02. doi: 10.1161/01.Cir.0000074209.17561.99. PubMed PMID: 12835229.
24. Schneeloch E, Mies G, Busch HJ, Buschmann IR, Hossmann KA. Granulocyte-macrophage colony-stimulating factor-induced arteriogenesis reduces energy failure in hemodynamic stroke. Proc Natl Acad Sci U S A. 2004;101(34):12730-5. Epub 2004/08/13. doi: 10.1073/pnas.0404880101. PubMed PMID: 15306685; PMCID: PMC514662.
25. Nakagawa T, Suga S, Kawase T, Toda M. Intracarotid injection of granulocyte-macrophage colony-stimulating factor induces neuroprotection in a rat transient middle cerebral artery occlusion model. Brain Res. 2006;1089(1):179-85. Epub 2006/05/09. doi: 10.1016/j.brainres.2006.03.059. PubMed PMID: 16678804.
26. Schabitz WR, Kruger C, Pitzer C, Weber D, Laage R, Gassler N, Aronowski J, Mier W, Kirsch F, Dittgen T, Bach A, Sommer C, Schneider A. A neuroprotective function for the hematopoietic protein granulocyte-macrophage colony stimulating factor (GM-CSF). J Cereb Blood Flow Metab. 2008;28(1):29-43. doi: 10.1038/sj.jcbfm.9600496. PubMed PMID: 17457367.
27. Schneider UC, Schilling L, Schroeck H, Nebe CT, Vajkoczy P, Woitzik J. Granulocyte-macrophage colony-stimulating factor-induced vessel growth restores cerebral blood supply after bilateral carotid

artery occlusion. Stroke. 2007;38(4):1320-8. doi: 10.1161/01.STR.0000259707.43496.71. PubMed PMID: 17332468.
28. Todo K, Kitagawa K, Sasaki T, Omura-Matsuoka E, Terasaki Y, Oyama N, Yagita Y, Hori M. Granulocyte-macrophage colony-stimulating factor enhances leptomeningeal collateral growth induced by common carotid artery occlusion. Stroke. 2008;39(6):1875-82. Epub 2008/04/05. doi: 10.1161/strokeaha.107.503433. PubMed PMID: 18388343.
29. Kong T, Choi JK, Park H, Choi BH, Snyder BJ, Bukhari S, Kim NK, Huang X, Park SR, Park HC, Ha Y. Reduction in programmed cell death and improvement in functional outcome of transient focal cerebral ischemia after administration of granulocyte-macrophage colony-stimulating factor in rats. Laboratory investigation. J Neurosurg. 2009;111(1):155-63. doi: 10.3171/2008.12.JNS08172. PubMed PMID: 19361262.
30. Sugiyama Y, Yagita Y, Oyama N, Terasaki Y, Omura-Matsuoka E, Sasaki T, Kitagawa K. Granulocyte colony-stimulating factor enhances arteriogenesis and ameliorates cerebral damage in a mouse model of ischemic stroke. Stroke. 2011;42(3):770-5. Epub 2011/01/25. doi: 10.1161/strokeaha.110.597799. PubMed PMID: 21257824.
31. Theoret JK, Jadavji NM, Zhang M, Smith PD. Granulocyte macrophage colony-stimulating factor treatment results in recovery of motor function after white matter damage in mice. Eur J Neurosci. 2016;43(1):17-24. Epub 2015/10/17. doi: 10.1111/ejn.13105. PubMed PMID: 26474338.
32. Dames C, Winek K, Beckers Y, Engel O, Meisel A, Meisel C. Immunomodulatory treatment with systemic GM-CSF augments pulmonary immune responses and improves neurological outcome after experimental stroke. J Neuroimmunol. 2018;321:144-9. Epub 2018/03/27. doi: 10.1016/j.jneuroim.2018.03.005. PubMed PMID: 29576323.
33. Ha Y, Kim YS, Cho JM, Yoon SH, Park SR, Yoon DH, Kim EY, Park HC. Role of granulocyte-macrophage colony-stimulating factor in preventing apoptosis and improving functional outcome in experimental spinal cord contusion injury. J Neurosurg Spine. 2005;2(1):55-61. Epub 2005/01/22. doi: 10.3171/spi.2005.2.1.0055. PubMed PMID: 15658127.
34. Hayashi K, Ohta S, Kawakami Y, Toda M. Activation of dendritic-like cells and neural stem/progenitor cells in injured spinal cord by GM-CSF. Neurosci Res. 2009;64(1):96-103. Epub 2009/05/12. doi: 10.1016/j.neures.2009.01.018. PubMed PMID: 19428687.
35. Park HC, Shim YS, Ha Y, Yoon SH, Park SR, Choi BH, Park HS. Treatment of complete spinal cord injury patients by autologous bone marrow cell transplantation and administration of granulocyte-macrophage colony stimulating factor. Tissue Eng. 2005;11(5-6):913-22. Epub 2005/07/07. doi: 10.1089/ten.2005.11.913. PubMed PMID: 15998231.
36. Bouhy D, Malgrange B, Multon S, Poirrier AL, Scholtes F, Schoenen J, Franzen R. Delayed GM-CSF treatment stimulates axonal regeneration and functional recovery in paraplegic rats via an increased BDNF expression by endogenous macrophages. Faseb j. 2006;20(8):1239-41. Epub 2006/04/26. doi: 10.1096/fj.05-4382fje. PubMed PMID: 16636109.
37. Huang X, Kim JM, Kong TH, Park SR, Ha Y, Kim MH, Park H, Yoon SH, Park HC, Park JO, Min BH, Choi BH. GM-CSF inhibits glial scar formation and shows long-term protective effect after spinal cord injury. J Neurol Sci. 2009;277(1-2):87-97. Epub 2008/11/27. doi: 10.1016/j.jns.2008.10.022. PubMed PMID: 19033079.
38. Kim HJ, Oh JS, An SS, Pennant WA, Gwak SJ, Kim AN, Han PK, Yoon DH, Kim KN, Ha Y. Hypoxia-specific GM-CSF-overexpressing neural stem cells improve graft survival and functional recovery in spinal cord injury. Gene Ther. 2012;19(5):513-21. Epub 2011/10/21. doi: 10.1038/gt.2011.137. PubMed PMID: 22011644.
39. Kim JY, Oh CH, Huang X, Kim MH, Yoon SH, Kim KH, Park H, Park HC, Park SR, Choi BH. Improvement in sensory function via granulocyte-macrophage colony-stimulating factor in rat spinal

