BRIEFING PACKAGE

FDA ADVISORY COMMITTEE MEETING
03 May 2013

STUDY DRUG: Leukine® (sargramostim)

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ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE

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LIST OF ABBREVIATIONS

Term	Definition
AE	Adverse event
ALL	Acute lymphoblastic leukemia
ANC	Absolute neutrophil count
AUC	Area under the curve
AML	Acute myelogenous leukemia
ARS	Acute radiation syndrome
BARDA	Biomedical Advanced Research and Development Authority
BMT	Bone marrow transplantation
^{o}C	Degrees Celsius
cGMP	Current good manufacturing practices
C_{max}	Maximum concentration
CRS	Cytokine release syndrome
CSF	Colony stimulating factor
CTCAE	Common Terminology Criteria for Adverse Events
d	Day
Da	Dalton
DC	Dendritic cell
DHBI	Double hemibody irradiation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FAC	Fluorouracil, doxorubicin, cyclophosphamide
G-CSF	Granulocyte-colony stimulating factor
GI	Gastrointestinal
GLP	Good laboratory practices
GM-CSF	Granulocyte-macrophage colony stimulating factor
GVHD	Graft vs. host disease
Gy	Gray
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HSA	Human serum albumin
HSCT	Hematopoietic stem cell transplantation
IAEA	International Atomic Energy Agency
IND	Investigational New Drug
IV	Intravenous
Kg	Kilogram
L	Liter

Term	Definition
LBI	Lower body irradiation
LD	Lethal dose
LGF	Leukocyte growth factor
µg	Microgram
µm	Micrometer(s)
mg	Milligram(s)
min	Minute(s)
mL	Milliliter(s)
mm	Millimeter(s)
mRNA	Messenger ribonucleic acid
NA	Not applicable
NE	Not evaluable
NHL	Non-Hodgkin's lymphoma
NHP	Non-human primate
nm	Nanometer(s)
nM	Nanomolar
NOAEL	No adverse effect level
PBS	Phosphate buffered saline
PK	Pharmacokinetic
Plt	Platelet
PBPC	Peripheral blood progenitor cells
PMN	Polymorphonuclear leukocyte
PSCT	Peripheral stem cell transplant
rhu GM-CSF	Recombinant human granulocyte macrophage-colony stimulating factor
rhIL-3	Recombinant human interleukin-3
RNA	Ribonucleic acid
RTI	Ready-to-inject
SAE	Serious adverse event
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of the mean
SNS	Strategic National Stockpile
$t_{1/2}$	Half-life
TBI	Total body irradiation
TPO	Thrombopoietin
U	Unit(s)
UBI	Upper body irradiation
US	United States

Term	Definition
USPI	United States Prescribing Information (i.e., for Leukine)
vs.	Versus
WBC	White blood cell

1 EXECUTIVE SUMMARY

Genzyme Corporation, a Sanofi company, ("Genzyme") is seeking approval for an additional indication for use of Leukine® (yeast derived, recombinant human granulocyte-macrophage colony-stimulating factor (rhu GM-CSF, sargramostim)) ("Leukine") to mitigate neutropenia in patients exposed to non-therapeutic ionizing radiation and to enable access of Leukine to patients in casualty settings under the purview of the United States Department of Health and Human Services. We will present existing non-clinical and clinical data supporting this indication, and are committed to continuing to work with the FDA and Biomedical Advanced Research and Development Authority (BARDA) based on the advice of the Advisory Committee.

1.1. Scientific Rationale for Use of Leukine in Treating Neutropenia

Neutrophils are an essential component of the immune system and are a critical line of defense against bacterial and fungal infections. Neutropenia develops following exposure of the bone marrow to myelosuppressive agents including chemotherapy and therapeutic and non-therapeutic ionizing radiation. While the severity, duration, and kinetics of the fall in absolute neutrophil count (ANC) is dependent on the dose of chemotherapy and radiation, both types of injury cause a decline in ANC. There is also a risk of severe and life-threatening infections when exposure to chemotherapy or radiation causes ANC to fall below 500/mm^3; in this setting leukocyte growth factors (LGFs) are used to shorten the duration of neutropenia and thereby decrease infectious morbidity.

LGFs are a class of growth factors that stimulate the clonal expansion and differentiation of hematopoietic progenitor cells. LGF nomenclature is reflective of this activity; granulocyte colony-stimulating factor (G-CSF) supports the differentiation of granulocytes alone whereas granulocyte macrophage colony-stimulating factor (GM-CSF), such as Leukine, stimulates the proliferation, differentiation and maturation of granulocytes, macrophages, and dendritic cells. GM-CSF exerts these biological activities (i.e., survival, division, maturation and activation) through binding to specific receptors expressed on the surface of both pluripotent and mature hematopoietic cells (Hercus 2012). An important consequence of GM-CSF receptor signaling on granulocyte precursors is the maturation of these progenitor cells into neutrophils. The stimulation of neutrophil production by LGFs, including Leukine, translates into clinically meaningful decrease in the duration of neutropenia in the setting of myelosuppression.

Acute exposure to non-therapeutic radiation causes dose-dependent myelosuppression with a mechanism similar to that following therapeutic radiation and/or chemotherapy (Waselenko 2004). Acute radiation-induced neutropenia models in non-human primates (NHPs; Rhesus monkeys) have been utilized to assess the ability of LGF to accelerate neutrophil recovery and reduce mortality. Data from studies in NHPs shows that LGFs, including Leukine, accelerates neutrophil recovery in NHP exposed to radiation.

1.2. Approved Indications:

The five approved indications for Leukine use are:

1) 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).

2) Following induction chemotherapy in older adult patients (>55-70) 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.
3) For acceleration of myeloid recovery in patients undergoing allogeneic BMT from HLA-matched related donors.
4) For patients who have undergone allogeneic or autologous BMT in whom engraftment is delayed or has failed.
5) For the mobilization of hematopoietic progenitor cells into peripheral blood for collection by leukapheresis.

Clinical Efficacy Data:

Data from Registration Studies Related to Myelosuppression

Three of Leukine's FDA approved indications are based on the demonstration of accelerated neutrophil recovery in prospective, placebo-controlled randomized Phase 3 studies and are summarized below.

- Efficacy of Leukine in patients (>55-70 years old) with AML following induction therapy with daunorubicin and cytarabine (Rowe 1995): The median time from initiation of Leukine or placebo to an ANC > 500/mm^3 was significantly shorter in the Leukine group. Clinically, episodes of infections ($\geq$ Grade 3) were reduced with Leukine.

- Efficacy of Leukine in patients receiving an autologous BMT (Nemunaitis 1991): patients receiving Leukine had a statistically significant acceleration of neutrophil recovery to an ANC > 500/mm^3 compared to placebo patients. In support of the clinical benefits of this accelerated neutrophil recovery, patients who received Leukine required fewer days of antibiotics and had fewer days of hospitalization. There was no significant difference in the time to neutrophil recovery between those who received chemotherapy alone compared to those who received both chemotherapy and total body irradiation

- Efficacy of Leukine in patients receiving allogeneic BMT (Nemunaitis 1995): patients who received Leukine experienced significantly earlier neutrophil recovery to an ANC >500 cells/mm^3. This acceleration of neutrophil recovery correlated with a decrease in infections. A post-hoc analysis showed neutrophil recovery was accelerated with Leukine compared to placebo regardless of the inclusion of total body irradiation (TBI) with chemotherapy as part of the pre-BMT preparative regimen.

These studies show that Leukine significantly shortens the time to neutrophil recovery (defined as ANC >500/mm^3) compared to placebo. In the setting of both autologous and allogeneic BMT, patients receiving myelosuppressive chemotherapy experienced accelerated neutrophil recovery with Leukine treatment regardless of the inclusion of TBI as part of the preparative regimen. While these patients did receive bone marrow cells that had not been exposed to radiation, these data support Leukine's ability to stimulate healthy bone marrow cells in a bone marrow environment exposed to radiation. The activity of Leukine in patients with AML treated with myelosuppressive chemotherapy indicates that Leukine can accelerate neutrophil recovery in the absence of an infusion of healthy bone marrow cells. In addition to the mitigation of neutropenia, Leukine treated patients in these studies demonstrated a statistically significant decrease in infectious morbidity. Overall, these studies address aspects of myelosuppression relevant to the

myelosuppression observed after exposure to acute ionizing radiation and indicate the ability of Leukine to accelerate neutrophil recovery.

Dose

All five Leukine indications were approved for use at the dose of 250 μg/m^2/day based on pharmacokinetic/pharmacodynamic (PK/PD) profiles and safety profiles established in dose ranging studies as discussed in Section 6.2.

Supplemental Clinical Data:

Conducting prospective clinical trials of LGFs for the mitigation of non-therapeutic ionizing radiation-induced neutropenia in healthy volunteers would be unethical. However, one published study of the development and treatment of clinically-important neutropenia caused by high dose therapeutic radiation in patients with multiple myeloma and treated with rhu GM-CSF was found and is discussed below.

Ten patients with stage III multiple myeloma ineligible for BMT underwent therapeutic double hemibody irradiation (DHBI) and were treated with non-Leukine (*E. coli*-derived) rhu GM-CSF (Troussard 1995). A historical cohort of 32 patients with stage III multiple myeloma who received the same radiation doses at the same treatment center without rhu GM-CSF treatment was used for comparison. Following DHBI, the historical controls showed greater granulocytopenia with granulocyte levels being at least three fold lower in the historical controls compared to the rhu GM-CSF group 15 days following irradiation. In addition, no severe infections were documented in the rhu GM-CSF group compared to 34% of the patients in the historical cohort.

This study provides data on the ability of rhu GM-CSF to prevent occurrence of neutropenia in patients with an irradiated bone marrow. While half of the body had intact marrow in this study, rhu GM-CSF did positively impact granulocyte numbers. Furthermore, the hemibody irradiation model more closely mimics the likely disproportionate exposure an individual would receive in a radiation event due to partial shielding behind objects and body position relative to the initial or subsequent radiation fallout from the explosion. These results suggest that rhu GM-CSF could accelerate neutrophil recovery and may reduce the incidence of infections following radiation exposure in the absence of chemotherapy and stem cell rescue.

CLINICAL SAFETY:

There is a large body of Leukine safety data from the use of Leukine in studies supporting its five FDA approved indications as well as in investigational settings. Since Leukine's first approval in1991, approximately 470,000 patients have received Leukine treatment in the post-marketing setting. Risks associated with use of the product are described in the U.S. Prescribing Information and the most frequent adverse events are fever, asthenia, headache, bone pain, chills and myalgia. These events were generally mild or moderate in severity and were usually prevented or reversed by the administration of analgesics and antipyretics.

Specific to the discussion of safety for the approved indications, this section includes safety data from the pivotal registration studies. Overall, the frequency and type of adverse events (AEs),

serious adverse events, fatal AEs and laboratory abnormalities reported for patients treated with Leukine were similar to the placebo-treated patients across the studied populations. The underlying comorbidities as well as the cytotoxic regimens involved in these populations under study should be noted when interpreting the reporting rates for these AEs. 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).

1.3. Non-Clinical Efficacy Data:

The existing non-clinical data using Leukine and non-Leukine rhu GM-CSF to treat irradiated NHPs (Rhesus monkeys) from published and unpublished studies are discussed below. Although these studies have certain limitations (e.g., not conducted under GLP conditions and relatively small group sizes), they suggest that rhu GM-CSF can accelerate neutrophil recovery after radiation. A summary of the main observations include:

- Leukine (at a dose approximating the proposed clinical dose) accelerated neutrophil recovery in NHPs (Rhesus monkeys) with radiation-induced neutropenia following exposure to TBI.
- Non-Leukine rhu GM-CSF accelerated neutrophil recovery in NHPs (Rhesus monkeys) with radiation-induced neutropenia after irradiation of partially shielded NHPs (Rhesus monkeys).
- Non-Leukine rhu GM-CSF improved neutrophil recovery in lethally irradiated NHPs (Rhesus monkeys) receiving a bone marrow transplant

1.4. Non-Clinical and Clinical Comparison of rhu GM-CSF and rhu G-CSF:

Both GM-CSF and G-CSF have been extensively studied in the setting of neutropenia, although studies directly comparing the two are limited.

Two non-clinical studies comparing rhu GM-CSF (one study using Leukine) to filgrastim found no difference in neutrophil recovery in NHPs (Rhesus monkeys) in a radiation-induced neutropenia model.

Several published reports that have evaluated the relative efficacy and safety of Leukine and filgrastim, however these studies were non-randomized, retrospective analyses, or in settings not related to mitigation of neutropenia.

There is, however, a single, prospective, randomized study compared the efficacy of Leukine to filgrastim in the setting of chemotherapy induced neutropenia (Beveridge 1998). In this study, patients with malignant lymphomas or other solid tumors receiving myelosuppressive chemotherapy were randomized to receive either Leukine or filgrastim. There was no statistical difference between Leukine and filgrastim in the mean number of days to reach to an ANC $>500/mm^3$. A statistically significant difference was reached for the mean time to an ANC $>1,000/mm^3$ with filgrastim accelerating recovery half a day earlier than Leukine. More importantly with regards to clinical relevant study endpoints, Leukine treated patients did not differ statistically from filgrastim in the number of patients hospitalized for fever or antibiotic therapy, mean length of stay, number of patients with positive blood cultures, or mean duration

of fever. Regarding safety, the authors concluded that "both growth factors were tolerated" and there were no differences in patients experiencing AEs between the two treatment groups.

1.5. Guidelines for the treatment of neutropenia:

The management of neutropenia in the cancer patients has been discussed in several guidelines with the American Society of Clinical Oncology's guideline (ASCO) being the most prominent (Smith 2006). This guideline refers to both LGFs in the management of neutropenia following myelosuppressive therapy. With respect to comparison of GM-CSF and G-CSF, the guidelines state *"No guideline recommendation can be made regarding the equivalency of the two colony-stimulating agents. As in 2000, further trials are recommended to study the comparative clinical activity, toxicity, and cost-effectiveness of G-CSF and GM-CSF."* In addition to the ASCO guidelines, the NCCN guidelines also advise the use of a LGF, but do not specify GM-CSF or G-CSF. While these guidelines recommend the use of LGF to accelerate neutrophil recovery, they do not recommend the use of one LGF over another.

With respect to the management of radiation-induced neutropenia, multiple guidelines that recommend the use of LGF in this setting have been published. The Strategic National Stockpile (SNS) Radiation Working Group recommends the use of cytokines, including sargramostim, filgrastim and pegfilgrastim, as options for the treatment of patients exposed to > 2 Gy radiation (Waselenko 2004). The United States Department of Health and Human Services (HHS) in their Radiation Event Medical Management (REMM) recommends these three LGFs. Finally, the Radiation Emergency Assistance Center/Training Site (REAC/TS) has provided the Department of Energy with their guidelines recommending the use of any one of the three LGF for patients with severe neutropenia. The recognition by these guidelines of the expected efficacy of rhu GM-CSF and rhu G-CSF in the treatment of radiation-induced neutropenia is reflective of the clinical data showing the acceleration of neutrophil recovery in multiple settings of neutropenia.

The recommendation of LGF treatment in the guidelines for acute radiation-induced neutropenia is corroborated by the use of LGF in accidental radiation exposures. Three reports of accidental exposures utilized rhu GM-CSF to treat acute radiation-induced neutropenia and a description of these experiences can be found in Appendix 8.

1.6. Conclusion:

Leukine is approved by the FDA for five indications, three of which relate to the acceleration of neutrophil recovery in patients with myelosuppression. The utilization of Leukine to treat neutropenia in these distinct clinical settings supports the use of Leukine in individuals exposed to non-therapeutic ionizing radiation. Treatment with rhu GM-CSF in patients with multiple myeloma receiving hemibody irradiation provides data on the efficacy of Leukine for the mitigation of radiation-induced neutropenia without stem cell rescue.

In addition, non-clinical studies present further evidence for the acceleration of neutrophil recovery by rhu GM-CSF in irradiated NHPs (Rhesus monkeys) and the similarity of these effects between Leukine and G-CSF.

Finally, multiple guidelines recommend the use of rhu GM-CSF or rhu G-CSF for neutropenia following myelosuppression induced by chemotherapy, radiotherapy, or non-therapeutic radiation exposure.

While no therapy is currently FDA-approved for use in mitigating neutropenia after exposure to non-therapeutic ionizing radiation, the utilization of Leukine to treat neutropenia in these distinct settings supports the expansion of Leukine's indication for its use in mitigation of neutropenia following exposure to non-therapeutic ionizing radiation.
Genzyme remains committed to continuing to work with the FDA and BARDA to define the appropriate development path for this indication based on the advice of this advisory committee.

2 INTRODUCTION

As part of the 2004 Project Bioshield Act, BARDA has identified leukocyte growth factors (LGFs) as a medical countermeasure to mitigate the morbidity and mortality associated with exposure to non-therapeutic ionizing radiation.

Leukine is currently approved in the United States (US) for a total of five indications with the first indication granted in 1991. In three of the five approved indications, Leukine was used for the mitigation of neutropenia following chemotherapy with or without radiation. The injury to the bone marrow caused by acute radiation exposure is qualitatively similar to that caused chemotherapy with or without radiation.

The FDA's objective from this Advisory Committee meeting is to obtain guidance on the appropriate evidence required for approval of LGF for the treatment of acute radiation-induced neutropenia, focusing on the potential adequacy of already available clinical and non-human primate (NHP) data. FDA has requested that the respective sponsors of the approved LGF (i.e., Amgen, Genzyme, Sicor) present the existing efficacy and safety data supporting their approved indications.

Leukine could confer clinical benefit in patients with acute radiation induced neutropenia similar to the benefit observed in the clinical settings where Leukine is already approved. Therefore, in this briefing book we are discussing the aggregate of the clinical and non-clinical data to support use of Leukine for acceleration of neutrophil recovery following exposure to non-therapeutic ionizing radiation.

3 NEUTROPENIA AND ITS CLINICAL CONSEQUENCES

Neutrophils are an important component of the immune system that mediates protection against infection. Neutropenia has been documented following exposure of the bone marrow to a variety of myelosuppressive agents including chemotherapy and radiation. While the magnitude and kinetics of the fall in absolute neutrophil count (ANC) is dependent on the dose of chemotherapy and radiation, both types of injury induce declines in ANC. Exposure to high doses of chemotherapy or radiation result in neutropenia (ANC<500/mm^3) and conveys a risk of severe and life-threatening infections. Given this increased risk of infectious morbidity that accompanies neutropenia, LGF were investigated for their ability to shorten the duration of severe neutropenia and thereby decrease the infectious consequences.

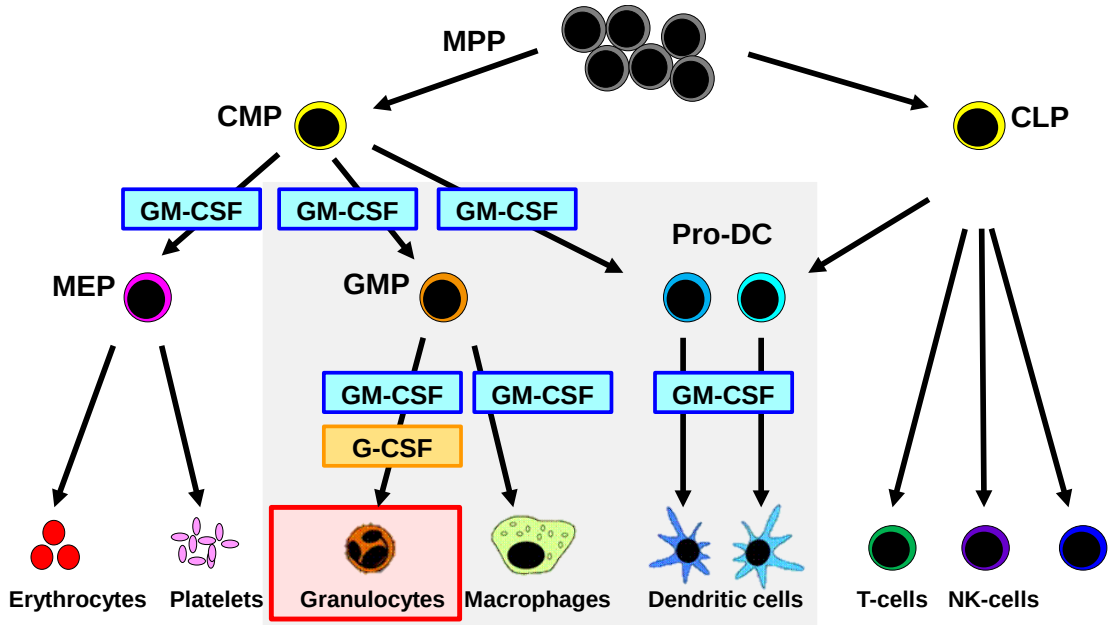
Neutropenia is recognized as a substantial risk factor for developing bacterial and fungal infection, especially when severe (ANC < 500/mm^3), and of sufficient duration (multiple days) (Brown 1984). Evidence supporting the clinical consequences of neutropenia was reported in a study of 52 patients with acute leukemia (Bodey 1966). For patients with an ANC less than 100/mm^3, infections were detected in 53% of those days compared to infections found in 37% of the days when the ANC was 100-500/mm^3. For patients with an ANC of 500-1,000/mm^3, infections occurred in 20% of the days and no infections were documented when the ANC was over 1,000/mm^3. Further, the number of days with an ANC under 500/mm^3 correlated with the presence and type of infection. Overall, this documents the infectious consequences of neutropenia and supports the rationale for limiting neutropenia.

