National Institute of Allergy and Infectious Diseases

Efficacy of Filgrastim in the Treatment of the Hematopoietic Syndrome of the Acute Radiation Syndrome

Briefing Document

Joint Meeting of the of the Medical Imaging Drugs Advisory Committee and Oncologic Drugs Advisory Committee

US Food and Drug Administration

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Division of Allergy, Immunology and Transplantation NIH/NIAID
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List of Abbreviations

ANC	absolute neutrophil count
ARS	acute radiation syndrome
BLA	Biologics License Application
CDC	Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
DAIT	Division of Allergy, Immunology and Transplantation
DMIP	Division of Medical Imaging Products, CDER, FDA
DNO	did not observe
DRR	dose response relationship
E. coli	*Escherichia coli*
FDA	Food and Drug Administration
FN	febrile neutropenia
G-CSF	granulocyte colony-stimulating factor
GLP	Good Laboratory Practice
GM-CSF	granulocyte macrophage colony-stimulating factor
Gy	Gray
H-ARS	Hematopoietic Syndrome of the Acute Radiation Syndrome
HGFs	hematopoietic growth factors
hr	hour
IL-3	interleukin-3
IND	Investigational New Drug Application
IV	intravenous
$LD_{50/60}$	lethal dose at sixty days in approximately 50% of the population
LGFs	leukocyte growth factors
LINAC	linear accelerator
MCM	medical countermeasures
MODS	multiple organ dysfunction syndrome
MOF	multiple-organ failure
MST	mean survival time
NE	nonestimable
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PD	pharmacodynamics
PK	pharmacokinetics
PLTs	platelets
RN	radiation nuclear
SC	subcutaneously
sBLA	supplemental Biologics License Application
SD	Study Day
SEM	standard error of the mean
SNS	Strategic National Stockpile
TBI	total body irradiation
^{60}Co	Cobalt-60
^{137}Cs	Cesium-137

^{192}Ir	Iridium-192

Executive Summary

This document has been prepared by the Division of Allergy, Immunology and Transplantation (DAIT) of the National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health (NIH), termed NIAID in this document. The document is intended to provide information in preparation for the Food and Drug Administration, Center for Drug Evaluation and Research, Joint Medical Imaging and Oncologic Drugs Advisory Committee Meeting on May 3, 2013, during which NIAID will present efficacy data for filgrastim (Amgen Inc., Thousand Oaks, CA) therapy for the treatment of individuals who develop the Hematopoietic Syndrome of the Acute Radiation Syndrome (H-ARS) following exposure to ionizing radiation.

Data from two animal studies are presented in this document. The first (Study AXR01) is a model characterization study, defining a rhesus macaque model of H-ARS. The second (Study AXG15) is an efficacy study, utilizing the rhesus macaque model of H-ARS that was characterized in Study AXR01.

Study AXG15 demonstrated a significant improvement in survival in rhesus macaques receiving filgrastim (10µg/kg/d, subcutaneous [SC], starting approximately 24 hours after exposure to a lethal dose at 60 days in approximately 50% of the population [$LD_{50/60}$] of ionizing radiation) and concomitant medical management compared to animals receiving medical management alone. Medical management included administration of fluids, antibiotics, antiemetics, antipyretics, antidiarrheals, pain medication, and blood products based on objective criteria as assessed by blinded study personnel. Filgrastim treatment also led to an improvement of neutrophil-related parameters and a decrease in the incidence of infection.

1.0 Introduction

The Division of Medical Imaging Products (DMIP) of the United States (US) Food and Drug Administration (FDA) is convening an Advisory Committee meeting on May 3, 2013 to discuss the safety and efficacy of currently approved leukocyte growth factors (LGFs) as potential treatments for radiation-induced myelosuppression associated with a radiological/nuclear incident. The DMIP defines myelosuppression as a reduction of blood cell production, which can be caused by radiation exposure. Currently approved LGFs include NEUPOGEN® (filgrastim, Amgen, Inc.), Neulasta® (pegfilgrastim, Amgen, Inc.), Leukine® (sargramostim, Genzyme, Inc.), and tbo-filgrastim (tbo-filgrastim, Sicor Biotech UAB). The DMIP invited NIAID to present efficacy data demonstrating the efficacy of filgrastim in improving survival in a rhesus macaque model of H-ARS. Safety and other supportive information are described in Amgen's labeling for NEUPOGEN® (filgrastim).

The DAIT has prepared this briefing document for the above referenced meeting and the contents address the following:

1. Background and regulatory history of this project,
2. A description of the clinical H-ARS and available treatments (see Section 2.0),
3. Characterization of a rhesus macaque (*Macaca mulatta*) model of H-ARS (Study AXR01) (see Section 2.0) and linkage to the clinical syndrome,
4. A rationale for selection of the dose of filgratim used in Study AXG15 (see Section 3.0),
5. Data from Study AXG15, demonstrating the efficacy of filgrastim in improving survival in lethally irradiated rhesus macaques manifesting H-ARS (see Section 4.0),
6. The Amgen Package Insert for NEUPOGEN® (filgrastim) which contains a summary of the safety profile of the product (see APPENDIX).

Currently, there are no FDA-approved drug products for the treatment of H-ARS. Treatment protocols for persons exposed to potentially lethal doses of radiation in the accident setting have been based on experience managing the symptoms arising from clinical experience with radiotherapy. Research using animal models of H-ARS has also added to the understanding of the treatment of H-ARS. A paradigm for treatment of H-ARS following unintended radiation exposure has emerged.

The traditional clinical development path leading to marketing authorization using human clinical trials for products to treat H-ARS resulting from unintentional radiation exposure is not possible due to the fact that such human clinical trials cannot be ethically conducted. Such products could only receive FDA approval via the FDA Animal Rule using data from efficacy studies in well-defined animal models of H-ARS. A drug product approved via the FDA Animal Rule and indicated for the treatment of H-ARS would allow rapid access and ease of distribution of the treatment to individuals with H-ARS.

1.1 Pharmacological Class

Filgrastim is a recombinant human granulocyte colony-stimulating factor (G-CSF) (175 amino acids with a molecular weight of 18,800 daltons) expressed in *Escherichia coli* (*E. coli*). Granulocyte colony-stimulating factor is a cytokine produced by monocytes, fibroblasts, and endothelial cells and regulates neutrophil production within the bone marrow and neutrophil progenitor proliferation, maturation, differentiation, and activation (phagocytosis, chemotaxis, respiratory burst, and antibody-dependent killing). Granulocyte colony-stimulating factor also mobilizes hematopoietic stem cells from the bone marrow to the periphery. Normal plasma levels of G-CSF in humans are approximately <10 pg/mL of plasma. The activity of G-CSF is neutrophil lineage-specific and human G-CSF cross reacts with other species, including mouse, dog, and nonhuman primate (NHP).

Endogenous G-CSF is a glycoprotein; however, because filgrastim is expressed in *E. coli*, it is nonglycosylated. Filgrastim also has an additional N-terminal methionine which is necessary for expression in *E. coli*.

1.2 Proposed Indication and Dosage

Study AXG15 was designed as a proof-of-concept Good Laboratory Practice (GLP), efficacy study conducted to demonstrate the efficacy of filgrastim in improving survival in support of the following proposed label indication: "Filgrastim is indicated for improvement of survival in individuals exposed to potentially lethal doses ionizing radiation leading to Hematopoietic Syndrome of Acute Radiation Syndrome (H-ARS)."