cord injury models. J Neurosurg Spine. 2013;18(1):69-75. Epub 2012/10/30. doi: 10.3171/2012.9.Spine1235. PubMed PMID: 23101949.
40. Chung J, Kim MH, Yoon YJ, Kim KH, Park SR, Choi BH. Effects of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor on glial scar formation after spinal cord injury in rats. J Neurosurg Spine. 2014;21(6):966-73. Epub 2014/10/04. doi: 10.3171/2014.8.Spine131090. PubMed PMID: 25279652.
41. Thomaty S, Pezard L, Xerri C, Brezun JM. Acute granulocyte macrophage-colony stimulating factor treatment modulates neuroinflammatory processes and promotes tactile recovery after spinal cord injury. Neuroscience. 2017;349:144-64. Epub 2017/03/10. doi: 10.1016/j.neuroscience.2017.02.035. PubMed PMID: 28274846.
42. Bombeiro AL, Pereira BTN, de Oliveira ALR. Granulocyte-macrophage colony-stimulating factor improves mouse peripheral nerve regeneration following sciatic nerve crush. Eur J Neurosci. 2018;48(5):2152-64. Epub 2018/08/14. doi: 10.1111/ejn.14106. PubMed PMID: 30099786.
43. Shultz SR, Tan XL, Wright DK, Liu SJ, Semple BD, Johnston L, Jones NC, Cook AD, Hamilton JA, O'Brien TJ. Granulocyte-macrophage colony-stimulating factor is neuroprotective in experimental traumatic brain injury. J Neurotrauma. 2014;31(10):976-83. Epub 2014/01/08. doi: 10.1089/neu.2013.3106. PubMed PMID: 24392832; PMCID: PMC4012635.
44. Kelso ML, Elliott BR, Haverland NA, Mosley RL, Gendelman HE. Granulocyte-macrophage colony stimulating factor exerts protective and immunomodulatory effects in cortical trauma. J Neuroimmunol. 2015;278:162-73. Epub 2014/12/04. doi: 10.1016/j.jneuroim.2014.11.002. PubMed PMID: 25468272; PMCID: PMC4297700.
45. Schallenberg M, Charalambous P, Thanos S. GM-CSF regulates the ERK1/2 pathways and protects injured retinal ganglion cells from induced death. Exp Eye Res. 2009;89(5):665-77. Epub 2009/06/30. doi: 10.1016/j.exer.2009.06.008. PubMed PMID: 19560459.
46. Schallenberg M, Charalambous P, Thanos S. GM-CSF protects rat photoreceptors from death by activating the SRC-dependent signalling and elevating anti-apoptotic factors and neurotrophins. Graefes Arch Clin Exp Ophthalmol. 2012;250(5):699-712. Epub 2012/02/03. doi: 10.1007/s00417-012-1932-9. PubMed PMID: 22297538.
47. Legacy J, Hanea S, Theoret J, Smith PD. Granulocyte macrophage colony-stimulating factor promotes regeneration of retinal ganglion cells in vitro through a mammalian target of rapamycin-dependent mechanism. J Neurosci Res. 2013;91(6):771-9. Epub 2013/03/05. doi: 10.1002/jnr.23205. PubMed PMID: 23456828.
48. Kim NK, Choi BH, Huang X, Snyder BJ, Bukhari S, Kong TH, Park H, Park HC, Park SR, Ha Y. Granulocyte-macrophage colony-stimulating factor promotes survival of dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced murine Parkinson's disease model. Eur J Neurosci. 2009;29(5):891-900. Epub 2009/02/28. doi: 10.1111/j.1460-9568.2009.06653.x. PubMed PMID: 19245369.
49. Mangano EN, Peters S, Litteljohn D, So R, Bethune C, Bobyn J, Clarke M, Hayley S. Granulocyte macrophage-colony stimulating factor protects against substantia nigra dopaminergic cell loss in an environmental toxin model of Parkinson's disease. Neurobiol Dis. 2011;43(1):99-112. Epub 2011/03/08. doi: 10.1016/j.nbd.2011.02.011. PubMed PMID: 21377529.
50. Choudhury ME, Sugimoto K, Kubo M, Nagai M, Nomoto M, Takahashi H, Yano H, Tanaka J. A cytokine mixture of GM-CSF and IL-3 that induces a neuroprotective phenotype of microglia leading to amelioration of (6-OHDA)-induced Parkinsonism of rats. Brain Behav. 2011;1(1):26-43. Epub 2012/03/09. doi: 10.1002/brb3.11. PubMed PMID: 22398979; PMCID: PMC3217672.
51. Kosloski LM, Kosmacek EA, Olson KE, Mosley RL, Gendelman HE. GM-CSF induces neuroprotective and anti-inflammatory responses in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

