COVER PAGE

Pilot Phase 2 Trial of the Safety & Efficacy of GM-CSF (Leukine®) in the Treatment of Alzheimer's Disease

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COMIRB # 12-1273

Pilot Phase 2 Trial of the Safety & Efficacy of GM-CSF (Leukine®) in the Treatment of Alzheimer's Disease

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TABLE OF CONTENTS

INTRODUCTORY STATEMENT

Trial Background:
GM-CSF (Granulocyte-Macrophage Colony-Simulating Factor) is a hematopoietic growth factor which stimulates the proliferation and differentiation of hematopoietic progenitor cells. Recombinant human GM-CSF, Leukine® (Sargramostim), 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, recombinant human GM-CSF, Leukine® 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 are usually rare mild-to-moderate pyrogenic effects that subside upon reducing the dosage of Leukine® by half or by halting administration. Leukine® has not been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness. In fact, in a briefing package, composed by Genzyme Corporation in May 2013 for an FDA Advisory Committee Meeting, it 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)*" ([1], attached as Appendix E).

Our preliminary preclinical results demonstrated that GM-CSF rapidly reduced cerebral amyloid deposition and completely reversed memory deficits in transgenic mouse models of Alzheimer's Disease (AD) ([2], attached as Appendix B). To assess the efficacy of GM-CSF in humans, we performed a retrospective analysis of a cognition study of human patients undergoing hematopoietic cell transplantation for cancer and who garner cognitive impairments from the chemotherapy or irradiation. In the patients that received a colony-stimulating factor (CSF) to stimulate the bone marrow and recover immune system function, we found that those who received GM-CSF (Leukine®/Sargramostim) plus G-CSF (Filigrastim) significantly improved in cognitive function as compared to those who received G-CSF alone ([3], attached as Appendix C). These findings combined with over two decades of accrued safety data using recombinant human GM-CSF, Leukine®/Sargramostim, in elderly leukopenic patients, suggested that Leukine® should be tested as a treatment to reverse cerebral amyloid pathology and cognitive impairment in AD.

Trial History:
Based upon the above findings, we initiated an ongoing clinical trial using Leukine® in mild-to-moderate AD subjects (NCT01409915), which began at the University of South Florida (USF) and which received an exemption from the IND regulations in October 2010. At USF, the trial progressed favorably, with 14 subjects completing the trial, and there were no incidences of any serious adverse events (AE's). In July 2012, Principal Investigator, Dr. Huntington Potter, relocated to the University of Colorado Denver (UCD), and the trial was halted while UCD Institutional Review Board approval was sought.

In July 2013, we submitted a revision to the NCT01409915 clinical trial protocol, so that a second clinical site, additional experimentation, and additional study subjects could be added, and the revised protocol received exemption from IND regulations on August 8, 2013 (Re: IND 119282, Reference ID: 3351332). In that revised protocol, we noted that the trial will now be based at UCD, and that subjects will continue to be recruited and enrolled at both the UCD and USF site. The Data

Safety Monitoring Board (DSMB) was also expanded to include safety oversight from individuals at both institutions. Additionally, our secondary endpoint of efficacy within our original 2010 protocol was divided into multiple separate secondary endpoints, so that our analysis plan could include details about how each secondary endpoint and its hypothesis will be tested, including the specific metric that will be compared between groups, the time point at which the comparison will be made, and any adjustments needed for multiple comparisons. Furthermore, in that revised protocol, we also added Dr. Jonathan Woodcock, MD, as a Co-Principal Investigator at the UCD site, and noted that Dr. Balebail Ashok Raj, MD will remain the Co-Principal Investigator at the USF site. Although we received exemption from IND regulations on August 8, 2013 (Re: IND 119282, Reference ID: 3351332), the revised protocol for the study at that time contained the usage of the Pittsburgh Compound B (^{11}C-PiB), a Positron Emission Tomography (PET) radioactive diagnostic agent that is used to estimate the β-amyloid neuritic plaque density in the brain of Alzheimer's disease subjects. However, it was subsequently determined by the Colorado Multiple Institutional Review Board that the ^{11}C-PiB compound was unable to be synthesized for use in this trial, and also that Dr. Peter Smith-Jones, the Sub-Investigator who would have been involved in the synthesis of ^{11}C-PiB for this trial, had announced his intent to resign from the University of Colorado. Therefore, we revised the protocol again to remove the ^{11}C-PiB- PET imaging and to remove Dr. Peter Smith-Jones from the list of Sub-Investigators. However, the rest of the protocol remained exactly the same as the protocol which received the Exemption from IND regulations on August 8, 2013. Thereafter, we resubmitted the IND and received Exemption again on November 15, 2013 (Re: IND 120445, Reference ID: 3407661).

Specifically, the November 15, 2013 revised protocol proposed to treat 20 mild-to-moderate AD subjects with Leukine® for five days a week for three weeks and to compare their clinical and cognitive responses to 20 untreated mild-to-moderate AD subjects, in order to determine the safety and potential efficacy of Leukine® for an indication of cognitive decline. There was proposed to be 10 treatment subjects and 10 placebo subjects recruited at each of the UCD and USF sites. Before this amended protocol, there had been no serious adverse events observed in either the half or full dose groups that had already completed the study at the University of South Florida, and the original DSMB had authorized the full FDA-recommended dosage of Leukine® for subsequent subjects (i.e. 250 μg /m^2/day/SC). Therefore the November 15, 2013 revised protocol proposed to continue at the full recommended dose of Leukine®. The DSMB was scheduled to review the data at the 3 week time point of the first 5 Leukine®-treated and 5 placebo subjects, and decide whether to continue at the same dose for each subsequent 5 Leukine®-treated and 5 placebo subjects. Following receipt of informed consent, a medical history, physical exam, complete blood cell count (CBC) with differential, complete metabolic panel (CMP), glomerular filtration rate (GFR) test, electrocardiogram (ECG), cognitive testing (MMSE only), and an MRI to determine medial temporal lobe atrophy (MTA) was scheduled to occur at screening. Following enrollment, blood biomarker assays, CBC with differential, and cognitive testing (all cognitive tests) was scheduled to be performed at baseline (i.e. Day 1 of treatment phase). The CBC with differential was scheduled to be performed approximately every three days during the treatment phase, and the CMP was scheduled to be performed again once per week. Vital signs, injection site review, and adverse event monitoring was scheduled to occur on each treatment day. A physical exam, CBC with differential, CMP, biomarker assays, MRI, and all cognitive tests was scheduled to be administered at the end of the treatment phase. A physical exam, CMP, biomarker assays, and all cognitive tests was scheduled to be administered again at the first (6-Week/45 day) follow-up visit. At the second (3-month/90 day) follow-up visit, a physical exam, CMP, biomarker assays, and all cognitive tests was again scheduled to be administered. Any adverse events throughout the complete study time was to be reported to the DSMB immediately.

On February 12, 2016, the treatment phase for the first cohort of randomized participants at the UCD site (5 Leukine treated at the 250 mcg/m^2/day/SQ and 5 placebo controls) was completed, in addition to 3 randomized participants from the USF site who had completed the trial. The DSMB

reviewed the data for all of these participants and recommended the following changes to the protocol going forward:

> "1) Based on review of the safety data reported to the DSMB, the current study dosing protocol does not present safety concerns and we have no recommended changes to dosing.
> 2) We recommend that the investigators establish specific criteria for terminating subjects from the study due to uncontrolled hypertension, in order to ensure standardized practices across subjects and treatment groups.
> 3) We recommend that with all scheduled DSMB data reviews in the future, the following data be included for review: 1) all vital sign data for each participant, 2) location of injection sites for each treatment and recorded outcomes from injection site review at each treatment visit, and 3) a tabulated list of the dates of treatment for each participant.
> 4) We request a copy at this point in time of the randomization assignment procedure currently being used by the University of Colorado site.
> 5) We recommend against altering the randomization procedure in any manner unless a protocol amendment with justification is approved by the IRB. This recommendation is prompted by the discovery that an investigator requested that the randomization assignment given to an enrolled participant who withdrew from the study be given to the next consecutively enrolled participant (i.e. that the same study drug be assigned to the next participant as had been given to the subject who withdrew).
> 6) The current protocol has no additional blood draws for complete blood count with differential (CBC with diff) beyond treatment day 15. We recommend a protocol change to include an additional CBC with diff on follow up day 90, at the same time as the day 90 scheduled blood draw for biomarkers and comprehensive metabolic panel. We recommend this change for all participants currently enrolled who have not reached day 90 as of April 7, 2016.
> 7) We recommend a correction to the protocol on page 19, to state that *serious adverse events* will be reported to the DSMB instead of *all adverse events*. In other locations it is specified that only *serious adverse events* will be reported to the DSMB, and we do not recommend that this be changed to all adverse events based on our review of the safety data."

A revised protocol came after the completion of treatment phase for the first cohort of randomized participants (5 Leukine®-treated at 250 µg /m2/day/SC, 5 placebo control), plus three participants from the University of South Florida (USF) site. The amendments within the revised protocol were in response to the recommendations of the April 6, 2016 Data Safety Monitoring Board (DSMB) Summary Report. The first DSMB Recommendation stated that "*Based on review of the safety data reported to the DSMB, the current study dosing protocol does not present safety concerns and we have no recommended changes to dosing*." This very positive conclusion gave us optimism for the successful completion of this trial, especially considering that there were no incidences in these participants of Amyloid-Related Imaging Abnormalities (ARIAs, including ARIA-E indicating vasogenic edema, and ARIA-H indicating hemorrhage), which have plagued all of the industry Alzheimer's disease (AD) clinical trials that have used antibodies against amyloid beta (Aβ) species or amyloid plaques.

The other DSMB Recommendations pertained to specific suggestions for the improvement of the trial's protocol. Specifically, the revised protocol removed USF as a site for this trial, due to the retirement of Dr. Balebail Ashok Raj, MD who was the Co-Principal Investigator at the USF site, and also because of the low recruitment rate at USF. We had already obtained all of the records and biological samples that were collected at USF during this trial, and the USF pharmacy had confidentially communicated only to the UCD pharmacy, who received Leukine and who received placebo amongst the 3 participants that completed the trial at UCD. The revised protocol also included new criteria for participants who presented with uncontrolled hypertension, improved scheduling of and

reporting to the DSMB, additional blood draws for complete blood count with differential (CBC with diff) on follow-up visits, and to request increased blood samples that will allow us to perform additional biomarker testing. The remaining amendments to the protocol pertained to clarifying and removing text related to the USF site, and to make other grammatical corrections (i.e. page numbers, correct tense, etc.).

Because our first cohort of randomized participants did not show any evidence of ARIA, and because our preliminary preclinical results had demonstrated that GM-CSF rapidly reduced cerebral amyloid deposition in transgenic mouse models of AD ([2], attached as Appendix B), we then needed to determine whether Leukine was indeed affecting amyloid load in the trial's remaining two cohorts of participants (10 Leukine®-treated and 10 placebo). We also needed to be certain that the determination of mild-to-moderate cognitive impairment and subsequent enrollment of potential study participants was due to Alzheimer's disease with cerebral amyloid deposition, and not due to other neurological disease. Amyloid Positron Emission Tomography (PET) observations from recent AD phase 3 trials of bapineuzumab and solanezumab had illuminated that 16% to 22% of enrolled subjects diagnosed with mild-to-moderate AD based on clinical criteria, were misclassified and did not have evidence of abnormal Aβ pathology on retrospective analyses of PET imaging (Sevigny et al., *Alzheimer Dis Assoc Discord,* 2016; 30:1-7). Therefore, we revised the protocol again to perform PET imaging at screening visit, as a new inclusion criterion, and at the first follow-up visit, or within a week thereof depending upon scheduling availability, utilizing the FDA-approved amyloid-binding PET imaging agent, Forbetapir F18 (Amyvid®). However, no treatment decisions were declared would be made based on the outcome of the follow-up PET scan. Additionally, we included another brain MRI at the first follow-up visit, to further assess any evidence of ARIA at that time point. Because all of the other AD clinical trials had shown the ability to reduce cerebral amyloid, but had also shown the very serious adverse effects of ARIAs, it was imperative that we determine whether Leukine was indeed affecting amyloid load and if so, whether it was doing so safely and without evidence of ARIAs. Thus, we submitted to the FDA a revised protocol using Amyvid® PET imaging and received Exemption from IND regulations on July 15, 2016 (IND 131238).

Update from last revision and current trial amendment:
Since the last amendment, we have completed another cohort of 10 randomized participants (i.e. 5 Leukine®-treated at 250 μg /m2/day/SC, and 5 placebo control). This brings the total number of participants to 30, since we relocated to Colorado and who have completed the trial at the full FDA-recommended dose of Leukine® (except for the 30th participant who still has their second follow-up visit remaining). Additionally to date, there still have NOT been any study-related Serious Adverse Events, especially NO evidence of onset of ARIA's among any trial participants. The DSMB has again convened after the 30th participant finished their treatment phase, and the DSMB has since approved the continuation of the trial at the current dosage of Leukine®. However, before we could resume enrollment after this last DSMB meeting, we received a letter from our regional representative at PETNET Solutions, Inc., a Siemens Medical Solutions, Inc.-owned subsidiary that is contracted with Eli Lilly/Avid Radiopharmaceuticals to manufacture and distribute Amyvid® in Colorado. In this letter, Eli Lilly stated that it had decided to terminate production of Amyvid® by the end of December, 2017 in 4 geographical locations within the United States, which includes Denver, Colorado. Because Amyvid® is not available to be made elsewhere and transported to Denver, CO, and because the other two FDA-approved PET tracers for imaging cerebral amyloid load (i.e. GE's Vizamyl® and Piramal's NeuroCeq®) are also not commercially made within Colorado, we have decided to replace the use of Amyvid® in this trial with ^{11}C-PiB. We currently make and use ^{11}C-PiB here at UCD for another ongoing study (IND 131985, COMIRB # 16-2064).

The replacement of Amyvid® with ^{11}C-PiB, along with an update of the Conflict of Interest section, are the only changes that we are proposing within this amended protocol.

SPECIFIC AIMS:

SPECIFIC AIM 1. To assess tolerability and safety of recombinant human GM-CSF, Leukine®, in mild-to-moderate AD patients *(Primary Endpoint)*.
Effects of Leukine® on mobilization of white blood cells will be determined by CBC with differential at screening, baseline Day 1 and on days 4, 6, 9, 11 and 15 of the treatment schedule (i.e. placebo or Leukine® at 250 mcg /m^2/day/SC for 5 days/ week for three weeks), and at the 45 and 90 day follow-up visits. Vital signs, injection site review, and adverse event monitoring will be performed at each treatment visit. Complete metabolic panels (CMP) will be performed at screen, and on days 4, 9, 15, 45, and 90 days after initiation of GM-CSF treatment (Table 1 below summarizes the time-table of procedures for the entire study). The follow up MRI at the end of the treatment phase will provide information on any adverse changes in the brain, such as vasogenic edema and microhemorrhage. The DSMB will review all available data after each group of 5 Leukine®-treated and 5 placebo subjects have finished the treatment phase and determine whether the remainder of the subjects (5 Leukine®-treated and 5 placebo) will continue to receive the same full recommended dose (250 mcg/m^2/day/SC).

> **HYPOTHESIS 1.** Recombinant human GM-CSF, Leukine®, will be tolerated by, and safe to use in mild-to-moderate AD patients.

SPECIFIC AIM 2. To test the hypothesis that Leukine® treatment will improve cognition in mild-to-moderate AD patients *(Secondary Endpoint)*.
A battery of cognitive tests will be performed at baseline and at approximately 15, 45, and 90 days after initiation of Leukine® treatment to determine whether any AD features reverse during or after Leukine® treatment compared to subjects receiving placebo. Primary cognitive tests will include the Mini Mental State Examination (MMSE) and the ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive subscale).

> **HYPOTHESIS 2.** Leukine® treatment in adults with mild-to-moderate AD will improve cognition as measured primarily by MMSE total score and ADAS-cog total score.

SPECIFIC AIM 3. To investigate whether Leukine® treatment has an effect on Medial Temporal Lobe Atrophy (MTA) in mild-to-moderate AD patients *(Tertiary Endpoint)*.
Atrophy in the Entorhinal cortex (ERC) and Hippocampus (HPC), measured on MRI scans, predicts future cognitive decline and conversion to AD among individuals with Mild Cognitive Impairment (MCI). 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.

> **HYPOTHESIS 3.** Leukine® treatment in adults with mild-to-moderate AD will reverse or stop the progression of MTA.

EXPLORATORY AIM 1. To assess whether Leukine® treatment will improve specific cognitive domains in mild-to-moderate AD patients.
A battery of cognitive tests will be performed at baseline Day 1 and at approximately 15, 45, and 90 days after initiation of Leukine® treatment to determine whether any AD features reverse during or after Leukine® treatment compared to subjects receiving placebo. Secondary cognitive domain tests will include the ADCS-ADL (Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory), Trail making tests (TRAILS) A, TRAILS B, Clinical Dementia Rating (CDR), and the Mohs Cancellation Task.