Anticipated use in a field setting

In the event of a public health emergency involving unintended exposure to radiation, a large number of casualties would require treatment from radiation exposure. As described in Section 2.0, acute radiation syndrome (ARS) occurs after whole-body or significant partial-body irradiation of greater than 1 Gray (Gy) delivered at a relatively high-dose rate. The bone marrow (hematopoietic stem cells and progenitor cells) of the hematopoietic system is one of the organs that is most sensitive to ARS, with increases in radiation exposure resulting in exponential loss of cells. (*1-3*)

The time of onset and severity of H-ARS depends on the level of radiation. Clinical manifestations of the H-ARS can be seen following radiation exposures of <2 Gy to 10 Gy. The H-ARS is characterized in humans by dose-dependent bone marrow depression leading to neutropenia, thrombocytopenia, and anemia. Neutropenia and thrombocytopenia begin approximately 2 days following radiation exposure, leading to other pathologies related to loss of immune and clotting function. The loss of platelets increases the chance of hemorrhage, slows down wound healing, and decreases the likelihood of survival. Loss of neutrophil function increases the risk of opportunistic

infection and patients are susceptible to febrile neutropenia. (*4*) Death due to H-ARS from infection or excessive bleeding occurs within 2 to 3 weeks after exposure

Treatment strategies for personnel exposed to potentially lethal doses of radiation have been the subject of several international conferences and working groups during the past 15 years. (*5-8*) In 2004, the Strategic National Stockpile (SNS) Radiation Working Group (*4*) recommended treatment of individuals inadvertently exposed to radiation using hematopoietic cytokines, such as filgrastim, based on the review of case studies (Section 2.0) where growth factors were given to increase neutrophil levels, and were found to be generally effective. An important component of treatment in these case studies was the noncytokine supportive care (medical management) provided to these patients.

The SNS Radiation Working group (*4*) recommended that cytokines be administered when the subject meets one or more of the following criteria:

- dosimetry indicates a 2.0 Gy exposure to otherwise healthy adults,
- onset of vomiting is within 2.6 hours of the radiation exposure, or
- lymphocyte depletion rate constant of ≥0.252 is calculated.

It is anticipated that filgrastim will be administered at its currently approved dose of 5 μg/kg/day, SC, and will be administered "to effect," defined as when the absolute neutrophil count (ANC) returns to 1000/μL for 3 consecutive days or when ANC exceeds 10,000/μL. Also, it is expected that filgrastim will be provided in the context of medical management including intravenous (IV) fluids, antibiotics, and blood products (red blood cells and platelets [PLTs]) as needed per medical management guidelines. (*4, 9*)

Treatment would need to be started soon after triage, with treatment depending on calculated radiation exposure and clinical symptoms. The use of filgrastim in such a scenario would follow the treatment decision plan described in Figure 1-1.

Figure 1-1. Treatment Decision Plan

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        ┌─────────────────────────────────────┐
        │  Radiation exposure estimated using │
        │  physical dosimetry and clinical    │
        │  symptoms                           │
        └─────────────────────────────────────┘
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Clinical signs and/or physical dosimetry indicate potential for H-ARS	Clinical signs and/or physical dosimetry DO NOT indicate potential for H-ARS
Administer filgrastim (daily up to 4 weeks OR "to effect" – after nadir ANC >1000/µL for 3 consecutive days or when ANC exceeds 10,000/µL). Monitor ALC and ANC	Monitor ALC and ANC. Initiate filgrastim if: lymphocyte depletion rate constant is ≥0.252 for 4 days and or ANC<500/µL

H-ARS: Hematopoietic Syndrome of the Acute
Radiation Syndrome
ANC: Absolute Neutrophil Count
ALC: Absolute Lymphocyte Count

1.3   Regulatory History

The events of September 11, 2001 led to the enactment of legislation, including those targeted to the development of medical countermeasures. The FDA issued the "Animal Rule" (21 Code of Federal Regulations [CFR] 314.600 subpart I and 21 CFR 601.90 subpart H) to allow for "Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible" in May 2002. The Project BioShield Act of 2004 (Public Law 108-276) provided funding for procurement of medical countermeasures for the SNS and new authorities to the Department of Health and Human Services (delegated to the NIH) to speed the research and development of medical countermeasures against chemical, biological, radiological and nuclear threats. The NIH delegated the responsibility for the development of radiation nuclear (RN) medical countermeasures (MCM) for to the NIAID, NIH.

The NIAID selected filgrastim as one of the first products to develop as an MCM in the event of a RN incident based on: (i) the recommendations of the SNS Working Group, described above; (ii) the fact that it is licensed in the US for multiple indications (see Amgen Package Insert for NEUPOGEN® in the APPENDIX); (iii) filgrastim is stockpiled by the Centers for Disease Control and Prevention (CDC) in the SNS; and (iv) the CDC has in place an Investigational New Drug Application (IND) (with the FDA) that contains a clinical protocol detailing how filgrastim would be administered in a RN incident.

To develop filgrastim as an MCM that could be indicated for improvement of survival in individuals exposed to potentially lethal doses ionizing radiation leading to H-ARS, the NIAID established a collaboration with Amgen, Inc, (the manufacturer of filgrastim) and a contract with the University of Maryland, Baltimore (for conduct of animal studies) to provide data in support of an Amgen supplemental BLA (sBLA) for the use of filgrastim in the treatment of H-ARS.

The NIAID and representatives from Amgen and the University of Maryland initiated meetings with the FDA (Office of Counter-Terrorism and Emergency Coordination and Division of Biological Oncology Products, Center for Drug Evaluation and Research [CDER], FDA) to discuss the requirements of the FDA Animal Rule and the studies (types, species, design, endpoints) that would provide the evidence of effectiveness for a sBLA to be submitted by Amgen. The development plan under NIAID's PreIND 100228 was based on these interactions and is delineated in Table 1-1.

Table 1-1. Proposal for Supplemental Biologics License Application Approval per the "Animal Rule" (21 CFR 314.600 subpart I and 21CFR 601.90 subpart H)

Final Rule Requirement	Supporting Data	Proposed Source
Reasonably well-understood pathophysiological **mechanism for the toxicity** of the chemical, biological, radiological, or nuclear substance and its **amelioration or prevention** by the product.	Mechanism of radiation-induced hematological toxicity	Literature
	Demonstration that filgrastim can ameliorate this toxicity.	Nonclinical studies in rhesus macaques and mouse Ex vivo assays to be determined
Effect is demonstrated in more than 1 animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model … for predicting the response in humans.	Data from GLP studies designed to show efficacy of filgrastim in rhesus macaques and mouse models of H-ARS	Nonclinical GLP pivotal studies in rhesus macaques and mouse Ex vivo assays performed by Amgen
Animal study **endpoint** is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity	Study endpoint in rhesus macaque and mouse studies is improved overall survival.	Nonclinical GLP pivotal studies in rhesus macaques and mouse
The data or information on the pharmacokinetics (PK) and pharmacodynamics (PD) of the product or other relevant data or information in animals and humans is sufficiently well understood to allow **selection of an effective dose in humans**, and it is therefore reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans	PK and PD measurements in rhesus macaque and mouse GLP studies	Ex vivo assays performed by Amgen
	PK and PD data from human clinical trials	Amgen's BLA 103353 for NEUPOGEN®.
Human Safety	Data from human clinical trials and postmarketing safety data	Amgen's BLA 103353 for NEUPOGEN®.
Postmarketing Studies (after sBLA approval)	To be determined	Amgen

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DAIT, NIAID, NIH
March 28, 2013

Following the conduct of the pilot study in rhesus macaques and prior to the initiation of the efficacy study in rhesus macaques, Amgen suspended their participation in this NIAID MCM development program. The loss of a corporate partner (and potential sBLA sponsor) led to changes in the goals of the studies to be conducted by NIAID and the University of Maryland. Instead of conducting the GLP animal studies that would provide the evidence of effectiveness for a sBLA to be submitted by Amgen for licensure per the FDA Animal Rule, the NIAID would conduct studies and submit the data to the FDA:

1. To support the use of filgrastim in a public health emergency, for example under CDC's IND for filgrastim, and
2. To support licensure for the use of filgrastim in the treatment of H-ARS for potential use by a corporate manufacturer of filgrastim.