intoxicated mice. J Neuroimmunol. 2013;265(1-2):1-10. Epub 2013/11/12. doi: 10.1016/j.jneuroim.2013.10.009. PubMed PMID: 24210793; PMCID: PMC3872482.
52. Gendelman HE, Zhang Y, Santamaria P, Olson KE, Schutt CR, Bhatti D, Shetty BLD, Lu Y, Estes KA, Standaert DG, Heinrichs-Graham E, Larson L, Meza JL, Follett M, Forsberg E, Siuzdak G, Wilson TW, Peterson C, Mosley RL. Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial. NPJ Parkinsons Dis. 2017;3:10. Epub 2017/06/27. doi: 10.1038/s41531-017-0013-5. PubMed PMID: 28649610; PMCID: PMC5445595.
53. Kim JK, Choi BH, Park HC, Park SR, Kim YS, Yoon SH, Park HS, Kim EY, Ha Y. Effects of GM-CSF on the neural progenitor cells. Neuroreport. 2004;15(14):2161-5. PubMed PMID: 15371725.
54. Huang X, Choi JK, Park SR, Ha Y, Park H, Yoon SH, Park HC, Park JO, Choi BH. GM-CSF inhibits apoptosis of neural cells via regulating the expression of apoptosis-related proteins. Neurosci Res. 2007;58(1):50-7. Epub 2007/03/03. doi: 10.1016/j.neures.2007.01.015. PubMed PMID: 17331604.
55. Choi JK, Choi BH, Ha Y, Park H, Yoon SH, Park HC, Park SR. Signal transduction pathways of GM-CSF in neural cell lines. Neurosci Lett. 2007;420(3):217-22. Epub 2007/06/09. doi: 10.1016/j.neulet.2007.03.065. PubMed PMID: 17556097.
56. Choi JK, Kim KH, Park H, Park SR, Choi BH. Granulocyte macrophage-colony stimulating factor shows anti-apoptotic activity in neural progenitor cells via JAK/STAT5-Bcl-2 pathway. Apoptosis. 2011;16(2):127-34. Epub 2010/11/06. doi: 10.1007/s10495-010-0552-2. PubMed PMID: 21052840.
57. Choi JK, Kim KH, Park SR, Choi BH. Granulocyte macrophage colony-stimulating factor shows anti-apoptotic activity via the PI3K-NF-kappaB-HIF-1alpha-survivin pathway in mouse neural progenitor cells. Mol Neurobiol. 2014;49(2):724-33. Epub 2013/09/12. doi: 10.1007/s12035-013-8550-3. PubMed PMID: 24022164.
58. Kruger C, Laage R, Pitzer C, Schabitz WR, Schneider A. The hematopoietic factor GM-CSF (granulocyte-macrophage colony-stimulating factor) promotes neuronal differentiation of adult neural stem cells in vitro. BMC Neurosci. 2007;8:88. doi: 10.1186/1471-2202-8-88. PubMed PMID: 17953750; PMCID: PMC2233634.
59. Krieger M, Both M, Kranig SA, Pitzer C, Klugmann M, Vogt G, Draguhn A, Schneider A. The hematopoietic cytokine granulocyte-macrophage colony stimulating factor is important for cognitive functions. Sci Rep. 2012;2:697. doi: 10.1038/srep00697. PubMed PMID: 23019518; PMCID: PMC3458247.
60. Kim J, Kim NK, Park SR, Choi BH. GM-CSF Enhances Mobilization of Bone Marrow Mesenchymal Stem Cells via a CXCR4-Medicated Mechanism. Tissue Eng Regen Med. 2019;16(1):59-68. Epub 2019/03/01. doi: 10.1007/s13770-018-0163-5. PubMed PMID: 30815351; PMCID: PMC6361095.
61. Park SR, Cho A, Kim JW, Lee HY, Hong IS. A Novel Endogenous Damage Signal, CSF-2, Activates Multiple Beneficial Functions of Adipose Tissue-Derived Mesenchymal Stem Cells. Mol Ther. 2019;27(6):1087-100. Epub 2019/04/10. doi: 10.1016/j.ymthe.2019.03.010. PubMed PMID: 30962162; PMCID: PMC6554530.
62. Gavino C, Cotter A, Lichtenstein D, Lejtenyi D, Fortin C, Legault C, Alirezaie N, Majewski J, Sheppard DC, Behr MA, Foulkes WD, Vinh DC. CARD9 deficiency and spontaneous central nervous system candidiasis: complete clinical remission with GM-CSF therapy. Clin Infect Dis. 2014;59(1):81-4. Epub 2014/04/08. doi: 10.1093/cid/ciu215. PubMed PMID: 24704721; PMCID: PMC4305130.
63. Bernasconi E, Favre L, Maillard MH, Bachmann D, Pythoud C, Bouzourene H, Croze E, Velichko S, Parkinson J, Michetti P, Velin D. Granulocyte-macrophage colony-stimulating factor elicits bone marrow-derived cells that promote efficient colonic mucosal healing. Inflamm Bowel Dis. 2010;16(3):428-41. Epub 2009/07/30. doi: 10.1002/ibd.21072. PubMed PMID: 19639560.
64. Dabritz J. GM-CSF and the role of myeloid regulatory cells in the pathogenesis and treatment of Crohn's disease. Mol Cell Pediatr. 2015;2(1):12. Epub 2015/12/03. doi: 10.1186/s40348-015-0024-4. PubMed PMID: 26626346; PMCID: PMC4666883.

65. Chousterman BG, Arnaud M. Is There a Role for Hematopoietic Growth Factors During Sepsis? Front Immunol. 2018;9:1015. Epub 2018/07/07. doi: 10.3389/fimmu.2018.01015. PubMed PMID: 29977234; PMCID: PMC6021493.
66. Ridwan S, Bauer H, Frauenknecht K, von Pein H, Sommer CJ. Distribution of granulocyte-monocyte colony-stimulating factor and its receptor alpha-subunit in the adult human brain with specific reference to Alzheimer's disease. J Neural Transm (Vienna). 2012;119(11):1389-406. Epub 2012/03/21. doi: 10.1007/s00702-012-0794-y. PubMed PMID: 22430742.
67. Daria A, Colombo A, Llovera G, Hampel H, Willem M, Liesz A, Haass C, Tahirovic S. Young microglia restore amyloid plaque clearance of aged microglia. Embo j. 2017;36(5):583-603. Epub 2016/12/23. doi: 10.15252/embj.201694591. PubMed PMID: 28007893; PMCID: PMC5331757.
68. Kiyota T, Machhi J, Lu Y, Dyavarshetty B, Nemati M, Yokoyama I, Mosley RL, Gendelman HE. Granulocyte-macrophage colony-stimulating factor neuroprotective activities in Alzheimer's disease mice. J Neuroimmunol. 2018;319:80-92. Epub 2018/03/27. doi: 10.1016/j.jneuroim.2018.03.009. PubMed PMID: 29573847; PMCID: PMC5916331.
69. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, Shen JC, Zou B, Xie XS, Tingle M, Hinkson IV, Angst MS, Wyss-Coray T. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature. 2017;544(7651):488-92. Epub 2017/04/21. doi: 10.1038/nature22067. PubMed PMID: 28424512; PMCID: PMC5586222.
70. Jim HS, Boyd TD, Booth-Jones M, Pidala J, Potter H. Granulocyte Macrophage Colony Stimulating Factor Treatment is Associated with Improved Cognition in Cancer Patients. Brain disorders & therapy. 2012;1(1). doi: 10.4172/bdt.1000101. PubMed PMID: 22905341; PMCID: 3422132.
71. Jacobs SR, Small BJ, Booth-Jones M, Jacobsen PB, Fields KK. Changes in cognitive functioning in the year after hematopoietic stem cell transplantation. Cancer. 2007;110(7):1560-7. doi: 10.1002/cncr.22962. PubMed PMID: 17685391.
72. Schliep G, Felgenhauer K. Serum-CSF protein gradients, the blood-GSF barrier and the local immune response. J Neurol. 1978;218(2):77-96. Epub 1978/05/18. doi: 10.1007/bf02402169. PubMed PMID: 78976.
73. Ji F, Pasternak O, Liu S, Loke YM, Choo BL, Hilal S, Xu X, Ikram MK, Venketasubramanian N, Chen CL, Zhou J. Distinct white matter microstructural abnormalities and extracellular water increases relate to cognitive impairment in Alzheimer's disease with and without cerebrovascular disease. Alzheimers Res Ther. 2017;9(1):63. Epub 2017/08/19. doi: 10.1186/s13195-017-0292-4. PubMed PMID: 28818116; PMCID: PMC5561637.
74. Li Y, Feng F, Lin P, Huang ZG, Liu T, Zhou B, Yao H, Zheng L, Li C, Wang P, Zhang Z, Guo Y, Wang L, An N, Zhu X, Zhang X, Wang J. Cognition-related white matter integrity dysfunction in Alzheimer's disease with diffusion tensor image. Brain Res Bull. 2018;143:207-16. Epub 2018/09/22. doi: 10.1016/j.brainresbull.2018.09.010. PubMed PMID: 30240840.
75. Mayo CD, Garcia-Barrera MA, Mazerolle EL, Ritchie LJ, Fisk JD, Gawryluk JR. Relationship Between DTI Metrics and Cognitive Function in Alzheimer's Disease. Front Aging Neurosci. 2018;10:436. Epub 2019/01/29. doi: 10.3389/fnagi.2018.00436. PubMed PMID: 30687081; PMCID: PMC6333848.
76. Mayo CD, Mazerolle EL, Ritchie L, Fisk JD, Gawryluk JR. Longitudinal changes in microstructural white matter metrics in Alzheimer's disease. Neuroimage Clin. 2017;13:330-8. Epub 2017/01/10. doi: 10.1016/j.nicl.2016.12.012. PubMed PMID: 28066707; PMCID: PMC5200876.
77. Strain JF, Smith RX, Beaumont H, Roe CM, Gordon BA, Mishra S, Adeyemo B, Christensen JJ, Su Y, Morris JC, Benzinger TLS, Ances BM. Loss of white matter integrity reflects tau accumulation in Alzheimer disease defined regions. Neurology. 2018;91(4):e313-e8. Epub 2018/07/01. doi: 10.1212/wnl.0000000000005864. PubMed PMID: 29959265; PMCID: PMC6070383.