Stemming from the data that neutropenia increases infectious risk, various therapeutic approaches were developed to stimulate neutrophil production and shorten the duration of neutropenia in the setting of myelosuppression. One approach involves the utilization of growth factors, such as LGFs, to stimulate the production of neutrophils thereby decreasing the incidence of infection. In support of this, Leukine has been used to shorten the time to neutrophil recovery in chemotherapy induced neutropenia in several indications.

4 SCIENTIFIC RATIONALE FOR THE USE OF LEUKINE

LGF are a class of growth factors that stimulate development of a variety of hematopoietic cells. LGF differ in their ability to stimulate the clonal expansion and differentiation of hematopoietic progenitor cells and their nomenclature is descriptive of this activity. For example, *in vitro* macrophage CSF (M-CSF) and granulocyte CSF (G-CSF) has shown activity in the proliferation and differentiation of macrophages and granulocytes, respectively, whereas GM-CSF stimulates the growth and differentiation of both macrophages and granulocytes (Figure 1).

Leukine is a recombinant human GM-CSF (rhu GM-CSF) that supports survival, clonal expansion, and differentiation of hematopoietic progenitor cells *in vitro*. Specifically, Leukine induces partially committed progenitor cells to proliferate and differentiate down the granulocyte-macrophage pathways leading to production of neutrophils, monocytes, macrophages, and dendritic cells *in vitro* (Figure 1). In addition, Leukine is also capable of activating mature granulocytes and monocytes increasing migration, oxidative metabolism, and antibody dependent phagocytosis *in vitro* (Metcalf 2010). Of particular importance for neutropenia is the ability of Leukine to stimulate the production of and enhance the function of neutrophils has been shown *in vitro*.



Adapted from Passegué E et al., 2003

Figure 1: Stimulation of hematopoiesis by GM-CSF and G-CSF. The impact of GM-CSF and G-CSF on the differentiation of hematopoietic progenitor cell to mature immune cells based on preclinical data.

GM-CSF exerts these biological activities (i.e., survival, division, maturation, activation) through GM-CSF binding to specific receptors expressed on the surface of target cells (Hercus 2012). This receptor is composed of an alpha and beta subunits that signals through Janus kinase 2 (JAK2) and the STAT, MAP kinase, and PI3 kinase pathways. This signaling causes transcriptional changes leading to cellular proliferation, maturation, and activation (Hercus

2012). An important consequence of GM-CSF receptor signaling on granulocyte precursors is the maturation of these progenitor cells into neutrophils.

Acute exposure to non-therapeutic radiation causes dose-dependent and predictable myelosuppression similar to that observed following therapeutic radiation and/or chemotherapy (Waselenko 2004). Non-human primates (NHPs) have been utilized to assess the ability of LGFs to accelerate neutrophil recovery and reduce mortality, and these studies provide data that LGFs, including Leukine, accelerate neutrophil recovery in NHPs (Rhesus monkeys) exposed to radiation.

While Leukine is a yeast-derived rhu GM-CSF, other forms of exogenous rhu GM-CSF have been produced using bacterial and mammalian expression systems. The different expression systems lead to differences in the degree and type of glycosylation (Dorr 1993). Comparing these different rhu GM-CSF in an *in vitro* functional assay has identified no significant differences in their activity. While one study did report differences in the mobilization of stem cells by these different rhu GM-CSF, no studies have compared differences in neutrophil recovery following treatment with the *E. coli*-derived or the yeast-derived rhu GM-CSF despite both therapies being extensively studied in the setting of neutropenia (Hussein 1995). A review of the literature by Dorr found a higher frequency of adverse events with the *E. coli*-derived rhu GM-CSF compared to the yeast-derived rhu GM-CSF (Dorr 1993). Leukine (a yeast-derived rhu GM-CSF) remains the only approved and marketed GM-CSF in the United States.

5 LEUKINE PRODUCT INFORMATION

5.1 Product Name:

Trade name	Leukine®
Generic name	Sargramostim
Common/established name	recombinant human granulocyte-macrophage colony-stimulating factor (rhu GM-CSF)

5.2 Pharmacokinetics:

Pharmacokinetic profiles have been analyzed in controlled studies of 24 normal volunteers. At the recommended dose of 250 μg/m^2, when Leukine was administered intravenously (IV) over two hours to normal volunteers, the mean beta half-life was approximately 60 minutes. The mean maximum concentration (Cmax) was 5.4 ng/mL, the mean clearance rate was approximately 431 mL/min/m2 and the mean AUC (0–inf) was 677 ng/mL•min. GM-CSF was last detected in blood samples obtained at three or six hours. When Leukine was administered subcutaneously (SC) at the same dose of 250 μg/m^2 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. For Leukine administered SC, the mean maximum concentration (Cmax) was 1.5 ng/mL, the mean clearance rate was approximately 529 mL/min/m2 and the mean AUC (0–inf) was 501 ng/mL•min.

5.3 Leukine: Approved Indications:

Leukine's first FDA approval was in 1991 for acceleration of neutrophil recovery following autologous bone marrow transplantation. Leukine is currently approved for the following five indications (see Appendix 1 for full indication language):

1) 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).

2) Following induction chemotherapy in older adult patients (55-70) 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.

3) For acceleration of myeloid recovery in patients undergoing allogeneic BMT from HLA-matched related donors.

4) For the mobilization of hematopoietic progenitor cells into peripheral blood for collection by leukapheresis.

5) For patients who have undergone allogeneic or autologous BMT in whom engraftment is delayed or has failed.

Three of these five indications are based on prospective, randomized studies that focus on accelerating myeloid recovery or shortening the duration of neutropenia following chemotherapy alone or in combination with total body irradiation (TBI), and thus, are most relevant to the potential use of Leukine for the mitigation of neutropenia following exposure to ionizing radiation. All Leukine indications are for use at a dose of 250 $\mu g/m^2$/day.

6 CLINICAL

6.1 Introduction

The utility of Leukine in accelerating neutrophil recovery has been evaluated in multiple clinical studies for indications similar to bone marrow damage likely to occur following acute radiation exposure. These include FDA approvals to shorten time to neutrophil recovery following induction chemotherapy in older adult patients with acute myelogenous leukemia (AML), and to accelerate neutrophil recovery following autologous or allogeneic bone marrow transplantation (BMT). All of these clinical settings share features that are relevant to acute radiation-induced neutropenia.

In addition to these efficacy data, Genzyme has extensive clinical and post-marketing safety data from a broad range of treated individuals, including males and females and ranging in age from pediatrics to the elderly, representing a well-characterized safety profile for Leukine. Studies have been performed in a range of clinical disorders, including neutropenia secondary to chemotherapy both with and without total body irradiation.

6.2 Dose of Leukine

The dose of Leukine utilized in all the pivotal studies for approval was 250 μg/m^2/d. This dose was derived, at least in part, from the results of eleven dose escalating studies including 215 patients with doses ranging up to 4000 μg/m^2/d, a dose 16-fold higher than the clinically approved dose. For 106 patients in the < 91 μg/m^2/day, two grade 3 AEs (one of asthenia and one of fever) and no grade 4 AEs were reported. The common side effects were fever, headache, malaise, myalgia vomiting and anorexia. For 102 patients treated in the dose range of 91-180 μg/m2/d, 12 Grade 3 AEs occurred with no Grade 4 AEs reported. The frequency of chills, asthenia, myalgia, malaise, and fever occurred at similar frequencies compared to the <91 mg/m2/d group (n=106). In 77 patients treated with a range of 180-320 μg/m2/d, one Grade 4 AE (dyspnea) and one grade 3 AE (bone pain) was reported. Otherwise, side effects were comparable to 91-180 μg/m2/d dose group. In 46 patients in the 321-899 μg/m2/d dose group, three Grade 4 AEs (dyspnea once and asthenia twice) and three Grade 3 AEs (all fever) were reported. For 22 patients in the >899 μg/m2/d, no Grade 4 AEs, three Grade 3 AEs (asthenia, headache, pain in extremity) were reported.

As expected for patients with various illnesses, side effects were frequently reported and in many instances were difficult to separate from disease symptoms. Fever was the most commonly reported side effect irrespective of relationship to study drug. Other common side effects were myalgia, chills, headache, malaise, and vomiting. By body system, side effects were most frequently reported for digestive, nervous, and musculoskeletal.

Leukine may lead to specific side effects such as fever, asthenia, anorexia, chills, and headache at the lowest dose level (less than 91 mcg/m2/day) with more severe toxicities occurring at higher dose levels. Fever occurred more frequently with lower cumulative doses. Frequency of chills, headache, malaise, myalgia, nausea, pain and rash is reduced at higher cumulative doses. This could be due to the underlying disease in different studies, or reflect an adaptive process.

This review of the PK/PD and safety profiles from these studies resulted in the selection of 250 μg/m^2/d dose for further clinical development in phases 2 and 3. Further information on dose-finding can be found in Appendix 6.

6.3 Clinical Efficacy of Leukine for Acceleration of Neutrophil Recovery

The approval for the three indications for acceleration of neutrophil recovery was based on randomized, prospective, placebo-controlled trials of Leukine. In addition to neutrophil recovery, clinical endpoints in these studies included outcomes such as infections, days of antibiotics, and hospital days. These studies represent distinct scenarios of myelosuppression due to chemotherapy with and without total body irradiation, and thus relate to the acute radiation-induced neutropenia setting.

6.3.1 Autologous Bone Marrow Transplantation

An initial area of investigation with Leukine was accelerating neutrophil recovery following autologous bone marrow transplantation (BMT). This culminated in three prospective, phase 2/3 double-blind and placebo-controlled trials of Leukine in patients undergoing autologous BMT that were conducted at three institutions (Nemunaitis 1991; Studies 301/302/303). A summary of the study design, endpoints, and statistical considerations can be in found in Appendix 9. The primary efficacy endpoint was time to ANC recovery to > 500/mm^3 defined as the number of days from BMT date to date of the first two consecutive ANC measurements greater than or equal to the target value. Time to ANC recovery was analyzed with the Kaplan-Meier method and compared between the 2 arms using the Wilcoxon procedure. Other endpoints included duration of hospitalization, duration of infection and duration of antibacterial therapy.

A total of one hundred twenty-eight patients were enrolled in these 3 studies; 65 randomized to Leukine and 63 to placebo. Patients were transplanted for acute lymphocytic leukemia (ALL; 17 patients), non-Hodgkin's lymphoma (NHL; 87 patients), Hodgkin's disease (23 patients) or acute myelogenous leukemia (AML; 1 patient). Leukine was administered at a dose of 250 μg/m^2/day by 2-hour IV infusion for 21 consecutive days starting within four hours of completion of the autologous bone marrow infusion. Preparative regimens varied by site; however, TBI (12-14.4 Gy fractionated over 6 days) was included in the preparative regimen in 50 of 65 (77%) of patients who received Leukine and 47 of 63 (75%) patients who received placebo.

Patients receiving Leukine had a statistically significantly more rapid neutrophil recovery to ANC > 500/mm^3 compared to placebo patients (19 vs. 26 days, p<0.001) (see Figure 2 and Table 1). Time to ANC > 1,000/mm^3 was also statistically significant in favor of Leukine treated patients (26 vs. 33 days, p=0.009) (Table 1). This indicates that the irradiation of the bone marrow did not impair the ability of Leukine to accelerate neutrophil recovery compared to placebo. These conditioning regimens for autologous BMT that include high doses of chemotherapy with and without TBI induce neutropenia that resembles that observed after exposure to acute radiation. Leukine's ability to accelerate neutrophil recovery in the setting of autologous BMT indicates that Leukine is able to stimulate the production of neutrophils from bone marrow that has been exposed to a combination of myelosuppressive agents.

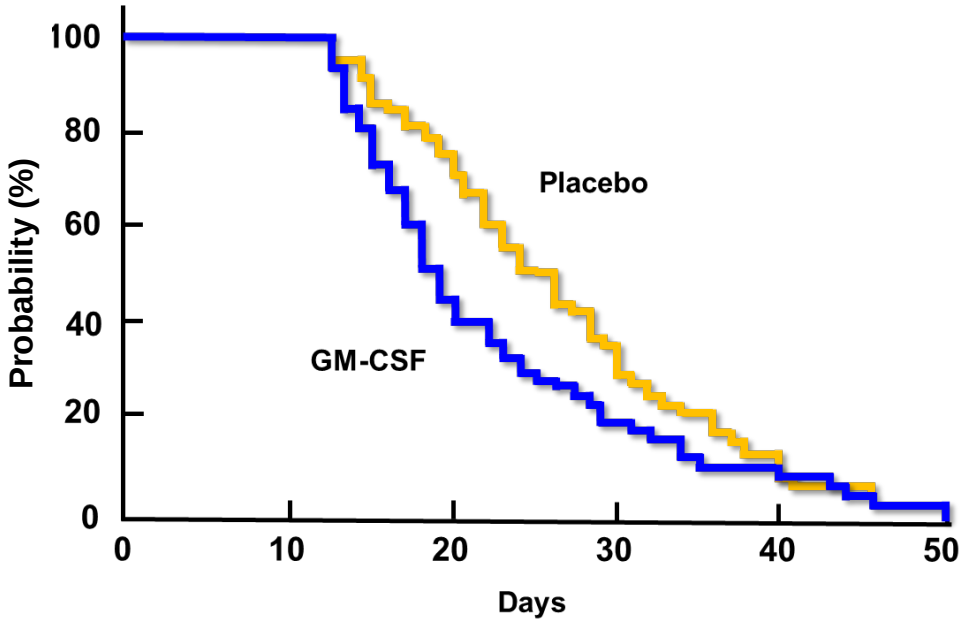


Figure 2: Kaplan-Meier curves of time to ANC recovery to > 500/mm^3. A Kaplan-Meier analysis of the time to ANC > 500/mm^3 for two consecutive days in patients receiving an autologous bone marrow transplant and treated with Leukine or placebo. (adapted from Nemunaitis 1991)

Table 1: Kaplan Meier Estimates of Time to ANC Recovery (in Days)

Time (median days)	**Leukine (n=65)**	**Placebo (n=63)**	**p value***
ANC > 500/mm^3	19	26	<0.001
ANC > 1000/mm^3	26	33	0.009

(Source: Nemunaitis 1991)
*By the Wilcoxon rank-sum test

In support of a clinical benefit of this accelerated neutrophil recovery, patients who received Leukine required significantly fewer days of antibiotics (24 vs. 27 days, p=0.009). Leukine treatment was also associated with a significantly shorter length of hospitalization (27 vs. 33 days, p=0.01). Owing to extensive radiation therapy and chemotherapy prior to transplant in the subset of patients with Hodgkin's disease and the inclusion of a single patient with AML, further analyses were conducted on the 104 patients with either ALL or NHL. In this population, the number of median days to ANC > 500/mm^3 remained significantly fewer with Leukine treatment compared to placebo (18 vs. 24; p<0.05) as seen in the larger analysis. Further analysis revealed Leukine treated patients had fewer hospitalizations, shortened duration of antibacterial therapy, and shortened duration of infection in febrile patients (Table 2).

Table 2: Clinical Outcomes in Patients with ALL or NHL undergoing Autologous BMT followed by Leukine or Placebo Treatment

Outcome (median days)	**Leukine (n=54)**	**Placebo (n=50)**	**p value***
Duration of infection	1	4	<0.05
Duration of antibacterial therapy	21	25	<0.05
Duration of hospitalization	25	31	<0.05

(Source: Leukine USPI, Appendix 1)
*Wilcoxon or CMH ridit chi-squared

Therefore, in patients undergoing autologous BMT for lymphoid neoplasia, Leukine significantly shortens the time to neutrophil recovery and lessens infection-related morbidity compared to placebo, whether the preparative regimen includes chemotherapy alone or chemotherapy plus TBI.

6.3.2 Leukine in Patients (>55-70 years of age) with Acute Myelogenous Leukemia

The efficacy of Leukine in accelerating neutrophil recovery following chemotherapy in AML was evaluated in a prospective, Phase 3, multi-center, double-blind, and placebo-controlled study conducted in 99 patients with de novo AML aged 55-70 (Rowe 1995; Study 305). A summary of the study design, endpoints, and statistical considerations can be in found in Appendix 9. Briefly, patients were randomized and then treated with a combination of standard doses of daunorubicin (days 1–3) and ara-C (days 1–7) as induction and high dose ara-C was administered for 6 days as a single course of consolidation, if given, based on bone marrow evaluation. Following a complete remission, patients received Leukine (250 µg/m^2/day IV over four hours) or placebo beginning four days following completion of chemotherapy and continuing until the ANC > 1,500/mm^3 for 3 consecutive days or for a maximum of 42 days. The primary endpoint for this study was the time to ANC > 500/mm^3 which was defined as the time from initiation of Leukine or placebo treatment to ANC > 500/mm^3. Time to ANC recovery was analyzed with the Kaplan-Meier method and compared between the two arms using the log rank and the Wilcoxon procedures.

Time to ANC recovery is summarized in Table 3 and Kaplan-Meier curves of time to ANC recovery > 500/mm^3 are provided in Figure 3. Median time to ANC recovery > 500/mm^3 was 13 days in the Leukine arm and 17 days in the placebo arm. The difference in time to ANC > 500/mm^3 and ANC>1,000/mm^3 between the two arms was statistically significant (p=0.009 and p=0.003, respectively).

Table 3: Kaplan-Meier Estimates of Time to ANC Recovery (in Days)

Time (median days, (25%, 75%)	**Leukine (n=52)**	**Placebo (n=47)**	**P Value****
ANC > 500/mm^{3a}	13 (11, 16)	17 (13,25)	0.009
ANC > 1,000/mm^{3b}	14 (12, 18)	21 (13, 34)	0.003

(Source: Leukine USPI, Appendix 1)

** p=generalized Wilcoxon

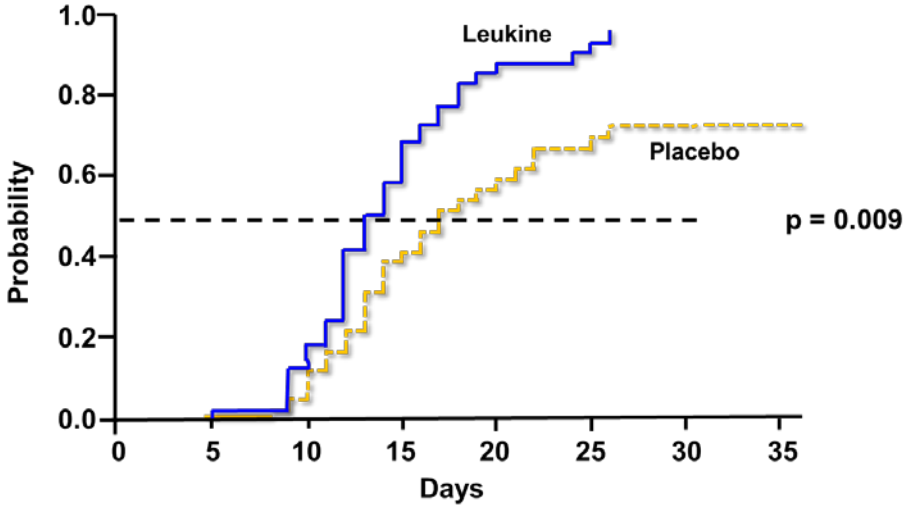


Figure 3: Kaplan-Meier curves of time to ANC recovery to > 500/mm3 in patients with AML. Adapted from Rowe 1995).

The clinical benefit of this accelerated neutrophil recovery was evidenced by fewer confirmed infections of Grade 3 or greater severity in the Leukine treated patients compared to placebo (Table 4). Microbiologically confirmed infections included routine surveillance cultures and cultures of clinically suspected sites of infection. This study indicated the ability of Leukine to shorten the duration of neutropenia in the setting of chemotherapy-induced myelosuppression without stem cell rescue which correlated with fewer infections. This impact of Leukine may have relevance to the management of radiation-induced myelosuppression.