> **EXPLORATORY HYPOTHESIS 1.** Leukine® treatment in adults with mild-to-moderate AD will improve specific cognitive domains as measured by the ADCS-ADL, TRAILS A,

TRAILS B, CDR, and the Mohs Cancellation Task.

EXPLORATORY AIM 2. To examine the effects of Leukine® treatment in mild-to-moderate AD patients on biomarkers associated with AD .
Biomarkers from peripheral blood collection include various forms of the hallmark proteins that form amyloid plaques in AD (i.e. amyloid-beta [Aβ] peptides), as well as other proteins which levels are known to be altered in AD (hyper-phosphorylated Tau and various inflammation-associated cytokines and chemokines).

> **EXPLORATORY HYPOTHESIS 2.** Leukine® treatment in adults with mild-to-moderate AD will induce changes in blood biomarkers toward published control levels.

EXPLORATORY AIM 3. To determine if Leukine® treatment will change amyloid load in mild-to-moderate AD patients.
Amyloid PET scans will be performed using Pittsburgh Compound B (^{11}C-PiB), produced at the UCD-Anschutz Medical Campus (AMC) cyclotron laboratory, which allows for amyloid-beta neuritic plaque density imaging, and will be used to test the hypothesis that Leukine® treatment of mild-to-moderate AD subjects reduces cerebral amyloid load.

> **EXPLORATORY HYPOTHESIS 3.** Leukine® treatment in adults with mild-to-moderate AD will change amyloid load.

BACKGROUND AND RESEARCH RATIONALE:

Alzheimer's Disease (AD) is a major cause of death and disability in the elderly, affecting about 12% of those over age 65 and about 40-50% of those over age 85. Although treatments slowing the course of the disease, such as cholinesterase inhibitors or a glutamate receptor agonist are available, their benefits are transient, are only effective for about half of the patients that take them, and they do not attack the etiology 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 treatments that are currently under investigation are designed to attack the formation of the Aβ peptide or its polymerization into neurotoxic oligomers/fibrils. Following encouraging results in animals, human trials have thus far not been very successful. Evidently a completely new approach may be necessary.

Amyloid deposition and cognitive decline in transgenic animals, perhaps because it develops over a relatively short period of time and in the absence of clear neuronal cell death, is fundamentally different from that which leads to AD in humans, and this concern always overshadows the use of animal models for developing AD therapies. We therefore sought a new approach that would identify a new mechanism that would provide protection against AD. Our investigation is based on the epidemiological evidence that the vast majority of people with Rheumatoid arthritis do not tend to develop onset of AD.

Rheumatoid arthritis (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) [4,5]. 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 [6,7]. Further enhancements of leukocyte populations come from increased expression of structurally-unrelated

colony-stimulating factors (CSFs): M-CSF (macrophage), G-CSF (granulocyte), and GM-CSF (granulocyte-macrophage) [8-11].

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, clinical trials with NSAIDs have proven unsuccessful in AD patients. We therefore reversed the approach and concluded that intrinsic factors within RA pathogenesis itself may underlie RA's protective effect. When, we examined the RA literature for evidence for circulating molecules that might enter or affect the brain and interfere with the Alzheimer pathogenic pathway, we were particularly struck by reports that several colony stimulating factors that induce phagocytic and other immune cells are released during RA. Therefore, we investigated the activity of these colony-stimulating factors on the pathology and behavior of transgenic AD mice ([2], attached as Appendix B).

To investigate the interplay of the innate immune system and AD, we studied the effects on AD pathology of the three hematopoietic colony-stimulating factors (macrophage, granulocyte, and granulocyte-macrophage colony-stimulating factors; M-CSF, G-CSF, and GM-CSF), which are up-regulated during RA pathogenesis. Those CSFs enhance the survival and function of their respective leukocytes and drive their proliferation and differentiation from myeloid lineage precursors. GM-CSF induces dendritic cells, macrophages, and granulocytes (neutrophils, basophils, and eosinophils), while M-CSF and G-CSF respectively induce the macrophage and granulocyte subsets of the innate immune system. These innate immune cells have the ability to diapedese from the circulatory system and to differentiate further into various specialized immune cells within organs (microglia, Langerhan's cells, etc.). GM-CSF and G-CSF are also known to also be involved in erythropoiesis, and GM-CSF and erythropoietin act synergistically in the maturation and proliferation of the burst-forming and colony-forming erythroid units to the normoblast stage of erythropoiesis [12]. Moreover, circulating $A\beta_{42}$, the β-sheet-prone misfolding pathogenic peptide that primarily comprises cerebral amyloid plaques in AD [13], binds to complement opsonin C3b in an antibody-independent fashion, and C3b-opsonized particles bind to the complement receptor, CR1, on erythrocytes and to CR1g on liver-resident kupfer macrophages, in classical immune adherence mechanisms that are key steps in the removal from the bloodstream of pathogens and foreign proteins, and wherein complement-opsonized pathogens and proteins bound to erythrocyte CR1 are stripped off and degraded by sessile macrophages in the liver [14,15]. Thus GM-CSF could potentially function in both, the peripheral clearance of toxic Aβ and in bone marrow-derived microglial activity, because it is involved in erythrocyte proliferation and also in the proliferation, differentiation, and maintenance of most innate immune system leukocytes.

PRELIMINARY PRECLINICAL RESULTS:

Intrahippocampal injections of colony-stimulating factors:

In the first experiment, 5µg bolus injections of M-CSF, G-CSF, and GM-CSF were injected into the hippocampus of the ipsilateral brain hemisphere with vehicle, artificial cerebrospinal fluid (aCSF), injected into the contralateral hippocampus as a control in aged cognitively-impaired transgenic AD (PS/APP) mice [2]. The mice were then sacrificed one week later and analyzed. While 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 confirmed by colleagues using daily peripheral G-CSF injections, which also led to reduced amyloidosis and cognitive deficits in the Radial Arm and Morris Water Mazes [16]. In contrast to the mild G-CSF effect, our GM-CSF injections, demonstrated pronounced decreases in amyloid deposition, as compared to control hemispheres (Fig 1a). Anterior to posterior quantification of amyloid plaques revealed significant reductions within individual mice and overall significant reductions for all plaque parameters measured (Fig 1b).

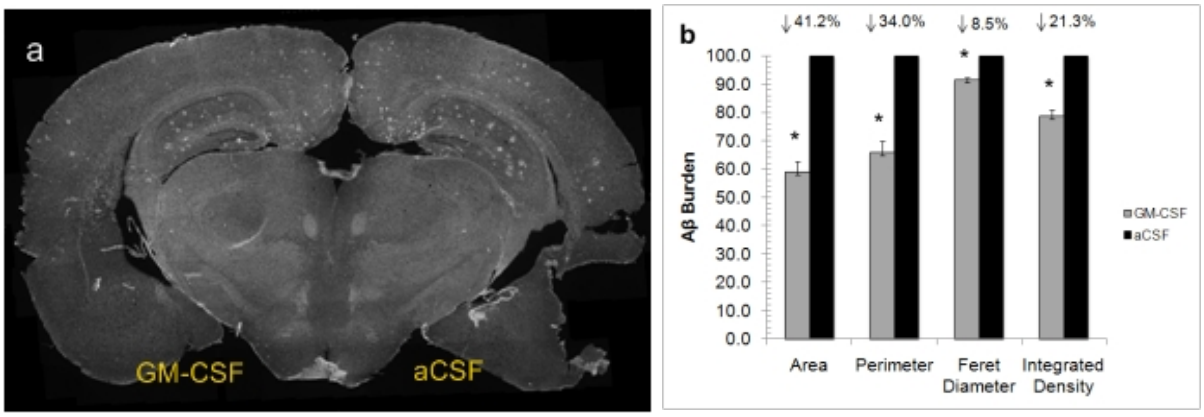

Figure 1. Intrahippocampal injection of GM-CSF on left and artificial cerebrospinal fluid (aCSF) on right. **(*a*)** *Representative coronal tissue cryo-sectioned at 14 µm and stained with MabTech α-Aβ/Alexa 546. Image is a montage of about 145 pictures taken at 10X.* ***(b)*** *Overall plaque reductions seen in all 4 plaque parameters measured from 5 quantified sections per mouse (n = 4 mice). Area and Perimeter data were calculated from the total number of plaque values in each hemisphere per section, and Feret Diameter and Integrated Density were calculated from the average values of the plaques measured in each hemisphere per section. (Area: $p < 1.11E-07$; Perimeter: $p < 1.41E-06$; Feret Diameter: $p < 2.36E-09$; Integrated Density: $p < 1.11E-07$)*

Daily subcutaneous injection of GM-CSF:

Based upon the remarkably-positive results of GM-CSF from only 7 days after intrahippocampal injections, we next investigated the effect of subcutaneous GM-CSF injection on AD pathology and cognitive function. Prior to GM-CSF treatment, both APPsw transgenic mice (Tg) were first confirmed by RAWM testing to be cognitively-impaired for working memory. Both, the non-transgenic control mice (NT) and the Tg mice were then sub-divided into two cognitively-balanced groups. Twenty days of daily subcutaneous injection of 5µg of GM-CSF (the most amyloid-reducing CSF in the bolus experiment) were administered to one of the cognitively balanced cohorts of AD mice. RAWM testing post-GM-CSF treatment re-confirmed that Tg control mice were substantially impaired compared to NT control mice. This impairment was evident in individual blocks of testing, as well as over all 4 days of testing (Fig 2a). In sharp contrast, GM-CSF-treated Tg mice now performed equally well or better than NT control mice, during individual blocks and overall. Even the GM-CSF-treated NT mice performed as well as, or slightly better than, NT controls (Fig 2a). An interference cognitive test that assessed the ability of the mice to switch from one water maze to another with different cues, also showed that GM-CSF restored normal cognitive function to the APP and PS/APP animals (not shown). Subsequent histological analysis of brains from the APPsw mice of this study revealed that GM-CSF treatment induced large reductions in amyloid burdens within Entorhinal cortex (↓55%) and the Hippocampus (↓57%) as compared to control Tg mice (Fig 2b).

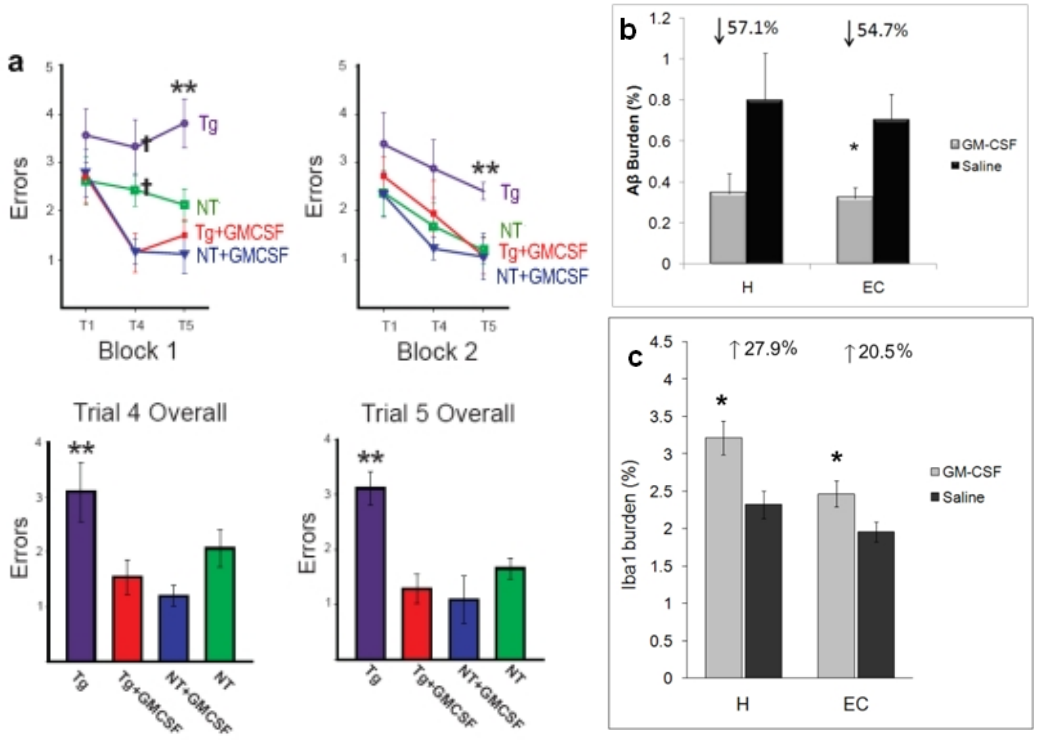

Figure 2. Behavioral and pathology analysis following daily subcutaneous GM-CSF injections. ***(a)*** *Tg control mice (n = 8) show substantial impairment on working memory trials T4 and T5 of the Radial Arm Water Maze compared to NT control mice (n = 8) in individual blocks of testing (upper), and over all 4 days of testing (lower). GM-CSF-treated Tg mice (n = 7) performed as well as or better than NT control mice on working memory trials T4 and T5 during individual blocks and over all.* ***(b)*** *Percent of amyloid burden from the average of five 5-µm sections (150-µm apart) through both anatomic regions of interest (hippocampus and entorhinal cortex) per mouse of GM-CSF-treated (n = 5) versus saline- treated (n = 6).* ***(c)*** *Percent of Iba1 burden from the average of five 5-µm sections (150 µm apart) through both anatomic regions of interest (H and EC) per mouse of GM-CSF-treated (n = 5) versus saline-treated (n = 6).*

The improved cognitive function and reduced cortical amyloidosis of GM-CSF-treated Tg mice were paralleled by increased microglial density as compared to saline-treated Tg mice (Fig 2c), implying an augmented ability to bind and remove amyloid deposition. Similarly, the GM-CSF-treated Tg mice demonstrated increased synaptophysin immunoreactivity in both CA1 and CA3 regions (Fig 3), indicating increased synaptic density in these hippocampal areas.

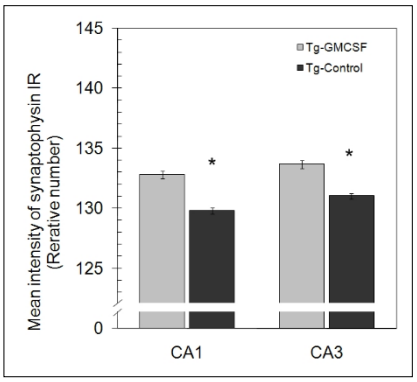

Figure 3. Synaptophysin immunostaining in subcutaneous GM-CSF-injected mice. *Percent of synaptophysin immunoreactivity from the average of 5 sections per mouse of GM-CSF-treated (n = 5) versus saline control-treated (n = 6). (CA1($p < 0.0013$), CA3($p < 0.0023$).*

Although our experimental mouse models did not allow for examination of other mechanistic effects from GM-CSF treatment, that may also help explain GM-CSF's reversal of cognitive impairment, GM-CSF has been shown in other studies to induce the proliferation and differentiation of neural stem cells, which are known to migrate to areas of damage in the brain [17,18]. It is also known from several stroke models that GM-CSF increases cerebral angiogenesis and is neuroprotective in the neurons and oligodendrocytes within the penumbras surrounding infarcts [19-22]. Neurogenesis and angiogenesis are known to occur together, with some of the same repulsive and attractive neuronal guidance factors that direct axonal growth, also directing migration of endothelial cells [23]. Because the Aβ peptide itself is anti-angiogenic [24], it is imperative that the amyloid plaques must be removed before any neovascularization and neurogenesis/synaptogenesis can occur. Studies have shown that truncated and occluded microvasculature surround amyloid plaques [25], and that cerebral amyloid angiopathy is found in almost all Alzheimer's disease patients [26]. Immunotherapy strategies that have been used to remove cerebral amyloid have all failed thus far in large randomized clinical trials, and they have also all been plagued with the induction of vasogenic edema and microhemorrhage [27,28]. These serious adverse events can be logically explained by the fact that these immunotherapies prevent the induction of bone marrow-derived leukocytes, and thus do not induce the conditions for neovascularization, which is necessary while simultaneously removing the amyloid and vascular-deposited Aβ. The mobilization from the bone marrow of peripheral blood progenitor cells is a critical step for any angiogenesis to occur, due to the production of endothelial progenitor cells [29], immature dendritic cells, myeloid-derived suppressor cells, and monocytes [30]. Recombinant human GM-CSF (Leukine®) has been FDA-approved and safely used over the last 20 years as a peripheral blood progenitor cell mobilizer, and a ongoing Phase II trial (NCT01041417) of GM-CSF in subjects with peripheral arterial disease (PAD) has already produced results showing it to be safe and that it is "*associated with mobilization of progenitor cells, improvement of endothelial dysfunction, and exercise capacity*" [31]. Since our peripheral administration of GM-CSF rapidly removed amyloid, while also increasing synaptic area and microglial density, we suspect that indeed neovascularization did occur. Furthermore, a subsequent study has found that targeted ablation of GM-CSF signaling in the hippocampus and amygdala results in spatial and fear memory deficits, and that the mice, carrying a null allele of the GM-CSF gene, have hippocampal neurons with significantly less dendritic tree arborization and reductions in spine densities and mature spines [32]. Combined, these pre-clinical (mouse) findings and ongoing PAD clinical trial suggest that improving cognition might be a new indication and function for GM-CSF.