78. Genc S, Steward CE, Malpas CB, Velakoulis D, O'Brien TJ, Desmond PM. Short-term white matter alterations in Alzheimer's disease characterized by diffusion tensor imaging. J Magn Reson Imaging. 2016;43(3):627-34. Epub 2015/08/01. doi: 10.1002/jmri.25017. PubMed PMID: 26228096.
79. Mak E, Gabel S, Su L, Williams GB, Arnold R, Passamonti L, Vazquez Rodriguez P, Surendranathan A, Bevan-Jones WR, Rowe JB, O'Brien JT. Multi-modal MRI investigation of volumetric and microstructural changes in the hippocampus and its subfields in mild cognitive impairment, Alzheimer's disease, and dementia with Lewy bodies. Int Psychogeriatr. 2017;29(4):545-55. Epub 2017/01/17. doi: 10.1017/s1041610216002143. PubMed PMID: 28088928; PMCID: PMC5819731.
80. Palesi F, De Rinaldis A, Vitali P, Castellazzi G, Casiraghi L, Germani G, Bernini S, Anzalone N, Ramusino MC, Denaro FM, Sinforiani E, Costa A, Magenes G, D'Angelo E, Gandini Wheeler-Kingshott CAM, Micieli G. Specific Patterns of White Matter Alterations Help Distinguishing Alzheimer's and Vascular Dementia. Front Neurosci. 2018;12:274. Epub 2018/06/21. doi: 10.3389/fnins.2018.00274. PubMed PMID: 29922120; PMCID: PMC5996902.
81. Rodriguez-Aranda C, Waterloo K, Johnsen SH, Eldevik P, Sparr S, Wikran GC, Herder M, Vangberg TR. Neuroanatomical correlates of verbal fluency in early Alzheimer's disease and normal aging. Brain Lang. 2016;155-156:24-35. Epub 2016/04/12. doi: 10.1016/j.bandl.2016.03.001. PubMed PMID: 27062691.
82. Colgan N, Siow B, O'Callaghan JM, Harrison IF, Wells JA, Holmes HE, Ismail O, Richardson S, Alexander DC, Collins EC, Fisher EM, Johnson R, Schwarz AJ, Ahmed Z, O'Neill MJ, Murray TK, Zhang H, Lythgoe MF. Application of neurite orientation dispersion and density imaging (NODDI) to a tau pathology model of Alzheimer's disease. Neuroimage. 2016;125:739-44. Epub 2015/10/28. doi: 10.1016/j.neuroimage.2015.10.043. PubMed PMID: 26505297; PMCID: PMC4692518.
83. Fu X, Shrestha S, Sun M, Wu Q, Luo Y, Zhang X, Yin J, Ni H. Microstructural White Matter Alterations in Mild Cognitive Impairment and Alzheimer's Disease : Study Based on Neurite Orientation Dispersion and Density Imaging (NODDI). Clin Neuroradiol. 2019. Epub 2019/06/09. doi: 10.1007/s00062-019-00805-0. PubMed PMID: 31175374.
84. Andre C, Tomadesso C, de Flores R, Branger P, Rehel S, Mezenge F, Landeau B, Sayette V, Eustache F, Chetelat G, Rauchs G. Brain and cognitive correlates of sleep fragmentation in elderly subjects with and without cognitive deficits. Alzheimers Dement (Amst). 2019;11:142-50. Epub 2019/02/23. doi: 10.1016/j.dadm.2018.12.009. PubMed PMID: 30788411; PMCID: PMC6369144.
85. Amorim L, Magalhaes R, Coelho A, Moreira PS, Portugal-Nunes C, Castanho TC, Marques P, Sousa N, Santos NC. Poor Sleep Quality Associates With Decreased Functional and Structural Brain Connectivity in Normative Aging: A MRI Multimodal Approach. Front Aging Neurosci. 2018;10:375. Epub 2018/12/14. doi: 10.3389/fnagi.2018.00375. PubMed PMID: 30524267; PMCID: PMC6257343.
86. Brzecka A, Leszek J, Ashraf GM, Ejma M, Avila-Rodriguez MF, Yarla NS, Tarasov VV, Chubarev VN, Samsonova AN, Barreto GE, Aliev G. Sleep Disorders Associated With Alzheimer's Disease: A Perspective. Front Neurosci. 2018;12:330. Epub 2018/06/16. doi: 10.3389/fnins.2018.00330. PubMed PMID: 29904334; PMCID: PMC5990625.
87. Spira AP, Gamaldo AA, An Y, Wu MN, Simonsick EM, Bilgel M, Zhou Y, Wong DF, Ferrucci L, Resnick SM. Self-reported sleep and beta-amyloid deposition in community-dwelling older adults. JAMA Neurol. 2013;70(12):1537-43. Epub 2013/10/23. doi: 10.1001/jamaneurol.2013.4258. PubMed PMID: 24145859; PMCID: PMC3918480.
88. Wu YH, Feenstra MG, Zhou JN, Liu RY, Torano JS, Van Kan HJ, Fischer DF, Ravid R, Swaab DF. Molecular changes underlying reduced pineal melatonin levels in Alzheimer disease: alterations in preclinical and clinical stages. J Clin Endocrinol Metab. 2003;88(12):5898-906. Epub 2003/12/13. doi: 10.1210/jc.2003-030833. PubMed PMID: 14671188.