Table 4: Infections in Patients with AML following Leukine or Placebo Treatment

	Leukine (n=52)	**Placebo (n=47)**	**P Value***
Number (%) of patients with Grade ≥ 3 Infection	27 (52%)	35 (74%)	0.02

(Source: Leukine USPI, Appendix 1)
* Fisher's exact test

6.3.3 Leukine following Allogeneic Bone Marrow Transplantation

Following the demonstration of Leukine efficacy in autologous BMT, Leukine was explored for the potential to accelerate neutrophil recovery following allogeneic BMT. A prospective, Phase 3, multi-center, randomized, and placebo-controlled trial of Leukine was conducted in 109 patients, including 23 pediatric patients <18 years of age, undergoing HLA-identical sibling BMT for a variety of lymphoid and myeloid malignancies (Nemunaitis 1995, Study 9002). A summary of the study design, endpoints, and statistical considerations can be in found in

Appendix 9. Primary objectives were to compare the effect of Leukine and placebo following allogeneic BMT on neutrophil recovery and length of hospitalization. The primary endpoint of time to ANC recovery to > 500/mm^3 was defined as the number of days from BMT date to date of the first two consecutive ANC measurements greater than or equal to the target value. Time to ANC recovery and duration of hospitalization were analyzed with the Kaplan-Meier method and compared between the two arms using the log rank and the Wilcoxon procedures stratified by site and baseline risk.

Fifty-three patients received Leukine (250 µg/m^2/day by 4-hour infusion) and 56 patients received placebo starting shortly after the end of bone marrow infusion (i.e. greater than 24 hours after completing chemotherapy and radiation) and continuing for 21 consecutive days. Preparative regimens varied by sites, but TBI was included in the preparative regimen in 32 of the 53 (60%) patients who received Leukine and 35 of 56 (59%) patients who received placebo.

Median time to ANC recovery to >500/mm^3 was 13 days in Leukine arm and 17 days in Placebo and this difference was statistically significant (p=0.0001) (Table 5). Kaplan-Meier curves of time to ANC recovery to >500/mm^3 are provided in Figure 4. This accelerated neutrophil recovery with Leukine was also supported by a statistically significant decrease in the median time to ANC>1,000/mm^3 (14 vs. 19 days, p=0.0001) (Table 5).

Table 5: Kaplan-Meier Estimates of Time to ANC Recovery (in Days)

Time (median in days)	**Leukine (n=53)**	**Placebo (n=56)**	**P Value***
ANC > 500/mm^3	13	17	0.0001
ANC > 1,000/mm^3	14	19	0.0001

(Source: Nemunaitis 1995 and Leukine USPI, Appendix 1)
* p=generalized Wilcoxon

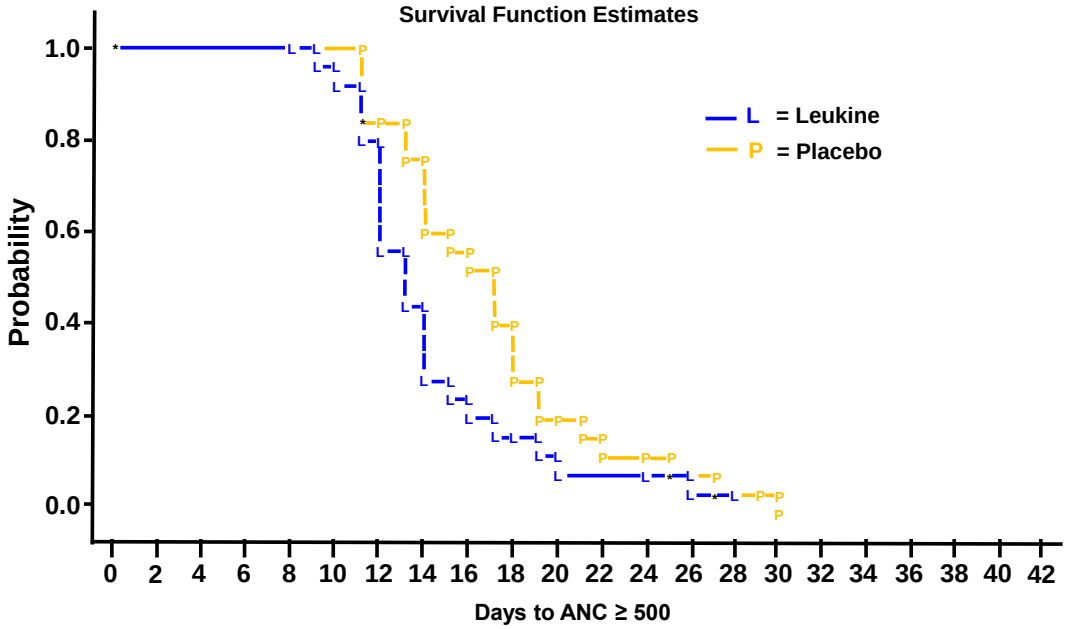


Figure 4: Kaplan-Meier curves of time to ANC recovery > 500/mm^3. Comparison of Leukine or placebo treated patients receiving an allogeneic BMT for median time to ANC < 500/mm^3.

Further, in patients exposed to radiation in addition to chemotherapy as part of their preparative regimen, a post-hoc analysis was performed to assess Leukine's ability to accelerate neutrophil recovery. In this analysis, Leukine accelerated neutrophil recovery in patients treated with TBI as evidenced by a median time to ANC >500/mm^3 of 13.5 days compared to 16 days in the placebo group (**Figure 5**). In addition to this being a post-hoc analysis, one of the limitations of this analysis is the transplanted stem cells were not exposed to radiation, thus limiting the applicability of these data to the acute radiation induced neutropenia. However, these data do help address the potential concern about damage to the bone marrow stroma by radiation impacting the ability of Leukine to accelerate neutrophil recovery in individuals exposed to radiation.

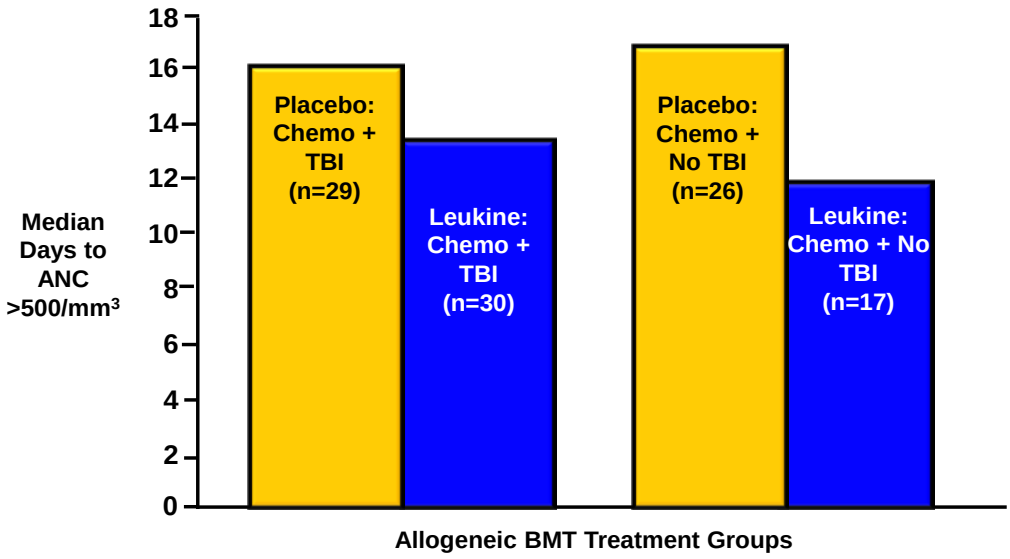


Figure 5: Neutrophil recovery based on the inclusion of TBI as part of the conditioning regimen prior to allogeneic bone marrow transplant. A post-hoc analysis assessed patients treated with chemotherapy with or without TBI as part of their BMT conditioning regimen BMT for the time to ANC > 500/mm^3.

Consistent with the previously discussed pivotal studies, the accelerated neutrophil recovery following allogeneic BMT was accompanied by clinical benefits. Total infections (bacterial, fungal and viral) were decreased in the Leukine group (34 infections) compared to the placebo group (51 infections). There was a difference in the number of patients with bacteremia in favor of Leukine (Table 6). In addition, the percentage of patients with infection was lower in the Leukine treated group (56.6% vs. 75%; p=0.001) (Table 6).

Table 6: Clinical Outcomes in Patients Undergoing Allogeneic BMT

	Leukine(n=53)	Placebo (n=56)	p value
Number (%) of patients with infections	30 (57%)	42 (75%)	<0.05*
Number (%) of patients with bacteremia	9 (17%)	19 (34%)	0.043**
Total Infections (bacterial, fungal, viral). Number (%) of patients	34 (64%)	51 (91%)	0.001

(Source: Nemunaitis 1995 and Leukine USPI, Appendix 1)
* generalized Wilcoxon test / ** simple chi-square test

In conclusion, Leukine accelerates neutrophil recovery in patients receiving an allogeneic BMT following chemotherapy with and without radiation.

6.3.4 Additional Indications

There are two other approved uses for Leukine. One indication is for mobilization of hematopoietic progenitor cells and following transplantation of hematopoietic progenitor cells. The other indication is for use in bone marrow transplantation failure or engraftment delay. A brief summary of each indication is provided below and additional information regarding these indications can be found in the package insert (Appendix 1). Data for these two indications is being presented for the purpose of complete disclosure as the relevance of these indications to radiation-induced neutropenia is limited.

6.3.4.1 Leukine for Peripheral Blood Progenitor Cell Mobilization and Engraftment

A retrospective review was conducted of data from patients with cancer undergoing collection of peripheral blood progenitor cells (PBPC) for autologous BMT at two transplant centers. Mobilization of PBPC and myeloid reconstitution post-transplant were compared between patients (n=196 and n=31 at each center) 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 at one center)]. While the main focus of this indication is mobilization of PBPC, there are data on neutrophil recovery with and without Leukine treatment post-transplant. Mobilized subjects had accelerated neutrophil recovery when Leukine was administered post-transplant compared to those who did not receive Leukine post-transplant (median time to ANC > 500/mm^3: 12 days vs. 21 days, p-value not reported) (Leukine USPI, Appendix 1).

6.3.4.2 Leukine for Engraftment Failure

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. 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. One hundred day survival was improved in favor of the patients treated with Leukine after graft failure following either autologous or allogeneic BMT (Leukine USPI, Appendix 1). 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.

6.4 Supplemental Clinical Efficacy

6.4.1 Additional Phase 3 Studies of Leukine in Neutropenic Setting

In addition to the registration studies described above, a literature review was performed to identify additional prospective, randomized, placebo-controlled studies using Leukine in the setting of neutropenia. This review lead to the identification of an additional prospective, Phase 3, randomized, placebo-controlled study to assess the ability of Leukine to accelerate neutrophil recovery following chemotherapy in patients with breast cancer receiving FAC (fluorouracil, doxorubicin, and cyclophosphamide) chemotherapy (Jones 1996). The median number of days with an ANC < 500/mm^3 was 2.8 days in the Leukine group compared to 6.8 days in the placebo group ($p<0.001$). The number of hospitalizations for febrile neutropenia (FN) was 6 in the Leukine arm versus 8 in the placebo arm, which did not reach statistical significance. This may be due to the relatively low myelosuppressive intensity of the FAC regimen or the use of ciprofloxacin in all patients when the ANC fell below 1,000/mm^3. This study provides data on the ability of Leukine to shorten the duration of neutropenia following myelosuppressive chemotherapy in the absence of stem cell rescue.

6.4.2 Double Hemibody Irradiation

Given that exposure to non-therapeutic radiation is extremely rare, it is not ethically feasible to conduct prospective studies of Leukine in humans for the management of neutropenia following exposure to ionizing radiation. However, published data on the use of non-Leukine rhu GM-CSF for the management of radiation-induced neutropenia is available from a study of sequential double hemibody therapeutic irradiation in patients with multiple myeloma. This report represents a population of patients with myelosuppression independent of chemotherapy or stem cell rescue and provides data regarding the neutrophil response to non-Leukine rhu GM-CSF treatment.

Patients with multiple myeloma who were not eligible for BMT were instead treated with double hemibody irradiation (DHBI) and given non-Leukine *E. coli*-derived rhu GM-CSF (Troussard 1995). Ten patients received either 8 Gy of radiation to the upper half of the body or 6.5 Gy to the lower half of the body followed by radiation treatment of the other half of the body, allowing for hematopoietic recovery between each radiation treatment. Radiation treatments were delivered over an average of 15 minutes. Patients were treated with 5 µg/kg/day (~ 200 µg/m^2/day) of rhu GM-CSF for 14 days starting one day after each course of hemibody irradiation.

These patients were compared to a historical cohort of 32 patients with stage III multiple myeloma who received the same treatment at the same institution, but without rhu GM-CSF

support. During the first hemi-body treatment, the non-Leukine rhu GM-CSF treated patients in this study experienced less granulocytopenia compared to the historical control (**Figure 6**) with median granulocyte counts of 7.7 x 10^9/L vs. 1.0 x 10^9/L at day 15. Granulocyte recovery was also improved following the second hemibody radiation treatment with median granulocyte counts of 5.0 x 10^9/L in the GM-CSF group vs. 0.4 x 10^9/L in the historical controls 15 days after the second hemibody irradiation. Tabulation of the granulocyte data can be found in Appendix 7. In support of a clinical benefit of this prevention of depressed granulocyte counts, severe infections were recorded in none of the non-Leukine rhu GM-CSF group and 11/32 (34%) in the historical cohort.

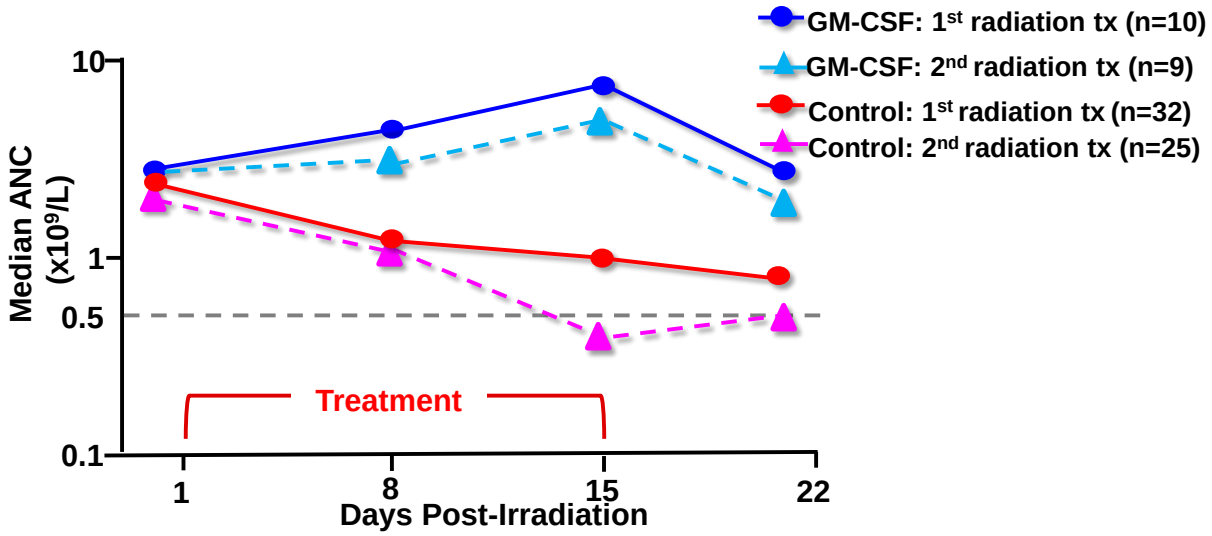


Figure 6: ANC of rhu GM-CSF treated patients compared to historical controls. Graph shows the ANC results following the 1st and 2nd hemibody irradiation treatments.

In conclusion, following hemibody irradiation in patients with multiple myeloma, treatment with non-Leukine rhu GM-CSF prevented the development of severe granulocytopenia. These data support the ability of non-Leukine rhu GM-CSF to impact myelopoiesis in patients with irradiated bone marrow. Furthermore, the hemibody irradiation model more closely mimics the likely disproportionate exposure an individual would receive in a radiation event due to partial shielding behind objects and body position relative to the explosion. While historical control studies must be interpreted carefully, these hemibody irradiation results indicate non-Leukine rhu GM-CSF can accelerate neutrophil recovery following radiation exposure in the absence of chemotherapy and stem cell rescue.

6.5 Efficacy Conclusion

Leukine has been widely studied and has been approved in three distinct settings of neutropenia. In these three settings, Leukine significantly shortens the time to neutrophil recovery (defined as ANC >500/mm^3) compared to placebo. Further, these studies all contain aspects of myelosuppression that are relevant to the myelosuppression observed after exposure to acute ionizing radiation. In the setting of both autologous and allogeneic BMT, patients receiving myelosuppressive chemotherapy experienced accelerated neutrophil recovery with Leukine

treatment regardless of whether TBI was included as part of the preparative regimen. While these patients did receive bone marrow cells that had not been exposed to radiation, these data support that the exposure of the bone marrow stoma to irradiation does not limit Leukine's ability to stimulate healthy bone marrow cells in this exposed bone marrow environment. In contrast to the BMT setting, the activity of Leukine in patients with AML indicates Leukine can accelerate neutrophil recovery in the absence of an infusion of healthy bone marrow cells. Therefore, Leukine accelerates neutrophil recovery in patients with neutropenia in a variety of disease states both with and without BMT rescue. In addition to accelerating neutrophil recovery, patients in all the pivotal registration studies demonstrated a statistically significant difference in infectious morbidity in favor of Leukine. Overall, these studies all contain aspects of myelosuppression that are relevant to the myelosuppression observed after exposure to acute ionizing radiation and indicate the ability of Leukine to accelerate neutrophil recovery across these distinct clinical settings.

6.6 Safety and Tolerability of Leukine

Leukine's safety profile has been characterized in company and investigator sponsored clinical trials and in post-marketing experience. Risks associated with the product use are described in the U.S. Prescribing Information (USPI, see Appendix 1). A summary of safety outcomes from the studies supporting the licensed indications and for the populations of interest (defined here as healthy volunteers, geriatric patients and pediatric patients) are described below and in Appendix 2.

All registration studies of Leukine were conducted at a dose of 250 µg/m^2/day. A number of studies administered Leukine at 6 µg/kg/day. For ease of evaluating the safety information, it should be noted that for an average 70 kg adult, 250 µg/m^2 would be approximately 6.3 µg/kg. Conversely, 6.0 µg/kg would be approximately 240 µg/m^2.

6.6.1 Adverse events reported in licensing studies

The adverse event profile of Leukine was consistent across the studied populations. The comparative safety experience with Leukine versus placebo including serious adverse events is provided in Table 7.

The frequency and type of adverse events, including serious adverse events, reported for patients treated with Leukine were similar to placebo across the studied populations (see USPI "Clinical Experience" section, Appendix 1). In addition, no difference was observed between Leukine and placebo-treated patients for laboratory abnormalities, including renal and hepatic parameters.

Table 7: Percent of patients with at least one adverse event in the trials for approved indications

Indication	Overall AEs (%)		Serious Adverse events (%)	
	Leukine	Placebo	Leukine	Placebo
Chemotherapy in Acute Myelogenous Leukemia (Leukine n = 52 Placebo n = 47)	100	100	32.7	42.5

Indication	Overall AEs (%)		Serious Adverse events (%)	
	Leukine	Placebo	Leukine	Placebo
Transplantation of Autologous Peripheral Blood Progenitor Cells (Leukine n = 196 historical controls n = 100)	54.1	Not reported[#]	1.5	Not reported[#]
Myeloid Reconstitution After Autologous Bone Marrow Transplantation (Leukine n = 79 Placebo n = 77)	100	100	78	86.2
Myeloid Reconstitution After Allogeneic Bone Marrow Transplantation (Leukine n = 53 Placebo n = 56)	100	100	11.3	14.3
Bone Marrow Transplantation Failure or Engraftment Delay (Leukine n =104 historical controls n= 103)	95.2	81.6[#]	45.2*	66.0*[#]
*Reported as severe and not serious. #historical control Source: Biologic License Application				

6.6.2 Adverse Events ≥ Grade 3 by Indication

Adverse events were also examined for their severity in accordance to Common Terminology Criteria for Adverse Events (CTCAE). They were graded from 1, which is considered mild, to 5 resulting in fatal outcome. In the following sections and referenced tables, the underlying comorbidities as well as the cytotoxic regimens involved in these populations under study should be noted when interpreting the reporting rates for these AEs.