PRELIMINARY CLINICAL RESULTS:

Moffitt Cancer Center:

Together with Dr. Heather Jim of the Moffitt Cancer Center, we performed a retrospecitive analysis of the data that was gathered from a study assessing cognition in Bone Marrow Transplant patients, who acquire cognitive deficits from the chemotherapy or irradiation procedures ([3], attached as Appendix C;[33]). Their cognitive function was measured

before treatments and then 6 months and 12 months after transplant and associated treatment, with a combination of either GM-CSF plus G-CSF or G-CSF alone. The neurological 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 hematopoietic cell transplantation (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 a high level of education (average of 13.89 years), the subjects displayed a statistically significant cognitive deficit at baseline.

The preliminary results show that patients who received GM-CSF plus G-CSF improved their cognitive functions (neuro minus motor features) between base line and six months, whereas subjects receiving only G-CSF did not improve (Figure 4 below). 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 propose to also use in this protocol. This human study also showed that a small number (less than 20) of patients only receiving part GM-CSF at the standard FDA-approved dose was sufficient to reveal that GM-CSF led to improved cognitive measures 6 months after HCT and CSF treatment, compared to the much larger group receiving G-CSF alone.

University of South Florida:
Before initiation of the current trial at the UCD site, fourteen participants enrolled in a preliminary study at USF. Of these 14 participants, 9 completed the entire study, including both follow-up visits; and the other 5 completed all treatment visits and at least one of the follow-up visits. Also, 3 of the 14 participants may have received the full Leukine® dose, but this number cannot be definite due to blinding of the study team at USF. Finally, the only AE's reported include injection site reactions seen in 6 patients (redness, raised, itching, bruising). No serious AE's were reported in these 14 participants.

The current trial (COMIRB #12-1273) was originally approved as a multi-site trial with recruitment efforts again, at USF, in Tampa, FL. USF's recruitment yielded 5 subjects who were enrolled into the current trial. Two of these subjects were screen failures and three completed the trial at USF. USF's last subject officially completed the trial on June 12th, 2015. However, due to USF's low recruitment, the decision was made to amend the protocol and close USF as a site. This amendment was approved by COMIRB on July 25th, 2016. Since the closure of the USF site, the research team at UCD has received all pertinent study data and study samples via secure HIPAA compliant practices.

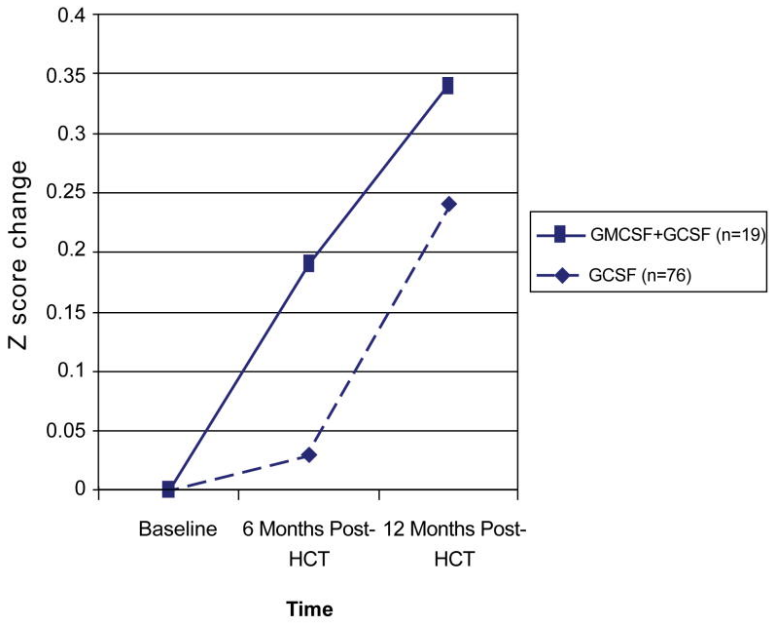

Figure 4. Total Neuropsychological Performance in Hematopoietic Cell Transplant (HCT) Recipients Receiving GM-CSF+G-CSF versus G-CSF Only.
The GM-CSF+GCSF group performed significantly better at 6 months post-HCT (p=.04), but there were no group differences at 12 months post-HCT (p=.24). The GM-CSF+G-CSF group improved significantly from baseline to 6 months post-HCT (p=.01) and from baseline to 12 months post-HCT (p<.01). The G-CSF group demonstrated no change from baseline to 6 months post-HCT (p=.33) but significant improvement from baseline to 12 months post-HCT (p<.01). Note: No patients received GM-CSF only. (GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; G-CSF: Granulocyte Colony-Stimulating Factor)

EXPERIMENTAL DESIGN AND METHODS:

Recruitment:
For recruitment at the UCD site, this pilot clinical trial of Leukine® in AD will utilize subjects with mild-to-moderate AD who are patients in the Memory and Dementia Clinic at the University of Colorado Hospital (UCH), of which the Co-Principal Investigator, Dr. Woodcock is the Clinical Director. Subjects will also be recruited from the Community. For recruitment at the USF site, this pilot clinical trial of GM-CSF utilized subjects with mild-to-moderate AD from the USF Byrd Alzheimer Institute Clinic patients and from the Tampa Bay Community. Recombinant human GM-CSF is commercially available from Sanofi/Genzyme Corporation as a recombinant molecule, i.e. Leukine®/Sargramostim, which is FDA-approved for use in elderly patients (> 55 years old) and has an outstanding safety record. To recruit from the community we will utilize study flyers posted at Anschutz Medical Campus, Clinicaltrials.gov and the UCD Clinical Trials Website. The consent process will emphasize the voluntary nature of the research study and the fact that declining participation will not alter clinical care. As help towards travel costs, patients will be informed that they will be receiving a $75.00 gift card at the end of the three week treatment period even if they cannot or choose not to complete the study, and will receive a $25.00 gift card after each of the follow up visits for a total of $125.00.

Inclusion/exclusion criteria:

Subjects must be

1. age 55 to 85 years;
2. should have a mild-to-moderate AD diagnosis (MMSE 10-26 inclusive);
3. should have qualitative evidence of elevated cortical amyloid by PET imaging.
4. if on anti-dementia treatment should be on stable treatment for at least 2 months (i.e. cholinesterase inhibitor and/or Memantine or Axona);
5. stable on all other medications for at least 30 days prior to screen;
6. should be fluent in English;
7. should be physically able to participate by medical history, clinical exam and tests;
8. should have a study partner to accompany them to scheduled visits.

Exclusion criteria are

1. clinically relevant arrhythmias or uncontrolled hypertension;
2. a resting pulse less than 50;
3. active cancer other than non-melanoma skin cancers;
4. use of another investigatory drug within 2 months of screening;
5. significant stroke or head trauma by history or MRI;
6. Contraindication for having a MRI;
7. DSM-IV criteria for a current major psychiatric disorder;
8. sensitivity to yeast or yeast products;
9. impaired kidney function as measured by a Glomerular Filtration Rate less than 60Ml/min;
10. preexisting fluid retention, pulmonary infiltrates, or congestive heart failure;
11. history of moderate-to-severe lung disease;
12. history of moderate-to-severe liver disease;
13. pregnant women, any women who feel they are likely to become pregnant during the study;
14. and prisoners

Leukine safety information and criteria for removal of trial subjects for cardiovascular events:
Leukine® (sargramostim) is indicated for the following uses: (i) following induction chemotherapy in older adult patients with acute myelogenous leukemia (AML) to shorten time to neutrophil recovery; (ii) for mobilization and following transplantation of autologous peripheral blood progenitor cells; (iii) for myeloid reconstitution after autologous or allogeneic bone marrow transplantation (BMT); (iv) for use in bone marrow transplantation failure or engraftment delay. Sanofi summarizes the studies on their website that led to FDA-approval for these indications. Of these indications, only two list reports of Cardiovascular System events:

> For AML (http://www.leukine.com/hcp-leukine-in-aml), Leukine is indicated for use following induction chemotherapy in older adult patients with acute myelogenous leukemia (AML) to shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections and infections resulting in death. The safety and efficacy of Leukine have not been assessed in patients with AML under 55 years of age. In a Phase III clinical trail, the safety and efficacy of Leukine® in the treatment of AML were evaluated in a multi-center, randomized, double-blind placebo-controlled trial of 99 newly diagnosed adult patients, 55–70 years of age, receiving induction with or without consolidation (52 Leukine®, 47 placebo). The percent of AML patients reporting cardiovascular system events of hypertension were 25% of the Leukine® group and 32% of the placebo group. Other cardiovascular system events reported in

each group include hypotension (13% Leukine®, 26% placebo), cardiac (23% Leukine®, 32% placebo), and hemorrhage (29% Leukine®, 43% placebo).

For the indication of Leukine® in the acceleration of myeloid recovery in patients undergoing allogeneic BMT from HLA-matched related donors (http://www.leukine.com/hcp-leukine-in-allogenic-bone-marrow-transplantation), Leukine® was found to be safe and effective in accelerating myeloid engraftment, reducing the incidence of bacteremia and other culture positive infections, and shortening the median duration of hospitalization. In a multi-center, randomized, placebo-controlled, and double-blinded study, a total of 109 patients (53 Leukine®, 56 placebo) were enrolled to evaluate the safety and efficacy of Leukine® for promoting hematopoietic reconstitution following allogeneic BMT. Hypertension was reported in 18 subjects receiving Leukine® (34%), and in 18 of the placebo subjects (32%).

On Sanofi's website under Important Safety Information for Leukine® (sargramostim), it is stated that "*Leukine® should be used with caution and monitored in patients with preexisting fluid retention, pulmonary infiltrates, or congestive heart failure, respiratory symptoms or disease; cardiac symptoms or disease; and renal or hepatic dysfunction.*" Sanofi's website states specifically for Cardiovascular Symptom that "*Occasional transient supraventricular arrhythmia has been reported in uncontrolled studies during Leukine® administration, particularly in patients with a previous history of cardiac arrhythmia. However, these arrhythmias have been reversible after discontinuation of Leukine®. Leukine® should be used with caution in patients with preexisting cardiac disease*."

Due to safety concerns regarding the potential of cardiac symptoms or disease, the Co-Principal Investigator and/or the DSMB may determine that a trial subject 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, the DSMB will be immediately notified, adverse events reporting protocols will be followed, and the following criteria will be used to determine whether the subject needs to be removed from the trial:

Inclusion Screening Criteria for hypertension:

- Should the potential subject have a screening blood pressure (BP) of more than 150 systolic or 95 diastolic, they will need to be under the care of a primary care provider for management of hypertension.
- The BP will have been well-controlled on a stable regimen for at least two months prior to study entry.
- The screening BP will be no greater than 160 systolic or 95 diastolic. (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, the patient will be removed from the study.

- The subject's hypertensive regimen may be adjusted by the primary care provider during the study as needed.

Sample size considerations:
Due to the well documented safety history of Leukine® **(**[1], attached as Appendix D**)** combined with additional patient information from the USF site, we believe that a sample size of 20 patients per research arm is adequate to assess our Primary Endpoint of measuring safety. Although the Moffitt BMT study was not specifically designed to distinguish the cognition of patients receiving GM-CSF plus G-CSF from those receiving G-CSF alone, it was possible to glean conclusions about the cognitive benefit of GM-CSF or G-CSF treatment. The study found highly significant improvement ($p<0.01$) in the scores of 19 patients receiving GM-CSF plus G-CSF, compared to patients receiving only G-CSF.

A Power analysis showed the following results:

Here is a table of the sample size needed per group with alpha at .20 and power at .60 for independent samples t tests:

Effect size	1 tailed test	2 tailed test
.3 - small	27	53
.5 - medium	10	20
.8 - large	4	8
1.22 – observed from BMT	3	4

Because the effect size of cognition improvement was so high with the BMT patients, that analysis provides clinical information as a reference to choose an appropriate sample size of AD subjects to receive GM-CSF as a potential cognition enhancer (one of our Secondary Endpoints). Essentially, only 3-4 subjects are needed for a power of 0.6. Therefore, the November 15, 2013 protocol proposed to recruit a total of 40 mild-to-moderate AD patients of either sex in a placebo-controlled protocol, with 20 subjects (10 Leukine®-treated and 10 placebo) recruited at the UCD site and 20 subjects (10 Leukine®-treated and 10 placebo) recruited at the USF site. However, with the revisions from the April 6, 2016 DSMB report, the revised protocol also removes active recruiting from the USF site, due to the retirement of Dr. Ashok Raj, and the remaining trial participants will be recruited at the UCD site.

The study will have two arms with subjects assigned in permuted blocks examining placebo and full recommended Leukine® dose. As mentioned above, in the trial subjects who have thus far received Leukine® at the University of South Florida, there were no serious adverse events reported by patients receiving either half dose or full dose Leukine® using the same methods proposed by this protocol. Therefore we will be starting patients at the full FDA-recommended Leukine® dose (250mcg/m^2/day SC). To assure that safety concerns of treating a novel population (AD patients) with GM-CSF are addressed, initially 5 subjects will be treated for 5 days/week for 3 weeks with Leukine® and 5 participants will be given placebo, followed by a DSMB review of the CBC+diff blood data, the MRI data, and the physical exam and adverse event monitoring at the end of the treatment phase. The DSMB will then recommend whether the dosage should be continued for the subsequent 5 Leukine® treatment subjects and 5 placebo controls or whether the Leukine® should be adjusted to a half dose. Recruitment and treatment initiation will be timed to allow for the DSMB interim assessments before further patients are treated, thus assuring maximum early knowledge of any safety concerns. Up to an additional 30 patients will be recruited at each study site, assuming that some screen failures and dropouts will occur, which will still allow us to treat 20 subjects with Leukine® and 20 with placebo, combined for a total of 40 mild-to-moderate AD subjects enrolled from both the UCD and the USF sites.

Data Analysis Plan:
Primary Endpoint*:* Generally, descriptive statistics will be utilized to examine safety of participants who received Leukine® injections compared to placebo injections. Safety will be assessed by measuring the toxicity grades of AE's. These toxicity grades have been standardized by the

National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE Version 4.03 attached as Appendix E). Differences in toxicity grades will be compared between treatments using a Wilcoxon test for ordered outcome.

Secondary Endpoint and Exploratory Aim 1*:* Neuropsychological assessments will be graded and scored using the standardized scoring methods associated with each of the neuropsychological tests. Our primary measures for cognitive assessments will be based on the participants' MMSE total score and ADAS-cog total scores. All other neuropsychometry tests will be used secondarily to examine changes in specific cognitive domains. Due to the multitude of both the neuropsychometry tests and the different time points these tests are administered, a Bonferroi correction method will be used to counteract the problem of multiple comparisons.

Tertiary Endpoint*:* Medial Temporal Lobe Atrophy (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 (ROI) in AD pathogenesis. FreeSurfer automatically computes cortical reconstruction and volumetric segmentation, including segmentation of the subcortical white matter and deep gray matter volumetric structures [34] and parcellation of the cortical surface [35] according to a previously published parcellation scheme [36]. 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; Pearson product–moment correlation coefficients evaluated the strength of relationships between of changes in regional volumes and thicknesses and cognitive measures. Receiver operator curve analyses will determine sensitivity and specificity of various MTA cut 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.

Exploratory Aim 2*:* Levels of AD-associated biomarkers in blood will be compared between placebo controls and Leukine® 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 [37]. 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 biomarker field of literature.

Exploratory Aim 3*:* Amyloid PET scans will be performed using Pittsburgh Compound B (^{11}C-PiB), produced at UCD-AMC, which allows for amyloid-beta neuritic plaque density imaging, and will be used to test the hypothesis that Leukine® treatment of mild-to-moderate AD subjects reduces cerebral amyloid load. ^{11}C-PiB PET scans will be performed at screening and at the first follow-up visit (+/- 7 days depending upon scheduling availability). For quantification of the brain amyloid load, assessments will evaluate the screening ^{11}C-PiB scan before the baseline visit and another ^{11}C-PiB scan within +/- 7 days of the first follow-up visit after the end of treatment with Leukine® or placebo. The change from the screening ^{11}C-PiB scan's SUVR to follow-up SUVR will be analyzed by a contracted central reading facility (i.e., BioClinica, a specialty clinical trial services provider) using the subject'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 ^{11}C-PiB 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. Data will be compared using parametric (SPM) and non-parametric (SnPM) statistical methods with family-wise error (FWE) and false discovery rate (FDR) corrections. Comparisons of SUVRs will be made between the first and second PET for each individual study participant. These differences will then be compared between placebo and treatments groups to

determine if Leukine® treatment changes amyloid load. The results of the ^{11}C-PiB PET quantitative analyses will not be used to make any treatment decisions.