89. Wu YH, Swaab DF. The human pineal gland and melatonin in aging and Alzheimer's disease. J Pineal Res. 2005;38(3):145-52. Epub 2005/02/24. doi: 10.1111/j.1600-079X.2004.00196.x. PubMed PMID: 15725334.
90. Maurizi CP. Choroid plexus portals and a deficiency of melatonin can explain the neuropathology of Alzheimer's disease. Med Hypotheses. 2010;74(6):1059-66. Epub 2010/01/16. doi: 10.1016/j.mehy.2009.12.026. PubMed PMID: 20074864.
91. Brunner P, Sozer-Topcular N, Jockers R, Ravid R, Angeloni D, Fraschini F, Eckert A, Muller-Spahn F, Savaskan E. Pineal and cortical melatonin receptors MT1 and MT2 are decreased in Alzheimer's disease. Eur J Histochem. 2006;50(4):311-6. Epub 2007/01/11. PubMed PMID: 17213040.
92. Cecon E, Chen M, Marcola M, Fernandes PA, Jockers R, Markus RP. Amyloid beta peptide directly impairs pineal gland melatonin synthesis and melatonin receptor signaling through the ERK pathway. Faseb j. 2015;29(6):2566-82. Epub 2015/03/12. doi: 10.1096/fj.14-265678. PubMed PMID: 25757565.
93. Zylinska K, Komorowski J, Robak T, Mucha S, Stepien H. Effect of granulocyte-macrophage colony stimulating factor and granulocyte colony stimulating factor on melatonin secretion in rats in vivo and in vitro studies. J Neuroimmunol. 1995;56(2):187-90. Epub 1995/02/01. PubMed PMID: 7532183.
94. Kunz D, Schmitz S, Mahlberg R, Mohr A, Stoter C, Wolf KJ, Herrmann WM. A new concept for melatonin deficit: on pineal calcification and melatonin excretion. Neuropsychopharmacology. 1999;21(6):765-72. Epub 2000/01/14. doi: 10.1016/s0893-133x(99)00069-x. PubMed PMID: 10633482.
95. Mahlberg R, Walther S, Kalus P, Bohner G, Haedel S, Reischies FM, Kuhl KP, Hellweg R, Kunz D. Pineal calcification in Alzheimer's disease: an in vivo study using computed tomography. Neurobiol Aging. 2008;29(2):203-9. Epub 2006/11/14. doi: 10.1016/j.neurobiolaging.2006.10.003. PubMed PMID: 17097768.
96. Beker-Acay M, Turamanlar O, Horata E, Unlu E, Fidan N, Oruc S. Assessment of Pineal Gland Volume and Calcification in Healthy Subjects: Is it Related to Aging? J Belg Soc Radiol. 2016;100(1):13. Epub 2016/02/01. doi: 10.5334/jbr-btr.892. PubMed PMID: 30038974; PMCID: PMC5854269.
97. Tan DX, Xu B, Zhou X, Reiter RJ. Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland. Molecules. 2018;23(2). Epub 2018/02/01. doi: 10.3390/molecules23020301. PubMed PMID: 29385085; PMCID: PMC6017004.
98. Beltrami D, Gagliardi G, Rossini Favretti R, Ghidoni E, Tamburini F, Calzà L. Speech Analysis by Natural Language Processing Techniques: A Possible Tool for Very Early Detection of Cognitive Decline? Front Aging Neurosci. 2018;10. doi: 10.3389/fnagi.2018.00369. PubMed PMID: 30483116; PMCID: PMC6243042.
99. Szatloczki G, Hoffmann I, Vincze V, Kalman J, Pakaski M. Speaking in Alzheimer’s Disease, is That an Early Sign? Importance of Changes in Language Abilities in Alzheimer’s Disease. Front Aging Neurosci. 2015;7. doi: 10.3389/fnagi.2015.00195. PubMed PMID: 26539107; PMCID: PMC4611852.
100. Clark LJ, Gatz M, Zheng L, Chen YL, McCleary C, Mack WJ. Longitudinal verbal fluency in normal aging, preclinical and prevalent Alzheimer disease. Am J Alzheimers Dis Other Demen. 2009;24(6):461-8. doi: 10.1177/1533317509345154. PubMed PMID: 19759254; PMCID: PMC2824246.
101. Gomez RG, White DA. Using verbal fluency to detect very mild dementia of the Alzheimer type. Arch Clin Neuropsychol. 2006;21(8):771-5. Epub 2006/10/03. doi: 10.1016/j.acn.2006.06.012. PubMed PMID: 17011743.
102. Kavé G, Goral M. Word retrieval in picture descriptions produced by individuals with Alzheimer's disease. J Clin Exp Neuropsychol. 2016;38(9):958-66. doi: 10.1080/13803395.2016.1179266. PubMed PMID: 27171756; PMCID: PMC4983450.
103. Study Drug: Leukine® (sargramostim): Hearing before the FDA Advisory Committee Meeting, May 3, 2013.

104. Leukine®/Sargramostim Product Monograph and Full Prescribing Information GC, a Sanofi Company.
105. Herrmann F, Schulz G, Lindemann A, Meyenburg W, Oster W, Krumwieh D, Mertelsmann R. Hematopoietic responses in patients with advanced malignancy treated with recombinant human granulocyte-macrophage colony-stimulating factor. J Clin Oncol. 1989;7(2):159-67. PubMed PMID: 2644395.
106. Mangan K, Mullaney M, Klumpp T, Goldberg S, Macdonald J. Mobilization of peripheral blood stem cells by subcutaneous injections of yeast-derived granulocyte macrophage colony stimulating factor: a phase I-II study. Stem Cells. 1993;11(5):445-54. doi: 10.1002/stem.5530110512. PubMed PMID: 8241955.
107. Vadhan-Raj S, Hittelman WN, Broxmeyer HE, Keating M, Urdal D, Gutterman JU. In vivo biologic activities of recombinant human granulocyte-macrophage colony-stimulating factor. Ann N Y Acad Sci. 1989;554:231-40. PubMed PMID: 2660675.
108. Bowman GL, Dayon L, Kirkland R, Wojcik J, Peyratout G, Severin IC, Henry H, Oikonomidi A, Migliavacca E, Bacher M, Popp J. Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. Alzheimers Dement. 2018;14(12):1640-50. Epub 2018/08/19. doi: 10.1016/j.jalz.2018.06.2857. PubMed PMID: 30120040.
109. Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535-62. Epub 2018/04/15. doi: 10.1016/j.jalz.2018.02.018. PubMed PMID: 29653606; PMCID: PMC5958625.
110. Appel J, Potter E, Bhatia N, Shen Q, Zhao W, Greig MT, Raj A, Barker WW, Potter H, Schofield E, Wu Y, Loewenstein DA, Duara R. Association of white matter hyperintensity measurements on brain MR imaging with cognitive status, medial temporal atrophy, and cardiovascular risk factors. AJNR American journal of neuroradiology. 2009;30(10):1870-6. doi: 10.3174/ajnr.A1693. PubMed PMID: 19643919.
111. Korf ES, Wahlund LO, Visser PJ, Scheltens P. Medial temporal lobe atrophy on MRI predicts dementia in patients with mild cognitive impairment. Neurology. 2004;63(1):94-100. PubMed PMID: 15249617.
112. Burton EJ, Barber R, Mukaetova-Ladinska EB, Robson J, Perry RH, Jaros E, Kalaria RN, O'Brien JT. Medial temporal lobe atrophy on MRI differentiates Alzheimer's disease from dementia with Lewy bodies and vascular cognitive impairment: a prospective study with pathological verification of diagnosis. Brain : a journal of neurology. 2009;132(Pt 1):195-203. doi: 10.1093/brain/awn298. PubMed PMID: 19022858.
113. Barker WW, Luis CA, Kashuba A, Luis M, Harwood DG, Loewenstein D, Waters C, Jimison P, Shepherd E, Sevush S, Graff-Radford N, Newland D, Todd M, Miller B, Gold M, Heilman K, Doty L, Goodman I, Robinson B, Pearl G, Dickson D, Duara R. Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. Alzheimer disease and associated disorders. 2002;16(4):203-12. PubMed PMID: 12468894.
114. Duara R, Loewenstein DA, Potter E, Appel J, Greig MT, Urs R, Shen Q, Raj A, Small B, Barker W, Schofield E, Wu Y, Potter H. Medial temporal lobe atrophy on MRI scans and the diagnosis of Alzheimer disease. Neurology. 2008;71(24):1986-92. doi: 10.1212/01.wnl.0000336925.79704.9f. PubMed PMID: 19064880; PMCID: 2676975.
115. Schinka JA, Raj A, Loewenstein DA, Small BJ, Duara R, Potter H. Cross-validation of the Florida Cognitive Activities Scale (FCAS) in an Alzheimer's disease research center sample. J Geriatr Psychiatry Neurol. 2010;23(1):9-14. doi: 10.1177/0891988709342724. PubMed PMID: 19703991.