Subjects enrolled in the pivotal studies for autologous and allogeneic bone marrow transplantation populations (studies number 301, 302, 303, and 9002) required peripheral stem cell, or autologous or allogeneic bone marrow cell support post ablative chemotherapy. Grade 3/4 adverse events reported in ≥ 5% of subjects randomized to Leukine are provided in Table 8. Diarrhea was the only event reported with >4% frequency in the Leukine treatment arm compared to placebo.

Table 8: Percent of patients with at least one Grade3/4 adverse events in autologous and allogeneic bone marrow transplantation indications by (≥5% frequency)

	Allogeneic BMT (Study 9002)		Autologous BMT (Study 301, 302, 303)	
	Leukine %	Placebo %	Leukine %	Placebo %
Alopecia	7.5	8.9	46.7	48.3
Anorexia	37.7	35.7	25.6	27.0
Diarrhea	18.9	7.1	31.1	33.7
Stomatitis	18.9	14.3	23.3	22.5
Nausea	26.4	28.6	12.2	12.4
Fever	9.4	7.1	17.8	16.9
Hemorrhage	1.9	NR*	15.6	18.0
Thrombocytopenia	18.9	30.4	NR*	NR*
Leukopenia	17.0	28.6	NR*	NR*

	Allogeneic BMT (Study 9002)		Autologous BMT (Study 301, 302, 303)	
	Leukine %	Placebo %	Leukine %	Placebo %
Respiratory Disease	1.9	5.4	8.9	11.2
Asthenia/Malaise	1.9	5.4	8.9	7.9
GI Disorder	1.9	NR*	8.9	11.2
Sepsis	3.8	5.4	7.8	7.9
Hypertension	15.1	23.2	NR*	1.1

*NR: Not reported

Subjects enrolled in the acute myelogenous leukemia (study 305) received induction and consolidation chemotherapy which resulted in severe bone marrow hypoplasia, but did not receive autologous or allogeneic bone marrow cell support in contrast to the populations above. There were no differences that were over 5% or more in grade 3/4 adverse events in the Leukine group vs placebo group (see Table 9).

Table 9: Percent of patients with at least one Grade 3/4 adverse events in acute myelogenous leukemia by event term (≥5% frequency)

AML (Study 305)	Leukine (n = 52)	Placebo(n = 47)
Event	%	%
Leukopenia	98.1	97.9
Thrombocytopenia	98.1	97.9
Anemia	50.0	48.9
Infection	23.1	40.4
Pulmonary	15.4	27.7
Liver	11.5	42.6
Metabolic	11.5	19.1
Neuroclinical	9.6	31.9
Skin	9.6	17.0
Cardiac	7.7	19.1
Fever (no infection)	5.8	4.3
Nausea	5.8	2.1
Neuro-psych	1.9	8.5
GU	1.9	8.5
Hemorrhage	0.0	8.5
Stomatitis	1.9	6.4
Diarrhea	0.0	6.4
Source: BLA		

Subjects enrolled in the pivotal study for bone marrow transplantation failure or engraftment delay (Study 501) involved three subgroups: autologous BMT, allogenic BMT and PSCT groups. The majority of the patients reported at least 1 severe AE. Severe events occurring by body system at 5% above historical control and/or twice the rate of historical control were as follows: body as a whole (including asthenia, chills, fever, infection, malaise, back pain) 31.7% vs 20.4%, metabolic and nutritional (including event term peripheral edema) 5.8 % vs 1.9%,

musculo-skeletal (including the event term bone pain) 4.8% vs 0.0%, skin and appendages (including the event term rash) 6.7% vs. 0.0% (Table 10).

Table 10: Percent of patients with at least one Grade 3/4 adverse events engraftment failure or delay following autologous or allogenic BMT by system

	Leukine (n = 104)	**Historical Control (n = 103)**
Events by System	%	%
Body as a whole*	31.7	20.4
Cardiovascular	7.7	14.6
Digestive	15.4	11.7
Endocrine	0.0	0.0
Hemic & Lymphatic	0.0	5.8
Metabolic and Nutritional	5.8	1.9
Musculoskeletal	4.8	0.0
Nervous	4.8	19.4
Respiratory	17.3	34.0
Skin & Appendages	6.7	0.0
Special Senses	0.0	1.0
Urogenital	1.9	23.3
*includes asthenia, chills, fever, infection, malaise, back pain		

A retrospective study supported approval of the mobilization and engraftment of peripheral blood progenitor cells (PBPC) indication. Adverse events were not classified by severity in the data collection set and, as such, AE ≥ Grade 3 are not presented in the appendices. Also, historical control comparison of AE data is not possible because adverse events were not systematically collected in the historical control (non-mobilized) patients.

6.6.3 Serious adverse events

The overall frequency of serious adverse events (SAE) was similar between Leukine treatment groups and placebo and historical controls. When examined in individual studies, the type of events was similar between the treatment groups. Fatal outcomes were also examined and were similar to placebo or historical controls.

Acute Myeloid Leukemia
In study 305 (Leukine N=52, Placebo N=47): In study 305 (Leukine N=52, Placebo N=47): SAEs were reported in 37 patients (17 in Leukine and 20 in placebo). Infection and respiratory distress were more common in the placebo group. Other reported SAEs were similar in distribution or occurred infrequently. Serious adverse events reported more commonly in Leukine group (≥ 2 events in excess of placebo group) were renal function abnormality (2 vs 0), progressive leukemia (2 vs 0) and hemorrhage (2 vs 0). There were four deaths in the Leukine (unrelated to study drug) versus 13 for the placebo group (Appendix 3).

Myeloid Reconstitution after Autologous Bone Marrow Transplantation
In Study 301 (Leukine N=16, Placebo N=14): SAE were reported in 12 patients (5 Leukine versus 7 placebo). Hepatic and renal disorders were reported in both treatment groups. Lung

disorders were more frequent in the Placebo group. There were no fatal events during the study (Appendix 3).

In Study 302 (Leukine N=30, Placebo N=32): SAE were reported for all subjects (with the exception of one subject in the placebo group). The most frequently reported events in both groups were alopecia, diarrhea, nausea, vomiting, mucous membrane disorder and fever. No fatal events were reported during the treatment period in the Leukine group (Appendix 3).

In Study 303 (Leukine N=13, Placebo N=12): SAE were reported with similar frequency in both treatment and placebo groups (84.6% for Leukine and 100% for placebo). Most frequent in both treatment groups were diarrhea, anorexia, alopecia and nausea. There were no fatal events during the treatment period of this study (Appendix 3).

Myeloid Reconstitution after Allogeneic Bone Marrow Transplantation
In Study 9002 (Leukine N=53, Placebo N=56): fourteen SAE were reported (6 Leukine group vs. 8 placebo group). There were two deaths reported in the Leukine group (unrelated by the investigator) vs. four deaths in the placebo group (Appendix 3).

Bone Marrow Transplantation Failure or Engraftment Delay
In Study 501 (Leukine N=104, Historical Controls N=103): 45 % vs. 66% of the subjects in the study reported a SAE. Frequently reported SAE (> 10% in either Leukine or **historical control** groups) were respectively; Body as whole (includes asthenia, chills, fever, infection, malaise, back pain) 31.7% vs. 20.4%, Respiratory symptoms 17.3% vs. 34.0% , digestive symptoms (15.4% vs. 20.4%) and Cardiovascular 7.7% vs. 14.6% (Appendix 3).

6.6.4 Leukine Safety Experience in Population of Interest

6.6.4.1 Healthy Volunteer

A total of 215 healthy volunteers received Leukine (Table 11). There were no reported SAE and no Grade 4 AE. Most AE were mild to moderate (Grade 1 or 2) and six patients discontinued due to adverse events (see Table 11).

Table 11: Adverse events leading to study discontinuation in healthy volunteers

Study	Dosing	Adverse event(s) leading to discontinuation	Treatment and Outcomes
Study 001.0004 (Grade 3)	250 µg/m^2/day	severe back pain and chest pain, hypotension, and shortness of breath	Intravenous normal saline and norepinephrine. Resolved
Study 001.0019 (Grade 3)	250 µg/m^2	Nausea and vomiting	Acetaminophen and prochlorperazine. Resolved
Study 308626 (grade not reported)	6 µg/kg (single dose)	Allergic reaction	Resolved without treatment
Study 308626 (Grade 2 and 1)	2 µg/kg	Grade 2 proteinuria and grade 1 hematuria	Resolved without treatment

Study	Dosing	Adverse event(s) leading to discontinuation	Treatment and Outcomes
Study 309901 -(Grade 2)	6 µg/kg	Non cardiac chest pain	Resolved without treatment
Study 309901 -(Grade 1)	6 µg/kg	Peripheral hematoma	Resolved without treatment

The type of adverse event(s) ≥ Grade 3 observed in healthy volunteers is consistent with those outlined and communicated in the USPI (Appendix 1). Treatment emergent adverse events were managed with symptomatic treatment.

Doses up to 100 µg/kg/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. AE reported were dyspnea, malaise, nausea, fever, rash, sinus tachycardia, headache and chills. All these events were reversible after discontinuation of Leukine. The maximum amount of Leukine that can be safely administered in single or multiple doses has not been determined. (USPI "Overdosage" Appendix 1).

6.6.4.2 Pediatric population

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. Pediatric experiences include subjects between the ages of 4 months and 18 years treated with Leukine in clinical trials at doses ranging from 60-1,000 µg/m2/day intravenously and 4-1,500 µg/m^2/day subcutaneously. The type and frequency of adverse events were comparable to those reported for the adult population (see USPI "Precautions" section, Appendix 1). These results are supported by a more recently completed pharmacokinetic study in Crohn's patients. Patients were assigned to 4 µg/kg/day or 6 µg/kg/day once daily for 8 weeks. (See USPI "Warnings" for premature infants).

Table 12: Incidence of AE and SAE in pediatric patients with Crohn's Disease

Protocol	Age in years	Number of subjects	Patients with at least one AE (%)	Patients with SAE (%)	Grade 3 AEs (N)	Grade 4 AEs (N)
308001	8-16	22	21(95%)	5 (23%)	3	0

Source: CSR

While the majority of the patients in this study reported an adverse event, only three were of severity Grade 3 and none were Grade 4 (Table 12). Five patients experienced serious events. The symptoms were back pain, nauseas, vomiting, headache, pyrexia, WBC increase, and abdominal pain. These events were similar to the experience the adult populations.

6.6.4.3 Geriatric population

In the registration studies, experience in older patients (age 65-70 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. In the placebo group, number of placebo patients in each age group were 13 and 33 patients respectively (data for one patient in the placebo group was not collected). This was not an adequate database for a complete safety assessment. Analyses of general trends in safety demonstrate similar patterns for older (65-70 yrs.) versus younger patients (55-64 yrs.). Greater sensitivity of some older individuals cannot be ruled out.

6.6.4.4 Safety Summary

A large body of safety data exists with Leukine, in both approved and investigational therapeutic settings, representing over 21 years of post-marketing experience. 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. This number was derived from an average of 10,870 µg of Leukine per patient for an average course of therapy, which is 500 µg/person/day for 20 days. Leukine is currently approved by the FDA for use in five indications at a dose of 250 µg/m^2 per day (see Sections 5.2 and 5.3, and the USPI, Appendix 1). 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).

7 NON-CLINICAL STUDIES

7.1 Overview

The non-clinical studies producing data regarding the use of Leukine and non-Leukine rhu GM-CSF in the mitigation of radiation induced neutropenia include:

1) Published literature in NHP (Rhesus monkeys) provide data on rhu GM-CSF's (Leukine and other rhu GM-CSF) efficacy (i.e., accelerated neutrophil recovery) following radiation exposure.
2) Unpublished mortality data with rhu GM-CSF in irradiated NHPs (Rhesus monkeys).
3) Head to head comparison of the ability of rhu GM-CSF and rhu G-CSF to accelerate neutrophil recovery in two studies with irradiated NHPs (Rhesus monkeys) provide data on similar neutrophil recovery kinetics for both LGF.
4) Toxicology studies in NHP (Cynomolgus monkeys) and rabbits with Leukine that were conducted to support the licensed indications (see Appendix 5 for toxicology study summary).

The published literature in irradiated NHPs (Rhesus monkeys) provides data for the accelerated recovery of neutrophils or granulocytes upon treatment with Leukine or other rhu GM-CSF (Farese 1993, MacVittie 1991, Neelis 1997, Monroy 1988, Monroy 1987). Although these studies have certain limitations (e.g., not conducted under GLP conditions and relatively small group sizes), they suggest biological activity of rhu GM-CSF-mediated acceleration of neutrophil recovery after radiation. Of particular importance is the efficacy studies conducted with NHPs (Rhesus monkeys) exposed to TBI (Farese 1993/MacVittie 1991, Neelis 1997). In these studies, daily administrations of 25 µg/kg (300 µg/m^2) of Leukine either by single daily or divided dose (twice per day) via subcutaneous injections (SC) reduced the number of days to neutrophil recovery compared to placebo. In two unpublished studies by Dr. Thomas MacVittie, treatment of NHPs (Rhesus monkeys) after TBI, using two different radiation sources, with 25 µg/kg (300 µg/m^2) rhu GM-CSF by daily SC injection suggests an increase in NHPs (Rhesus monkeys) survival compared to placebo control. Finally, two studies (Neelis 1997 and MacVittie unpublished) show that treatment with rhu GM-CSF or rhu G-CSF to irradiated monkeys stimulated neutrophil recovery in a similar time course.

A summary of 5 published studies performed in irradiated NHPs (Rhesus monkeys) is provided in Table 13 and the studies are more extensively described below. Studies with non-Leukine rhu GM-CSF and rhu G-CSF are included as they share similar pharmacology with Leukine and provide additional data for cytokine-mediated acceleration of neutrophil recovery after irradiation.

Table 13: Summary of Animal Studies in Irradiated NHP (Rhesus monkeys) Models

Study	Species and # of animals	Treatment		ANC Recovery	Mortality
		TBI	Rhu GM-CSF		
Farese 1993, MacVittie 1991	Control: 5 Leukine: 4	4.5 Gy	Leukine initiated 24 h after irradiation	• Leukine significantly shortened duration of neutropenia by approximately 5 days (p=0.009)	Survival was not reported
Neelis 1997	Control: 4 Leukine: 4 rhu G-CSF: 3	5 Gy	Leukine initiated 24 h after irradiation	• Leukine significantly shortened duration of neutropenia by approximately 5 days (p<0.05) • Leukine and G-CSF stimulated neutrophil recovery with similar kinetics	Survival was not reported
Monroy 1988	Control unshielded: 7 Control shielded: 5 Non-Leukine rhu GM-CSF treated, shielded: 5	8 Gy	Non-Leukine rhu GM-CSF treatment started on Day 3 or 4 after radiation	• Granulocyte recovery accelerated by 4 days with GM-CSF treatment	Survival was not reported
Monroy 1987	Saline: 2 Non-Leukine rhu GM-CSF: 5	9 Gy	Non-Leukine rhu GM-CSF immediately after bone marrow transplantation	• Shortened time to neutrophil recovery by 4 days with GM-CSF treatment	Survival was not reported
Unpublished data (MacVittie)	Control: 14 Non-Leukine rhu GM-CSF: 5 rhu G-CSF: 5	7 Gy	Non-Leukine rhu GM-CSF or rhu G-CSF initiated 24 h after irradiation	• Non-Leukine rhu GM-CSF shortened duration of neutropenia by approximately 3-4 days • Non-Leukine rhu GM-CSF and G-CSF stimulated neutrophil recovery with similar kinetics	14% of animals (2/14) in control group died, whereas all animals treated with GM-CSF survived
Unpublished data (MacVittie)	Control: 10 Non-Leukine rhu GM-CSF: 5	4.5 Gy	Non-Leukine rhu GM-CSF or rhu G-CSF initiated 24 h after irradiation	• Non-Leukine rhu GM-CSF shortened duration of neutropenia by approximately 3-4 days	10% of animals (1/10) in control group died, whereas all animals treated with GM-CSF survived

7.2 Literature Assessing Efficacy of Leukine and non-Leukine rhu GM-CSF

7.2.1 Leukine: Studies Providing Data for Neutrophil Recovery in NHPs (Rhesus monkeys)

Leukine's activity in stimulating neutrophil recovery after radiation exposure comes from the published literature describing irradiation experiments in NHPs (Rhesus monkeys). Two studies evaluating the effects of Leukine on hematopoietic parameters after TBI have been published and are summarized below [Farese 1993, MacVittie 1991, Neelis 1997 (the Farese and MacVittie publications include data from the same study)]. These TBI studies were conducted with radiation exposure protocols leading to significant myelosuppression as noted by the degree and duration of neutropenia and time to recovery. In both studies, Leukine provided data of accelerated neutrophil recovery.

The effects of Leukine were evaluated in NHPs (Rhesus monkeys) receiving 4.5 Gy TBI. One day after irradiation, four NHPs received 25 µg/kg/day (300 µg/m^2) of Leukine divided into two daily SC injections for 21 consecutive days (Farese 1993, MacVittie 1991). Control NHPs (n=5) received human serum albumin (HSA) during the same time period.

As a single agent, Leukine was associated with shorter periods of neutropenia in the irradiated animals compared to the human serum albumin-treated animals (p=0.009) (**Figure 7**). Functional cellular assays showed that neutrophil hydrogen peroxide production was enhanced following the administration of Leukine as compared to pre-irradiated neutrophils (p<0.05). Survival was not evaluated in this study.

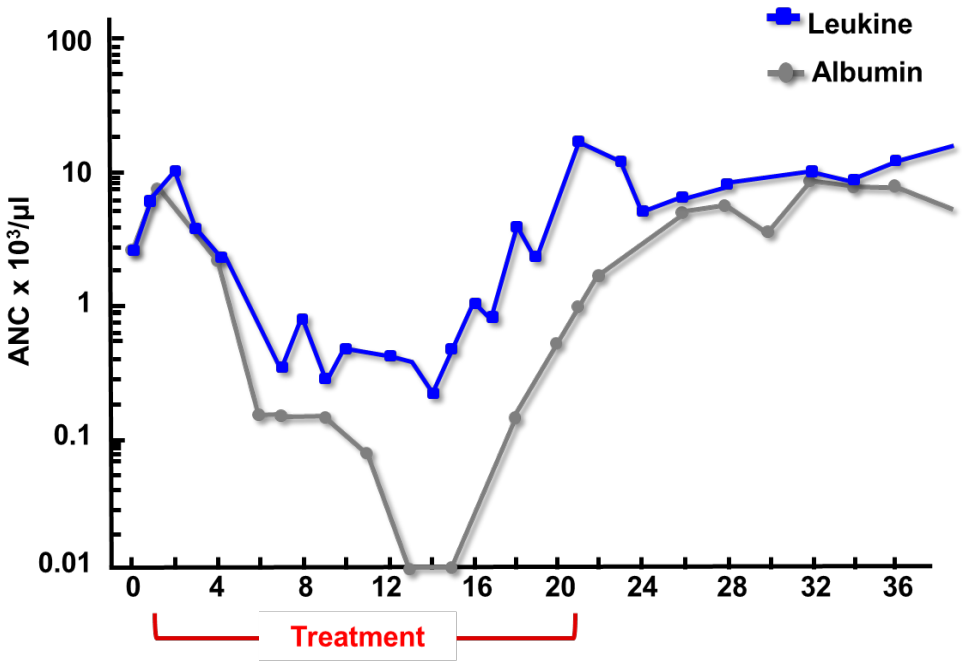


Figure 7: Neutrophil recovery in irradiated NHPs (Rhesus monkeys). Rhesus monkeys receiving 4.5 Gy TBI were treated with Leukine (n=4) or alumin control (n=5). ANC in days following radiation exposure is shown. ANC is absolute neutrophil count (mean), treatment is in days (adapted from Figure 1B in Farese 1993).

The effects of Leukine treatment were evaluated in NHPs (Rhesus monkeys) receiving 5 Gy TBI (Neelis 1997). One day after irradiation, NHPs (4 animals/group) received 25 µg/kg/day (300 $\mu g/m^2/d$) of Leukine given as a single daily injection for 14 consecutive days (n=4). Control NHPs (n=4 plus n=8 historical controls) received placebo during the same time period.

Leukine significantly reduced the mean number of days to neutrophil recovery (to > 0.5 X 10^9/L) from 22.5 ± 2.4 days for control to 17.7 ± 2.2 days for Leukine ($p<0.05$). Neutrophil recovery over time (days) is displayed in Figure 8. Survival was not reported. This study, combined with the previous study, provides data on the ability of Leukine to accelerate neutrophil recovery in NHP (Rhesus monkey) models of radiation induced neutropenia. These neutrophil recovery results are consistent with the results in NHPs (Rhesus monkeys) reported for G-CSF (Neelis 1997 and MacVittie unpublished).