In addition, the ability to conduct certain ex vivo assays associated with our animal studies (such as pharmacokinetics [PK, both human and animal species], immunogenicity, and bioactivity of filgrastim), was lost as these were originally to be conducted by Amgen.

The NIAID completed 2 studies in the rhesus macaques and the data from these 2 studies are presented in this briefing package. Study AXR01 was conducted to characterize the rhesus macaque model of H-ARS. Study AXG15, designed as a proof-of-concept GLP efficacy study conducted to demonstrate the efficacy of filgrastim in the rhesus macaque model of H-ARS defined in Study AXR01, was terminated early for efficacy after the interim analysis showed a statistically significant improvement in survival of filgrastim-treated animals compared to controls.

In July 2011, the NIAID submitted final study reports for studies AXR01 and AXG15 to the FDA (Division of Biologic Oncology Products, CDER).

In November 2011, the NIAID received a request for information from the Division of Medical Imaging Products (DMIP), CDER, FDA, for additional data from study AXG15 which suggested that oversight of PreIND 100228 now resided with DMIP.

In October 2012, the NIAID was invited to present the data from Study AXG15 at this Advisory Committee Meeting and data were requested in electronic format to allow FDA to conduct its own analyses of the data from AXG15.

In December 2012, the FDA audited the GLP labs of the Preclinical Radiobiology Laboratory at the University of Maryland, School of Medicine, in Baltimore.

In February 2013, the date of May 03, 2013 for the Advisory Committee Meeting was scheduled.

2.0 Hematopoietic Syndrome of the Acute Radiation Syndrome

Acute radiation syndrome occurs after whole-body or significant partial-body irradiation of greater than 1 Gy delivered at a relatively high-dose rate. The clinical components of the ARS are dose dependent and include the hematopoietic, gastrointestinal, cerebrovascular, and central nervous system syndromes as depicted in Figure 2-1. (*1, 4, 10*) These syndromes are often listed separately; however, at the higher radiation exposures, patients will experience multiple organ dysfunction syndrome (MODS) or multiple-organ failure (MOF). (*11*)

Figure 2-1. Acute Radiation Syndromes Relative to Exposure Dose
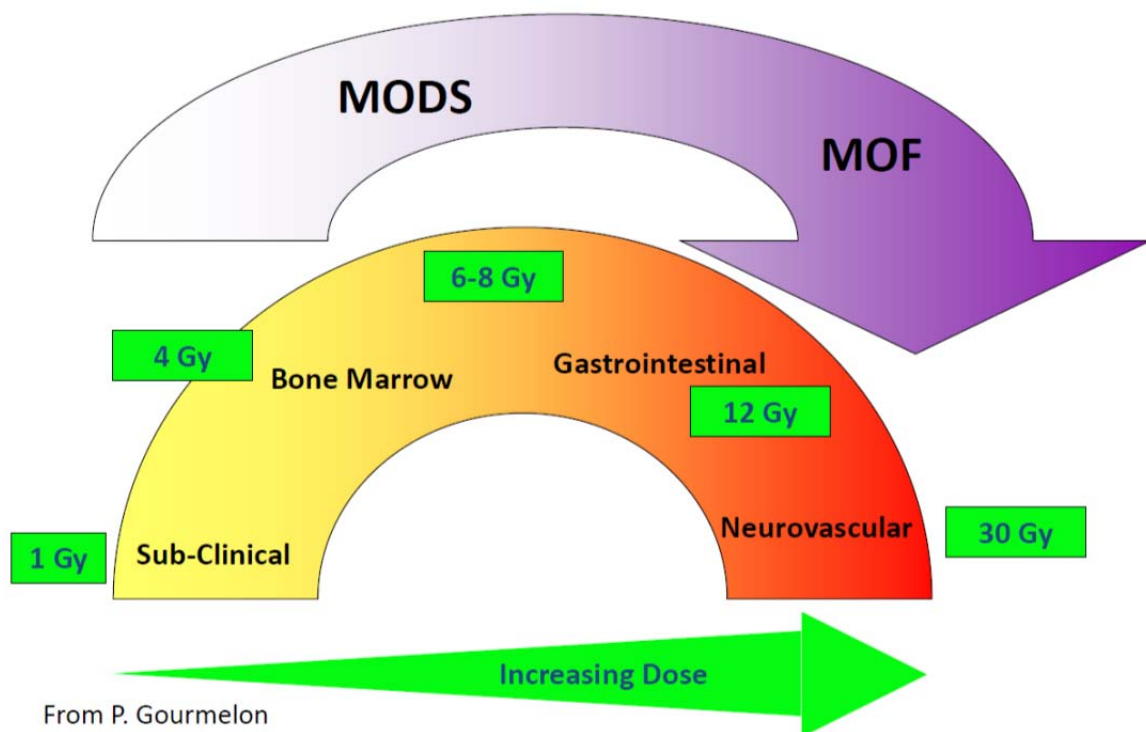
From P. Gourmelon

The bone marrow (hematopoietic stem cells and progenitor cells) of the hematopoietic system is one of the organs that is most sensitive to ionizingradiation. The H-ARS is dose- and time-dependent, with increasing mortality and morbidity as exposure dose increase. (*1-3*)

The time of onset and severity of H-ARS depends on the level of radiation exposure. Clinical manifestations of the H-ARS can be seen following radiation exposures of <2 Gy to 10 Gy. The approximate $LD_{50/60}$ for humans is 3.25 to 4.50 Gy without medical management. (*4*) However, when medical management (such as intravenous fluids, antibiotics, blood products) is provided, the $LD_{50/60}$ can increase to 6.0 to 7.0 Gy. (*4, 10, 12*)

The H-ARS in humans is characterized by dose-dependent bone marrow depression leading to neutropenia, thrombocytopenia, and anemia. Neutropenia and thrombocytopenia begin approximately 2 days following radiation exposure, leading to other pathologies related to loss of immune and clotting function. The loss of platelets increases the chance of hemorrhage, slows down wound healing, and decreases the likelihood of survival. Loss of neutrophil function increases the risk of opportunistic infection and patients are susceptible to febrile neutropenia. (*4*) Death due to H-ARS from infection or excessive bleeding occurs within 2 to 3 weeks after exposure. Survival following H-ARS is dependent on the recovery of the hematopoietic stem and progenitor cells so that production of mature, functional neutrophils, and PLTs can occur. Neutrophil recovery is inversely related to radiation exposure and can occur 3 to 4 weeks following radiation exposure. (*4, 13, 14*)

It is anticipated that radiation exposures in a public health emergency would be heterogeneous, thus sparing some areas of the body. Despite the radiation-induced cell death, some hematopoietic progenitor cells may escape destruction, either because they are in a noncycling state and are, therefore, more radio-resistant or may be in a bone marrow compartment that has been shielded. The cells that are spared can act as targets for cytokines, which could hasten leukocyte recovery.