116. Baldwin GC, Benveniste EN, Chung GY, Gasson JC, Golde DW. Identification and characterization of a high-affinity granulocyte-macrophage colony-stimulating factor receptor on primary rat oligodendrocytes. Blood. 1993;82(11):3279-82. Epub 1993/12/01. PubMed PMID: 8241500.
117. Nishihara T, Ochi M, Sugimoto K, Takahashi H, Yano H, Kumon Y, Ohnishi T, Tanaka J. Subcutaneous injection containing IL-3 and GM-CSF ameliorates stab wound-induced brain injury in rats. Exp Neurol. 2011;229(2):507-16. Epub 2011/04/26. doi: 10.1016/j.expneurol.2011.04.006. PubMed PMID: 21515263.
118. Klunk WE. Biopsy support for the validity of Pittsburgh compound B positron emission tomography with a twist. Arch Neurol. 2008;65(10):1281-3. doi: 10.1001/archneur.65.10.1281. PubMed PMID: 18852340; PMCID: PMC2637565.
119. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Annals of neurology. 2004;55(3):306-19. doi: 10.1002/ana.20009. PubMed PMID: 14991808.
120. Klunk WE, Bacskai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT. Imaging Abeta plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. J Neuropathol Exp Neurol. 2002;61(9):797-805. PubMed PMID: 12230326.
121. Klunk WE, Wang Y, Huang GF, Debnath ML, Holt DP, Mathis CA. Uncharged thioflavin-T derivatives bind to amyloid-beta protein with high affinity and readily enter the brain. Life Sci. 2001;69(13):1471-84. PubMed PMID: 11554609.
122. Villain N, Chetelat G, Grassiot B, Bourgeat P, Jones G, Ellis KA, Ames D, Martins RN, Eustache F, Salvado O, Masters CL, Rowe CC, Villemagne VL, Group AR. Regional dynamics of amyloid-beta deposition in healthy elderly, mild cognitive impairment and Alzheimer's disease: a voxelwise PiB-PET longitudinal study. Brain : a journal of neurology. 2012;135(Pt 7):2126-39. doi: 10.1093/brain/aws125. PubMed PMID: 22628162.
123. Daud AI, Mirza N, Lenox B, Andrews S, Urbas P, Gao GX, Lee JH, Sondak VK, Riker AI, Deconti RC, Gabrilovich D. Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. J Clin Oncol. 2008;26(19):3235-41. doi: 10.1200/JCO.2007.13.9048. PubMed PMID: 18591558.
124. Evans S, McRae-McKee K, Wong MM, Hadjichrysanthou C, De Wolf F, Anderson R. The importance of endpoint selection: How effective does a drug need to be for success in a clinical trial of a possible Alzheimer's disease treatment? Eur J Epidemiol. 2018;33(7):635-44. Epub 2018/03/25. doi: 10.1007/s10654-018-0381-0. PubMed PMID: 29572656; PMCID: PMC6061129.
125. Cohen AD, Klunk WE. Early detection of Alzheimer's disease using PiB and FDG PET. Neurobiol Dis. 2014;72 Pt A:117-22. doi: 10.1016/j.nbd.2014.05.001. PubMed PMID: 24825318; PMCID: PMC4226742.
126. Kobylecki C, Langheinrich T, Hinz R, Vardy ER, Brown G, Martino ME, Haense C, Richardson AM, Gerhard A, Anton-Rodriguez JM, Snowden JS, Neary D, Pontecorvo MJ, Herholz K. 18F-florbetapir PET in patients with frontotemporal dementia and Alzheimer disease. J Nucl Med. 2015;56(3):386-91. doi: 10.2967/jnumed.114.147454. PubMed PMID: 25655625.
127. Bhamra MS, Ashton NJ. Finding a pathological diagnosis for Alzheimer's disease: are inflammatory molecules the answer? Electrophoresis. 2012;33(24):3598-607. doi: 10.1002/elps.201200161. PubMed PMID: 23161254.
128. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: automated

labeling of neuroanatomical structures in the human brain. Neuron. 2002;33(3):341-55. PubMed PMID: 11832223.
129. Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM. Automatically parcellating the human cerebral cortex. Cereb Cortex. 2004;14(1):11-22. PubMed PMID: 14654453.
130. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage. 2006;31(3):968-80. doi: 10.1016/j.neuroimage.2006.01.021. PubMed PMID: 16530430.
131. Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. Neurology. 2007;68(21):1809-14. Epub 2007/05/23. doi: 10.1212/01.wnl.0000262031.18018.1a. PubMed PMID: 17515542; PMCID: PMC2668699.
132. Matsumoto Y, Yanase D, Noguchi-Shinohara M, Ono K, Yoshita M, Yamada M. Cerebrospinal fluid/serum IgG index is correlated with medial temporal lobe atrophy in Alzheimer's disease. Dement Geriatr Cogn Disord. 2008;25(2):144-7. Epub 2007/12/22. doi: 10.1159/000112555. PubMed PMID: 18097142.
133. Janelidze S, Hertze J, Nagga K, Nilsson K, Nilsson C, Wennstrom M, van Westen D, Blennow K, Zetterberg H, Hansson O. Increased blood-brain barrier permeability is associated with dementia and diabetes but not amyloid pathology or APOE genotype. Neurobiol Aging. 2017;51:104-12. Epub 2017/01/07. doi: 10.1016/j.neurobiolaging.2016.11.017. PubMed PMID: 28061383; PMCID: PMC5754327.
134. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. J Neural Transm (Vienna). 2002;109(5-6):813-36. Epub 2002/07/12. doi: 10.1007/s007020200068. PubMed PMID: 12111471.
135. Sperling R, Salloway S, Brooks DJ, Tampieri D, Barakos J, Fox NC, Raskind M, Sabbagh M, Honig LS, Porsteinsson AP, Lieberburg I, Arrighi HM, Morris KA, Lu Y, Liu E, Gregg KM, Brashear HR, Kinney GG, Black R, Grundman M. Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. Lancet Neurol. 2012;11(3):241-9. Epub 2012/02/07. doi: 10.1016/s1474-4422(12)70015-7. PubMed PMID: 22305802; PMCID: PMC4063417.
137. Lawton M, Kasten M, May MT, et al. Validation of conversion between mini-mental state examination and Montreal cognitive assessment. *Mov Disord*. 2016; **31**(4): 593-596. doi:**10.1002/mds.26498**
138. Bergeron D, Flynn K, Verret L, et al. Multicenter validation of an MMSE-MoCA conversion table. *J Am Geriatr Soc*. 2017; **65**(5): 1067-1072. doi:**10.1111/jgs.14779**
139. Saczynski JS, Inouye SK, Guess J, et al. The Montreal cognitive assessment: creating a crosswalk with the mini-mental state examination. *J Am Geriatr Soc*. 2015; **63**(11): 2370-2374. doi:**10.1111/jgs.13710**