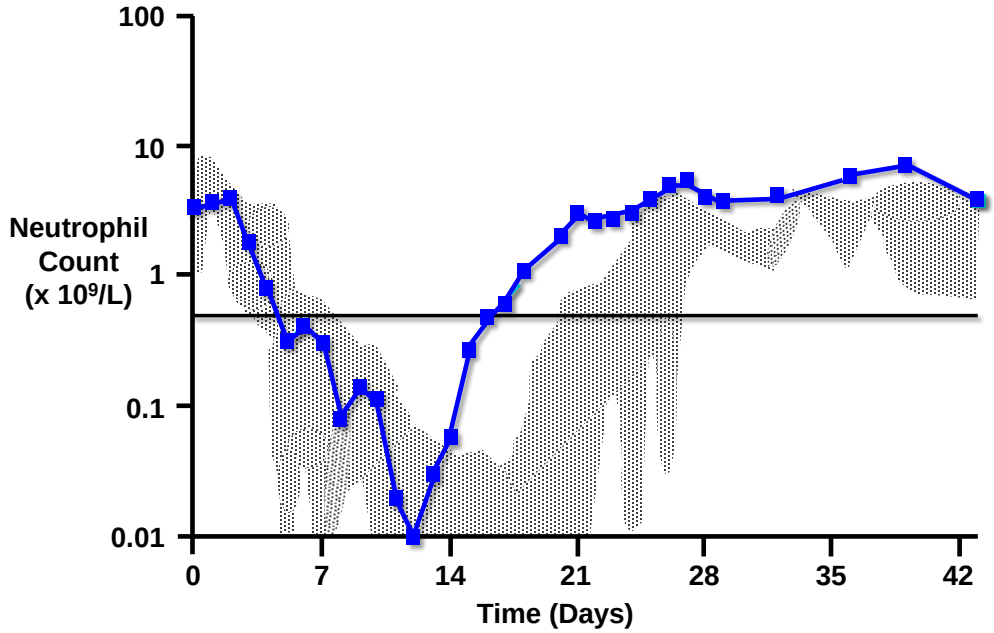


Figure 8: Neutrophil recovery after Leukine after irradiation. Neutrophil counts (log scale) after 5 Gy TBI (Day 0) for NHPs (Rhesus monkeys) treated with Leukine in dark blue (n=4) Gray shade is placebo control (n=12). Data represent the arithmetic mean ± SD. The horizontal line defines the level of 0.5 x 10^9/L (adapted from Neelis, 1997).

7.2.2 Non-Leukine rhu GM-CSF: Studies Evaluating Neutrophil Recovery in NHPs (Rhesus Monkeys)

In addition to the studies using Leukine, two studies with non-Leukine rhu GM-CSF have provided data of a similar acceleration of neutrophil recovery in NHPs (Rhesus monkeys) following non-uniform (i.e., partially shielded) radiation exposure or TBI and subsequent bone marrow transplant (Monroy 1988, 1987).

Granulocyte counts were evaluated following administration of non-Leukine rhu GM-CSF to shielded male NHPs (Rhesus monkeys) who had a lethal, non-uniform radiation dose of 8 Gy (Monroy 1988). Lead shielding of the tibia was utilized to mimic partial shielding that is likely to

occur in exposure to non-therapeutic radiation. Non-Leukine rhu GM-CSF was administered intravenously at a dose of 50,000 U/kg (8 µg/kg, 96 µg/m^2) given on Day 3 or 4 after radiation, followed by 72,000 U/kg (12 µg/kg, 144 µg/m^2) via continuous SC infusion for seven consecutive days. The non-Leukine rhu GM-CSF-treated, shielded group (n=5) was compared to unshielded (n=7) and shielded (n=5) groups not receiving rhu GM-CSF.

Granulocyte recovery was accelerated by 4 days when the shielded NHPs were treated with non-Leukine rhu GM-CSF, compared to the shielded/untreated group (Figure 9). In addition, a two-fold increase in peripheral granulocyte counts, compared to pre-irradiation granulocyte levels, was observed in non-Leukine rhu GM-CSF treated animals at Day 30. In contrast, shielded/untreated NHPs had peripheral granulocyte counts that were 25 to 30% of pre-irradiation levels at Day 30. Survival was not reported.

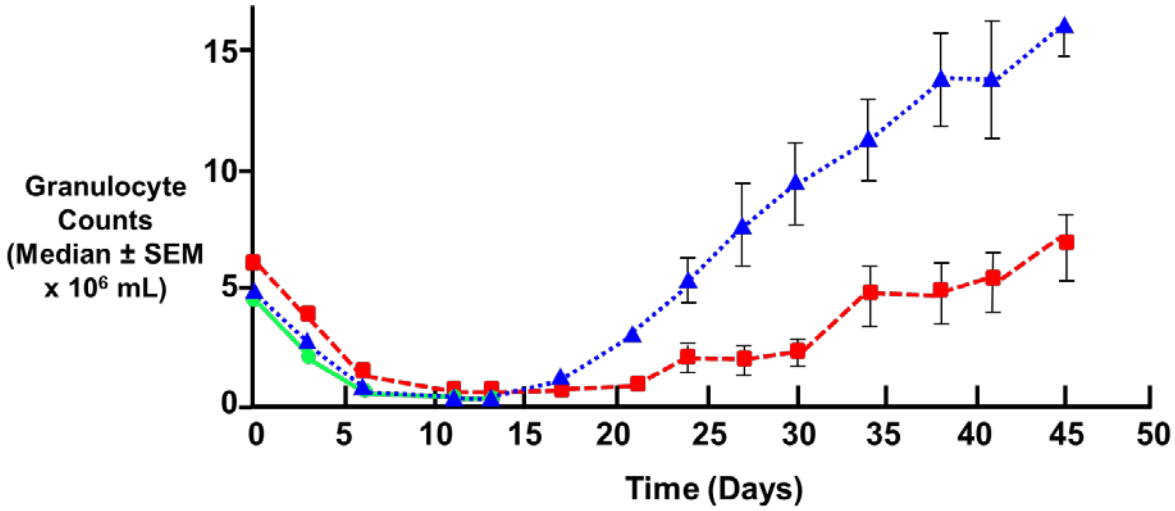


Figure 9: Granulocyte recovery in irradiated NHPs (Rhesus monkeys). The effect of shielding and rhu GM-CSF treatment on the recovery of peripheral blood granulocytes. Each point is a median ± SEM for the groups: no shielding (●, green), shielded (■, red) and shielded plus rhu GM-CSF treatment (▲, blue). (adapted from Monroy 1988).

In another study, neutrophil counts were evaluated following administration of non-Leukine rhu GM-CSF to male NHPs (Rhesus monkeys) receiving a TBI dose of 9 Gy followed by bone marrow transplant (Monroy 1987). Saline (n=2) or non-Leukine rhu GM-CSF (loading dose of 50,000 U/kg followed by 50,400 units/kg/day; n=5), was administered by continuous subcutaneous infusion for seven days via mini-pump implanted between Days 0 and 5.

Neutrophil recovery is shown in **Figure 10** below. Treatment with non-Leukine rhu GM-CSF led to a shortened time to ANC > 1,000 /mm^3 compared to saline control animals (17 days vs. 20 days). By day 20, neutrophil levels were 80% and 33% of the normal pre-irradiation levels in NHPs treated with non-Leukine rhu GM-CSF and saline, respectively. In addition, non-Leukine rhu GM-CSF enhanced the neutrophil response to levels greater than baseline values by day 31, whereas control neutrophil levels were 70% of the pre-irradiated levels. Upon termination of non-Leukine rhu GM-CSF therapy, the enhanced neutrophil level was sustained. Survival was not reported.

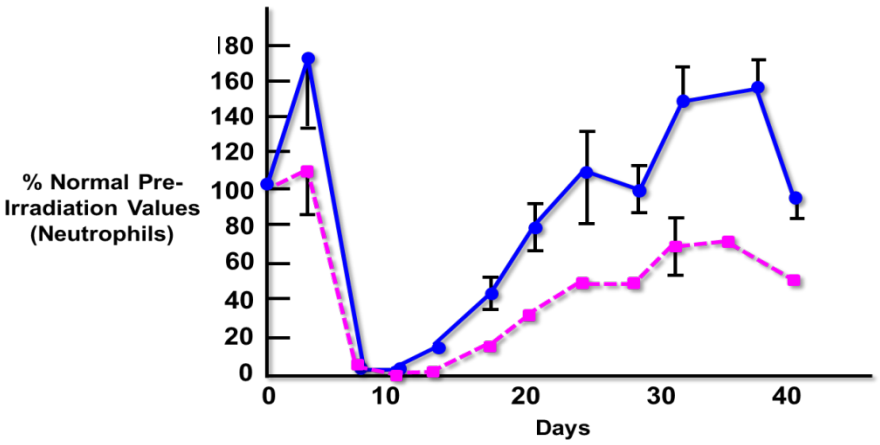


Figure 10: Recovery of neutrophil production in animals treated with non-Leukine rhu GM-CSF following bone marrow transplantation. Each point is a mean ± SE for the groups: rhu GM-CSF treated (●, blue) and saline treated (■, pink) (adapted from Monroy 1997).

7.2.3 Studies in NHPs (Rhesus Monkeys) Providing Data for Decreased Mortality

Two unpublished studies performed by Dr. Thomas MacVittie (Professor and Director, Preclinical Radiobiology Laboratory, MCART, University of Maryland School of Medicine) were provided through personal communication. These studies evaluated the neutrophil recovery and survival of NHPs (Rhesus Monkeys) following TBI with and without non-Leukine rhu GM-CSF treatment. NHPs (Rhesus Monkeys) were exposed to TBI by two different radiation sources (see Table 14). The next day, animals were administered either human serum albumin (n=14 for 7 Gy exposure or n=10 for 4.5 Gy exposure) or 25 µg/kg (300 µg/m^2) rhu GM-CSF (n= 5 for 7 Gy exposure and n=5 for 4.5 Gy exposure) SC daily for 23 days. Neutrophil counts were evaluated throughout each study and survival was determined after 60 days.

Consistent with the studies described above, rhu GM-CSF treatment resulted in a diminished duration of neutropenia (ANC < 500/µL) by approximately 4 days and shortened time to neutrophil recovery (ANC > 500/µL) by approximately 4 days. With respect to survival, 10-14% of the control animals died after irradiation, whereas all animals treated with rhu GM-CSF survived (Table 14).

Table 14: Survival and Neutrophil Recovery of Irradiated NHPs (Rhesus Monkeys) Treated with and without Non-Leukine rhu GM-CSF[a]

Radiation (Type and dose)	Treatment (n)	Neutropenia (mean days)	ANC recovery > 500/uL (mean days)	Survival (%)
^{60}Co 7 Gy	Control (14)	14.7	19.8	86
^{60}Co 7 Gy	Non-Leukine rhu GM-CSF (5)	10.8	16.2	100
Fn:γ 4.5 Gy	Control (10)	14.4	20.5	90
Fn:γ 4.5 Gy	Non-Leukine rhu	10.0	17.6	100

	GM-CSF (5)			

[a]Non-Leukine rhu GM-CSF dose = 25 μg/kg, divided dose daily from day 1 to day 23 after TBI

7.3 Conclusions of NHP (Rhesus Monkey) Studies

From the studies presented above, the following conclusions can be made:

- Leukine, at a dose approximating the proposed clinical dose of 250 $\mu g/m^2/d$, accelerates neutrophil recovery in NHPS (Rhesus monkeys) with radiation-induced neutropenia when therapy was initiated one day following TBI.
- Non-Leukine rhu GM-CSF accelerated neutrophil recovery in NHPs (Rhesus monkeys) with radiation-induced neutropenia when therapy was initiated 3-4 days following non-uniform irradiation.
- Non-Leukine rhu GM-CSF was associated with significantly improved neutrophil recovery in lethally irradiated NHPs (Rhesus Monkeys) receiving a bone marrow transplant compared to those receiving only bone marrow transplantation.
- Non-Leukine rhu GM-CSF treatment accelerated neutrophil recovery and resulted in no deaths compared to 1 death at 7 Gy dose and 2 deaths at 4.5 Gy dose in control NHPs (Rhesus monkeys) when therapy was initiated one day following TBI.

8 LEUKOCYTE GROWTH FACTORS: COMPARISONS OF GM-CSF AND G-CSF

Both GM-CSF and G-CSF have been extensively studied in the setting of neutropenia, however studies directly comparing the two are limited. Two non-clinical studies in NHPs (Rhesus monkeys) exposed to TBI compare rhu GM-CSF (one study using Leukine) to filgrastim and are described below.

Several published reports that have evaluated the relative clinical efficacy and safety of Leukine and filgrastim, however these studies were non-randomized, retrospective analyses, or in settings not related to mitigation of neutropenia. A single, prospective, randomized study that compared the efficacy of Leukine to filgrastim in the setting of neutropenia recovery is described below (Beveridge, 1998).

8.1 Non-Clinical Data

8.1.1 Neutrophil Recovery in NHPs (Rhesus monkeys) with GM-CSF and G-CSF after 5 Gy Irradiation

In section *7.2.1* (Neelis 1997), we previously discussed the effect of Leukine on neutrophil recovery. The same study compared the efficacy of Leukine and rhu G-CSF to accelerate neutrophil recovery in NHPs (Rhesus monkeys) receiving 5 Gy TBI (Neelis 1997). One day after irradiation, NHPs received 25 μg/kg/day (300 μg/m^2) of Leukine (n=4) or 10 μg/kg/day rhu G-CSF (n=3) given as a single daily injection for 14 consecutive days. Control NHPs (n=4, plus n= 8 historical controls) received placebo during the same time period.

Leukine and rhu G-CSF therapy stimulated neutrophil recovery with similar kinetics in NHPs (Rhesus monkeys) exposed to TBI (**Figure 11**). Both Leukine and rhu G-CSF shortened the time to neutrophil recovery (Table 15)

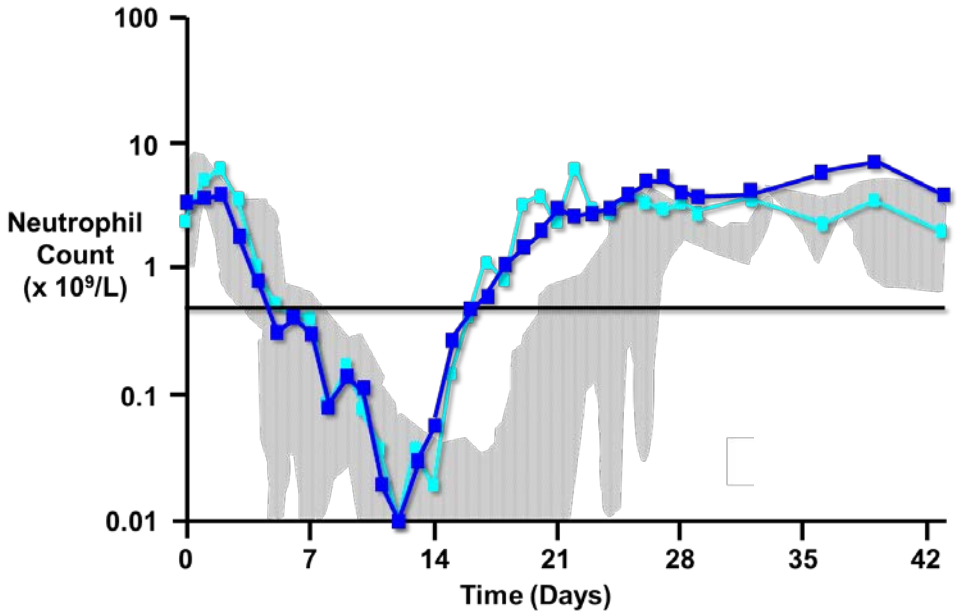


Figure 11: Leukine and G-CSF effect on neutrophil recovery in NHPs (Rhesus monkeys) exposed to TBI. Dark blue line represents GM-CSF; light blue line represents rhu G-CSF. Gray shade is placebo control (n=12). (Adapted from Figure 4 in Neelis 1997).

Table 15: Time to Neutrophil Recovery in Irradiated NHPs (Rhesus monkeys)

Treatment	**Days to Neutrophil recovery > 500/mm^3**
Control	22.5 ± 2.4
GM-CSF (Leukine)	17.7 ± 2.2*
G-CSF	19.7 ± 3.2

*statistically significantly different from placebo treated monkeys (P<0.05)

8.1.2 Neutrophil Recovery in NHPs (Rhesus Monkeys) with GM-CSF and G-CSF Following 7 Gy Irradiation

In an unpublished study by Dr. Thomas MacVittie (Professor and Director, Preclinical Radiobiology Laboratory, MCART, University of Maryland School of Medicine, provided through personal communication), **NHPs (Rhesus monkeys)** were exposed to a TBI dose of 7 Gy (LD14/60). One day after irradiation, NHPs (5 animals/group) received 25 µg/kg/d (300 µg/m^2) of non-Leukine rhu GM-CSF or 10 µg/kg/d of rhu G-CSF as daily subcutaneous injections for 23 consecutive days. Control NHPs (n=14) received human serum albumin during the same time period.

Neutrophil recovery is shown in **Figure 12** below. Treatment with non-Leukine rhu GM-CSF or rhu G-CSF led to a shortened time to ANC > 500 /mm^3 compared to saline control by approximately 3-4 days with a similar neutrophil recovery profile.

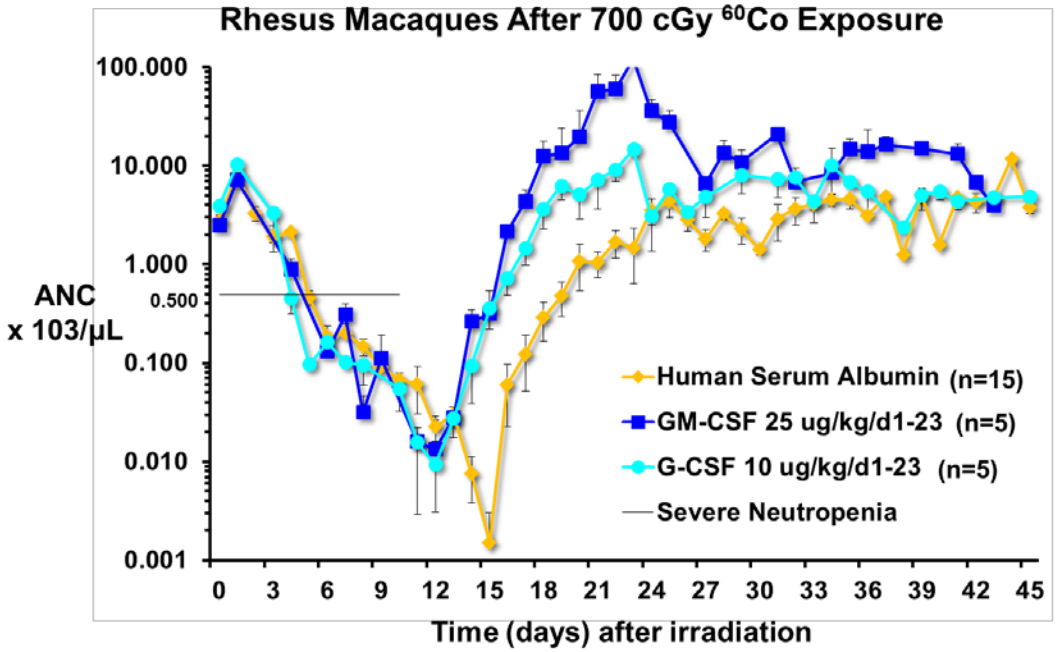


Figure 12: Neutrophil count kinetics with GMCSF and G-CSF treatment accelerates neutrophil recovery in NHPs (Rhesus Macaques) after 7 Gy 60Co exposure. The mean ANC +/- SD is shown for days following irradiation. ANC is absolute neutrophil count; horizontal line denotes ANC of 500/mm^3.

In conclusion, rhu GM-CSF and rhu G-CSF stimulate neutrophil recovery with similar kinetics in irradiated NHPS (Rhesus monkeys) when therapy is initiated one day following TBI.

8.2 Clinical Data

8.2.1 A Randomized, Prospective Study Comparing Leukine and filgrastim for Chemotherapy Induced Neutropenia

The single, prospective, randomized study comparing the efficacy of Leukine and filgrastim was performed by Beveridge et al (Beveridge1998). In this study, patients with malignant lymphomas or other solid tumors receiving myelosuppressive therapy were randomized to receive either Leukine (n=79) or filgrastim (n=102) and monitored for time to ANC recovery. Key results are summarized in Table 16.