2.1 Previous Human Experience with Treatment of H-ARS and Unmet Medical Need

Data generated from humans exposed to radiation either during radiation therapy, accidents, or nuclear weapons have served as the source of information to determine the human radiation dose-response relationship (DRR) and its modification by medical management and hematopoietic growth factors (HGFs). (*1, 4, 10, 15*)

A great deal of knowledge has been gained by studying the victims of the Chernobyl radiation accident and other accident cases treated by the Clinical Department, Institute of Biophysics, Moscow, Russia and documented in the database created in collaboration with the Department of Clinical Physiology, Occupational and Social Medicine, University of Ulm, Germany. These data showed that after total body irradiation (TBI), severity of ARS could be predicted by measuring time to peripheral blood neutrophil decrease to $500/\mu L$ (*9, 16-18*). Twenty-eight patients died from the Chernobyl accident, of these 28 patients with, 14 demonstrated spontaneous recovery of hematopoiesis even in the absence of treatment with growth factors. The recovery of hematopoiesis in the 14 of 28 patients who died, suggested that spontaneous regeneration of hematopoiesis can occur after total body exposure up to 8 Gy.

Evaluation of the neutrophil curves from 18 patients that experienced estimated TBI of 4.7 to 8.3 Gy showed that each patient experienced an ANC $<100/\mu L$ within a range of 1 to 4 days after the ANC $<500/\mu L$. (*15*) It was also noted that fever and infection coincided with neutropenia in the exposure doses of 4 to 5 Gy and was noted as being more "aggressive" in all patients in the 5 to 6 Gy exposure range.

In subsequent radiation accidents, cytokines such as G-CSF, granulocyte macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3) have been used to treat ARS in individuals exposed to radiation accidentally and provide some anecdotal evidence for the safety and efficacy of cytokines in the treatment of ARS. The use of HGFs in radiation accidents is limited to approximately 40 patients and is summarized below along with information from other radiation accidents where no HGFs were administered (Table 2-1). Of these patients, some received G-CSF or GM-CSF and others received various combinations of HGFs and bone marrow transplants. Few patients received HGFs immediately after exposure; most received the growth factors weeks later, and treatment was often started only after precipitous drops in neutrophil counts. Despite the delay in HGF administration, ANC increased after treatment; however, not all patients survived

Table 2-1. Sources of Information on the Relationship Between Radiation Dose and Lethality

Source of Radiation	Time/Place	# Exposed/ Estimated Exposure	Treatments	Reference
15-kiloton nuclear device	Hiroshima, Japan 1945	N=150,000 casualties and 75,000 fatalities, estimated that all survivors had less than 3 Gy exposure	Medical management (limited)	(19)
Iridium-192 (^{192}Ir) source	Gilan, Iran 1986	N=1 with exposure estimate of 2.8 to 4.7 Gy	N=1, G-CSF (400 μg bid SC, 300 μg/day SC)	(20)
Nuclear Reactor	Chernobyl Ukraine 1986	N=214 with exposure estimates of 1 to >13 Gy	Medical management	(15-18, 21)
Cesium-137 (^{137}Cs) radiotherapy unit	Goiania, Brazil, 1987	N=10, estimated total body doses of 2.5 to 7.0 Gy	GM-CSF (500 μg/m^2/day, IV infusion)	(22)
Cobalt-60 (^{60}Co) medical sterilizer	San Salvador, 1989	N=3, estimates ranged from 3to 10Gy total body with localized exposures (feet, legs) of 20 Gy, at least in 2 of the 3 workers	GM-CSF (240 μg/m^2, IV)	(23)
^{60}Co from atomic reactor	Israel, 1990	N=1, estimated dose of >10 Gy	IL-3 and GM-CSF on day 1 after a bone marrow transplant	(24)
^{60}Co	Nyasvizh, Belarus,	N=1, estimated dose 12 to 15 Gy	GM-CSF early (days 3-6) and then the combination of	(25)

Table 2-1. Sources of Information on the Relationship Between Radiation Dose and Lethality

Source of Radiation	Time/Place	# Exposed/ Estimated Exposure	Treatments	Reference
	1991		IL-3 and GM-CSF (days 6-31)	
^{60}Co source	Istanbul, Turkey, 1998	N=10, body doses range from 10 to 20 Gy (5 patients) and 20 to 40 Gy (5 patients)	N=5, G-CSF in 7 patients: 5-8 µg/kg/day, platelets, antibiotics	(26)
Mixed neutron:γ radiation	Tokaimura, Japan, 1999	N=3, 8 to 13 Gy	N=2, Stem cell transplant and G-CSF, erythropoietin, thrombopoietin	(27, 28)
^{192}Ir - source	Yanango, Peru, 1999	N=1, total body dose was <3 Gy with 80 Gy to the right thigh area	N=1, G-CSF (300 µg/day)	(29)
Teletherapy Head ^{60}Co	Samut Prakarn Province, Thailand, 2000	N=10, estimated dose of ≥2 Gy and of the 10, at least 4 received doses >6 Gy	N=9, G-CSF (10 µg/kg/day) and GM-CSF (300 µg/day)	(30)
^{192}Ir - source	Meet Halfa, Egypt, 2000	N=7, estimated doses of 3.4 to 8.4 Gy	N=4, G-CSF (10 µg/kg/day)	(31)
^{60}Co medical sterilizer	Fleurus, Belgium	N=1, estimated dose of 4.4 to 4.8 Gy	G-CSF, EPO, stem cell factor	(32)

In all of the above, where colony-stimulating factors were administered, medical management was also provided. Medical management includes: fluid replacement, nutritional support, pain management, and administration of antiemetics, antipyretics, antidiarrheals, and antibiotics. In the case of bleeding or thrombocytopenia, blood or platelets would be administered.

The goal of antibiotic treatment administered after exposure to radiation, is to reduce the presence of Enterobacteriaceae species while preserving the anaerobic gut flora, targeting primarily the gram-negative bacteria that could translocate across the intestinal epithelium and cause sepsis. Targeting gram-negative bacteria should be the first line of defense with secondary targeting of gram- positive bacteria; therefore, the recommendation is for the use of a fluoroquinolone with streptococcal coverage or fluoroquinolone without streptococcal coverage plus penicillin. The treatment should continue until the patient recovers or develops febrile neutropenia. In the latter case, the combination of ciprofloxacin plus amoxicillin-clavulanate should be used. (33) Brook, et al. specify the use of the fluoroquinolones ciprofloxacin or levofloxacin as the

antimicrobials of choice, noting the expanded gram-positive coverage of levofloxacin. (*4, 34*)

Medical management of patient symptoms is an important component of the response to radiation exposure; however, these treatments are symptom-based and do not address the underlying injury. Currently, in the US, there are no licensed products available for the treatment of the underlying injury associated with H-ARS and, therefore, treatments that mitigate damage or hasten recovery given in conjunction with medical management are needed.

Treatment strategies for personnel exposed to potentially lethal doses of radiation have been the subject of several international conferences and working groups during the past 15 years. (*5-8*) Based on the consensus for treatment of radiation injuries developed at these meetings as well as the meeting of the SNS Radiation Working Group (*4*), the CDC has developed a protocol entitled "NEUPOGEN for the treatment of ARS following a radiological incident" under BB IND #11,510. In the CDC's protocol submitted to the FDA under its IND, individuals who are exposed to radiation in the range between 300 and 1000 cGy and have a diagnosis of the hematopoietic syndrome as manifested by neutropenia (ANC $\leq 500/\mu L$) will be treated with filgrastim at 5 μg/kg/day SC in combination with medical management (IV fluids and antibiotics). Treatment will continue until ANC is >1000/μL for 2 to 3 consecutive days.