> **Important Note**: Full-dose data from the two sites will be combined and analyzed together for both safety and cognitive outcomes. Also, other statistical tests that may be utilized include: independent t-tests to examine differences between means; the Chi-square test to assess association between variables; and logistical regression to calculate the ratio of the odds of an event occurring in one group compared to another group.

Dosage:
Recombinant human GM-CSF (Leukine®) is currently marketed in the US by Genzyme Corporation, a subsidiary of Sanofi. 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, Leukine® 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. ([38], attached as Appendix D). The FDA-approved recommended dosage is 250 mcg/m^2/day SC for up to 42 days until absolute neutrophil count (ANC) reaches 1500 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 which resulted in cognitive improvement and amyloid plaque reduction was about twice the recommended dosage of Leukine® (~500 mcg/m^2/day SC or ~167 mcg/kg/day) for a total of 20 injections (cumulative amount per mouse = 100 mcg). However, several clinical trials with Leukine® have shown no serious adverse events at a dosages of $\geq$500 mcg/m^2/day [39-41], and the maximum amount of Leukine® that can safely by administered by single or multiple dose has yet to be determined. Doses up to 100 mcg/kg/day (4000 mcg/m^2/day or 16 times the recommended dose) were administered to 4 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 adverse events (dyspnea, malaise, nausea, fever, rash, sinus tachycardia, headache and chills) were reversible upon discontinuation of the drug, as discussed above ([38], attached as Appendix D). These and other results, from the approximately 470,000 subjects that have received Leukine® during its post-marketing setting ([1]; attached as Appendix E), indicate that Leukine® has an extremely good safety profile.

More importantly, the Moffitt Cancer Center study, shown in the Preliminary Clinical Results section above, used the recommended FDA-approved dosage (250 mcg/m^2/day) and found highly significant ($p<0.01$) cognitive enhancement in patients treated with GM-CSF plus G-CSF compared to G-CSF alone ([3], attached as Appendix C). This allowed us to calculate the Power analysis presented above.

Finally, as mentioned, in our current Leukine® Safety Trial at the University of South Florida there were no serious adverse events reported by patients receiving either half dose or full dose Leukine® and using the same methods proposed by this study. Therefore we will be starting patients at the full FDA-approved recommended Leukine® dose. Nonetheless because of safety concerns, the go-slow approach of the proposed trial of assessing the subjects regularly and providing interim DSMB analyses (after each 10 subjects: 5 Leukine®-treated and 5 placebo) will allow us to determine quickly if there are any unexpected toxic effects and for the DSMB to halt the trial as per protocol. Each subject's first injection will be administered at the time of baseline (randomization) visit in the Clinical and Translational Research Center (CTRC) Outpatient Clinic on the 3rd floor of the UCH Leprino Building for the UCD site or at the USF Health Byrd Alzheimer Center clinic for the USF site. Subsequent

injections will also be administered in the CTRC Outpatient Clinic or at the USF Health Byrd Alzheimer Center clinic.

Data Storage:
All data collected at UCH will be stored in a REDCap, a HIPPA compliant database. Data collected will include general patient information (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. For the general manipulation of data, the clinical data will be transformed into a de-identified data set using the unique study IDs. This patient clinic data will be stored in a REDCap, HIPPA compliant database. Any paper record of the data will be kept under lock and key with the Co-Principal Investigator at the University of Colorado Hospital or with the Co-Principal Investigator at the USF Health Byrd Alzheimer Center. The co-investigators at the University of South Florida only had access to the de-identified clinic data. The statistician at the University of South Florida will also have access to the de-identified clinic data to conduct statistical analysis.

Specimen Storage:
The blood samples collected will only be used for this study and will not be provided to any third party. Samples will be stored for at least 7 years, or the entire course of this study. Excess sample will be added to biobanks for future studies, but only with consent of the subject. These biobanks will be located in the Linda Crnic Institute for Down Syndrome and the Colorado Intellectual & Developmental Disabilities Research Center. If a participant withdraws from the study, their samples will be discarded at the written request of the subject or appropriate consenting party. To confirm destruction of the samples at the Colorado Intellectual & Developmental Disabilities Research Center site, the site will send the research team written documentation confirming disposal of the sample. Otherwise, the sample will be stored at the Linda Crnic Institute for Down Syndrome and the Colorado Intellectual & Developmental Disabilities Research Center. The blood samples collected from the USF Health Byrd Alzheimer Center were de-identified and only coded with the unique study ID, and shipped for analysis and storage to the Linda Crnic Institute for Down Syndrome and the Colorado Intellectual & Developmental Disabilities Research Center.

STUDY EVENTS:

A summary of all study events are presented in Table 1. All interviews with subjects and potential subjects will be carried out in a private room. At the initial visit (screen), after informed consent is obtained, the MMSE is performed. If the subject qualifies on the MMSE entry criteria then vital signs are recorded, an ECG and a physical exam are performed, and 8 ml of blood is drawn for a CBC with differential, and a complete metabolic panel (CMP). A brain MRI will also be done during the screening period to exclude other causes of dementia and to assess for Medial Temporal Lobe Atrophy (MTA). Also, the first positron emission tomography (PET) scan, using ^{11}C-PiB, will be performed during the screening period to assess evidence of cerebral amyloid load. If the PET scan is performed on the same day as the MRI scan, the PET scan will be performed after the MRI scan. A qualitative read of the ^{11}C-PiB PET scan will be made to determine evidence of β-amyloid neuritic plaques. For women enrolled, who feel that there is a chance they may be pregnant, a pregnancy test will be administered. Clinically, if the patient cannot name their last menstrual period or is uncertain whether they are pregnant or not, a urine dipstick test is performed. The creatinine level from the CMP will also

be used to assess the Glomular Filtration Rate (GFR). Also a list of all patient medications will be recorded. Patients will have to meet the inclusion/exclusion criteria (stability on AD medications for 2 months prior to screen and all other meds must be stable 30 days prior to screen). Particular caution will be paid to individuals taking Lithium or corticosteroids, but they will not be excluded from the study.

Once the results are available, if the subject continues to meet entrance criteria they will return for their baseline Day 1 (randomization) visit. This visit takes place about 1 to 4 weeks after the screen visit. At this visit, vital signs are recorded, 33 ml of blood will be drawn for a CBC with differential, a complete metabolic panel (CMP), and for biomarker assays (for example, GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-α, Eotaxin, IP-10, MCP-1, MCP-4, MIP-1β, TARC, P-Tau, Total Tau, and Aβ species), and tests of cognition, such as the MMSE, ADAS-cog, the ADCS-ADL scale, Trails A, Trails B, CDR, and the Mohs Cancellation task, are administered. The test article will then delivered by the unblinded pharmacist, administered subcutaneously by the (blinded) study nurse or clinician, and the subject then goes home.

Subsequent injections will also be administered at the clinic. About 4 ml of blood will be drawn for CBC plus differential approximately every three days during the treatment phase to monitor for leukocytosis (see safety monitoring plan),and an addition 4 ml of blood will be drawn for CMPs once every week of the treatment phase. Vital signs, injection site review, and adverse events will be recorded at each visit. At the last treatment visit approximately three weeks later, we will record vitals, perform a physical exam, review for adverse events, administer cognitive tests and draw 33 ml blood for a CBC with differential, a CMP, and biomarker assays. A brain MRI will be done for comparison with the initial screen MRI to assess for any changes in the Medial Temporal lobe and for any incidence of vasogenic edema and microhemorrhage.

The first follow-up visit is about 6-weeks/45 days after the last treatment phase visit. At this visit the vital signs are recorded, 33 ml of blood for a CBC plus differential, CMP and biomarkers is drawn, a physical exam is performed, adverse events are reviewed, and cognitive tests are administered. Also, a brain MRI and second ^{11}C-PiB PET scan will be performed at the 6-weeks/45 day follow-up visit, or within a week before or after, depending upon scheduling availability. If the PET scan is performed on the same day as the MRI scan, the PET scan will be performed after the MRI scan. No treatment decisions will be made based on the outcome of the follow-up ^{11}C-PiB PET scan. The end of study and the second follow-up visit is performed approximately 6-weeks/45 days after the first follow-up visit (e.g. 3 months after the end of the treatment phase). At this visit, vital signs are recorded, adverse events are reviewed, a physical exam is performed, 33 ml of blood is drawn for a CBC plus differential, CMP and biomarker assays, and cognitive tests are administered.

COGNITIVE TESTS:

MMSE (Mini-Mental State Exam)
Education corrected Folstein Mini-Mental State Exam is a standard cognitive assessment used by all ADRCs including the FADRC to identify and monitor AD subjects.

ADAS-cog (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 is the most popular cognitive testing instrument used in clinical trials of nootropics (drugs or agents that improve cognitive function). It consists of 11 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 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.

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

TRAILS B
Executive function will be assessed using Trail Making Test-B, a sensitive test of cognitive flexibility and psychomotor speed in which subjects must connect a series of alternating numbers and letters placed randomly on a page.

Mohs Cancellation Task
Psychomotor speed and cognitive vigilance will be assessed using Mohs Cancellation Task, a timed task involving cancellation of target stimuli in a larger array of distractors.

CDR (Clinical Dementia Rating)
The CDR is a caregiver and subject 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). All raters in this trial will have completed the Washington University Alzheimer's Disease Research Center CDR Training.

LABS:

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. At the UCD site, phlebotomy will be performed by the University of Colorado Hospital Clinical and Translational Research Center (CTRC) nurses and at the USF site, by the USF Health Byrd Alzheimer Center clinic nurses.

Complete blood count (CBC) with differential and complete metabolic panel (CMP):
These labs will processed by an outside independent test center.

Biomarkers:
This testing will be performed in Dr. Potter's lab.

MRI PROCEDURE:

Atrophy in the Entorhinal cortex (ERC) and Hippocampus (HPC), measured on MRI scans, predict future cognitive decline and conversion among individuals with Mild Cognitive Impairment (MCI) to AD [42,43]. 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 [44]. 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 [45].

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 and perirhinal cortex (PRC), which are specific regions

of interest (ROI) in AD pathogenesis. We have shown that these individual and summed MTA scores distinguish AD and acute MCI subjects from normal elderly control subjects and predict progression from acute MCI to dementia/AD [46,47].

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 subjects may have difficulty lying motionless during the scan or may experience anxiety while in the scanner. This can be mitigated by administering a sedative prior to scanning.

Brain MRI scans at the UCD trial site will be performed in Building 400 on the Anschutz Medical Campus (AMC) at the University of Colorado and Denver VA Medical Center Brain Imaging Center, a component of the AMC's comprehensive Colorado Translational Research Imaging Center (C-TRIC). Scans may also be done in the University of Colorado Hospital Anschutz Outpatient Pavilion radiology department. Brain MRI scans at the USF trial site were performed at University Diagnostic Institute on the campus of the University of South Florida.

PET PROCEDURE – Pittsburgh Compound B (^{11}C-PiB):

The amyloid PET procedure will utilize the C-TRIC research PET scanner for human imaging studies. The Philips Gemini 64TF imaging system is located in Building 400 on the Anschutz Medical Campus at the University of Colorado and shares patient handling facilities with the Department of Psychiatry.

Pittsburgh Compound B (^{11}C-PiB) 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 for inclusion into the trial, subjects will receive a screening ^{11}C-PiB PET scan before the baseline visit that will be assessed by a Nuclear Medicine Radiologist (Michael Mueller, PhD) within the University of Colorado School of Medicine. The Nuclear Medicine Radiologist will remain blinded to subject identity and randomized test article group, and qualitative results, including any incidental findings, will reported to the Study Coordinator and Study Physician. 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 C-TRIC (located in Building 400 at CU-AMC). For quantification of the brain amyloid load, assessments will evaluate the screening ^{11}C-PiB scan before the baseline visit and another ^{11}C-PiB scan within +/- 7 days of the first follow-up visit after the end of treatment with Leukine® or placebo. The change from the screening ^{11}C-PiB scan's SUVR to follow-up SUVR will be analyzed by a contracted central reading facility (i.e., BioClinica, a specialty clinical trial services provider) using the subject'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 ^{11}C-PiB 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 ^{11}C-PiB PET quantitative analyses will not be revealed to the trial participants or used to make any treatment decisions. MRI scans scans will also be performed in conjunction with the ^{11}C-PiB PET imaging, so that any evidence of ARIA can be determined.

PET Imaging

Prior to the visit, subjects will be asked to abstain from food or drinks for 4 hours before the procedure. These procedures will be performed on a Philips Gemini 64TF scanner at the Colorado Translational Research Imaging Center (C-TRIC). Each study visit will take approximately 2 hours. The subject will be comfortably positioned in a chair. An intravenous catheter will be inserted using standard aseptic technique. A 10-15 mCi intravenous injection of ^{11}C-PiB will be administered. The subject will be placed on the scanning table and moved into the scanner. A preliminary, 3-5 minute head CT scan will

precede the PET scan to aid in alignment and calibration, and adjust for attenuation effects. ^{11}C-PiB PET images will be acquired for approximately 20 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

Pittsburgh Compound B (^{11}C-PiB) 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 ^{11}C-PiB 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 ^{11}C-PiB 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 of Pittsburgh Compound B has become a widely accepted amyloid imaging method in human subjects research. As of 2008, PiB imaging was being performed at over 38 sites worldwide and had become a part of the Alzheimer's Disease Neuroimaging Initiative (48). Therefore, it has substantiated potential as a quantitative imaging biological marker for detecting amyloid plaques in subjects.

Mechanism of Action

Pittsburgh Compound B (^{11}C-PiB, or N-methyl-[^{11}C]-2-(4′-methylaminophenyl)-6-hydroxy-benzothiazole) is an ^{11}C-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 (49). Klunk and colleagues developed a number of derivatives of Congo Red, a known beta-sheet binding molecule (50). These derivatives were made lipophilic so as to be able to cross the blood brain barrier after intravenous injection and could be shown to be non-toxic. Similarly, uncharged derivatives of thioflavin T were also shown to bind amyloid *in situ* (51). Klunk and colleagues were able to demonstrate strong target engagement of the radiotracer (i.e. sensitivity), and cross-labeling studies further demonstrated that ^{11}C-PiB does not label structures that do not also preferentially label amyloid specific dye (i.e. specificity). Comprehensive toxicology tests were conducted on pre-clinical models, including tests of acute vascular irritation in rabbits, bacterial reverse mutation assay, *in vitro* mammalian cell gene mutation test, mammalian erythrocyte micronucleus test, *in vitro* mammalian chromosome aberration test, expanded acute intravenous toxicity study in rats, an expanded acute toxicity study in dogs, an evaluation of the cardiovascular and pulmonary effects in dogs, a bridging study on the acute intravenous toxicity of ^{11}C-PiB in rats, and a bridging study on the cardiovascular and pulmonary effects in dogs. Based on these studies, no deleterious toxicology results have been noted in animals.

Early clinical research studies with humans demonstrated significant and consistent ^{11}C-PiB retention in frontal cortex, precuneus/posterior cingulate, striatal, temporal, and parietal cortices in the brains of patients diagnosed with AD dementia relative to healthy controls. ^{11}C-PiB was reported in 2004 as successfully able to bind to amyloid and thus remain in the brain of AD patients, while quickly clearing the brain of normal subjects (49). These findings were subsequently confirmed in other studies (52-54), with the retention pattern consistent with the cortical distribution of neuropathological findings of Aβ plaques on autopsy (55), suggesting that *in vivo* imaging of amyloid deposition parallels the underlying neuropathology. Since then, many studies have reported on the use of PiB for safely imaging amyloid in AD brain by PET, and the compound has become the method of choice for longitudinal studies of amyloid development in AD and for demonstrating and quantitating the efficacy of amyloid-reducing therapies (56).

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 subject will be exposed to per (^{11}C-PiB) 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 year. We will perform PET imaging at the screening visit and at the first follow-up visit, or within a week thereof, depending upon scheduling availability. Our administered dose of (^{11}C-PiB) will be 10-15 mCi. For a (^{11}C-PiB) dose of 15 mCi (555 MBq), the expected whole body effective dose is 2.9 mSv. In addition, the effective dose for a lose 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 (^{11}C-PiB) scans, we expect the trial subjects to be exposed to about 7 mSv, well below the 50 mSv maximum annual dose of radiation exposure. At the first screening visit, subjects and/or subject's partner/caregiver will be asked to provide information whether the subject 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 subject exceeding maximum allowed levels of radiation exposure.