Appendix B: GM-CSF Upregulated in Rheumatoid Arthritis Reverses Cognitive Impairment and Amyloidosis in Alzheimer Mice (Boyd et al., 2010).

Appendix C: Granulocyte Macrophage Colony Stimulating Factor Treatment is Associated with Improved Cognition in Cancer Patients (Jim et al., 2012).

Appendix D: Briefing Package – FDA Advisory Committee Meeting May 3, 2013; Study Drug: Leukine® (Sargramostim).

Appendix E: Leukine® (Sargramostim) Package Insert and Full Prescribing Information.

Appendix F: Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

- https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Appendix G: Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial (Gendelman et al., 2017).

Appendix H

General Rules for Excluded Concomitant Medications

This list is intended to be comprehensive, but not exhaustive. If a concomitant medication is in doubt, the decision of the Principal Investigator (PI), or Sponsor as specified, shall be the final determinant for allowance or not.

The PI will review all prior and concomitant medications, and review concomitant medication changes on an ongoing basis. If disallowed concomitant medications with exceptions for PRN use exceed frequencies as specified, the PI will be consulted prior to participant continuation in the study.

The PI and/or Sponsor may allow a participant to continue in the study while taking an excluded concomitant medication (or at an excluded frequency) on a case by case basis to ensure the safety of participants and the scientific validity of study data. Exceptions to excluded concomitant medications may be needed for situations such as treatment of an adverse event. Any exceptions to excluded concomitant medications or frequencies will result in a protocol deviation.

.

EXCLUDED CONCOMITANT MEDICATIONS1. Participation in any other investigational drug study within 4 weeks of screening (individuals may not participate in any other drug study while participating in this protocol).

- Any prior use of study drug that was investigated for anti-amyloid or anti-tauopothy effects, or AD vaccine, unless it can be documented that the participant was on placebo.
- Any prior use of sargramostim within 6 months of screening.

2. Diuretic drugs should not be started or discontinued within 4 weeks prior to screening. Any change in diuretic medication during the study should be reported.

3. As a class, chronic use of systemic corticosteroids are prohibited from within 4 weeks of screening through the follow-up visit due to possible risk of myeloproliferation.

4. Lithium is prohibited from within 4 weeks of screening through the follow-up visit due to possible risk of myeloproliferation.

5. Systemic immunosuppressive or immunomodulatory agents/drugs (other than corticosteroids, see below) are not permitted for a period of 60 days before screening. Rules for PRN use of select drugs that have a systemic effect, such as corticosteriods, are listed below.

6. Immunoglobulin therapy and biologic drugs are not permitted within 6 months before screening.

7. Off label or non-standard of care usage of leukotriene inhibitors, such as montelukast, are not allowed from before screening until after the final study visit.

8. AChEI and/or memantine therapy should follow standard of care parameters, and be a stable dosage for 60 days prior to and during screening. Initiation of, or changes to, AChEI or memantine therapy is strongly discouraged during the treatment period, and must be approved by the PI prior to change for participant continuation in the study. If changes occur without prior PI approval, the Sponsor must be consulted to approve of continuation in the study.

9. If a medication is found in more than one category, the rule for the most strict category will be used for use/exclusion.

ANTICOAGULATION THERAPY: Not allowed within 4 weeks prior to screening through follow-up visit (*with exception of 81 mg aspirin daily therapy)*

GENERIC NAME	BRAND NAME
Apixaban	Eliquis
Dabigatran	Pradaxa
Edoxaban	Savaysa
Enoxaparin	Lovenox
Fondaparinux	Arixtra
Heparin	
Rivaroxaban	Xarelto
Warfarin	Coumadin

NARCOTIC ANALGESICS: Chronic use not allowed within 4 weeks prior to screening through follow-up visit PRN prescribed narcotic analgesic use is acceptable (Note definition of chronic use is weekly usage of a narcotic analgesic drug for three or more times per week AND for two or more weeks within any four-week period)

GENERIC NAME	BRAND NAME
Hydromorphone	Dilaudid
Oxycodone/Acetaminophen	Percocet
Oxycodone/Aspirin	Percodan
Propoxyphene/Darvon and its variations	
Narcotics that contain codeine or morphine	

NEUROLEPTICS: Not allowed within 4 weeks prior to screening through follow-up visit

GENERIC NAME	BRAND NAME
Chlorpromazine	Thorazine
Fluphenazine	Prolixin
Loxapine	Loxitane
Perphenazine	Etrafon, Trilafon
Thioridazine	Mellaril
Thiothixene	Navane
Trifluoperazine	Stelazine
Clozapine	Clozaril
Haloperidol	Haldol
Olanzapine	Zyprexa
Quetiapine	Seroquel

NEUROLEPTICS: Use of the following is permitted if dose is stable for 4 weeks prior to screening:

GENERIC NAME	BRAND NAME
Aripiprazole	Abilify
Risperidone	Risperdal (up to 2mg/day)
Ziprasidone	Geodon

ANTIPARKINSONIAN MEDICATIONS: Not allowed within 4 weeks prior of screening through follow-up visit

GENERIC NAME	BRAND NAME
Amantadine	Symmetrel

Bromocriptine Deprenyl/Selegilene	Parlodel Eldepry
Levodopa Pergolide Pramipexole	Sinemet Permax Mirapex

SEDATIVES/BENZODIAZEPINES: Chronic use allowed if on stable doses 4 weeks prior to screening or PRN use if prescribed for a specific procedure.