2.2   Study AXR01: Characterization of a Rhesus Macaque Model of H-ARS

An essential component of the FDA Animal Rule is the use of a well-characterized animal model to demonstrate the efficacy of the drug product being developed. The determination of the efficacy of candidate MCMs for mitigation of H-ARS required the development and characterization of a reliable rhesus macaque model of H-ARS. Nonhuman primates exhibit radiation responses similar to what has been observed in people; and the manifestations H-ARS following radiation exposure are described below. A critical component of defining the rhesus macaque model was the determination of the radiation exposure levels which define H-ARS in this species.

Study AXR01 (*35*) was conducted to define a rhesus macaque model of H-ARS. This well-characterized model of H-ARS would be used to assess the efficacy of drug products proposed for the treatment of H-ARS. Forty-eight male rhesus macaques (*Macaca mulatta)* were exposed to 6 MV of bilateral, TBI administered by a photon linear accelerator (LINAC). Animals were exposed to lethal radiation exposures ranging from 7.2 Gy to 8.9 Gy at an approximate dose rate of 0.80 Gy/min with the objective of defining the lethal radiation exposure curve that would define H-ARS in the rhesus macaque. The radiation dose was delivered at midline tissue at the xiphoid process.

After irradiation, the animals were provided with medical management (antibiotics, fluids, nutritional support, and blood products) in order to mirror the clinical scenario. Provision of medical management was based on objective criteria such as cage-side

observations, clinical signs, etc. Animals were followed for 60 days after irradiation with the intent of determining the lethal doses at 30 days after irradiation with a 30-day follow-up.

After irradiation, animals were monitored daily. Complete blood counts were performed daily between Study Day (SD) 0-21, and at least every third day thereafter. Body weights and core body temperatures were collected at the same time points. Blood cultures were obtained if febrile neutropenia (FN) [defined as core body temperature >103°F and an ANC <500/μL], was observed. Parameters measured included survival at 60 days (d) after TBI; secondary endpoints included hematopoietic parameters (neutrophil and platelet nadirs, respective durations of cytopenia and time to recovery to cellular thresholds), number of transfusions, incidence of documented infection, febrile neutropenia, and mean survival time (MST) of decedents.

In this study, the antibiotic used as first line of defense was the fluoroquinolone enrofloxacin, which was given when the ANC was <500/μL and was continued until the animal maintained an ANC >500/μL for 48 hours. Additionally, gentamicin sulfate was administered in combination with enrofloxacin when the body temperature ≥103°F and was continued for 24 hours. If FN persisted or microbial resistance was demonstrated to either enrofloxacin or gentamicin, ceftriaxone was administered. Imipenem/cilastatin was administered when microbial resistance was demonstrated to enrofloxacin, gentamicin, and ceftriaxone.

Rhesus macaques in Study AXR01 were exposed to lethal doses of ionizing radiation and were euthanized if they met one or a combination of the criteria described below:
   a. An observation of <u>any one</u> of the following signs is justification for euthanasia:
       1. Inactivity: recumbent in the cage with decreased or absent responsiveness to touch.
       2. Seizure activity that is either not responsive to medication or that continues despite medication, as determined by a veterinarian.
       3. Hemorrhage from the gastrointestinal tract or other orifice estimated to be in excess of 20% of estimated blood volume in any 24-hour period.
       4. Hyperthermia (rectal temperature ≥106°F).
       5. Loss of body weight >25% for 72 hours.
       6. Hypothermia (rectal body temperature <96°F) for >6 hours.
       7. Self-mutilation: If not responsive to increased "enrichment efforts" over a 2-week period or if repeated surgical intervention is required from such trauma.
   b. Observations of a <u>combination of two</u> of the following signs are justification for euthanasia:
       1. Respiratory distress: Labored breathing (pulse of <90 and >40 breaths/min).
       2. Abnormal activity: difficulty with ambulation, decreased food and water intake, self-mutilation, reluctance to move for >24 hours.
       3. Clinical conditions:

    i. Severe dehydration,
    ii. Shallow respiration or
    iii. Hyperthermia (rectal temperature >105.5°F), which is unresponsive to antipyretic therapy over 72 hours.
    iv. Evidence of eye or upper respiratory infections that are nonresponsive to antibiotic therapy over 96 hours.
  4. Loss of body weight >20% of baseline body weight for >72 hours.
  5. Abnormal appearance: rough coat, head down, tucked abdomen, pallor, exudates around eyes and/or nose.
c. Observations of a severe injury or condition, such as but not limited to, bone fracture, progressive tissue necrosis, or severe internal bleeding, are justification for euthanasia.

Observation: Survival

Table 2-2. Study AXR01: 60-Day Mortality of Rhesus Macaques After 6 MV Photon Radiation With Medical Management			
Radiation Dose (Gy)	**Mortality**	**Survival time of decedents (days)**	
		Mean ± SEM	**Median**
7.20	3/8 (38%)	20.0 ± 5.5	15.0
7.55	4/8 (50%)	18.3 ± 2.1	18.5
7.85	6/8 (75%)	22.2 ± 6.0	16.5
8.05	5/8 (63%)	16.2 ± 3.0	14.0
8.40	6/8 (75%)	17.5 ± 1.6	17.5
8.90	8/8 (100%)	21.1 ± 3.6	18.0

Radiation dose was a significant predictor of mortality (P=0.01) with increasing mortality rates at the higher doses (Table 2-2). The $LD_{30/30}$, $LD_{50/30}$, $LD_{70/30}$, and $LD_{80/30}$ with their respective confidence intervals (CI) were calculated from the logistic regression model and are shown in Table 2-3 and Figure 2-2.

Table 2-3. Estimated Radiation Dose in Rhesus Macaques After 6 MV Photon Radiation With Medical Management – Study AXR01			
	LD estimate (Gy)	**Lower 95% CI (Gy)**	**Upper 95% CI (Gy)**
$LD_{30/30}$	7.06	5.01	7.50
$LD_{50/30}$	7.53	6.50	7.88
$LD_{70/30}$	7.99	7.60	8.65

Figure 2-2 shows the data from Study AXR01 (open squares, blue dotted line) and also includes historical data sets showing the dose response and calculated $LD_{50/30}$ values (95% CI) of rhesus macaques exposed to TBI from ^{60}Co gamma radiation (Eltringham, personal communication to T. MacVittie) or 2 Mev x-radiation. (36)