There were no statistical significant difference between Leukine and filgrastim in the mean number of days to reach ANC of 500/mm^3 (3.3 days vs 3.6 days; p=0.32, Table 16). A statistically significant difference was reached in the mean number of days to reach ANC >1,000/mm^3 with 5.1 days in the Leukine group vs. 4.5 days with filgrastim (p=0.009). However, with respect to clinical study endpoints, there were no statistical significant difference between the two groups in hospitalizations for fever or antibiotic therapy (6.3% vs 7.8% of patients), mean length of stay (4.8 vs 5.6 days), positive blood cultures (0 vs 2), or mean duration of fever (1.6 versus 3.6 days).

Table 16: Beveridge (1998): Summary of key patient characteristics and efficacy results

	Leukine (N=79)	Neupogen (N=102)	p-value
Patient characteristics			
Median Age (years)	57.2	54.2	
Gender (Male / Female), %	22%/78%	27%/72%	
Primary neoplasm, %			
Solid tumor[a]	45%	53%	
Lymphoma	33%	23%	
Other	22%	23%	
Efficacy			
Mean (± SEM days) to ANC of			
500/µL	3.3 ± 0.16	3.6 ± 0.16	0.32
1000/µL	5.1 ± 0.22	4.5 ± 0.13	0.009
1500/µL	5.7 ± 0.23	4.6 ± 0.14	0.0001
Hospitalization with neutropenic fever or for IV antibiotic, %	6.3%	7.8%	0.46
Length of stay in days (mean ± SEM)	4.8 ± 0.58	5.6 ± 1.1	0.58
Duration of fever in days (mean ± SEM)	1.6 ± 0.6	3.6 ± 0.92	0.14

a: includes breast, lung and ovarian

There was no statistical difference between the two arms in the mean number of days to reach the clinically relevant level of ANC >500/mm^3 and while there was a statistically significant difference in time to ANC > 1,000/mm^3 (with a difference in means less than 1 day), this did not result in statistically significant differences in hospitalization, fever, or infection rates. Regarding safety, the authors concluded that "both growth factors were tolerated" and there were no differences in patients experiencing AEs between the two treatment groups.

The lack of robust data collected in clinical prospective, randomized studies limit the ability to directly compare Leukine and filgrastim. While there was a difference in time to ANC >1,000/mm^3, the NHP (Rhesus monkey) studies and the single clinical study did not detect differences between Leukine and filgrastim in their ability to stimulate neutrophils to an ANC >500/mm^3 or clinical endpoints.

9 GUIDELINES

The management of neutropenia in the oncology setting has been discussed in several guidelines with the American Society of Clinical Oncology (ASCO) being a prominent guideline (Smith 2006). This guideline refers to both LGF in the management of neutropenia following myelosuppressive therapy. With respect to comparison of GM-CSF and G-CSF, the 2005 guidelines state "No guideline recommendation can be made regarding the equivalency of the two colony-stimulating agents. As in 2000, further trials are recommended to study the comparative clinical activity, toxicity, and cost-effectiveness of G-CSF and GM-CSF." The guidelines do note that G-CSF has been shown to result in statistically significant improvements in stem cell mobilization for bone marrow transplantation compared to GM-CSF. However, mobilization of stem cells is distinct from the treatment of neutropenia. In addition to the ASCO guidelines, the 2009 NCCN guidelines also advise the use of a LGF, but do not specifiy GM-CSF or G-CSF.

With respect to the management of radiation-induced neutropenia, multiple guidances have been published. In all cases, LGF are recommended to accelerate neutrophil recovery as a means to prevent or treat neutropenia following exposure to life-threatening doses of ionizing radiation (e.g., $\geq$ 2 Gy). The ASCO guideline recommends the prompt administration of a LGF for patients exposed to radiation (Smith 2006). The Strategic National Stockpile (SNS) Radiation Working Group also recommends the use of cytokines, including sargramostim (Leukine), filgrastim and pegfilgrastim, as options for the treatment of patients exposed to > 2 Gy of radiation (Waselenko 2004). The Department of Health and Human Services (DHHS) in their Radiation Event Medical Management (REMM) recommends these three LGF for those likely to have been exposed to $\geq$ 2 Gy, are likely to develop an ANC < 0.5 X 10^9/L, or are likely to have prolonged periods of significant neutropenia. The Radiation Emergency Assistance Center/Training Site (REAC/TS) has provided the Department of Energy with expertise related to the medical management of radiation accidents and their guidelines recommend the use of any one of the three LGFs for patients with severe neutropenia.

The recommendation of LGF treatment in these guidelines also relates to the use of LGF in accidental radiation exposures. Consistent with these recommendations, three accidental exposures have utilized rhu GM-CSF to treat acute radiation induced neutropenia and a description of these experiences can be found in Appendix 8. The recognition by these guidelines of the efficacy of both GM-CSF and G-CSF in the treatment of neutropenia as well as use of LGF in radiation accidents reflects upon the relevance of the clinical data showing an acceleration of neutrophil recovery in multiple setting of neutropenia.

10 CONCLUSIONS

The majority of clinical experience with Leukine comes from its use to shorten the duration of neutropenia following administration of myelosuppressive chemotherapy with or without TBI. Three of Leukine's five indications are based on the efficacy of Leukine in shortening neutropenia and decreasing infectious morbidity. In the setting of both autologous and allogeneic BMT, patients receiving myelosuppressive chemotherapy experienced accelerated neutrophil recovery with Leukine treatment regardless of whether TBI was included as part of the preparative regimen. While these patients did receive bone marrow cells that had not been exposed to radiation, these data support that the exposure of the bone marrow stoma to radiation does not limit Leukine's ability to stimulate healthy bone marrow cells in this exposed bone marrow environment. In contrast to the BMT setting, the activity of Leukine in patients with AML indicates Leukine can accelerate neutrophil recovery in the absence of an infusion of healthy bone marrow cells. Therefore, Leukine accelerates neutrophil recovery in patients with neutropenia in a variety of disease states both with and without BMT rescue as represented in the three indications.

Regarding the myelosuppression that follows exposure to ionizing radiation, evidence that rhu GM-CSF is able to stimulate neutrophil recovery comes from two sources, non-clinical data with NHPs (Rhesus monkeys) and clinical data from patients with multiple myeloma treated with sequential hemibody radiation therapy without chemotherapy. In the NHP (Rhesus monkey) studies, Leukine and non-Leukine rhu GM-CSF both accelerated neutrophil recovery following myelosuppressive doses of radiation. Furthermore, in the NHP (Rhesus monkey) model of acute radiation-induced neutropenia, all non-Leukine rhu GM-CSFs treated animals survived compared to 2 deaths in the 7 Gy dose and 1 death at the 4.5 Gy dose. With respect to the clinical data, the study in hemibody irradiated patients with multiple myeloma shows rhu GM-CSF administration prevented the development of granulocytopenia and no infections developed when compared to historical controls that developed granulocytopenia and 34% developed severe infections.

In support of a class effect, GM-CSF and G-CSF have both demonstrated a similar propensity to accelerate neutrophil recovery following myelosuppression in both preclinical models and clinical settings.

Neutrophil decline and recovery are the critical parameters regarding the risk of developing infections, regardless of causation of neutropenia. The inability to conduct a prospective, randomized clinical study of Leukine for radiation-induced neutropenia, forces the reliance on existing clinical and non-clinical data. At present, while the role of Leukine as well as G-CSFs is recognized in all relevant guidelines for the treatment of patients exposed to non-therapeutic ionizing radiation, no therapy is FDA-approved for such an indication. The potential efficacy of Leukine in this setting is supported by Leukine's demonstrated clinical efficacy and safety in accelerating neutrophil recovery in the approved indications, the ability of Leukine to accelerate neutrophil recovery in NHP (Rhesus monkey) models of acute radiation injury, and the similarity of these effects between Leukine and G-CSFs. Genzyme remains committed to continuing to work with the FDA and BARDA to define the appropriate development path for this indication based on the advice of this advisory committee.

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12 APPENDICES

APPENDIX 1: LEUKINE PRESCRIBING INFORMATION

Leukine® (sargramostim) United States Prescribing Information (USPI, revision date 07/09).

6060 Revision date (07/09)
US License 1791
Rx only

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 Progenitor 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 ε1500/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 posttransplant 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 nonmobilized 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/mm³	ANC ≥1000/mm³	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] ε 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 ε65 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.

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			

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**.

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 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.

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.*

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

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 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 (2.8×10^6 IU/mL) sargramostim. Lyophilized LEUKINE is available in vials containing 250 mcg (1.4×10^6 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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APPENDIX 2: SUPPLEMENTAL SAFETY INFORMATION

12.1 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 µg/m^2/day intravenously and 4-1,500 µg/m^2/day subcutaneously. In 53 pediatric patients enrolled in controlled studies at a dose of 250 µg/m^2/day by 2-hour IV infusion, the type and frequency of adverse events were comparable to those reported for the adult population (USPI Appendix 1).

Table 17 below outlines SAEs from a phase 1/2 PK/PD and safety study in pediatric Crohn's disease (Study 308001) administered 4 or 6 µg/kg/day SC (liquid EDTA) in 22 subjects. Five subjects experienced a total of 14 SAEs.

Table 17: SAEs from Phase 1/2 PK/PD and Safety Study in Pediatric Crohn's Disease (Study 308001)

Treatment Group	Subject	Preferred Term	CTCAE[a]	Relationship to Study Drug[a]
4 µg SGR/CS$^-$	104005	Full blood count abnormal	Grade 2	Unlikely
6 µg SGR/CS$^-$	105002	Abdominal pain	Grade 3	Possible
		Duodenal ulcer	Grade 2	Possible
	107002	Anemia	Grade 3	Unlikely
4 µg SGR/CS$^+$	101007	Abdominal pain, increased[b,c]	Grade 2	Unlikely
		Abdominal pain[c] (secondary to strep throat)	Grade 2	Unlikely
		Vomiting (secondary to strep throat)	Grade 1	Unlikely
		Pyrexia (secondary to strep throat)	Grade 1	Unlikely
		Pharyngitis streptococcal	Grade 2	Unlikely
		Therapeutic procedure[d]	Grade 1	No
		Vomiting[b,c]	Grade 2	Possible
6 µg SGR/CS$^+$	101008	Abdominal pain[e]	Grade 2	Unlikely
		Nausea[e]	Grade 2	Unlikely
		Pyrexia[e]	Grade 2	Unlikely

Patients received sargramostim with (+) or without (-) corticosteroids (CS)
CS = corticosteroid; CTCAE = Common Toxicity Criteria for Adverse Events; SAE = serious adverse events; SGR = sargramostim
[a] Investigator's assessment.
[b] SAE led to premature discontinuation of the study drug.
[c] These SAEs were not resolved at the time of reporting; all other SAEs were resolved.
[d] Hospitalization for an elective procedure, placement of percutaneous gastric feeding tube.
[e] These SAEs occurred during the 30-day follow-up period; all other SAEs occurred during treatment

Of note, there were no deaths, two subjects experienced grade 3 AE (1 abdominal pain and one anemia) and none reported a grade 4 AE (USPI Appendix 1).

12.2 Geriatric Use

In the clinical trials, experience in older patients (age ≥ 65 years), was limited to the acute myelogenous leukemia (Study 305) study discussed above. 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 was 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 (USPI Appendix 1).

12.3 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-Leukine 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 is 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 (USPI Appendix 1).

12.4 Overdosage

The maximum amount of Leukine that can be safely administered in single or multiple doses has not been determined. Doses up to 100 µg/kg/day (4,000 µg/m^2/day or 16 times the recommended dose) were administered to four patients in a Phase I uncontrolled 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 (USPI Appendix 1).

12.5 Post-Marketing Experience with Leukine

Over 470,000 patients have received Leukine treatment in the post-marketing setting from the time of product launch in March 1991 through December 2012 (estimate based on average course of therapy of 10,870 µg per patient). The most commonly reported adverse events

attributable to Leukine administration are fever, injection site reactions, non-cardiac chest pain, dyspnea, nausea, vomiting, bone pain, and back pain.

Review of periodic adverse drug experience reports (PADERs) from 2002 to 2012 did not identify any required updates to the product label. In summary, the post-marketing adverse events reported to the company have been consistent with the information stated in the U.S. Prescribing Information (Appendix 1).

12.6 Supplemental Safety Tables

Table 18: Percent of AuBMT Patients Reporting Events (USPI Appendix 1)

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			
Malaise	57	51	**Respiratory System**		
Sepsis	11	14	Dyspnea	28	31
			Lung disorder	20	23
Digestive System					
Nausea	90	96	**Hemic and Lymphatic System**		
Diarrhea	89	82	Blood dyscrasia	25	27
Vomiting	85	90			
Anorexia	54	58	**Cardiovascular System**		
GI disorder	37	47	Hemorrhage	23	30
GI hemorrhage	27	33			
Stomatitis	24	29	**Urogenital System**		
Liver Damage	13	14	Urinary tract disorder	14	13
			Kidney function abnormal	8	10
Skin and Appendages					
Alopecia	73	74	**Nervous System**		
Rash	44	38	CNS disorder	11	16

Table 19: Percent of Allogeneic BMT Patients Reporting Events (USPI Appendix 1)

Events by Body System	Leukine (n=53)	Placebo (n=56)	Events by Body System	Leukine (n=53)	Placebo (n=56)
Body, General			**Metabolic, Nutritional Disorder**		
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	Increase 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			
Dyspepsia	17	20	**Hemic and Lymphatic System**		
Hematemesis	13	7	Thrombocytopenia	19	34
Dysphagia	11	7	Leukopenia	17	29
GI hemorrhage	11	5	Petechia	6	11
Constipation	8	11	Agranulocytosis	6	11
Skin and Appendages			**Urogenital System**		
Rash	70	73	Hematuria	9	21
Alopecia	45	45			
Pruritis	23	13	**Nervous System**		
			Paresthesia	11	13
Musculo-skeletal System			Insomnia	11	9
Bone pain	21	5	Anxiety	11	2
Arthralgia	11	4			
			Laboratory Abnormalities*		
Special Senses			High glucose	41	49
Eye hemorrage	11	0	Low albumin	27	36
			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.

Table 20: Percent of AML Patients Reporting Events (USPI Appendix 1)

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			
Weight gain	8	21	**Respiratory System**		
Chills	19	26	Pulmonary	48	64
Allergy	12	15			
Sweats	6	13	**Hemic and Lymphatic System**		
			Coagulation	19	21
Digestive System					
Nausea	58	55	**Cardiovascular System**		
Liver	77	83	Hemorrhage	29	43
Diarrhea	52	53	Hypertension	25	32
Vomiting	46	34	Cardiac	23	32
Stomatitis	42	43	Hypotension	13	26
Anorexia	13	11			
Abdominal distension	4	13	**Urogenital System**		
			GU	50	57
Skin and Appendages					
Skin	77	45	**Nervous System**		
Alopecia	37	51	Neuro-clinical	42	53
			Neuro-motor	25	26
			Neuro-psych	15	26
			Neuro-sensory	6	11

Table 21: Studies of Leukine in Healthy Volunteers

Summary of study design	Grade 3/4 AEs:
001.0004 (1994) Phase 1 randomized, open-label, crossover study Healthy males Liquid sargramostim • Dose: 250 μg/m^2 • Route of administration: Intravenous (2 hr) • Duration of treatment: Single dose Lyophilized sargramostim • Dose: 250 μg/m^2 • Route of administration: Intravenous (2 hr) • Duration of treatment: Single dose Liquid sargramostim • Dose: 250 μg/m^2 • Route of administration: Subcutaneous • Duration of treatment: Single dose Lyophilized sargramostim • Dose: 250 μg/m^2 • Route of administration: Subcutaneous • Duration of treatment: Single dose	• Grade 3: One subject experienced grade 3 AE of back and chest pain, hypotension, and shortness of breath • Grade 4: None
001.0019 (1999) Phase 1 randomized, open-label, crossover study Healthy males Liquid sargramostim • Dose: 250 μg/m^2 • Route of administration: Subcutaneous • Duration of treatment: Single dose Prefilled sargramostim syringes with EDTA • Dose: 250 μg/m^2 • Route of administration: Subcutaneous • Duration of treatment: Single dose	• Grade 3: One subject experienced grade 3 AE of vomiting and nausea

Summary of study design	Grade 3/4 AEs:
308626 (2004) Phase 1 randomized, open-label, crossover study Healthy Japanese males Test product: Liquid sargramostim vial, with EDTA • Dose: 2, 6, and 8 µg /kg • Route of administration: Subcutaneous • Duration of treatment: Single dose Healthy Caucasian males Test product: Liquid sargramostim vial, with EDTA • Dose: 2, 6, and 8 µg /kg • Route of administration: Subcutaneous • Duration of treatment: Single dose	• Grade 3: Three subjects experienced grade 3 AEs with back pain • Grade 4: None
309404 (2005) Part 1: Phase 1 randomized double-blind crossover Healthy males Test product: Liquid vial with EDTA • Doses: 6 µg/kg • Route of administration: Subcutaneous • Duration of treatment: Single dose Reference therapy: Lyophilized • Dose: 6 µg/kg • Route of administration: Subcutaneous • Duration of treatment: Single dose Part 2: Phase 1 randomized open-label crossover Healthy males Test product: Liquid vial with EDTA • Dose: 500 µg • Route of administration: Subcutaneous • Duration of treatment: Single dose Reference therapy: Liquid vial with EDTA • Dose: 500 µg • Route of administration: IV infusion (2 hr) • Duration of treatment: Single dose	• Grade 3: Two patients experienced grade 3 AEs with back pain • Grade 4: None

Summary of study design	Grade 3/4 AEs:
309901 (2006) Phase 1 randomized, open-label, crossover study Healthy adults Test product: Liquid sargramostim 1,000 µg/mL • Dose: 6 µg /kg • Route of administration: Subcutaneous • Duration of treatment: Single dose Reference therapy: Liquid sargramostim 500 µg/mL • Dose: 6 µg/kg • Route of administration: Subcutaneous • Duration of treatment: Single dose	• Grade 3: Three patients experienced grade 3 AEs: One patient with back pain, one low back pain, and one bone pain • Grade 4: None

APPENDIX 3: SERIOUS ADVERSE EVENTS BY STUDY

Table 22: Types of SAEs in Study 305 (AML)

	Leukine N=52	**Placebo N=47**
Number of patients experience SAEs	17	20
Infection	4	10
Liver function abnormality	3	2
Renal function abnormality	2	0
Progressive leukemia	2	0
Multi-organ failure	0	1
Seizures	1	0
Depression	1	0
Pulmonary		
Edema	1	0
Dyspnea	0	1
Respiratory failure/distress	1	1
Hemorrhage	2	0
Cardiovascular		
Arrest	1	1
Hypertension	0	1
Subdural hematoma	0	1
Metabolic	1	1
Accident	0	1
One patient can experience multiple events		

Table 23: Types of SAEs in Study 301 (Autologous BMT)

	Leukine N=16	**Placebo N=14**
Number of patients with SAEs	5	7
Kidney failure	1	1
Liver damage	3	4
Subcutaneous hematoma	1	0
Dyspnea	1	2
Renal	1	0

	Leukine N=16	Placebo N=14
Dizziness	1	0
Headache	1	0
Hemorrhage	1	1
GI disorder	0	1
Lung disorder	1	4
Hypertension	0	1
Central nervous system	0	1
Sepsis	0	1
Chills and fever	0	1
Fever	0	2
Blood dyscrasia	0	1
Intracranial hemorrhage	1	0
Edema	1	0
Kidney function abnormal	2	1
One patient can have multiple events		

Source: CSR

Table 24: Types of SAEs in Study 302 (Autologous BMT)

	Leukine-AuBMT N=18	Leukine-PSCT N=12	Placebo-AuBMT N=19	Placebo-PSCT N=13
Number of patients with SAEs	18	12	18	13
Abdomen enlarged	0	0	1	0
Asthenia	1	3	0	1
Back pain	0	0	1	1
Fever	5	6	7	2
Infection	1	0	1	0
Malaise	2	1	0	0
Mucous membrane disorder	8	5	7	5
Sepsis	1	4	2	0
Hemorrhage	1	0	0	1
Hypotension	0	1	0	1
Pericarditis	0	0	0	1
Anorexia	0	1	3	1