RANDOMIZATION AND MAINTENACE OF BLIND

The drug will be purchased with funds for this study and study participants will not be billed for study related costs. At the UCD site, the Leukine® and the placebo samples for injection will be prepared by the UCH Pharmacy, 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 which 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 which 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 Alzheimer's Clinic will not know which study participants are receiving the study drug or the placebo.

At the USF site, the Leukine® and the placebo samples for injection were randomized, prepared, and supplied to the USF Health Byrd Alzheimer's Institute's Comprehensive Clinic by the USF College of Pharmacy Manager of the Investigational Research Pharmacy, which is located within the Carol and Frank Morsani Center for Advanced Healthcare, and which is a component of USF's Clinical and Translational Research Institute (CTSI).

DATA SAFETY MONITORING PLAN AND PROTECTION OF HUMAN SUBJECTS

Because of the extensive safety history of Leukine® in elderly patients, we do not anticipate any serious safety problems, but we must be vigilant in assessing safety. A review of published adverse events for GM-CSF and the Genzyme Leukine® Product Insert and the papers it references will be used to guide the safety monitoring protocol ([38], attached as Appendix D). The greatest safety concern noted with Leukine® is excessive leukocytosis. Therefore, as advised by Sanofi/Genzyme Corporation, we will obtain a CBC with differential twice a week during treatment and the dosage will be reduced by half if total ANC rises above 20,000/mm^3. In addition, the DSMB described below will monitor laboratory results, the MRI 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® [57], although the latest version of the NCI-CTCAE (Version 4.03) will also be referenced for all AE's (attached as Appendix F):

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

Data and Safety Monitoring Board (DSMB):

The DSMB will be comprised of members of relevant Departments at UCH, USF and the Moffitt Cancer Center. The DSMB will be charged with overseeing patient safety and compliance. An initial meeting of the DSMB, via teleconference or video-conference, will be held before dual enrollment occurs between the two sites in order for the members to review the study protocol and the study/participant termination guidelines. Data will be reviewed immediately if a serious adverse event is reported. Otherwise, all data will be reviewed after the first 10 participants (5 Leukine®-treatment, 5 placebo control) have completed the initial treatment phase, and again, after each next 10 participants (5 Leukine®-treatment, 5 placebo control) have completed the initial treatment phase from both UCD and USF clinical sites. Additionally, review of data will occur in a similar manner as above at 45 and 90 days after treatment for each group of 10 participants (5 Leukine®-treatment, 5 placebo control). Furthermore, because this was a multi-center study, serious AE's will be reported immediately to the DSMB, which includes members from both UCD and USF. If necessary, DSMB members from both sites will convene via teleconference or video-conference to discuss the serious AE's and to decide whether the study should continue. Of note, if serious AE's Grade 3 or 4 occur in 20% of subjects between the two sites following Leukine® administration the study will be stopped. All DSMB meetings will be documented.

The members of the DSMB include:

DSMB Members:

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Dr. Rasouli is Professor of Medicine at the University of Colorado. She received her M.D. from the University of Tehran and did her Internship and Residency at Michigan State University. Dr. Rasouli's expertise is in endocrinology and she heads many clinical trials in diabetes, obesity and heart failure and she is a member of the DSMB for "Neuroendocrine dysfunction after TBI" at the Craig Rehabilitation Hospital, CO.

Donald Leung, MD, PhD
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Dr. Leung is Professor of Pediatrics at the University of Colorado. He received his Ph.D. and M.D. from the University of Chicago and is an expert on allergy and clinical immunology, which is very appropriate expertise for membership on the DSMB of a trial on an immune modulator, GM-CSF. He has also served on multiple NIH advisory panels, has given many national and international invited lectures and was Editor in Chief of the Journal of Allergy and Clinical immunology form 1998-2015. He is currently Chair of the Aimmune Peanut Immunotherapy DSMB.

Theresa Zesiewicz, MD
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Dr. Zesiewicz received her M.D. from the University of Medicine and Dentistry of New Jersey, is Professor of Neurology at the University of South Florida, and is Director of the USF Ataxia Research Center. She has served on the DSMB for the 'Pilot phase 2 Trial of the Safety and Efficacy of GM-CSF (Leukine) in the Treatment of Mild-Moderate Alzheimer's Disease' since its inception at the University of South Florida through its new IND and IRB application at the University of Colorado Anschutz Medical Campus. Thus, she is well aware of the safety data of this drug in this trial. She has also great experience leading and/or participating in multiple safety and efficacy trials of interventions in Friedreich Ataxia (i.e. PMID: 30656180, 30051753, 29624723).

Heather Jim, PhD (Statistician)
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Dr. Heather Jim is a medical researcher of psychosocial and behavioral aspects of cancer control at the Moffitt Cancer Center in Tampa, FL, with research that specifically focuses on the adjustment to diagnosis and treatment, management of symptoms and side effects, and quality of life. Because of her interest in assessment, measurement issues are a recurrent theme in her research, which is augmented by an expertise in biostatistics. She has recently been involved in the investigation of cognitive functioning following treatment for breast and prostate cancer. Thus, due to her strong background in statistical analyses, cognitive testing, and immunology, Dr. Jim was recruited and has agreed to be the statistician for this DSMB.

Assurance of Independence of Judgment: Members of the DSMB are employed by several different departments and institutions.

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 Co-Principal Investigator, Dr. Woodcock, will review, edit and submit the AE forms at the UCD site and the Co- Principal Investigator, Dr. Raj, reviewed, edited and submitted the AE forms at the USF site. Laboratory results and data from case report forms will be entered into a computerized data registry.

Data Verification: Dr. Woodcock and Dr. Raj will discuss the AE's with the study coordinators, and verify AE data entry.

Data to be monitored, assessed, and reported to/by the DSMB:

Common but non-life threatening side effects include:

- Fatigue or asthenia
- Malaise
- Rash
- Itching (pruritis)
- 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 the 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

Serious Adverse Events (AE's) will be determined according to the most recent National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Event (CTCAE) Version 4.03 (attached as Appendix E).

Frequency: An initial meeting of the DSMB, via teleconference or video-conference, will be held before dual enrollment occurs between the two sites in order for the members to review the study protocol and study/participant termination guidelines. Data will be reviewed immediately if a serious adverse event is reported. Otherwise, all data will be reviewed after the first 10 participants (5 Leukine®-treatment, 5 placebo control) have completed the initial treatment phase, and again, after each next 10 participants (5 Leukine®-treatment, 5 placebo control) have completed the initial treatment phase from both UCD and USF clinical sites. Additionally, review of data will occur in a similar manner as above at 45 and 90 days after treatment for each 10 participants (5 Leukine®-treatment, 5 placebo control) from both UCD and USF clinical sites. Furthermore, because this was a multi-center study, serious AE's will be reported immediately to the DSMB, which includes members from both UCD and USF.

Report of Unexpected Problems to the DSMB: The study coordinator will notify the Co-Principal Investigator if there are any unexpected problems. The Co-Principal Investigator will call 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 serious adverse event will be reported by the Co-Principal Investigator, Dr. Woodcock, to COMIRB using the on-line reporting system. If 2 of 5 subjects experience serious AE's, the FDA will also be notified.

Stopping rules: If serious AE's Grade 3 or 4 occur in 20% of subjects following GM-CSF administration the study will be stopped. For individual subjects, the **DSMB** will discontinue any subject that reaches grade 3 or 4 in any category, or if they decline more than 25% in any of the cognitive tests from baseline. Subjects, are, of course free to withdraw from the study at any time for any or no reason.

Reporting:

Timeframe for reporting: Serious AE's will be reported immediately to the **DSMB** for review and, within 48 hrs (2 business days) to COMIRB. A persistent or significant disability will be reported within 5 business days. The FDA will be notified by written report within one or two weeks if 2 of 5 subjects experience serious AE's.

Reporting mechanisms: AE forms will be completed and submitted on-line using the UCH 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.

Responsibility for preparing and submitting reports: The study coordinator will prepare the AE report and the Co-Prinicipal Investigator will review/edit as appropriate and 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 cabinet when not in use. Data entered into the computerized data registry will only be accessible to those members of the research team.

Control of Conflict of Interest:

Dr. Potter was one of the conceivers of the Arthritis/GM-CSF/G-CSF approach to AD therapy and, along with Dr. Timothy Boyd, is one of the inventors on a University of South Florida patent on the use of GM-CSF in AD (US Patent 9,132,168) that is subject to the standard University of South Florida royalty sharing agreement. According to that agreement and although there are no current or immediately expected financial benefits that would attend the results of the proposed research, the University of Colorado Anschutz Medical Campus Conflict of Interest Committee (UCD-AMC COIC) has determined that Drs. Huntington Potter and Timothy Boyd each have a significant financial interest due to the issuance of the patent, and the UCD-AMC COIC has approved management plans, which Drs. Potter and Boyd will be glad to share upon request, and that allow the continued involvement of Drs. Potter and Boyd in the clinical trials of Leukine. Additionally, the conduct of this trial will be further managed as follows: The Co-Principal Investigators, Dr. Woodcock and Dr. Raj, who each have no real or perceived conflict, will carry out the clinical assessments of the subjects. Furthermore, the following steps will be used to help manage any potential conflict of interest:

1) The investigators will be blinded to the treatment group.
2) Endpoints are quantitative/objective (improvement in cognition by assessment with a battery of neuropsychological and clinical assessments used in the UCH clinic and changes in blood biomarkers towards normal).
3) If interested investigator(s) do participate in data collection and recording of data, they will be blinded and will not be the sole evaluator of the results/endpoints.
4) Financial interest has not been licensed and has no current commercial value.
5) Additional controlled studies would be done if outcome of study indicates results that would be favorable to the financial interest.
6) An independent **DSMB** has been established to oversee patient safety.
7) There are numerous other non-interested investigators involved in the study who will be collecting data.
8) Notification of Dr. Potter's and Dr. Boyd's financial interests in the study will be made to editors of journals publishing study results and to the public in the context of communication of any research results.

AVAILABLE RESOURCES:

University of Colorado Denver:

Laboratory: The Principal Investigator, Dr. Potter, has 1800 square feet of space in the Linda Crnic Institute for Down Syndrome where the biomarker levels of cytokines, chemokines, Tau and Aβ peptides from the plasma of the trial's participants will be assessed.

Clinic – UCH: The Clinical and Translational Research Center (CTRC) Outpatient Clinic on the 3rd floor of the UCH Leprino Building will be the location for subject evaluation in private rooms. The clinic contains 11 Exam Rooms, 3 Metabolic Testing Rooms, an Echocardiogram Testing Room with ECG services, a Cognitive Testing Room, a 5-Chair

Infustion Center, and a Phlebotomy Room. There is also a patient registration area and waiting room.

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 with necessary software, printer and internet access. The server is administered and backed-up daily by UCH IT.

Office: Dr. Potter and Dr. Woodcock each have 100-200 Sq. Ft. offices with telephone, fax access, and voice mail. The study coordinator has office space in the LCI and in the Academic Office 1 Building, and the psychometrist will utilize space in the Cognitive Testing Room of the CTRC Outpatient Clinic. Within each office are PC's, telephones, fax access, and voice mail.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.

Equipment:

- Two -80C freezers, plus other refigerators and -20C freezers,
- Cryo Locator 6 liquid nitrogen storage sytem,
- Cell culture room with BSL2 tissue culture and sample hood,
- CO_2 incubators,
- A new Zeiss flourescence microscope with Z stage and ccd cameras for analyzing immunostained and immunofuorescent samples,
- A Meso Scale Discovery system with ccd camera for imaging electrochemiluminescent immunoassays,
- Preparative and ultra centrifuges with refrigeration and rotors for plates, conicals and ultrafuge tubes,
- Real-time PCR machine,
- Autoclave,
- Spectrophotometer,
- Biotek microtitre plate reader with luminometer, and plate washing system,
- Electroporation and electrophoretic power supplies and associated equipment,
- Stereo microscope for array QC,
- Designated sets of drummond and micropipettors.

University of South Florida:

Clinic – USF Health Byrd Alzheimer's Center: The USF Health Byrd Alzheimer's Institute's Comprehensive Clinic, will be the location for subject evaluation in private rooms. The clinic comprises over 8000 square feet. There are 6 exam rooms, 5 patient consult rooms and 4 offices shared by associated clinical staff. There is also a patient registration area, waiting room and patient library.

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 operating systems with necessary software, printer and internet access. The server is administered and backed-up daily by USF Health IT.

Office: Dr Raj has 100-200 Sq. Ft. office with telephone, fax access, and voice mail. The study

coordinator and the psychometrist, each have offices within the Byrd Institute. Within each office are PC's, telephones, fax access, and voice mail.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.

Equipment:

- Preparative and ultra-centrifuges,
- -80C freezer, plus other refigerators and -20C freezers,
- Cell culture room with BSL2 tissue culture and sample hood.

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STUDY SCHEDULE

VISITS	SCREEN	TREATMENT PHASE																			Follow up 1	End of study
DAYS	7-28	1	2	3	4	5			6	7	8	9	10			11	12	13	14	15	45 days post (PET +/- 7 days)	90 days post
Informed consent	X																					
Pregnancy test	X																					
GFR	X																					
MMSE	X	X																		X	X	X
Medical history	X																					
concomitant meds	X	X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X	X
Adverse events		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X	X
Physical exam	X																			X	X	X
Injection site review		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X		
ECG	X																					
MRI	X																			X	X	
PET	X																				X	
LABS																						
Biomarkers		X																		X	X	X
Quest CBC+diff	X	X			X				X			X				X				X	X	X
CMP	X				X							X								X	X	X
Cognitive testing		X																		X	X	X
Study drug injections		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X		

PHARMACOLOGY, TOXICOLOGY, AND PREVIOUS HUMAN EXPERIENCE

DESCRIPTION

LEUKINE® (sargramostim) is a recombinant human granulocyte-macrophage colony stimulating factor (rhu GM-CSF) produced by recombinant DNA technology in a yeast (*S. cerevisiae*) expression system. GM-CSF is a hematopoietic growth factor which stimulates proliferation and differentiation of hematopoietic progenitor cells. LEUKINE is a glycoprotein of 127 amino acids characterized by three primary molecular species having molecular masses of 19,500, 16,800 and 15,500 daltons. The amino acid sequence of LEUKINE differs from the natural human GM-CSF by a substitution of leucine at position 23, and the carbohydrate moiety may be different from the native protein. Sargramostim has been selected as the proper name for yeast-derived rhu GM-CSF.

The liquid LEUKINE presentation is formulated as a sterile, preserved (1.1% benzyl alcohol), injectable solution (500 mcg/mL) in a vial. Lyophilized LEUKINE is a sterile, white, preservative-free powder (250 mcg) that requires reconstitution with 1 mL Sterile Water for Injection, USP or 1 mL Bacteriostatic Water for Injection, USP. Liquid LEUKINE has a pH range of 6.7 - 7.7 and lyophilized LEUKINE has a pH range of 7.1 - 7.7.

Liquid LEUKINE and reconstituted lyophilized LEUKINE are clear, colorless liquids suitable for subcutaneous injection (SC) or intravenous infusion (IV). Liquid LEUKINE contains 500 mcg (2.8×10^6 IU/mL) sargramostim and 1.1% benzyl alcohol in a 1 mL solution. The vial of lyophilized LEUKINE contains 250 mcg (1.4×10^6 IU/vial) sargramostim. The liquid LEUKINE vial and reconstituted lyophilized LEUKINE vial also contain 40 mg/mL mannitol, USP; 10 mg/mL sucrose, NF; and 1.2 mg/mL tromethamine, USP, as excipients. Biological potency is expressed in International Units (IU) as tested against the WHO First International Reference Standard. The specific activity of LEUKINE is approximately 5.6×10^6 IU/mg.

CLINICAL PHARMACOLOGY

General GM-CSF belongs to a group of growth factors termed colony stimulating factors which support survival, clonal expansion, and differentiation of hematopoietic progenitor cells. GM-CSF induces partially committed progenitor cells to divide and differentiate in the granulocyte-macrophage pathways which include neutrophils, monocytes/macrophages and myeloid-derived dendritic cells.