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Figure 2-2. Logistic Regression Probability of 30-Day Mortality of Rhesus Macaques After ^{60}Co, 2 Mev X-Ray, or 6 MV LINAC Radiation With and Without Medical Management - Study AXR01 (With Historical Data)
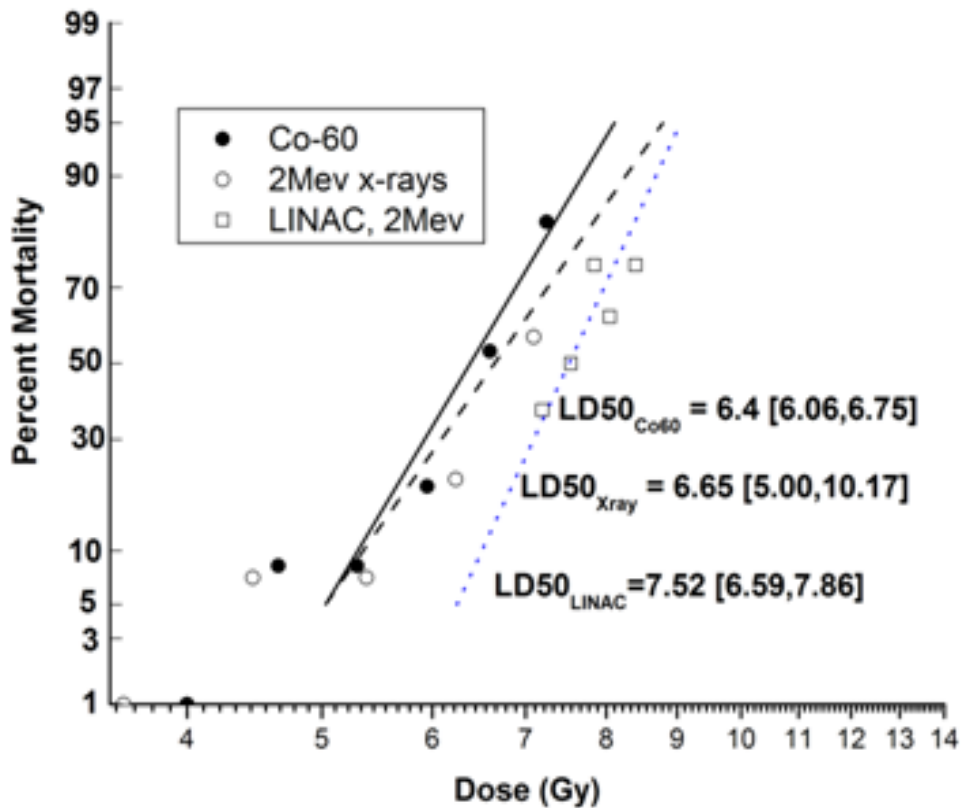
Radiation dose was a significant predictor of mortality (*P*=0.01) with increasing mortality rates at the higher doses. The LD$_{30/30}$, LD$_{50/30}$, and LD$_{70/30}$ levels of radiation in this study were determined with confidence intervals around each dose. The slope of the dose-response curve (20.42 probits/log$_{10}$ dose) appears to be more similar to what was observed in 2 other historical rhesus macaque studies in which medical management was not provided. One study (solid circles, solid black line) used ^{60}Co gamma radiation and the other (open circles, dashed black line), a 2 Mev x-radiation source; each had a respective DRR with slopes of 15.86 and 13.57 probits/log$_{10}$ dose. Another measure of the steep slope is the respective LD$_{90}$:LD$_{10}$. These were 1.48, 1.42, and 1.36 for the ^{60}Co gamma radiation, 2 Mev x-radiation, and 6 MV LINAC-derived photons, respectively, used herein. The respective DRRs and LD$_{90}$:LD$_{10}$ were not significantly different from each other and are a measure of support for predictive validity of the DRR reported herein relative to the historical NHP database for the H-ARS.

The MST of decedents for each radiation dose cohort in study AXR01 ranged from 16.2 to 22.2 days with the overall MST of decedents across all dose cohorts being 19.4 days. A review of the literature indicated that the MST for NHP exposed to TBI in the mid-lethal dose range and not provided medical management was 14.5 days. This comparison

with historical studies indicates that medical management enhances the LD_{50} value and survival across the lethal hematopoietic syndrome range.

Similar to humans, H-ARS in rhesus macaques increases in severity with radiation exposure. Human radiation dose-response relationships (*10*) were calculated from (i) the atom bomb explosions in Hiroshima and Nagasaki, after which victims received limited medical care, and (ii) from individuals exposed during the Chernobyl accident, where medical management (antibiotics, blood products) was provided. (*37*)

The improvement in survival of rhesus macaques that received medical management compared to historical data mirrors the improvement in survival in the Chernobyl victims, who received medical management, compared to the Hiroshima and Nagasaki victims. In rhesus macaques, the LD_{50} increased from about 6.5 Gy to about 7.5 Gy. In people, the increase was from 3.25-4 Gy to 6-7 Gy.

Observation: Neutrophils

Neutrophils provide the first line of defense against opportunistic infections and neutropenia is associated with greater risk of infection; therefore, various neutrophil-related parameters were determined:
- First day to Grade 3 (ANC <500/μL) and Grade 4 neutropenia (ANC <100/μL),
- Duration of neutropenia (ANC <500/μL, ANC <100/μL)
- Neutrophil nadir
- Time to recovery to ANC ≥500/μL and ANC ≥1000/μL
- Incidence and first day of FN (core temp 103°F, 39.4°C, ANC <500/μL)

Neutrophil recovery is shown in Figure 2-3. For this figure, radiation exposures that were closest to the calculated $LD_{30/30}$, $LD_{50/30}$, and $LD_{70/30}$ are shown. Despite the differences in radiation, neutrophil decline, nadir, and recovery for the different radiation exposure groups were similar.

Figure 2-3. Mean Absolute Neutrophil Counts in Rhesus Macaques Following Irradiation (6 MV LINAC) and Medical Management
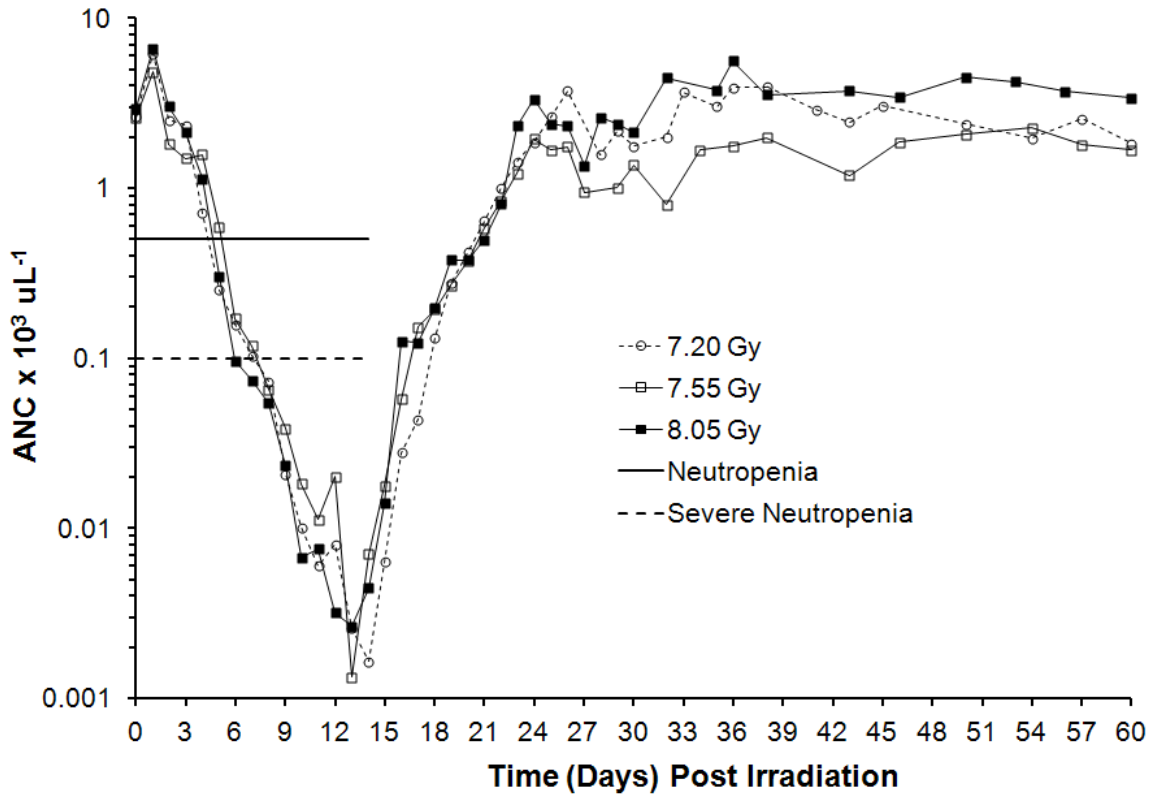
Neutrophil data are shown in Table 2-4 through Table 2-6.