	Leukine-AuBMT N=18	Leukine-PSCT N=12	Placebo-AuBMT N=19	Placebo-PSCT N=13
Diarrhea	7	4	4	6
Dysphagia	0	1	0	0
Esophagitis	0	0	0	1
Gastrointestinal disorder	5	2	5	4
Gastrointestinal hemorrhage	1	0	0	1
Gastrointestinal pain	1	0	1	0
Hepatosplenomegaly	0	0	0	1
Jaundice	0	1	0	0
Liver damage	0	2	0	1
Nausea	5	1	5	2
Stomatitis	4	3	3	3
Vomiting	3	0	3	2
Blood dyscrasia	5	4	6	2
Edema	2	2	0	0
Bone pain	1	0	0	1
Central nervous system disorder	2	4	2	1
Dyspnea	3	4	1	2
Hyperventilation	0	0	1	0
Lung disorder	2	4	1	2
Respiratory disorder	1	3	0	1
Alopecia	16	12	18	13
Rash	0	2	2	0
Kidney failure	0	2	1	0
Kidney function abnormal	0	0	1	0
Urinary tract disorder	1	1	1	2
One patient can experience multiple events				

Source: CSR

Table 25: Types of SAEs in Study 303 (Autologous BMT)

	Leukine N=13	Placebo N=12

Number of patients with SAEs	11	12
Alopecia	5	4
Anorexia	8	9
Asthenia	1	2
Diarrhea	8	10
Dyspnea	1	1
Nausea	3	5
Urticaria	0	1
Vomiting	0	2
One patient can experience multiple events		

Table 26: Types of SAEs in Study 9002 (Allogeneic BMT)

	Leukine N=53	**Placebo N=56**
Number of patients with SAEs	**6**	**8**
Seizure activity	1	0
Leukemia relapse	1	0
Sepsis	1	1
Septic shock	1	0
Hypoxemia	1	0
Flushing	1	0
Rigors	1	0
Anxiety	1	0
First dose response	1	0
Spasmodic movement	1	0
GVHD-skin	0	1
Mucositis	0	1
Respiratory distress	0	1
Acute respiratory distress syndrome	0	1
Endotoxin shock	0	1
Kleb. pneumonia	0	1
Dehydration	0	1
Hyperglycemia	0	2
Hyperkalemia	0	2
Chest pain	0	1
Neurologic deterioration	0	1

	Leukine N=53	Placebo N=56
One patient can experience multiple events		

Source: CSR

Table 27: Types of SAEs in Study 501 (Engraftment Failure)

	Leukine (N= 104)	Historical controlled (N= 103)
Body as a whole*	31.7%	20.4%
Respiratory	17.3	34.0%
Digestive	15.4%	11.7%
Cardiovascular	7.7%	14.6%
Skin/Appendages	6.7%	0.0%
Metabolic/Nutritional	5.8%	1.9%
Nervous	4.8%	19.4%
Musculoskeletal	4.8%	0.0%
Urogenital	1.9%	23.3%
Special Senses	0.0%	1.0%
Hemic/Lymphatic	0.0%	5.8%
*Body as a whole – includes asthenia, chills, fever, infection, malaise, back pain One person can experience multiple events		

Source: CSR

APPENDIX 4: RADIATION-INDUCED NEUTROPENIA

Exposure to total body ionizing radiation is known to lead to bone marrow hypoplasia or aplasia in a dose-dependent manner. Radiation exposure in excess of 2 Gray (Gy) results in significant bone marrow suppression with neutropenia being one of the consequences of greatest clinical significance (see Table 28 below). High-dose accidental whole body radiation exposure (> 6-10 Gy) can lead to a rapid decrease in neutrophil counts within 5 to 8 days of exposure as well as damage to other organ systems such as the gastrointestinal tract and lungs, which contributes to a decreased likelihood of survival. By contrast, a less severe degree of hematopoietic injury occurs after lower doses of radiation (e.g., 2-6 Gy) with neutropenia developing between 6 and 10 days after exposure with neutrophil nadir occurring between 20 and 30 days following exposure. The primary concern in these neutropenic patients is their increased propensity for infection.

Table 28: Pathophysiological events of acute radiation syndrome in humans

Dose Range, G*y*	**Prodrome**	**Manifestation of Illness**	**Prognosis (without Therapy)**
0.5-1.0	Mild	Slight decrease in blood cell counts	Almost certain survival
1.0-2.0	Mild to moderate	Early signs of bone marrow damage	Highly probable survival (>90% of victims)
2.0-3.5	Moderate	Moderate to severe bone marrow damage	Probable survival
3.5-5.5	Severe	Severe bone marrow damage; slight GI damage	Death within 3.5-6 wk (50% of victims)
5.5-7.5	Severe	Pancytopenia and moderate GI damage	Death probable within 2-3 wk
7.5-10.0	Severe	Marked GI and bone marrow damage, hypotension	Death probable within 1-2.5 wk
10.0-20.0	Severe	Severe GI damage, pneumonitis, altered mental status, cognitive dysfunction	Death certain within 5-12 d
20.0-30.0	Severe	Cerebrovascular collapse, fever, shock	Death certain within 2-5 d

Table adapted from Waselenko 2004

Guidances have been for the management of radiation-induced neutropenia (see table below). In all cases LGFs are recommended to accelerate neutrophil recovery as a means to prevent or treat neutropenia following exposure to $\geq$ 2 Gy of ionizing radiation. The most commonly referenced neutropenia guideline comes from the American Society of Clinical Oncology (ASCO) and was updated in 2005. This guideline specifies that LGFs should be used to accelerate neutrophil recovery when neutrophil levels drop below 1000 mm^3 to help reduce infectious morbidity and mortality regardless of neutropenia cause *(*ASCO Guidelines; Smith 2006). In addition, the guideline states that "the management of patients exposed to lethal doses of total body radiotherapy, but not doses high enough to lead to certain death … should include the prompt administration of a LGF…"

Table 29: [Federal Guidances on Radiation-Induced Neutropenia]

Agency	Leukine	Indication
Health & Human Services (2010)	Yes	≥ 2 Gy or ANC < 500
Depart of Energy (REAC/TS) (2010)	Yes	Not specified
Homeland Security (2009)	Cytokines (not specified)	≥ 2 Gy
Department of Defense (2003)	Yes	Not specified

APPENDIX 5: TOXICOLOGY STUDIES

Since Leukine has no activity in rodents, toxicity studies were limited to Cynomolgus monkeys and rabbits. The nonclinical safety program of Leukine consists of GLP-compliant, single-dose and 14-day, 30-day and 42-day repeat-dose general toxicity studies in Cynomolgus monkeys and a complete series of GLP-compliant, reproductive and developmental studies in rabbits that include assessments of fertility, embryo-fetal development and peri- and post-natal development (Table 30). Due to the production of anti- Leukine antibodies that reduce drug exposure (as measured by C_{max} and AUC) and neutralize Leukine activity, which is reflected by an inhibition of Leukine's pharmacodynamic markers (i.e., stimulation of WBC count and specifically neutrophil count), studies greater than 6 weeks in duration were not conducted.
This program has sufficiently supported the licensure of Leukine for 5 indications, and per FDA communication on November 11, 2012, is adequate to support the proposed indication.

Table 30: Toxicity Studies Conducted with Leukine

Study type	Route of administration, regimen and dose levels	Species	Study Number
General Toxicity Studies			
Acute intravenous toxicology study (GLP-compliant)	intravenous injection 0 and 300 µg/kg	Cynomolgus monkey	2423-103
14-day intravenous toxicology study with 14-day recovery period (GLP-compliant)	intravenous injection once daily for 14 days 0, 1, 10 and 300 µg/kg	Cynomolgus monkey	2423-105
30-day subcutaneous toxicology study with 14-day recovery period (GLP-compliant)	Subcutaneous injection once daily for 30 days 0, 20 and 200 µg/kg	Cynomolgus monkey	2423-111
Orienting 42-day subcutaneous toxicology study (GLP-compliant)	Subcutaneous injection once daily for 42 days 0, 20, 63 and 200 µg/kg	Cynomolgus monkey	TXST20040246/ A24993
42-day subcutaneous toxicology study with 12-13 weeks recovery period (GLP-compliant)	Subcutaneous injection once daily for 42 days 0, 20, 63 and 200 µg/kg	Cynomolgus monkey	TXST20050052/ A27294
Reproductive and Developmental Toxicity Studies			
Maximum tolerated dose study (GLP-compliant)	Subcutaneous injection once daily for 14 days 0, 20, 60, 110 and 200 µg/kg	Nonpregnant female New Zealand White Rabbit	AA23118/ TXEX20040041/ A28816

Study type	Route of administration, regimen and dose levels	Species	Study Number
Maximum tolerated dose study (GLP-compliant)	Subcutaneous injection once daily for 14 days 0, 50, 200 and 400 µg/kg	Nonpregnant female New Zealand White Rabbit	A31774/ 543001/ TXEX20050009
Dose range-finding fertility and embryo-/fetotoxicity study (GLP-compliant)	Subcutaneous injection once daily from day 6 before mating to day 6 of gestation, from day 6 – 18 of gestation or days 17-28 of gestation 0, 50 and 200 µg/kg	Pregnant female New Zealand White Rabbit	A39389/ TXEX20040037/ AA22578
Dose range-finding study for effects on embryo/fetal development and early postnatal survival (GLP-compliant)	Subcutaneous injection once daily from gestation days 6-19 (collective A) and gestation days 6- parturition (collective B) A: 0, 25, 200 and 400 µg/kg B: 0, 25, 100 and 200 µg/kg	Pregnant female New Zealand White Rabbit	A33918/ TXEX20050016/ 543002
Study of fertility and early embryonic development to implantation (GLP-compliant)	Subcutaneous injection once daily from day 6 before artificial insemination to day 7 of gestation 0, 25, 70, and 200 µg/kg	Pregnant female New Zealand White Rabbit	A38192/ 543009/ TXEX20050038
Embryo fetal development study (GLP-Compliant)	Subcutaneous injection once daily from gestation days 6-19 or gestation days 19- 28 0, 25, 70 and 200 µg/kg	Pregnant female New Zealand White Rabbit	A38193/ TXEX20050039/ 543012
Pre- and postnatal development, including maternal function study (GLP-Compliant)	Subcutaneous injection once daily from gestation day 6 to 19, gestation day 19 to parturition or lactation day 1 to 14 0, 25, 70 and 200 µg/kg	Pregnant female New Zealand White Rabbit	A43883/ TXEX20050040/ 543013

The main target organ in Cynomologous monkeys was the lympho-hematopoietic system; effects consisted of increases in white blood cell counts (particularly neutrophils, eosinophils, basophils and lymphocytes) and platelets, hypercellularity/hyperplasia of bone marrow, spleen and lymph nodes and inflammatory cell infiltrates in numerous organs (e.g., liver, heart, lung). Inflammatory reactions were observed at the injection site. There was evidence for reversibility of these effects during post-dosing recovery periods. The presence of anti- Leukine antibodies that neutralized the activity of Leukine was observed in the 30-Day and 6-Week studies.

In fertility and embryofetal development studies in rabbits, Leukine's effects included decreased embryonic survival, increased pre- and post-implantation loss, increased late resorptions and increased abortions. The ability of rabbits to conceive was unaffected. In a pre- and post-natal

development study in rabbits, Leukine had no effect on kit survival, F1 reproductive parameters or F2 survival. No malformations were observed.

APPENDIX 6: CLINICAL DOSE-FINDING STUDIES

Three phase 1/2 dose-finding studies were conducted in patients undergoing autologous BMT patients, which preceded the pivotal phase 3 study presented in Section 6.3.1.

Nemunaitis and co-workers (Nemunaitis 1988) conducted a dose-escalation study of Leukine given as a 2-hour infusion daily for 14 days following bone marrow infusion. Fifteen consecutive patients with lymphoid malignancies were included in the study and compared to 86 disease-matched and treatment-matched historic controls. TBI was included in the preparative regimen in all but one of the 15 patients. Doses of Leukine ranged from 15 to 240 μg/m^2/day.

Table 31: ANC Recovery vs. Leukine Dose

Dose (μg/m^2/d)	Number of Evaluable Patients	Mean (Median) Days to > 500/mm^3
0*	86	25 (Range 10 – 60)
15	3	20.7 (18)
30	3	23.3 (15)
60	3	13.3 (13)
120	2	15.5 (15.5)
240	3	14.3 (13)

*Historical control

Doses of Leukine at or above 60 μg/m^2/day were associated with fewer days of temperatures ≥ 38°C: 6 ± 3 vs. 11 ± 6 at lower doses vs. 12 ± 7 in historical controls (mean ± SD). No patient failed to complete their planned 14 days of treatment because of toxicity.

In conclusion, patients receiving Leukine at ≥ 60 μg/m^2/day recovered neutrophil counts more rapidly and had fewer febrile days.

In a study by Blazar and colleagues (Blazar 1989), 25 patients with ALL undergoing autologous BMT received increasing doses of Leukine and were compared to 27 historical controls with similar disease status and type. TBI was included in all 3 different preparative regimens. Doses of Leukine ranged from 16 to 256 μg/m^2/day.

Table 32: ANC Recovery vs. Leukine Dosing Regimen

Dose (μg/m^2/d)	Number of Evaluable Patients	Mean (Median) Days to ≥ 500/mm^3
0*	27	(24)*
16 (X 14d)	2	104.5 (Values 23, > 186)
32 (X 14d)	3	24.7 (21)
64 (X 21d)	2	21 (Values 16, 26)
128 (X 21d)	5	24.2 (26)
256 (X 21 d)	9	20.7 (22)

*Historical control, mean value not reported

No differences in median values for neutrophil recovery were noted in comparisons of controls to Leukine-treated patients. No consistent Leukine dose-related effects were evident. Sepsis

was documented in 40% (10 of 25) of the Leukine-treated patients vs. 44% (11 of 25) of control patients who were analyzed for infectious episodes post-BMT. It was noted that all septic episodes in the Leukine treated group occurred by 9 days following BMT, when the ANC remained below 100/mm^3.

Overall, 18 of 25 patients completed the entire course, but no patient failed to complete their course owing to Leukine toxicity.

In conclusion, no clear dose-dependent effect of Leukine on time to neutrophil recovery was evident. Further studies are needed.

A third phase 1/2 dose escalation study was conducted in 31 patients with Hodgkin's disease undergoing autologous BMT (Devereaux 1989). Seventeen of these were treated by a consistent preparative regimen prior to rhu GM-CSF becoming available. The subsequent 14 patients were to receive the same preparative regimen and were offered rhu GM-CSF; 12 agreed to participate. The remaining two subjects were included with the above 17 as the control group. Seven of the 31 received Leukine by continuous infusion at 100 µg/m^2/day for 10 to 20 days. Three received *E. coli*-derived rhu GM-CSF at 165 µg/m^2/day. Two patients received 400 µg/m^2/day, but both died prior to achieving neutrophil recovery (colitis Day 7; progressive disease Day 17).

Table 33: ANC Recovery vs. Leukine Dose

Dose (µg/m^2/d)	**Number of Evaluable Patients**	**Mean (Median) Days to ≥ 500/mm^3**
0*	19	24.9 (25)
100	7	18.3 (17)
165	3	15.7 (15)

*Historical control

Clinical outcomes were similar between the rhu GM-CSF groups and historical controls, including infection rate (58% vs. 68%), number of febrile days (5.0 vs. 4.7) and hospital days (30.1 vs. 30.2).

In conclusion, the mean time to attain a neutrophil count greater than 500/mm^3 was approximately 7 days less with rhu GM-CSF compared to control subjects.

APPENDIX 7: DOUBLE HEMIBODY IRRADIATION STUDY RESULTS TABLE

Double Hemi-body irradiation in patients with and without rhu GM-CSF support

	Historical data	Range of Granulocytes (x 10^9/L)	Patients treated with rhu GM-CSF:	Range of Granulocytes (x 10^9/L)
No. of patients	32		10	
DBHI as first-line therapy	19		2	
DBHI as second-line therapy	13		8	
Median (and range) granulocytes (x10^9/L) after first hemibody irradiation				
Day 0	2.4	(0.6-6.3)	2.7	(0.5-5.9)
Day 8	1.2	(0.0-1.4)	4.5	(0.7-10.4)
Day 15	1.0	(0.0-1.4)	7.7	(1.3-16.7)
Day 21	0.8	(0.2-2.1)	2.9	(0.6-6.4)
Median (and range) days between UBI and LBI	108	(28-482)	41	(28-50)
Median granulocytes (x10^9/L) after second hemibody irradiation				
Day 0	2.0	(0.7-5.9)	2.8	(0.07-5.4)
Day 8	1.1	(0.03-1.6)	3.0	(0.04-6.9)
Day 15	0.4	(0.03-0.4)	5.0	(0.8-14.7)
Day 21	0.5	(0.1-1.6)	1.9	(0.5-5.6)
Treatment achieved	25/32		9/10	

APPENDIX 8: RHU GM-CSF USE IN HUMAN ACCIDENTALLY EXPOSED TO RADIATION

Genzyme identified three published anecdotal reports of patients treated with rhu GM-CSF (Leukine or bacterially-derived rhu GM-CSF) without concomitant use of other CSFs following accidental radiation exposure. All patients in these case series experienced severe neutropenia. These anecdotal cases are not placebo-controlled, but do suggest accelerated neutrophil recovery following rhu GM-CSF treatment in the context of ionizing radiation-induced neutropenia.

Leukine was used to treat eight myelosuppressed individuals following accidental exposure to Cesium-137 in Gioania, Brazil (Butturini 1988). Leukine therapy was initiated in granulocytopenic patients ($\leq 0.5 \times 10^9$/L) in a range of 24 to 47 days following radiation exposure. The estimated radiation exposure dose ranged from 2.5 to 6.0 Gy. Leukine was dosed at 500 μg/m^2 IV daily until the granulocyte count exceeded 2.0×10^9/L, and subsequently continued at half the dose for an additional three days. Overall, four of eight exposed individuals survived. The report noted that Leukine was initiated in the four surviving patients within five days of developing granulocytopenia and prior to onset of infectious complications. By contrast, four individuals who did not survive were colonized with drug resistant Klebsiella prior to receiving Leukine and had severe granulocytopenia by the time Leukine was initiated ($0.0\text{-}0.2 \times 10^9$/L; 3-10 days from the granulocyte count dropping below 1.0×10^9/L). Based on this observation, the next two patients began Leukine when their granulocyte count was 0.5 and 0.7 $\times 10^9$/L (4 and 1 days from the granulocyte count dropping below 1.0×10^9/L) and both subsequently survived. Survival and granulocyte recovery data are presented in Table 34. The authors concluded that Leukine contributed to the granulocyte recovery based upon the rapid rise in granulocytes within 12 hours in several individuals, the decline in granulocytes after dose reduction or discontinuation and the difference in pattern of recovery in the 8 treated patients compared to the two non-treated patients.

In San Salvador, three individuals developed severe neutropenia following accidental exposure to 3.0 to 8.1 Gy of radiation from a Cobalt-60 radiation source (IAEA 1990). At 24, 26, and 32 days following exposure, each patient received rhu GM-CSF (non-Leukine) at a daily dose of 240 μg/m^2 IV over 2 hours until the ANC reached 1,500/mm^3. All three patients responded to rhu GM-CSF such that they had an ANC > 1,500/mm^3 within 9 to 20 days after starting rhu GM-CSF. None experienced severe infections. Two of the three patients ultimately survived; one patient (8.1 Gy) died 197 days following exposure, thought to be related to radiation pneumonitis. While the rise in neutrophil counts correlated with initiation of rhu GM-CSF treatment and the authors considered this related to rhu GM-CSF, spontaneous recovery could not be excluded.

Three individuals in Henan Province, China were accidentally exposed to high dose Cobalt-60 (mean dose estimates: 6.1, 3.4 and 2.4 Gy) (Liu 2008). All three received rhu GM-CSF starting when the total white cell count was below 1.0×10^9/L (ANC not reported). The first subject received rhu GM-CSF beginning on day 9 following exposure at a dose of 400 μg/m^2 per day, then tapered to 200 μg/m^2 on days 33-37 following exposure. The second patient started rhu GM-CSF on day 18 following exposure at a dose of 200 μg/m^2/day, then decreased to 50 μg/m^2/day for days 33-37. The third patient began receiving rhu GM-CSF at 400 μg/m^2/day on

day 26 following exposure with the last dose administered on day 35. All three patients showed an increase in leukocyte counts. Furthermore, all patients survived this radiation exposure.