GM-CSF is also capable of activating mature granulocytes and macrophages. GM-CSF is a multilineage factor and, in addition to dose-dependent effects on the myelomonocytic lineage, can promote the proliferation of megakaryocytic and erythroid progenitors.[1] However, other factors are required to induce complete maturation in these two lineages. The various cellular responses (i.e., division, maturation, activation) are induced through GM-CSF binding to specific receptors expressed on the cell surface of target cells.[2]

***In vitro* Studies of LEUKINE in Human Cells** The biological activity of GM-CSF is species-specific. Consequently, *in vitro* studies have been performed on human cells to characterize the pharmacological activity of LEUKINE. *In vitro* exposure of human bone marrow cells to LEUKINE at concentrations ranging from 1–100 ng/mL results in the proliferation of hematopoietic progenitors and in the formation of pure granulocyte, pure macrophage and mixed granulocyte-macrophage colonies.[3] Chemotactic, anti-fungal and anti-parasitic[4] activities of granulocytes and monocytes are increased by exposure to LEUKINE *in vitro*. LEUKINE increases the cytotoxicity of monocytes toward certain neoplastic cell lines[3] and activates polymorphonuclear neutrophils to inhibit the growth of tumor cells.

***In vivo* Primate Studies of LEUKINE** Pharmacology/toxicology studies of LEUKINE were performed in cynomolgus monkeys. An acute toxicity study revealed an absence of treatment-related toxicity following a single IV bolus injection at a dose of 300 mcg/kg. Two subacute studies were performed using IV injection (maximum dose 200 mcg/kg/day x 14 days) and subcutaneous injection (SC) (maximum dose 200 mcg/kg/day x 28 days). No major visceral organ toxicity was documented. Notable histopathology findings included increased cellularity in hematologic organs and heart and lung tissues. A dose-dependent increase in leukocyte count, which consisted primarily of segmented neutrophils, occurred during the dosing period; increases in monocytes, basophils, eosinophils and lymphocytes were also noted. Leukocyte counts decreased to pretreatment values over a 1-2 week recovery period.

Pharmacokinetics Pharmacokinetic profiles have been analyzed in controlled studies of 24 normal male volunteers. Liquid and lyophilized LEUKINE, at the recommended dose of 250 mcg/m^2, have been determined to be bioequivalent based on the statistical evaluation of AUC.[5]

When LEUKINE (either liquid or lyophilized) was administered IV over two hours to normal volunteers, the mean beta half-life was approximately 60 minutes. Peak concentrations of GM-CSF were observed in blood samples obtained during or immediately after completion of LEUKINE infusion. For liquid LEUKINE, the mean maximum concentration (Cmax) was 5.0 ng/mL, the mean clearance rate was approximately 420 mL/min/m^2 and the mean AUC (0–inf) was 640 ng/mL•min. Corresponding results for lyophilized LEUKINE in the same subjects were mean Cmax of 5.4 ng/mL, mean clearance rate of 431 mL/min/m^2, and mean AUC (0-inf) of 677 ng/mL•min. GM-CSF was last detected in blood samples obtained at three or six hours.

When LEUKINE (either liquid or lyophilized) was administered SC to normal volunteers, GM-CSF was detected in the serum at 15 minutes, the first sample point. The mean beta half-life was approximately 162 minutes. Peak levels occurred at one to three hours post injection, and LEUKINE remained detectable for up to six hours after injection. The mean Cmax was 1.5 ng/mL. For liquid LEUKINE, the mean clearance was 549 mL/min/m^2 and the mean AUC (0–inf) was 549 ng/mL•min. For lyophilized LEUKINE, the mean clearance was 529 mL/min/m^2 and the mean AUC (0-inf) was 501 ng/mL•min.

INDICATIONS AND USAGE

Use Following Induction Chemotherapy in Acute Myelogenous Leukemia LEUKINE is indicated for use following induction chemotherapy in older adult patients with acute myelogenous leukemia (AML) to shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections and infections resulting in death. The safety and efficacy of LEUKINE have not been assessed in patients with AML under 55 years of age.

The term acute myelogenous leukemia, also referred to as acute non-lymphocytic leukemia (ANLL), encompasses a heterogeneous group of leukemias arising from various non-lymphoid cell lines which have been defined morphologically by the French-American-British (FAB) system of classification.

Use in Mobilization and Following Transplantation of Autologous Peripheral Blood Progentior Cells LEUKINE is indicated for the mobilization of hematopoietic progenitor cells into peripheral blood for collection by leukapheresis. Mobilization allows for the collection of increased numbers of progenitor cells capable of engraftment as compared with collection without mobilization. After myeloablative chemotherapy, the transplantation of an increased number of progenitor cells can lead to more rapid engraftment, which may result in a decreased need for supportive care. Myeloid reconstitution is further accelerated by administration of LEUKINE following peripheral blood progenitor cell transplantation.

Use in Myeloid Reconstitution After Autologous Bone Marrow Transplantation LEUKINE is indicated for acceleration of myeloid recovery in patients with non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukemia (ALL) and Hodgkin's disease undergoing autologous bone marrow transplantation (BMT). After autologous BMT in patients with NHL, ALL, or Hodgkin's disease, LEUKINE has been found to be safe and effective in accelerating myeloid engraftment, decreasing median duration of antibiotic administration, reducing the median duration of infectious episodes and shortening the median duration of hospitalization. Hematologic response to LEUKINE can be detected by complete blood count (CBC) with differential cell counts performed twice per week.

Use in Myeloid Reconstitution After Allogeneic Bone Marrow Transplantation LEUKINE is indicated for acceleration of myeloid recovery in patients undergoing allogeneic BMT from HLA-matched related donors. LEUKINE has been found to be safe and effective in accelerating myeloid engraftment, reducing the incidence of bacteremia and other culture positive infections, and shortening the median duration of hospitalization.

Use in Bone Marrow Transplantation Failure or Engraftment Delay LEUKINE is indicated in patients who have undergone allogeneic or autologous bone marrow transplantation (BMT) in whom engraftment is delayed or has failed. LEUKINE has been found to be safe and effective in prolonging survival of patients who are experiencing graft failure or engraftment delay, in the presence or absence of infection, following autologous or allogeneic BMT. Survival benefit may be relatively greater in those patients who demonstrate one or more of the following characteristics: autologous BMT failure or engraftment delay, no previous total body irradiation, malignancy other than leukemia or a multiple organ failure (MOF) score ≤ two (see CLINICAL EXPERIENCE). Hematologic response to LEUKINE can be detected by complete blood count (CBC) with differential performed twice per week.

CLINICAL EXPERIENCE

Acute Myelogenous Leukemia The safety and efficacy of LEUKINE in patients with AML who are younger than 55 years of age have not been determined. Based on Phase II data suggesting the best therapeutic effects could be achieved in patients at highest risk for severe infections and mortality while neutropenic, the Phase III clinical trial was conducted in older patients. The safety and efficacy of LEUKINE in the treatment of AML were evaluated in a multi-center, randomized, double-blind placebo-controlled trial of 99 newly diagnosed adult patients, 55–70 years of age, receiving induction with or without consolidation.[6] A combination of standard doses of daunorubicin (days 1–3) and ara-C (days 1–7) was administered during induction and high dose ara-C was administered days 1–6 as a single course of consolidation, if given. Bone marrow evaluation was performed on day 10 following induction chemotherapy. If hypoplasia with <5% blasts was not achieved, patients immediately received a second cycle of induction chemotherapy. If the bone marrow was hypoplastic with <5% blasts on day 10 or four days following the second cycle of induction chemotherapy, LEUKINE (250 mcg/m^2/day) or placebo was given IV over four hours each day, starting four days after the completion of chemotherapy. Study drug was continued until an ANC ≥1500/mm^3 for three consecutive days was attained or a maximum of 42 days. LEUKINE or placebo was also administered after the single course of consolidation chemotherapy if delivered (ara-C 3–6 weeks after induction following neutrophil recovery). Study drug was discontinued immediately if leukemic regrowth occurred.

LEUKINE significantly shortened the median duration of ANC <500/mm^3 by 4 days and <1000/mm^3 by 7 days following induction (see **Table 1**). 75% of patients receiving LEUKINE achieved ANC >500/mm^3 by day 16, compared to day 25 for patients receiving placebo. The proportion of patients receiving one cycle (70%) or two cycles (30%) of induction was similar in both treatment groups; LEUKINE significantly shortened the median times to neutrophil recovery whether one cycle (12 versus 15 days) or two cycles (14 versus 23 days) of induction chemotherapy was administered. Median times to platelet (>20,000/mm^3) and RBC transfusion independence were not significantly different between treatment groups.

Table 1

Hematological Recovery (in Days): Induction

Dataset	sargramostim n=52* Median (25%, 75%)	Placebo n=47 Median (25%, 75%)	p-value**
ANC>500/mm^3 [a]	13 (11, 16)	17 (13, 25)	0.009
ANC>1000/mm^3 [b]	14 (12, 18)	21 (13, 34)	0.003
PLT>20,000/mm^3 [c]	11 (7, 14)	12 (9, >42)	0.10
RBC[d]	12 (9, 24)	14 (9, 42)	0.53

** Patients with missing data censored.*
[a] 2 patients on sargramostim and 4 patients on placebo had missing values.
[b] 2 patients on sargramostim and 3 patients on placebo had missing values.
[c] 4 patients on placebo had missing values.
[d] 3 patients on sargramostim and 4 patients on placebo had missing values.
*** p=Generalized Wilcoxon*

During the consolidation phase of treatment, LEUKINE did not shorten the median time to recovery of ANC to 500/mm^3 (13 days) or 1000/mm^3 (14.5 days) compared to placebo. There were no significant differences in time to platelet and RBC transfusion independence.

The incidence of severe infections and deaths associated with infections was significantly reduced in patients who received LEUKINE. During induction or consolidation, 27 of 52 patients receiving LEUKINE and 35 of 47 patients receiving placebo had at least one grade 3, 4 or 5 infection (p=0.02). Twenty-five patients receiving LEUKINE and 30 patients receiving placebo experienced severe and fatal infections during induction only. There were significantly fewer deaths from infectious causes in the LEUKINE arm (3 versus 11, p=0.02). The majority of deaths in the placebo group were associated with fungal infections with pneumonia as the primary infection.

Disease outcomes were not adversely affected by the use of LEUKINE. The proportion of patients achieving complete remission (CR) was higher in the LEUKINE group (69% as compared to 55% for the placebo group), but the difference was not significant (p=0.21). There was no significant difference in relapse rates; 12 of 36 patients who received LEUKINE and five of 26 patients who received placebo relapsed within 180 days of documented CR (p=0.26). The overall median survival was 378 days for patients receiving LEUKINE and 268 days for those on placebo (p=0.17). The study was not sized to assess the impact of LEUKINE treatment on response or survival.

Mobilization and Engraftment of PBPC A retrospective review was conducted of data from patients with cancer undergoing collection of peripheral blood progenitor cells (PBPC) at a single transplant center. Mobilization of PBPC and myeloid reconstitution post-transplant were compared between four groups of patients (n=196) receiving LEUKINE for mobilization and a historical control group who did not receive any mobilization treatment [progenitor cells collected by leukapheresis without mobilization (n=100)]. Sequential cohorts received LEUKINE. The cohorts differed by dose (125 or 250 mcg/m^2/day), route (IV over 24 hours or SC) and use of LEUKINE post-transplant. Leukaphereses were initiated for all mobilization groups after the WBC reached 10,000/mm^3. Leukaphereses continued until both a minimum number of mononucleated cells (MNC) were collected (6.5 or 8.0 x 10^8/kg body weight) and a minimum number of phereses (5-8) were performed. Both minimum requirements varied by treatment cohort and planned conditioning regimen. If subjects failed to reach a WBC of 10,000 cells/mm^3 by day five, another cytokine was substituted for LEUKINE; these subjects were all successfully leukapheresed and transplanted. The most marked mobilization and post-transplant effects were seen in patients administered the higher dose of LEUKINE (250 mcg/m^2) either IV (n=63) or SC (n=41).

PBPCs from patients treated at the 250 mcg/m^2/day dose had significantly higher number of granulocyte-macrophage colony-forming units (CFU-GM) than those collected without mobilization. The mean value after thawing was 11.41 x 10^4 CFU-GM/kg for all LEUKINE-mobilized patients, compared to 0.96 x 10^4/kg for the non-mobilized group. A similar difference was observed in the mean number of erythrocyte burst-forming units (BFU-E) collected (23.96 x 10^4/kg for patients mobilized with 250 mcg/m^2 doses of LEUKINE administered SC vs. 1.63 x 10^4/kg for non-mobilized patients).

After transplantation, mobilized subjects had shorter times to myeloid engraftment and fewer days between transplantation and the last platelet transfusion compared to non-mobilized subjects. Neutrophil recovery (ANC >500/mm^3) was more rapid in patients administered LEUKINE following PBPC transplantation with LEUKINE-mobilized cells (see **Table 2**). Mobilized patients also had fewer days to the last platelet transfusion and last RBC transfusion, and a shorter duration of hospitalization than did non-mobilized subjects.

Table 2

ANC and Platelet Recovery after PBPC Transplant

	Route for Mobilization	Post-transplant LEUKINE	ENGRAFTMENT (median value in days)	
			ANC>500/mm^3	Last platelet transfusion
No Mobilization	—	no	29	28
LEUKINE 250 mcg/m^2	IV	no	21	24
	IV	yes	12	19
	SC	yes	12	17

A second retrospective review of data from patients undergoing PBPC at another single transplant center was also conducted. LEUKINE was given SC at 250 mcg/m^2/day once a day (n=10) or twice a day (n=21) until completion of the phereses. Phereses were begun on day 5 of LEUKINE administration and continued until the targeted MNC count of 9 x 10^8/kg or CD34+ cell count of 1 x 10^6/kg was reached. There was no difference in CD34+ cell count in patients receiving LEUKINE once or twice a day. The median time to ANC>500/mm^3 was 12 days and to platelet recovery (>25,000/mm^3) was 23 days.

Survival studies comparing mobilized study patients to the non-mobilized patients and to an autologous historical bone marrow transplant group showed no differences in median survival time.

Autologous Bone Marrow Transplantation[7] Following a dose-ranging Phase I/II trial in patients undergoing autologous BMT for lymphoid malignancies,[8, 9] three single center, randomized, placebo-controlled and double-blinded studies were conducted to evaluate the safety and efficacy of LEUKINE for promoting hematopoietic reconstitution following autologous BMT. A total of 128 patients (65 LEUKINE, 63 placebo) were enrolled in these three studies. The majority of the patients had lymphoid malignancy (87 NHL, 17 ALL), 23 patients had Hodgkin's disease, and one patient had acute myeloblastic leukemia (AML). In 72 patients with NHL or ALL, the bone marrow harvest was purged prior to storage with one of several monoclonal antibodies. No chemical agent was used for *in vitro* treatment of the bone marrow. Preparative regimens in the three studies included cyclophosphamide (total dose 120-150 mg/kg) and total body irradiation (total dose 1,200-1,575 rads). Other regimens used in patients with Hodgkin's disease and NHL without radiotherapy consisted of three or more of the following in combination (expressed as total dose): cytosine arabinoside (400 mg/m^2) and carmustine (300 mg/m^2), cyclophosphamide (140-150 mg/kg), hydroxyurea (4.5 grams/m^2) and etoposide (375-450 mg/m^2).

Compared to placebo, administration of LEUKINE in two studies (n=44 and 47) significantly improved the following hematologic and clinical endpoints: time to neutrophil engraftment, duration of hospitalization and infection experience or antibacterial usage. In the third study (n=37) there was a positive trend toward earlier myeloid engraftment in favor of LEUKINE. This latter study differed from the other two in having enrolled a large number of patients with Hodgkin's disease who had also received extensive radiation and chemotherapy prior to harvest of autologous bone marrow. A subgroup analysis of the data from all three studies revealed that the median time to engraftment for patients with Hodgkin's disease, regardless of treatment, was six days longer when compared to patients with NHL and ALL, but that the overall beneficial LEUKINE treatment effect was the same. In the following combined analysis of the three studies, these two subgroups (NHL and ALL vs. Hodgkin's disease) are presented separately.

Table 3

Autologous BMT: Combined Analysis from Placebo-Controlled Clinical Trials of Responses in Patients with NHL and ALL
Median Values (days)

	ANC ≥500/mm^3	ANC ≥1000/mm^3	Duration of Hospitalization	Duration of Infection	Duration of Antibacterial Therapy
LEUKINE (n=54)	18*#	24*#	25*	1*	21*
Placebo (n=50)	24	32	31	4	25

** p <0.05 Wilcoxon or CMH ridit chi-squared # p <0.05 Log rank*
Note: The single AML patient was not included.

Patients with Lymphoid Malignancy (Non-Hodgkin's Lymphoma and Acute Lymphoblastic Leukemia)

Myeloid engraftment (absolute neutrophil count [ANC] ≥ 500 cells/mm^3) in 54 patients receiving LEUKINE was observed 6 days earlier than in 50 patients treated with placebo (see **Table 3**). Accelerated myeloid engraftment was associated with significant clinical benefits. The median duration of hospitalization was six days shorter for the LEUKINE group than for the placebo group. Median duration of infectious episodes (defined as fever and neutropenia; or two positive cultures of the same organism; or fever >38°C and

one positive blood culture; or clinical evidence of infection) was three days less in the group treated with LEUKINE. The median duration of antibacterial administration in the post-transplantation period was four days shorter for the patients treated with LEUKINE than for placebo-treated patients. The study was unable to detect a significant difference between the treatment groups in rate of disease relapse 24 months post-transplantation. As a group, leukemic subjects receiving LEUKINE derived less benefit than NHL subjects. However, both the leukemic and NHL groups receiving LEUKINE engrafted earlier than controls.