Table 2-4. Neutrophil-Related Parameters for Rhesus Macaques Following Total-Body Irradiation							
TBI Dose (Gy)		First Day (d) and Range ANC <500/µL or 100/µL (n=8)		Duration (days and range) ANC <500/µL or 100/µL		Recovery to ANC ≥1000/µL	ANC Nadir (per µL) (n=8)
		<500/µL	<100/µL	<500/µL	<100/µL	≥1000/µL	
7.20	Mean[a]	4.6±0.3	7.3±0.3	16.0±0.5[b]	11.5±1.3[c]	23.4±0.8[b]	0
	Median	NA	NA	16.0[b]	10.5[c]	24.0[b]	0
	Range	4-6	6-9	15-18	9-18	21-24	NA
7.55	Mean[a]	5.5±0.6	7.1±0.4	24.0±7.3[d]	9.8±1.0[d]	26.7±3.7[e]	0
	Median	NA	NA	17.0[d]	9.5[d]	23.0[e]	0
	Range	3-6	5-8	16-46	8-12	22-23[f]	NA
7.85	Mean[a]	4.6±0.3	6.5±0.4	14.3±1.5[e]	10.3±0.9[e]	21.7±1.2[e]	0.5±0.5
	Median	NA	NA	14.0[e]	10.0[e]	21.0[e]	0
	Range	4-6	6-8	12-17	9-12	20-24	0-4
8.05	Mean[a]	5.0±0.0	6.5±0.3	15.0±1.0[e]	10.3±0.9[e]	24.7±2.7[e]	0
	Median	NA	NA	16.0[e]	10.0[e]	22.0[e]	0
	Range	NA	6-8	13-16	9-12	22-23	NA
8.40	Mean[a]	5.0±0.3	6.4±0.4	17.0±2.0[g]	12.7±2.3[e]	42.0±18[g]	0
	Median	NA	NA	NA	NA	NA	NA
	Range	4-6	5-8	15 or d19	8-15	24 or d27	NA
8.90	Mean[a]	4.5±0.2	6.3±0.3	DNO	DNO	DNO	0
	Median	NA	NA	NA	NA	NA	0
	Range	4-5	5-8	DNO	DNO	DNO	NA

[a]Mean data shown with the standard error of the mean (±SEM); [b]n=5; [c]n=6; [d]n=4; [e]n=3;
[f]One animal survived but the ANC after the nadir did not attain ≥1000/µL at study day 60; [g]n=2; ANC= absolute neutrophil count; DNO= did not observe; NA= all numbers were equal to the mean value or in the case of the median where it is not applicable due to n=2; TBI=total body irradiation

Table 2-5. Incidence and Severity of Neutropenia in Total-Body Irradiated Rhesus Macaques

TBI Dose (Gy)		ANC (d) <100/µL		ANC (d) <500/µL	
		First Day (n=8)	Final Day	First Day (n=8)	Final Day
7.20	Day	6	23[a]	4	22[b]
	Range	6-9	15-23	4-6	18-22
7.55	Day	5	18[b]	3	46[c]
	Range	5-8	15-18	3-6	20-46
7.85	Day	5	17[d]	4	20[d]
	Range	5-8	14-17	4-6	15-20
8.05	Day	6	18[d]	5	20[d]
	Range	6-8	15-18	NA	17-20
8.40	Day	6	21[d]	4	23[e]
	Range	6-8	15-21	4-6	20,23
8.90	Day	5	DNO	4	DNO
	Range	5-8	DNO	4-5	DNO

[a]n=6; [b]n=5; [c]n=4; [d]n=3 [e]n=2; NA=all numbers equal to the mean; DNO=did not observe

Table 2-6. Febrile Neutropenia and Antibiotic Requirements for Rhesus Macaques Following Total-Body Irradiation

TBI Dose (Gy)	First day FN (n=8)	Duration (d) FN	Days on Antibiotics	Days CBT ≥103.0°F (n=8)
7.20	8.0±0.7	6.4±2.0[a]	19.6±1.4[a]	11.1±1.8
7.55	10.0±1.3	8.5±2.1[b]	27.3±5.9[b]	12.5±3.7
7.85	7.1±1.1	7.5±0.5[c]	16.0±0[c]	13.1±3.0
8.05	7.6±2.1	1.0±0.6[d]	18.0±0[d]	5.5±1.0
8.40	10.9±0.9	6.5±3.5[c]	19.0±2.0[c]	7.0±1.4
8.90	8.5±0.8	DNO	DNO	8.9±1.9

[a]n=5; [b]n=4; [c]n=2; [d]n=3; DNO=did not observe.

The ANC decreased to <500/µL within 5 days across the H-ARS dose range. The ANC continued to decrease to <100/µL within the next 1.4 to 2.7 days after TBI. The mean duration of Grade 4 neutropenia for all dose cohorts (survivors and nonsurvivors) ranged from 9.8 to 12.7 days. The range for the mean duration of ANC <500/µL, Grade 3 neutropenia, was 14.3 to 24.0 days. The time to recovery to an ANC ≥1000/µL ranged from SD 21 to 24 after TBI for all radiation dose cohorts.

Antibiotics were administered when the ANC <500/µL with the expectation that the ANC will be <100/µL within 2 to 3 days and remain at that level or lower for a 7- to 10-day duration. Antibiotic use in the neutropenic animals followed the recommendations of the Infectious Disease Society of America (*38*) for treatment of people experiencing severe neutropenia. The first day of FN ranged from mean values of 7.1 to 10.9 across all exposure cohorts. The duration of FN ranged from 6.4 to 8.5 days and varied across the cohorts. The cohort exposed to 7.5 Gy, the approximate $LD_{50/60}$

value, experienced FN on Day 10, and had an 8.5-day duration of FN and was administered antibiotics for an average 27.3 days after TBI.

Information about the human response (neutropenia and recovery) to high radiation exposures was obtained from studying the time course of five cleanup workers (liquidators) who were exposed to radiation at Chernobyl and survived for at least 30 days. (*39*) ANC in these patients dropped to <500/µL 6-8 days after exposure, and reached nadir about 18 days after exposure, recovery of ANC to 500/µL occurred 30-38 days after exposure. The irradiated rhesus macaques also experienced neutropenia followed by recovery of ANC, albeit in a shorter time frame compared to the exposed workers. In the rhesus macaques, ANC dropped to <500/µL in 3-5 days, reached nadir in about 13 days and recovered to 500/µL about 20 days after exposure.

Observation: Platelets

Lethal doses of TBI induced a severe decrease in PLT levels for all radiation dose cohorts (Figure 2-4 and Table 2-7). Exposure of animals to TBI over the dose range for $LD_{30/60}$, $LD_{50/60}$, and $LD_{70/60}$ resulted in similar values for nadir, duration of PLT <20,000/µL, and recovery of PLT to >20,000/µL. The first day of PLT levels at 20,000 or 10,000/µL ranged from 8.6 to 9.7 after TBI. The duration of PLT levels <20,000/µL was 8.7 to 20.5 days. The animals recovered to PLT level >20,000/µL over a range of 18 to 30 days. The cohort exposed to 7.5 Gy, the approximate $LD_{50/60}$ value, had a mean first day to PLT <20,000/µL of 8.0 with the duration of PLT <20,000/µL of 19.8 days. The PLT recovery to 20,000/µL took on average 29.5 days with an average of 2.0 transfusions per animal. The first day to a transfusion was a mean of 11 days.