Table 34: Neutrophil recovery and survival in accidental radiation exposure victims

Results of Therapy with Leukine

No	Sex	Age	Estimated dose (range)*	Days from exposure to therapy	Granulocytes (<1 x 10^9/L) to therapy (days)	Granulocytes (x 10^9/L)†		Outcome
						Pretreat-ment	Maximum	
3	F	37	6.0 (5.1-6.8)	30	10	0	0.6	Died, hemorrhage‡
4	F	6	6.0 (5.1-7.3)	24	4	0	NE	Died, hemorrhage §
5	M	22	4.0 (3.2-4.8)	34	3	0.2	0.5	Died, infection¶
6	M	18	5.3 (4.4-6.5)	30	4	<0.1	23.1	Died, infection
7	M	19	2.5 (1.7-3.4)	35	5	<0.1	19.7	Alive
8	F	57	4.3 (3.0-5.0)	25	5	0	21.4	Alive
9	M	42	4.4 (3.5-5.5)	40	1	0.7	9.9	Alive
10	M	21	3.0 (2.0-4.0)	47	4	0.5	7.3	Alive

*Integrated sum of external and internal doses. Calculation based on neutrophil and lymphocyte kinetics, dicentric chromosomes, and physical measurements of Cesium-137 incorporation.
†Includes myelocytes, metamyelocytes, and bands.
‡This patient also had cerebral oedema and pre-existing hepatic cirrhosis.
§Diffuse pulmonary haemorrhage was immediate cause of death.
¶Bronchopneumonia.
NE – not evaluable

In summary, rhu GM-CSF has been used to treat 14 accidental radiation victims (range of 2.4-8.1 Gy), of whom 9 survived. Treatment with rhu GM-CSF was initiated from 9 to 47 days after radiation exposure and was given for a range of 9 to 29 days. The authors of these reports noted that rhu GM-CSF may offer a benefit for the management of radiation-induced neutropenia even when rhu GM-CSF is initiated several days to weeks following exposure.

APPENDIX 9: CLINICAL TRIAL SYNOPSES

CLINICAL TRIAL SUMMARY: Autologous Bone Marrow Transplantation (AuBMT)

Multicenter versus one center study: Performing a multicenter study, event with fast patient enrollment and a carefully designed sample, would have led to differences in preparative regimens, the stage of disease at which the autologous bone marrow transplantation (AuBMT) was performed, and the supportive therapy given. On this basis, the decision was made to design three Phase 2/3 studies with a smaller sample size, each with clear and homogeneous guidelines for the indication of AuBMT, preparative regimen, and supportive therapy. If the investigators indicated that particular groups should be distinguished in their patients population, substratification was established before randomization of that study.

COMPOUND: Sargramostim / Leukine® STUDY No: 301	
TITLE	Safety and effectiveness of Recombinant Human Granulocyte-Macrophage Stimulating Factor (Rhu GM-CSF; Sargramostim) compared with Placebo following autologous bone marrow transplantation in subject with lymphoid malignancies
INVESTIGATOR/TRIAL LOCATION	United States
PHASE OF DEVELOPMENT	Phase 2/3
STUDY OBJECTIVES	This study was conducted to assess the efficacy and safety of Sargramostim, compared with placebo, for promoting myeloid engraftment following purging and autologous bone marrow transplantation (AuBMT) in subjects with various lymphoid malignancies. Efficacy measurements were to include time to engraftment, need for transfusions, infection experience, and duration of hospitalization. Safety was to be monitored by recording of adverse events and blood chemistry variables.
STUDY DESIGN	This was a prospective, double-blind, randomized, placebo-controlled study conducted in a single institution. Patients were randomized according to two factors: - whether the subject had ALL, or HD, or NHL of favorable prognostic. or HD or NHL of unfavorable prognostic - whether the subject's bone marrow was purged or not purged before transplant The study consisted of the following parts: 1. Bone marrow harvest, with or without purging, followed by cryopreservation 2. Preparative chemoradiotherapy 3. AuBMT 4. Sargramostim or placebo therapy (treatment Day 1-21) 5. Follow-up
STUDY POPULATION **Main selection criteria**	Subjects with acute lymphoblastic leukemia (ALL), Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) considered as eligible for AuBMT were eligible for admission to this study. There were no restriction for admission regarding age or sex or disease state/stage and prior therapy. However, subjects with HIV positive or active

	infections immediately before the marrow infusion were not eligible.
STUDY TREATMENTs	Preparative regimen The preparative regimen used was dependent on the diagnostic group. Preparative treatment included high dose cyclophosphamide and fractionated total body irradiation (TBI). For some subjects, bone marrow was purged to remove tumor cells before transplantation. Study medication After AuBMT, patients received study drug, 250μg/m2 Sargramostim or placebo, as a 2-hour intravenous infusion for a maximum of 21 days.
ENDPOINTS	**Efficacy endpoints** Hematology variables evaluated to measure to time engraftment were: - Time to ANC≥100/mm3, ANC≥500/mm3 and WBC>1000/mm3, - Time to final platelet transfusion and total packed cell units transfused The following clinical outcomes influenced by engraftment were also monitored - Frequency, duration and severity of infections - Days febrile and days febrile and neutropenic - Antimicrobial usage - Duration of hospitalization - Survival to day 100 **Safety endpoints :** Adverse event recording, laboratory tests, physical examination, vital signs.
STATISTICAL CONSIDERATIONS	Sample size determination Twenty subjects were to be randomized to each treatment group. This would provide 80% power to detect a difference in the percentage of subject with infection between 50% in the placebo group and 10% in the group using a one-sided type I error of 5%. Analysis population All patients were included in efficacy and safety analyses. Efficacy analysis Time to ANC or WBC recovery, duration of hospitalization and survival were analyzed with the Kaplan-Meier method and compared between groups using Wilcoxon and log-rank statistics.

COMPOUND: Sargramostim / Leukine® STUDY No: 302	
TITLE	A controlled study of the safety and effectiveness of Recombinant Human Granulocyte-Macrophage Stimulating Factor (Rhu GM-CSF; Sargramostim) compared with Placebo following autologous bone marrow transplantation (AuBMT) or peripheral stem cell transplantation (PSCT) in subject with lymphoid malignancies
INVESTIGATOR/TRIAL LOCATION	United States
PHASE OF DEVELOPMENT	Phase 3
STUDY OBJECTIVES	This study was conducted to assess the efficacy and safety of Sargramostim, compared with placebo, in patients undergoing AuBMT or PSCT following intensive chemotherapy or chemo-radiotherapy for lymphoid malignancy. Efficacy measurements were to include time to engraftment, need for transfusions, infection experience, antimicrobial usage and duration of hospitalization. Safety was to be monitored by recording of adverse events and blood chemistry values
STUDY DESIGN	This was a single-center, prospective, double-blind, randomized, placebo-controlled study. Patients were randomized according to two factors (total 8 strata): - Preparative regimen (total of 4) - Type of transplantation The study consisted of the following parts: 1. Bone marrow or peripheral stem cell harvest followed by cryopreservation of the harvest 2. Preparative chemotherapy or chemo-radiotherapy 3. Bone marrow or peripheral stem cell transplantation 4. Sargramostim or placebo therapy (treatment Day 1-21) beginning the day of transplantation 5. Follow-up
STUDY POPULATION **Main selection criteria**	Patients with Hodgkin's disease (HD) were required to have progressive disease or relapse after previous standard therapy without BMT. Criteria for patients with non-Hodgkin's lymphoma (NHL) were defined in the protocol. There were no restriction for admission regarding age or sex or extent of lymphoma, or type of prior therapy for lymphoma.
STUDY TREATMENTs	Preparative regimen The preparative regimen (total of 4) used was dependent on the diagnostic group. Study medication Beginning within 1 hour after AuBMT or PSCT, patients received study drug, 250μg/m2 Sargramostim or placebo, as a 2-hour intravenous infusion for a maximum of 21 days.
ENDPOINTS	**Efficacy endpoints** Hematology variables: - Time to ANC≥100/mm3, ANC≥500/mm3 and WBC>1000/mm3, - Time to final platelet transfusion and total packed cell units

	transfused The following clinical outcomes influenced by engraftment were also monitored - Duration of hospitalization - Survival to day 100 - Frequency, duration and severity of infections - Days febrile and days febrile and neutropenic - Antimicrobial usage **Safety endpoints :** Adverse event recording, laboratory tests, physical examination, vital signs.
STATISTICAL CONSIDERATIONS	Sample size determination A total of 60 subjects were to be randomized: 40 patients undergoing AuBMT and 20 patients undergoing PSCT. This would provide 80% power to detect a difference in the percentage of subject with infection between 50% in the placebo group and 10% in the group using a one-sided type I error of 5%. Analysis population All patients were included in efficacy and safety analyses. Efficacy analysis Patients with AuBMT and PSCT were analyzed separately. Time to ANC or WBC recovery, duration of hospitalization and survival were analyzed with the Kaplan-Meier method and compared between groups using Wilcoxon and log-rank statistics.

COMPOUND: Sargramostim / Leukine® STUDY No: 303	
TITLE	Safety and effectiveness of Recombinant Human Granulocyte-Macrophage Stimulating Factor (Rhu GM-CSF; Sargramostim) compared with Placebo following autologous bone marrow transplantation in subject with B-cell non-Hodgkin's Lymphoma (NHL)
INVESTIGATOR/TRIAL LOCATION	United States
PHASE OF DEVELOPMENT	Phase 2/3
STUDY OBJECTIVES	This study was conducted to assess the efficacy and safety of Sargramostim, compared with placebo, for promoting engraftment after autologous bone marrow transplantation (AuBMT) in subjects with B-cell lymphoma. Efficacy measurements were to include time to engraftment, need for transfusions, infection experience, and duration of hospitalization. Safety was to be monitored by recording of adverse events and blood chemistry variables.
STUDY DESIGN	This was a prospective, double-blind, randomized, placebo-controlled study conducted in a single institution. There was no stratification The study consisted of the following parts: 1. Bone marrow harvest with purging followed by cryopreservation 2. Preparative chemoradiotherapy 3. AuBMT 4. Sargramostim or placebo therapy (treatment Day 1-21) 5. Follow-up
STUDY POPULATION **Main selection criteria**	Subject with histologically proven B-cell non-Hodgkin's lymphoma (NHL) were eligible for admission to this study. Subjects had to be more than 18 and less than 65 years old with and ECOG PS≤2. Subjects were required to have normal hepatic and renal excretion.
STUDY TREATMENTs	Preparative regimen The preparative regimen included high dose cyclophosphamide and fractionated total body irradiation (TBI). Bone marrow was purged to remove tumor cells and cryopreserved before transplantation. Study medication After AuBMT, patients received study drug, 250μg/m2 Sargramostim or placebo, as a 2-hour intravenous infusion for a maximum of 21 days.
ENDPOINTS	**Efficacy endpoints** Hematology variables evaluated to measure to time engraftment were: - Time to ANC≥100/mm3, ANC≥500/mm3 and WBC>1000/mm3, - Time to final platelet transfusion and total packed cell units transfused The following clinical outcomes influenced by engraftment were also monitored - Frequency, duration and severity of infections - Days febrile and days febrile and neutropenic - Antimicrobial usage - Duration of hospitalization

	- Survival to day 100 **Safety endpoints :** Adverse event recording, laboratory tests, physical examination, vital signs.
STATISTICAL CONSIDERATIONS	Sample size determination Twenty subjects were to be randomized to each treatment group. This would provide 80% power to detect a difference in the percentage of subject with infection between 50% in the placebo group and 10% in the group using a one-sided type I error of 5%. Analysis population All patients were included in efficacy and safety analyses. Efficacy analysis Time to ANC or WBC recovery, duration of hospitalization and survival were analyzed with the Kaplan-Meier method and compared between groups using Wilcoxon and log-rank statistics.

CLINICAL TRIAL SUMMARY: Acute Myeloid Leukemia (AML)

COMPOUND: Sargramostim / Leukine® - STUDY No: 305	
TITLE	A Randomized Placebo-Controlled Phase 3 Study of Granulocyte-Macrophage Colony Stimulating Factor (rhu-GM-CSF) in Adult Patients (>55-70 Years) with Acute Non-Lymphocytic Leukemia (ANLL)
INVESTIGATOR/TRIAL LOCATION	United States
PHASE OF DEVELOPMENT	Phase 3
STUDY OBJECTIVES	To evaluate the safety and efficacy of administering rhu-GM-CSF (S. cerevisiae) Sargramostim as a daily 4-hour intravenous infusion following induction and consolidation chemotherapy in elderly patients with de novo ANL To evaluate the ability of Sargramostim to accelerate hematopoietic recovery following induction and consolidation chemotherapy in elderly patients with de novo ANLL To evaluate whether the administration of Sargramostim decreases the morbidity and mortality from infections complications following induction and consolidation chemotherapy in elderly patients with de novo ANLL To estimate the rate and duration of complete response, the duration of survival of elderly patients with de novo ANLL who received induction hemotherapy with Daunorubicin and Cytarabine and a single course of consolidation chemotherapy with high-dose Cytarabine
STUDY DESIGN	This was a multi-site, randomized double-blind placebo controlled Phase 3 study in adult subjects, >55-70 years of age, with ANLL receiving induction and consolidation chemotherapy. - Subject received one or two courses of induction chemotherapy with Daunorubicin and Cytarabine - Bone marrow aspiration and biopsy was performed 3 days after completion of first and second induction chemotherapy courses (day 10 of each course)

	- A single consolidation therapy course with Cytarabine was given 3-6 weeks after induction therapy following neutrophil recovery in those subjects achieving complete remission who were re-registered for this phase of therapy - Sargramostim or placebo was administered IV over a 4-hour period starting approximately on Day 11, if the bone marrow on day 10 or 4 after completion of the second course of induction chemotherapy contained <5% blasts if a second cycle of induction was necessary. The same study drug was delivered after the single course of consolidation chemotherapy
STUDY POPULATION **Main selection criteria**	Eligibility criteria for Induction: Patients must have had >55 and ≤70 years old, morphologic proof of acute myeloid leukemia (AML), no prior cytotoxic or radiation therapy and adequate renal and hepatic functions. Eligibility criteria for Post-remission therapy Patient must have had complete remission, ECOG 0 or 1 and adequate renal and hepatic functions.
STUDY TREATMENTS	Induction Drug administration Schedule Up to 2 cycles of induction therapy were permitted in the attempt to gain a complete remission. Patients not attaining a complete remission after 2 cycles went off study. - Daunorubicin: 60mg/m2 per day by IV injection through a freshly established free flowing IV line on days 1, 2 and 3 - Cytosine arabinoside : 25 mg/m2 push followed by continuous infusion of 100mg/m2 per day IV on days 1 through 7 (seven 24 hr periods of infusion) Sargramostim or placebo was initiated on day 11, or 4 days after completion of the second course of induction therapy if the bone marrow aspirate or biopsy touch preps are clear. Sargramostim or placebo was administered intravenously at 250µg/m2 over 4 hours and was administered until the absolute neutrophil count (ANC) was ≥1,500/mm3 for 3 consecutive days or for a maximum of 43 days. Consolidation drug administration schedule One course of consolidation therapy was given 3-6 weeks after completion of the induction regimen. - Cytosine arabinoside: 1.5g/m2 administered intravenously over 1 hour every 12 hours for 12 doses. On day 11 of consolidation therapy, patients received the identical blinded study medication (Sargramostim or placebo) that they had received during induction therapy.
ENDPOINTS	The primary efficacy endpoint, as per sample size calculation, was time to neutrophil recovery >500/mm3 defined as time from initiation of Sargramostim or placebo to recovery of ANC > 500/mm3. If neutrophil recovery was not observed, patients were censored at the date of last evaluation without neutrophil recovery. In case of death prior to neutrophil recovery, data were censored at day of death. The other efficacy endpoints included time to neutrophil recovery >1000/mm3, duration of thrombocytopenia and duration of anemia. Same approach than for primary efficacy endpoints was used for definition of other efficacy endpoints. Survival was calculated from the date of randomization (start of induction) to the date of death, with censoring at the date of last know alive.

	Adverse events were graded using the common toxicity criteria. Adverse events collected during the Sargramostim/placebo period (i.e. after or on the date of first double-blinded treatment) were analyzed using the worst grade of each event. Laboratory abnormalities were graded using the common toxicity criteria.
STATISTICAL CONSIDERATIONS	Sample size determination The sample size for this study was calculated to provide greater than 80% to detect a 7- to 9-day reduction in the median time to ANC>500/mm3 recovery. Analysis population Efficacy and safety analyses were performed on patients who have received one dose of Sargramostim or placebo. Primary efficacy endpoint analysis Time to ANC recovery was analyzed with the Kaplan-Meier method and compared using Wilcoxon and log-rank procedures.

CLINICAL TRIAL SUMMARY: Allogeneic Bone Marrow Transplantation (BMT)

COMPOUND: Sargramostim / Leukine® STUDY No: 9002	
TITLE	A phase 3 study of recombinant human GM-CSF following allogeneic bone marrow transplantation
INVESTIGATOR/TRIAL LOCATION	United States and Canada
PHASE OF DEVELOPMENT	Phase 3
STUDY OBJECTIVES	**Primary objective** To compare the effect of Sargramostim and placebo following allogeneic bone marrow transplantation (BMT) on: - Neutrophil recovery - Length of hospitalization **Secondary objectives** To compare the effect of Sargramostim and placebo on: - Platelet recovery - Red blood cell recovery - Incidence of infection - Severity of renal disease - Incidence of interstitial pneumonia - Incidence and severity of acute graft versus host disease (GVHD) - Relapse rates - Survival
STUDY DESIGN	This was a prospective multicenter, randomized double-blinded placebo controlled Phase 3 study to evaluate the efficacy of Sargramostim versus placebo following allogeneic bone marrow transplantation (BMT) in patients with hematologic malignancy. All patients were treated with standard preparative regimens for their institution, including optional total body irradiation (TBI), followed by human leukocyte antigen (HLA) identical sibling BMT. Study drug was started at Day 0 after the bone marrow infusion. Patients were randomized according to the risk category for their disease (low- or high-risk).
STUDY POPULATION **Main selection criteria**	Patients of any age and of either sex were eligible for this study if they were undergoing a HLA identical sibling BMT utilizing standard preparative regimens. They could have had the following disease or states: CML in accelerated or chronic phase, AML in first or second remission, ALL in first or second remission, ALL in first or second relapse, NHL of any disease state, HD of any disease state, multiple myeloma, myelodysplatic syndrome, adult aplastic anemia (>16 years) that failed ATG or ALG therapy or pediatric aplastic anemia (<16 years) with or without prior ATG or ALG therapy
STUDY TREATMENTs	Preparative regimen for BMT Patients received regimens specific for each institution. Chemotherapy included busulfan cyclophosphamide, cytosine arabinoside, etoposide, methotrexate, corticosteroids and asparaginase. Some patients also received

	total body, splenic or testicular irradiation. Bone marrow was harvested according to institutional protocols. Day 0 was defined as the date of bone marrow infusion. Study medication Patients received study drug, 250μg/m2 Sargramostim or placebo, as a 4-hour intravenous infusion beginning on the day of bone marrow infusion. Daily doses of study medications were given from Day 0 to Day 20 (total of 21 doses) unless significant toxicity or a patient achieved an absolute neutrophil count (ANC)>10x10^{9}cells/l for 2 consecutive days.
ENDPOINTS	**Primary endpoints** - Time to ANC recovery of 100, 500 and 1,000/mm3: Time to ANC recovery of 100, 500 and 1000/mm3 was defined as the number of days from BMT date to date of the first two consecutive ANC measurements greater than or equal to the target value. If this is recovery was not achieved by Day 42, the subject was censored in the analysis at Day 42. If the subject died or dropped from the study before Day 42, the subject was censored on the day of the last available ANC measurement. Time to WBC recovery was similarly defined. - Length of hospitalization: Time to hospital discharge was defined per protocol as the number of days from BMT date to date of first discharge from the hospital. This day could occur before or after Day 42. If the subject died, the time to discharge was censored on the day of death, before or after Day 42. **Secondary endpoints :** - Date of last platelet transfusion and day of platelet independence: Time to platelet independence was defined as the number of days from BMT date until platelets reached 20,000/mm3 or above with no transfusion for 7 days, and platelets never went below 20,000/mm3 again in the window from Day 0 to Day 42. - Number of units of Red blood cell recovery over first 42 days - Incidence of infection - Severity of mucositis - Incidence and severity of VOD - Severity of renal disease - Incidence of interstitial pneumonia - Incidence and severity of acute graft versus host disease (GVHD) - Relapse rates - Survival For any variable measuring time to an event, if that event never occurred in the Day 0 to Day 42 window (e.g. ANC never fell below 500/mm3), then the subject was set to zero and set to missing in separate analyses for that variable. Laboratory toxicity grades were calculated using standard common toxicity criteria issued by the national cancer institute to define these grades. Adverse events were graded according to a scale of 1 to 4 as the most severe and classified by term and body system with the COSTART dictionary.
STATISTICAL CONSIDERATIONS	Analysis population All patients were included in efficacy and safety analyses.

	Efficacy analysis Time to ANC or WBC recovery, duration of hospitalization and survival were analyzed with the Kaplan-Meier method and compared between groups using Generalized Wilcoxon test, controlling for site and baseline risk as strata.