Patients with Hodgkin's Disease

If patients with Hodgkin's disease are analyzed separately, a trend toward earlier myeloid engraftment is noted. LEUKINE-treated patients engrafted earlier (by five days) than the placebo-treated patients (p=0.189, Wilcoxon) but the number of patients was small (n=22).

Allogeneic Bone Marrow Transplantation A multi-center, randomized, placebo-controlled, and double-blinded study was conducted to evaluate the safety and efficacy of LEUKINE for promoting hematopoietic reconstitution following allogeneic BMT. A total of 109 patients (53 LEUKINE, 56 placebo) were enrolled in the study. Twenty-three patients (11 LEUKINE, 12 placebo) were 18 years old or younger. Sixty-seven patients had myeloid malignancies (33 AML, 34 CML), 17 had lymphoid malignancies (12 ALL, 5 NHL), three patients had Hodgkin's disease, six had multiple myeloma, nine had myelodysplastic disease, and seven patients had aplastic anemia. In 22 patients at one of the seven study sites, bone marrow harvests were depleted of T cells. Preparative regimens included cyclophosphamide, busulfan, cytosine arabinoside, etoposide, methotrexate, corticosteroids, and asparaginase. Some patients also received total body, splenic, or testicular irradiation. Primary graft-versus-host disease (GVHD) prophylaxis was cyclosporine A and a corticosteroid.

Accelerated myeloid engraftment was associated with significant laboratory and clinical benefits. Compared to placebo, administration of LEUKINE significantly improved the following: time to neutrophil engraftment, duration of hospitalization, number of patients with bacteremia and overall incidence of infection (see **Table 4**).

Table 4

Allogeneic BMT: Analysis of Data from Placebo-Controlled Clinical Trial
Median Values (days or number of patients)

	ANC ≥ 500/mm^3	ANC ≥ 1000/mm^3	Number of Patients with Infections	Number of Patients with Bacteremia	Days of Hospitalization
LEUKINE (n=53)	13*	14*	30*	9**	25*
Placebo (n=56)	17	19	42	19	26

** p <0.05 generalized Wilcoxon test* *** p <0.05 simple chi-square test*

Median time to myeloid engraftment (ANC ≥ 500 cells/mm^3) in 53 patients receiving LEUKINE was 4 four days less than in 56 patients treated with placebo (see **Table 4**). The number of patients with bacteremia and infection was significantly lower in the LEUKINE group compared to the placebo group (9/53 versus 19/56 and 30/53 versus 42/56, respectively). There were a number of secondary laboratory and clinical endpoints. Of these, only the incidence of severe (grade 3/4) mucositis was significantly improved in the LEUKINE group (4/53) compared to the placebo group (16/56) at p<0.05. LEUKINE-treated patients also had a shorter median duration of post-transplant IV antibiotic infusions, and shorter median number of days to last platelet and RBC transfusions compared to placebo patients, but none of these differences reached statistical significance.

Bone Marrow Transplantation Failure or Engraftment Delay A historically-controlled study was conducted in patients experiencing graft failure following allogeneic or autologous BMT to determine whether LEUKINE improved survival after BMT failure.

Three categories of patients were eligible for this study:

1) patients displaying a delay in engraftment (ANC ≤ 100 cells/mm^3 by day 28 post-transplantation);
2) patients displaying a delay in engraftment (ANC ≤ 100 cells/mm^3 by day 21 post-transplantation) and who had evidence of an active infection; and
3) patients who lost their marrow graft after a transient engraftment (manifested by an average of ANC ≥ 500 cells/mm^3 for at least one week followed by loss of engraftment with ANC < 500 cells/mm^3 for at least one week beyond day 21 post-transplantation).

A total of 140 eligible patients from 35 institutions were treated with LEUKINE and evaluated in comparison to 103 historical control patients from a single institution. One hundred sixty-three patients had lymphoid or myeloid leukemia, 24 patients had non-Hodgkin's lymphoma, 19 patients had Hodgkin's disease and 37 patients had other diseases, such as aplastic anemia, myelodysplasia or non-hematologic malignancy. The majority of patients (223 out of 243) had received prior chemotherapy with or without radiotherapy and/or immunotherapy prior to preparation for transplantation.

One hundred day survival was improved in favor of the patients treated with LEUKINE after graft failure following either autologous or allogeneic BMT. In addition, the median survival was improved by greater than two-fold. The median survival of patients treated with LEUKINE after autologous failure was 474 days versus 161 days for the historical patients. Similarly, after allogeneic failure, the median survival was 97 days with LEUKINE treatment and 35 days for the historical controls. Improvement in survival was better in patients with fewer impaired organs.

The MOF score is a simple clinical and laboratory assessment of seven major organ systems: cardiovascular, respiratory, gastrointestinal, hematologic, renal, hepatic and neurologic.[10] Assessment of the MOF score is recommended as an additional method of determining the need to initiate treatment with LEUKINE in patients with graft failure or delay in engraftment following autologous or allogeneic BMT (see **Table 5**).

Table 5

Median Survival by Multiple Organ Failure (MOF) Category
Median Survival (days)

	MOF ≤ 2 Organs	MOF > 2 Organs	MOF (Composite of Both Groups)
Autologous BMT			
LEUKINE	474 (n=58)	78.5 (n=10)	474 (n=68)
Historical	165 (n=14)	39 (n=3)	161 (n=17)
Allogeneic BMT			
LEUKINE	174 (n=50)	27 (n=22)	97 (n=72)
Historical	52.5 (n=60)	15.5 (n=26)	35 (n=86)

Factors that Contribute to Survival

The probability of survival was relatively greater for patients with any one of the following characteristics: autologous BMT failure or delay in engraftment, exclusion of total body irradiation from the preparative regimen, a non-leukemic malignancy or MOF score ≤ two (zero, one or two dysfunctional organ systems). Leukemic subjects derived less benefit than other subjects.

CONTRAINDICATIONS

LEUKINE is contraindicated:

1) in patients with excessive leukemic myeloid blasts in the bone marrow or peripheral blood (≥ 10%);
2) in patients with known hypersensitivity to GM-CSF, yeast-derived products or any component of the product;
3) for concomitant use with chemotherapy and radiotherapy.

Due to the potential sensitivity of rapidly dividing hematopoietic progenitor cells, LEUKINE should not be administered simultaneously with cytotoxic chemotherapy or radiotherapy or within 24 hours proceeding or following chemotherapy or radiotherapy. In one controlled study, patients with small cell lung cancer received LEUKINE and concurrent thoracic radiotherapy and chemotherapy or the identical radiotherapy and chemotherapy without LEUKINE. The patients randomized to LEUKINE had significantly higher incidence of adverse events, including higher mortality and a higher incidence of grade 3 and 4 infections and grade 3 and 4 thrombocytopenia.[11]

WARNINGS

Pediatric Use Benzyl alcohol is a constituent of liquid LEUKINE and Bacteriostatic Water for Injection diluent. Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants. **Liquid solutions containing benzyl alcohol (including liquid LEUKINE) or lyophilized LEUKINE reconstituted with Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol) should not be administered to neonates** (see **PRECAUTIONS** and **DOSAGE AND ADMINISTRATION**).

Fluid Retention Edema, capillary leak syndrome, pleural and/or pericardial effusion have been reported in patients after LEUKINE administration. In 156 patients enrolled in placebo-controlled studies using LEUKINE at a dose of 250 mcg/m^2/day by 2-hour IV infusion, the reported incidences of fluid retention (LEUKINE vs. placebo) were as follows: peripheral edema, 11% vs. 7%; pleural effusion, 1% vs. 0%; and pericardial effusion, 4% vs. 1%. Capillary leak syndrome was not observed in this limited number of studies; based on other uncontrolled studies and reports from users of marketed LEUKINE, the incidence is estimated to be less than 1%. In patients with preexisting pleural and pericardial effusions, administration of LEUKINE may aggravate fluid retention; however, fluid retention associated with or worsened by LEUKINE has been reversible after interruption or dose reduction of LEUKINE with or without diuretic therapy. LEUKINE should be used with caution in patients with preexisting fluid retention, pulmonary infiltrates or congestive heart failure.

Respiratory Symptoms Sequestration of granulocytes in the pulmonary circulation has been documented following LEUKINE infusion[12] and dyspnea has been reported occasionally in patients treated with LEUKINE. Special attention should be given to respiratory symptoms during or immediately following LEUKINE infusion, especially in patients with preexisting lung disease. In patients displaying dyspnea during LEUKINE administration, the rate of infusion should be reduced by half. If respiratory symptoms worsen despite infusion rate reduction, the infusion should be discontinued. Subsequent IV infusions may be administered following the standard dose schedule with careful monitoring. LEUKINE should be administered with caution in patients with hypoxia.

Cardiovascular Symptoms Occasional transient supraventricular arrhythmia has been reported in uncontrolled studies during LEUKINE administration, particularly in patients with a previous history of cardiac arrhythmia. However, these arrhythmias have been reversible after discontinuation of LEUKINE. LEUKINE should be used with caution in patients with preexisting cardiac disease.

Renal and Hepatic Dysfunction In some patients with preexisting renal or hepatic dysfunction enrolled in uncontrolled clinical trials, administration of LEUKINE has induced elevation of serum creatinine or bilirubin and hepatic enzymes. Dose reduction or interruption of LEUKINE administration has resulted in a decrease to pretreatment values. However, in controlled clinical trials the incidences of renal and hepatic dysfunction were comparable between LEUKINE (250 mcg/m^2/day by 2-hour IV infusion) and placebo-treated patients. Monitoring of renal and hepatic function in patients displaying renal or hepatic dysfunction prior to initiation of treatment is recommended at least every other week during LEUKINE administration.

PRECAUTIONS

General Parenteral administration of recombinant proteins should be attended by appropriate precautions in case an allergic or untoward reaction occurs. Serious allergic or anaphylactic reactions have been reported. If any serious allergic or anaphylactic reaction occurs, LEUKINE therapy should immediately be discontinued and appropriate therapy initiated.

A syndrome characterized by respiratory distress, hypoxia, flushing, hypotension, syncope, and/or tachycardia has been reported following the first administration of LEUKINE in a particular cycle. These signs have resolved with symptomatic treatment and usually do not recur with subsequent doses in the same cycle of treatment.

Stimulation of marrow precursors with LEUKINE may result in a rapid rise in white blood cell (WBC) count. 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. Excessive blood counts have returned to normal or baseline levels within three to seven days following cessation of LEUKINE therapy. Twice weekly monitoring of CBC with differential (including examination for the presence of blast cells) should be performed to preclude development of excessive counts.

Growth Factor Potential LEUKINE is a growth factor that primarily stimulates normal myeloid precursors. However, the possibility that LEUKINE can act as a growth factor for any tumor type, particularly myeloid malignancies, cannot be excluded. Because of the possibility of tumor growth potentiation, precaution should be exercised when using this drug in any malignancy with myeloid characteristics.

Should disease progression be detected during LEUKINE treatment, LEUKINE therapy should be discontinued.

LEUKINE has been administered to patients with myelodysplastic syndromes (MDS) in uncontrolled studies without evidence of increased relapse rates.[13, 14, 15] Controlled studies have not been performed in patients with MDS.

Use in Patients Receiving Purged Bone Marrow LEUKINE is effective in accelerating myeloid recovery in patients receiving bone marrow purged by anti-B lymphocyte monoclonal antibodies. Data obtained from uncontrolled studies suggest that if *in vitro* marrow purging with chemical agents causes a significant decrease in the number of responsive hematopoietic progenitors, the patient may not respond to LEUKINE. When the bone marrow purging process preserves a sufficient number of progenitors (>1.2 x 10^4/kg), a beneficial effect of LEUKINE on myeloid engraftment has been reported.[16]

Use in Patients Previously Exposed to Intensive Chemotherapy/Radiotherapy In patients who before autologous BMT, have received extensive radiotherapy to hematopoietic sites for the treatment of primary disease in the abdomen or chest, or have been exposed to multiple myelotoxic agents (alkylating agents, anthracycline antibiotics and antimetabolites), the effect of LEUKINE on myeloid reconstitution may be limited.

Use in Patients with Malignancy Undergoing LEUKINE-Mobilized PBPC Collection When using LEUKINE to mobilize PBPC, the limited *in vitro* data suggest that tumor cells may be released and reinfused into the patient in the leukapheresis product. The effect of reinfusion of tumor cells has not been well studied and the data are inconclusive.

Information for Patients LEUKINE should be used under the guidance and supervision of a health care professional. However, when the physician determines that LEUKINE may be used outside of the hospital or office setting, persons who will be administering LEUKINE should be instructed as to the proper dose, and the method of reconstituting and administering LEUKINE (see **DOSAGE AND ADMINISTRATION**). If home use is prescribed, patients should be instructed in the importance of proper disposal and cautioned against the reuse of needles, syringes, drug product, and diluent. A puncture resistant container should be used by the patient for the disposal of used needles.

Patients should be informed of the serious and most common adverse reactions associated with LEUKINE administration (see **ADVERSE REACTIONS**). Female patients of childbearing potential should be advised of the possible risks to the fetus of LEUKINE (see **PRECAUTIONS, Pregnancy Category C**).

Laboratory Monitoring LEUKINE can induce variable increases in WBC and/or platelet counts. In order to avoid potential complications of excessive leukocytosis (WBC >50,000 cells/mm^3; ANC >20,000 cells/mm^3), a CBC is recommended twice per week during LEUKINE therapy. Monitoring of renal and hepatic function in patients displaying renal or hepatic dysfunction prior to initiation of treatment is recommended at least biweekly during LEUKINE administration. Body weight and hydration status should be carefully monitored during LEUKINE administration.

Drug Interaction Interactions between LEUKINE and other drugs have not been fully evaluated. Drugs which may potentiate the myeloproliferative effects of LEUKINE, such as lithium and corticosteroids, should be used with caution.

Carcinogenesis, Mutagenesis, Impairment of Fertility Animal studies have not been conducted with LEUKINE to evaluate the carcinogenic potential or the effect on fertility.

Pregnancy (Category C) Animal reproduction studies have not been conducted with LEUKINE. It is not known whether LEUKINE can cause fetal harm when administered to a pregnant woman or can affect reproductive capability. LEUKINE should be given to a pregnant woman only if clearly needed.

Nursing Mothers It is not known whether LEUKINE is excreted in human milk. Because many drugs are excreted in human milk, LEUKINE should be administered to a nursing woman only if clearly needed.

Pediatric Use Safety and effectiveness in pediatric patients have not been established; however, available safety data indicate that LEUKINE does not exhibit any greater toxicity in pediatric patients than in adults. A total of 124 pediatric subjects between the ages of 4 months and 18 years have been treated with LEUKINE in clinical trials at doses ranging from 60-1,000 mcg/m^2/day intravenously and 4-1,500 mcg/m^2/day subcutaneously. In 53 pediatric patients enrolled in controlled studies at a dose of 250 mcg/m^2/day by 2-hour IV infusion, the type and frequency of adverse events were comparable to those reported for the adult population. **Liquid solutions containing benzyl alcohol (including liquid LEUKINE) or lyophilized LEUKINE reconstituted with Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol) should not be administered to neonates** (see **WARNINGS**).

Geriatric Use In the clinical trials, experience in older patients (age ≥65 years), was limited to the acute myelogenous leukemia (AML) study. Of the 52 patients treated with LEUKINE in this randomized study, 22 patients were age 65-70 years and 30 patients were age 55-64 years. The number of placebo patients in each age group were 13 and 33 patients respectively. This was not an adequate database from which determination of differences in efficacy endpoints or safety assessments could be reliably made and this clinical study was not designed to evaluate difference between these two age groups. Analyses of general trends in safety and efficacy were undertaken and demonstrate similar patterns for older (65-70 yrs) vs younger patients (55-64 yrs). Greater sensitivity of some older individuals cannot be ruled out.

ADVERSE REACTIONS

Autologous and Allogeneic Bone Marrow Transplantation LEUKINE is generally well tolerated. In three placebo-controlled studies enrolling a total of 156 patients after autologous BMT or peripheral blood progenitor cell transplantation, events reported in at least 10% of patients who received IV LEUKINE or placebo were as reported in Table 6.