GENERIC NAME	BRAND NAME
Alprazolam	Xanax
Buspirone	Buspar
Chloral Hydrate	Noctec
Chlordiazepoxide	Librium
Clonazepam	Klonopin
Diazepam	Valium
Flurazepam	Dalmane
Lorazepam	Ativan
Meprobamate	Miltown
Oxazepam	Serax
Temazepam	Restoril
Trazodone	Desyrel
Trizolam	Halcion
Zaleplon	Sonata
Zolpidem	Ambien

RHEUMATOID ARTHRITIS MEDICATIONS: Not allowed within 4 weeks of screening through follow-up visit

GENERIC NAME	BRAND NAME
Abatacept	Orencia
Adalimumab	Humira
Anakirna	Kineret
Baricitinib	Olumiant
Betamethasone	Celestone
Certolizmab	Cimzia
Etanercept	Enbrel
Golimumab	Simponi
Hydroxychloroquine sulfate	Plaquenil
Infliximab-dyyb	Inflectra, Remicade
Leflunomide	Arava
Methotrexate	Rheumatrex/Trexall
Prednisone	
Rituximab	Rituxan
Sarilumab	Kevzara
Tocilizumab	Actemra
Tofacitinib	Xeljanz

ANTIHYPERTENSIVE AGENTS WITH FREQUENT CNS SIDE-EFFECTS: Not allowed within 4 weeks of screening through follow-up visit

Clonidine	Catapres

SYSTEMIC CORTICOSTEROIDS: Not allowed within 4 weeks of screening through follow-up visit excepting PRN or short course therapy as a necessary prescribed therapeutic for an acute condition. PI must be notified of use. (Note definition of chronic use is weekly usage of a systemic corticosteriod drug for three or more times per week AND for two or more weeks within any four-week period)

GENERIC NAME	BRAND NAME
Cortisone	Cortone Acetate
Prednisone	Rayos, Sterapred, Deltasone
Prednisolone	Flo-Pred, Millipred, Orapred, Pediapred, Veripred 20, Prelone, Hydeltra, Hydeltrsol, Key-Pred, Predacort, Predalone, Predate, Predaject, Medicort, Pri-Cortin, Predcor, AsmalPred
Methylprednisolone	Medrol, MethylPREDNIsolone
Dexamethasone	Baycadron, Decadron, Dexamethasone, Intensol, DexPak, TaperDex, Zema-Pak, ZoDex, Zonacort
Betamethasone	Celestone Soluspan, Pod-Care
hydrocortisone	Cortef

OTHER SYSTEMIC IMMUNOSUPPRESSANTS AND/OR IMMUNOMODULATORS NOT LISTED ELSEWHERE: Not allowed within 60 days of screening through follow-up visit.

GENERIC NAME	BRAND NAME
Cyclosporine	Neoral, Sandimmune, SangCya
Tacrolimus	Astagraf XL, Envarsus XR, Prograf
Sirolimus	Rapamune
Everolimus	Afinitor, Zortress
Azathioprine	Azasan, Imuran
Lefunomide	Avara
Mycophenolate	CellCept, Myfortic
Ixekizumab	Taltz
Natalizumab	Tysabi
Secukinumab	Consentyx
Ustekinumab	Stelara
Vedolizumab	Entyvio
Basiliximab	Simulect
Daclizumab	Zinbryta

Appendix I

The DSMB will be chartered previous to the initiation of enrollment activities of the clinical trial.
The Colorado Clinical and Translational Sciences Institute (CCTSI) will set up and manage an independent DSMB for this study. No staff at CCTSI work as personnel on this study, but they will recruit qualified scientific and medical professionals from inside and outside of the University of Colorado scientific community to review the patient safety data generated by this study. The appropriate DSMB member oversight will be managed by the leader of the CCTSI DSMB. The DSMB will meet at a regularly scheduled interval (every six months) to review participant safety data.

Assurance from Conflicts of Interest (COI): If recruited from the at-large CU-AMC professional community, members of the DSMB will be employed within different departments on the CU Anschutz Medical Campus, and no members have any real or perceived conflicts. All other members will be recruited from outside the University of Colorado scientific community. In addition, DSMB members will provide annual CU-mandated disclosures of any perceived or real COIs, and will notify fellow members of the DSMB, the trial's PI and/or Sponsor of any potential COIs that may arise during the trial.

Appendix J

AVAILABLE RESOURCES:

CU-AMC and UCH:

Research Clinic – UCH: The Clinical and Translational Research Center (CTRC) Outpatient Clinic on the 3rd floor of the UCH Leprino Building, and Building 400 will be the location for participant evaluation in private rooms. The CTRC clinic contains 11 Exam Rooms, three Metabolic Testing Rooms, an Echocardiogram Testing Room with ECG services, a Cognitive Testing Room, a 5-Chair Infusion Center, and a Phlebotomy Room. There is also a patient registration area and waiting room. Building 400 has an examinination room available that may be used for procedures.

The University of Colorado Alzheimer's and Cognition Center (CUACC) Memory Disorders Clinic: The Memory Disorders Clinic includes examination rooms, procedure and consultation rooms, and four neuropsychometric testing rooms. Participants will be recruited from the Memory Disorders Clinic, but the informed consent, screening, and study-related tests will be performed at the CTRC Outpatient Clinic or Building 400.

The University of Colorado Research Imaging Center (CU-RIC)
and Brain Imaging Center (BIC): The CU-RIC in building Biosciences 3houses the research PET/CT, PET/MRI and cyclotron laboratory managed by Pharmalogic. The BIC houses a research MRI in Building 400..

CUACC Laboratory: Located in the Research Center 2 (RC2) building, a short walk from the clinic. Two -80 degree freezers located within Dr. Huntington Potter's laboratory will be utilized for banking of blood samples. Biomarker freezers are connected to continuous power supplies. Available cores include a state-of-the-art microscopy core with stereologer, fluorescent, confocal, and near IR microscopes, flow cytometry, qRT-PCR, ultra centrifuges, cryostat, flow cytometer/cell sorter, plate washer, dark room and developer, gel doc system, Meso Scale Discovery (MSD) Biomarker System, and Fuidigm Cell Sorting and Analysis System.

Data Management: Includes REDCap, a secure, HIPPA compliant database, database software (FileMaker Pro), PrismGraphPad, SPS, SAS, AnyMaze, MetaMorph, and FreeSurfer and NeuroQuant MRI analysis programs are all available. University of Pittsburgh collaborators have agreed to share their PET imaging analysis software.

Computer: The laboratories and clinic are adequately equipped with a PC and Apple computers with access to the Internet and literature search programs. All staff members also have computers running Windows XP or more recent, with necessary software, printer and internet access. The server is administered and backed-up daily by UCH IT.

Office: The Sponsor, Huntington Potter, PhD, and the PI each have 100–200 sq. ft. offices with telephone, fax access, and voice mail. The study coordinator(s) has office space in Building 400 of the CU-AMC, and the psychometrist will utilize space in the Cognitive Testing Room of the CTRC Outpatient Clinic and Building 400. Within each office or adjacent are PC's, telephones, fax access, and voice mail. Additional office space is available on the CU-AMC campus as needed. Various large and small conference/lecture rooms are available for reservation for meetings, seminars, and outreach events. All conference space is equipped with tele-communication equipment for tele-conferencing and audio visual equipment and marker boards for presentations.