No significant differences were observed between LEUKINE and placebo-treated patients in the type or frequency of laboratory abnormalities, including renal and hepatic parameters. In some patients with preexisting renal or hepatic dysfunction enrolled in uncontrolled clinical trials, administration of LEUKINE has induced elevation of serum creatinine or bilirubin and hepatic enzymes (see **WARNINGS**). In addition, there was no significant difference in relapse rate and 24 month survival between the LEUKINE and placebo-treated patients.

In the placebo-controlled trial of 109 patients after allogeneic BMT, events reported in at least 10% of patients who received IV LEUKINE or placebo were as reported in Table 7.

Table 6

Percent of AuBMT Patients Reporting Events					
Events by Body System	LEUKINE (n=79)	Placebo (n=77)	Events by Body System	LEUKINE (n=79)	Placebo (n=77)
Body, General			**Metabolic, Nutritional Disorder**		
Fever	95	96	Edema	34	35
Mucous membrane disorder	75	78	Peripheral edema	11	7
Asthenia	66	51	**Respiratory System**		
Malaise	57	51	Dyspnea	28	31
Sepsis	11	14	Lung disorder	20	23
Digestive System			**Hemic and Lymphatic System**		
Nausea	90	96	Blood dyscrasia	25	27
Diarrhea	89	82	**Cardiovascular System**		
Vomiting	85	90	Hemorrhage	23	30
Anorexia	54	58	**Urogenital System**		
GI disorder	37	47	Urinary tract disorder	14	13
GI hemorrhage	27	33	Kidney function abnormal	8	10
Stomatitis	24	29	**Nervous System**		
Liver damage	13	14	CNS disorder	11	16
Skin and Appendages					
Alopecia	73	74			
Rash	44	38			

There were no significant differences in the incidence or severity of GVHD, relapse rates and survival between the LEUKINE and placebo-treated patients.

Adverse events observed for the patients treated with LEUKINE in the historically-controlled BMT failure study were similar to those reported in the placebo-controlled studies. In addition, headache (26%), pericardial effusion (25%), arthralgia (21%) and myalgia (18%) were also reported in patients treated with LEUKINE in the graft failure study.

In uncontrolled Phase I/II studies with LEUKINE in 215 patients, the most frequent adverse events were fever, asthenia, headache, bone pain, chills and myalgia. These systemic events were generally mild or moderate and were usually prevented or reversed by the administration of analgesics and antipyretics such as acetaminophen. In these uncontrolled trials, other infrequent events reported were dyspnea, peripheral edema, and rash.

Reports of events occurring with marketed LEUKINE include arrhythmia, fainting, eosinophilia, dizziness, hypotension, injection site reactions, pain (including

Table 7

Percent of Allogeneic BMT Patients Reporting Events					
Events by Body System	LEUKINE (n=53)	Placebo (n=56)	Events by Body System	LEUKINE (n=53)	Placebo (n=56)
Body, General			**Metabolic/Nutritional Disorders**		
Fever	77	80	Bilirubinemia	30	27
Abdominal pain	38	23	Hyperglycemia	25	23
Headache	36	36	Peripheral edema	15	21
Chills	25	20	Increased creatinine	15	14
Pain	17	36	Hypomagnesemia	15	9
Asthenia	17	20	Increased SGPT	13	16
Chest pain	15	9	Edema	13	11
Back pain	9	18	Increased alk. phosphatase	8	14
Digestive System			**Respiratory System**		
Diarrhea	81	66	Pharyngitis	23	13
Nausea	70	66	Epistaxis	17	16
Vomiting	70	57	Dyspnea	15	14
Stomatitis	62	63	Rhinitis	11	14
Anorexia	51	57	**Hemic and Lymphatic System**		
Dyspepsia	17	20	Thrombocytopenia	19	34
Hematemesis	13	7	Leukopenia	17	29
Dysphagia	11	7	Petechia	6	11
GI hemorrhage	11	5	Agranulocytosis	6	11
Constipation	8	11	**Urogenital System**		
Skin and Appendages			Hematuria	9	21
Rash	70	73	**Nervous System**		
Alopecia	45	45	Paresthesia	11	13
Pruritis	23	13	Insomnia	11	9
Musculo-skeletal System			Anxiety	11	2
Bone pain	21	5	**Laboratory Abnormalities***		
Arthralgia	11	4	High glucose	41	49
Special Senses			Low albumin	27	36
Eye hemorrhage	11	0	High BUN	23	17
Cardiovascular System			Low calcium	2	7
Hypertension	34	32	High cholesterol	17	8
Tachycardia	11	9			

**Grade 3 and 4 laboratory abnormalities only. Denominators may vary due to missing laboratory measurements.*

abdominal, back, chest, and joint pain), tachycardia, thrombosis, and transient liver function abnormalities.

In patients with preexisting edema, capillary leak syndrome, pleural and/or pericardial effusion, administration of LEUKINE may aggravate fluid retention (see **WARNINGS**). Body weight and hydration status should be carefully monitored during LEUKINE administration.

Adverse events observed in pediatric patients in controlled studies were comparable to those observed in adult patients.

Acute Myelogenous Leukemia

Adverse events reported in at least 10% of patients who received LEUKINE or placebo were as reported in Table 8.

Nearly all patients reported leukopenia, thrombocytopenia and anemia. The frequency and type of adverse events observed following induction were similar between LEUKINE and placebo groups. The only significant difference in the rates of these adverse events was an increase in skin associated events in the LEUKINE group (p=0.002). No significant differences were observed in laboratory results, renal or hepatic toxicity. No significant differences were observed between the LEUKINE and placebo-treated patients for adverse events following consolidation. There was no significant difference in response rate or relapse rate.

In a historically-controlled study of 86 patients with acute myelogenous leukemia (AML), the LEUKINE treated group exhibited an increased incidence of weight gain (p=0.007), low serum proteins and prolonged prothrombin time (p=0.02) when compared to the control group. Two LEUKINE treated patients had progressive increase in circulating monocytes and promonocytes and blasts in the marrow

Table 8

Percent of AML Patients Reporting Events					
Events by Body System	LEUKINE (n=52)	Placebo (n=47)	Events by Body System	LEUKINE (n=52)	Placebo (n=47)
Body, General			**Metabolic/Nutritional Disorder**		
Fever (no infection)	81	74	Metabolic	58	49
Infection	65	68	Edema	25	23
Weight loss	37	28	**Respiratory System**		
Weight gain	8	21	Pulmonary	48	64
Chills	19	26	**Hemic and Lymphatic System**		
Allergy	12	15	Coagulation	19	21
Sweats	6	13	**Cardiovascular System**		
Digestive System			Hemorrhage	29	43
Nausea	58	55	Hypertension	25	32
Liver	77	83	Cardiac	23	32
Diarrhea	52	53	Hypotension	13	26
Vomiting	46	34	**Urogenital System**		
Stomatitis	42	43	GU	50	57
Anorexia	13	11	**Nervous System**		
Abdominal distention	4	13	Neuro-clinical	42	53
Skin and Appendages			Neuro-motor	25	26
Skin	77	45	Neuro-psych	15	26
Alopecia	37	51	Neuro-sensory	6	11

which reversed when LEUKINE was discontinued. The historical control group exhibited an increased incidence of cardiac events (p=0.018), liver function abnormalities (p=0.008), and neurocortical hemorrhagic events (p=0.025).[15]

Antibody Formation Serum samples collected before and after LEUKINE treatment from 214 patients with a variety of underlying diseases have been examined for immunogenicity based on the presence of antibodies. Neutralizing antibodies were detected in five of 214 patients (2.3%) after receiving LEUKINE by continuous IV infusion (three patients) or subcutaneous injection (SC)(two patients) for 28 to 84 days in multiple courses. All five patients had impaired hematopoiesis before the administration of LEUKINE and consequently the effect of the development of anti-GM-CSF antibodies on normal hematopoiesis could not be assessed. Antibody studies of 75 patients with Crohn's disease receiving LEUKINE by subcutaneous injection with normal hematopoiesis and no other immunosuppressive drugs showed one patient (1.3%) with detectable neutralizing antibodies. The clinical relevance of the presence of these antibodies are unknown. Drug-induced neutropenia, neutralization of endogenous GM-CSF activity and diminution of the therapeutic effect of LEUKINE secondary to formation of neutralizing antibody remain a theoretical possibility. Serious allergic and anaphylactoid reactions have been reported with LEUKINE but the rate of occurrence of antibodies in such patients has not been assessed.

Overdosage The maximum amount of LEUKINE that can be safely administered in single or multiple doses has not been determined. Doses up to 100 mcg/kg/day (4,000 mcg/m^2/day or 16 times the recommended dose) were administered to four patients in a Phase I uncontrolled clinical study by continuous IV infusion for 7 to 18 days. Increases in WBC up to 200,000 cells/mm^3 were observed. Adverse events reported were dyspnea, malaise, nausea, fever, rash, sinus tachycardia, headache and chills. All these events were reversible after discontinuation of LEUKINE.

In case of overdosage, LEUKINE therapy should be discontinued and the patient carefully monitored for WBC increase and respiratory symptoms.

To report SUSPECTED ADVERSE REACTIONS, contact Genzyme Corporation at 1-888-4RX-LEUKINE or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

DOSAGE AND ADMINISTRATION

Neutrophil Recovery Following Chemotherapy in Acute Myelogenous Leukemia The recommended dose is 250 mcg/m^2/day administered intravenously over a 4 hour period starting approximately on day 11 or four days following the completion of induction chemotherapy, if the day 10 bone marrow is hypoplastic with <5% blasts. If a second cycle of induction chemotherapy is necessary, LEUKINE should be administered approximately four days after the completion of chemotherapy if the bone marrow is hypoplastic with <5% blasts. LEUKINE should be continued until an ANC >1500 cells/mm^3 for 3 consecutive days or a maximum of 42 days. LEUKINE should be discontinued immediately if leukemic regrowth occurs. If a severe adverse reaction occurs, the dose can be reduced by 50% or temporarily discontinued until the reaction abates.

In order to avoid potential complications of excessive leukocytosis (WBC > 50,000 cells/mm^3 or ANC > 20,000 cells/mm^3) a CBC with differential is recommended twice per week during LEUKINE therapy. LEUKINE treatment should be interrupted or the dose reduced by half if the ANC exceeds 20,000 cells/mm^3.

Mobilization of Peripheral Blood Progenitor Cells The recommended dose is 250 mcg/m^2/day administered IV over 24 hours or SC once daily. Dosing should continue at the same dose through the period of PBPC collection. The optimal schedule for PBPC collection has not been established. In clinical studies, collection of PBPC was usually begun by day 5 and performed daily until protocol specified targets were achieved (see **CLINICAL EXPERIENCE, Mobilization and Engraftment of PBPC**). If WBC > 50,000 cells/mm^3, the LEUKINE dose should be reduced by 50%. If adequate numbers of progenitor cells are not collected, other mobilization therapy should be considered.

Post Peripheral Blood Progenitor Cell Transplantation The recommended dose is 250 mcg/m^2/day administered IV over 24 hours or SC once daily beginning immediately following infusion of progenitor cells and continuing until an ANC>1500 cells/mm^3 for three consecutive days is attained.

Myeloid Reconstitution After Autologous or Allogeneic Bone Marrow Transplantation The recommended dose is 250 mcg/m^2/day administered IV over a 2-hour period beginning two to four hours after bone marrow infusion, and not less than 24 hours after the last dose of chemotherapy or radiotherapy. Patients should not receive LEUKINE until the post marrow infusion ANC is less than 500 cells/mm^3. LEUKINE should be continued until an ANC >1500 cells/mm^3 for three consecutive days is attained. If a severe adverse reaction occurs, the dose can be reduced by 50% or temporarily discontinued until the reaction abates. LEUKINE should be discontinued immediately if blast cells appear or disease progression occurs.

In order to avoid potential complications of excessive leukocytosis (WBC > 50,000 cells/mm^3, ANC > 20,000 cells/mm^3) a CBC with differential is recommended twice per week during LEUKINE therapy. LEUKINE treatment should be interrupted or the dose reduced by 50% if the ANC exceeds 20,000 cells/mm^3.

Bone Marrow Transplantation Failure or Engraftment Delay The recommended dose is 250 mcg/m^2/day for 14 days as a 2-hour IV infusion. The dose can be repeated after 7 days off therapy if engraftment has not occurred. If engraftment still has not occurred, a third course of 500 mcg/m^2/day for 14 days may be tried after another 7 days off therapy. If there is still no improvement, it is unlikely that further dose escalation will be beneficial. If a severe adverse reaction occurs, the dose can be reduced by 50% or temporarily discontinued until the reaction abates. LEUKINE should be discontinued immediately if blast cells appear or disease progression occurs.

In order to avoid potential complications of excessive leukocytosis (WBC > 50,000 cells/mm^3, ANC > 20,000 cells/mm^3) a CBC with differential is recommended twice per week during LEUKINE therapy. LEUKINE treatment should be interrupted or the dose reduced by half if the ANC exceeds 20,000 cells/mm^3.

Preparation of LEUKINE

1. Liquid LEUKINE is formulated as a sterile, preserved (1.1% benzyl alcohol), injectable solution (500 mcg/mL) in a vial. Lyophilized LEUKINE is a sterile, white, preservative-free powder (250 mcg) that requires reconstitution with 1 mL Sterile Water for Injection, USP, or 1 mL Bacteriostatic Water for Injection, USP.
2. Liquid LEUKINE may be stored for up to 20 days at 2-8°C once the vial has been entered. Discard any remaining solution after 20 days.
3. Lyophilized LEUKINE (250 mcg) should be reconstituted aseptically with 1.0 mL of diluent (see below). The contents of vials reconstituted with different diluents should not be mixed together.

   Sterile Water for Injection, USP (without preservative): Lyophilized LEUKINE vials contain no antibacterial preservative, and therefore solutions prepared with Sterile Water for Injection, USP should be administered as soon as possible, and within 6 hours following reconstitution and/or dilution for IV infusion. The vial should not be re-entered or reused. Do not save any unused portion for administration more than 6 hours following reconstitution.

   Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol): Reconstituted solutions prepared with Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol) may be stored for up to 20 days at 2–8°C prior to use. Discard reconstituted solution after 20 days. Previously reconstituted solutions mixed with freshly reconstituted solutions must be administered within 6 hours following mixing. **Preparations containing benzyl alcohol (including liquid LEUKINE and lyophilized LEUKINE reconstituted with Bacteriostatic Water for Injection) should not be used in neonates** (see **WARNINGS**).
4. During reconstitution of lyophilized LEUKINE the diluent should be directed at the side of the vial and the contents gently swirled to avoid foaming during dissolution. Avoid excessive or vigorous agitation; do not shake.
5. LEUKINE should be used for SC injection without further dilution. Dilution for IV infusion should be performed in 0.9% Sodium Chloride Injection, USP. If the final concentration of LEUKINE is below 10 mcg/mL, Albumin (Human) at a final concentration of 0.1% should be added to the saline prior to addition of LEUKINE to prevent adsorption to the components of the drug delivery system. To obtain a final concentration of 0.1% Albumin (Human), add 1 mg Albumin (Human) per 1 mL 0.9% Sodium Chloride Injection, USP (e.g., use 1 mL 5% Albumin [Human] in 50 mL 0.9% Sodium Chloride Injection, USP).
6. An in-line membrane filter should NOT be used for intravenous infusion of LEUKINE.
7. Store liquid LEUKINE and reconstituted lyophilized LEUKINE solutions under refrigeration at 2-8°C (36-46°F); DO NOT FREEZE.
8. In the absence of compatibility and stability information, no other medication should be added to infusion solutions containing LEUKINE. Use only 0.9% Sodium Chloride Injection, USP to prepare IV infusion solutions.
9. Aseptic technique should be employed in the preparation of all LEUKINE solutions. To assure correct concentration following reconstitution, care should be exercised to eliminate any air bubbles from the needle hub of the syringe used to prepare the diluent. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used.

HOW SUPPLIED

Liquid LEUKINE is available in vials containing 500 mcg/mL (2.8 x 10^6 IU/mL) sargramostim. Lyophilized LEUKINE is available in vials containing 250 mcg (1.4 x 10^6 IU/vial) sargramostim.

Each dosage form is supplied as follows:

Lyophilized LEUKINE	
Carton of five vials of lyophilized LEUKINE 250 mcg	(NDC 58468-0180-2)
Liquid LEUKINE	
Carton of one multiple-use vial; each vial contains 1 mL of preserved 500 mcg/mL liquid LEUKINE	(NDC 58468-0181-1)
Carton of five multiple-use vials; each vial contains 1 mL of preserved 500 mcg/mL liquid LEUKINE.	(NDC 58468-0181-2)

STORAGE

LEUKINE should be refrigerated at 2-8°C (36-46°F). Do not freeze or shake. Do not use beyond the expiration date printed on the vial.

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