Figure 2-4. Mean Absolute Platelet Counts in Rhesus Macaques Following Irradiation (6 MV LINAC) and Medical Management
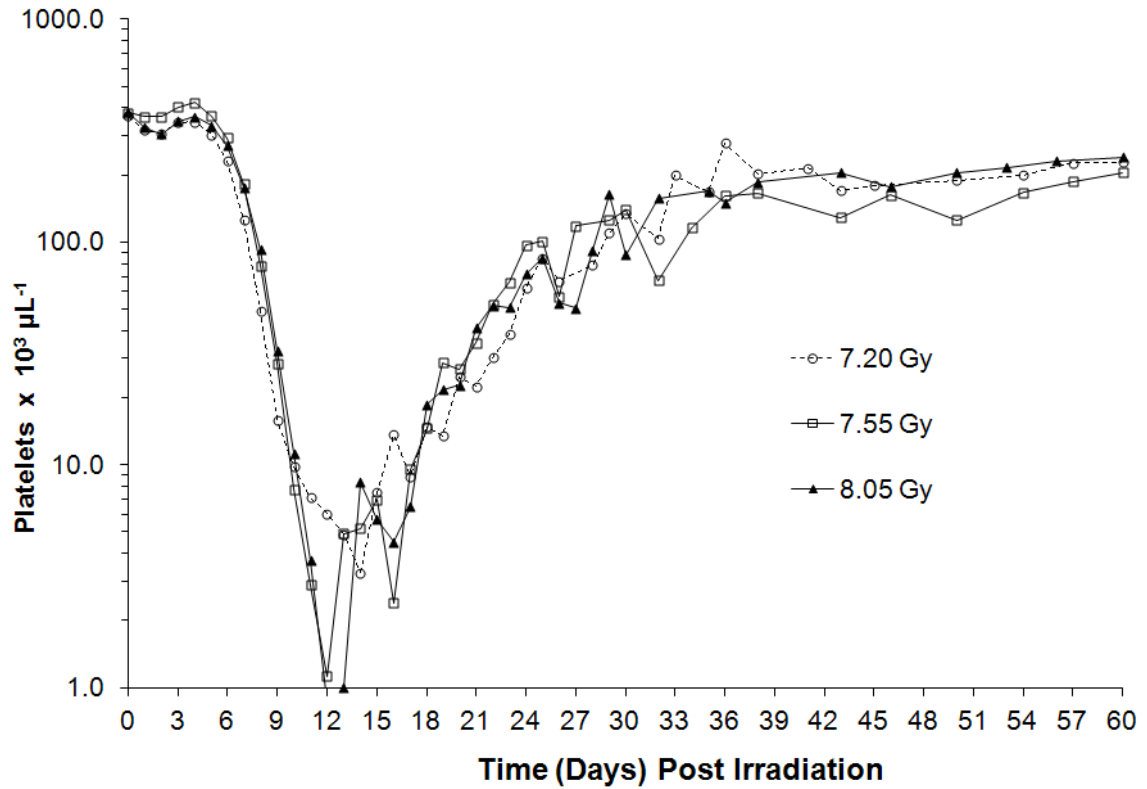
Table 2-7. Incidence and duration of thrombocytopenia and transfusion requirements in total-body irradiated rhesus macaques.

TBI Dose (Gy)		First Day PLT (day) <20,000/µL (n=8)	Duration (days)[a] <20,000/µL	Nadir[b] (PLT/µL) (n=8)	Recovery to PLT ≥ 20,000/µL	# Trans-fusions[b] (54 mL) (n=8)	First Day (d) Transfusion Occurred Within Radiation Cohort (n=8)
	Mean±SEM	8	12.6±1.8[c]	0.5±0.4	24.3±1.4[c]	2.3±0.6	9
7.20	Range	8-10	8-12	0-3	18-27	0.5-5.5	9-15
7.55	Mean±SEM	8	19.8±9.5[d]	0.4±0.2	29.5±10.2[d]	2.0±0.7	11
	Range	8-11	8-48	0-1	18-60	0.5-6.5	11-15
7.85	Mean±SEM	7	13.7±3.2[e]	0±0	22.7±3.2[e]	1.8±0.3	11
	Range	7-9	11-20	NA	19-29	0.5-3	11-13
8.05	Mean±SEM	9	8.7±0.3[e]	0.4±0.2	19.0±0.8[e]	1.5±0.5	10
	Range	9-11	8-9	0-1	18-20	0-4	10-14
8.40	Mean±SEM	8	20.5±1.5[f]	0.1±0.1	30.0±2.0[f]	2.3±0.6	11
	Range	8-10	19, 22	0-1	28, 32	0.5-5.5	11-13
8.90	Mean±SEM	9	DNO	0.1±0.1	DNO	3.2±0.9	11
	Range	9-10	DNO	0-1	DNO	1-9	11-15

[a]Durations do not include data from decedent animals unless recovery occurred to that level, e.g. PLT ≥20,000/µL prior to death; [b]The platelet nadir and number of transfusions includes both survivors and non-survivors; [c]n=5; [d]n=4; [e]n=3; [f]n=2; DNO=did not observe due to 100% lethality in the 8.90 Gy cohort.

A review of the System for Evaluation and Archiving of Radiation Accidents based on Case Histories (SEARCH), a database of people accidentally exposed to total-body irradiation showed that platelet levels reached nadir in 20-30 days with spontaneous recovery in 30-55 days. (*40*) Platelet kinetics in irradiated rhesus macaques in Study AXR01 showed a more rapid time course compared with humans exposed to radiation with nadir at 12-15 days and recovery in 18-30 days.

In Study AXR01following exposure of rhesus macaques to lethal doses of radiation, leukocyte levels dropped, resulting in severe neutropenia and thrombocytopenia. Neutrophil nadir was observed approximately 13 days after exposure and surviving animals underwent immune system regeneration, with neutrophils reaching 500/µL approximately 3 weeks after exposure. Furthermore, damage to the immune system increased susceptibility to opportunistic infection, with animals experiencing febrile neutropenia, and bacteremia. (*35*) Clinical signs such as dehydration, loss of appetite, weight loss, hemorrhage, infection, and diarrhea were also noted. All of these observations were similar to what would be seen in humans following radiation exposure leading to H-ARS. The similarities between the human disease and the rhesus macaque model described above indicate similar pathophysiological mechanisms and the triggers to treat (to determine the components of medical management) are similar to those that are observed in people exposed to radiation.

Managing the effects of radiation exposure through antibiotic administration, fluid and nutritional support, and providing blood products increased the survival in rhesus macaques compared to animals not provided any medical management. These observations mirror the human experience.

3.0 Filgrastim Dose Selection for Study AXG15

Selection of an efficacious dose of a drug product for human H-ARS will depend on the PK and pharmacodynamics (PD) of that drug product in the animal species used for the animal efficacy studies compared to the same parameters in humans.

NEUPOGEN® (filgrastim, Amgen, Inc.) is a marketed product that is approved in the US for a number of indications. The details of clinical studies conducted with filgrastim, including PK studies are summarized in the Amgen's US package insert for filgrastim (see APPENDIX).

Per Amgen's Package Insert for NEUPOGEN®, the currently approved dose, route, and schedule of administration of NEUPOGEN® for the treatment of cancer patients receiving myelosuppressive chemotherapy is 5 μg/kg/day SC, administered daily for up to 2 weeks, until postnadir ANC surpasses 10,000/μL. (*41*) In 2004, the SNS Radiation Working Group recommended administration of filgrastim at a dosing regimen of 5 μg/kg/day SC in patients exposed to radiation in the case of a radiation event. (*4*)

Pharmacokinetics

The NIAID conducted efficacy Study AXG15 in rhesus macaques to demonstrate the efficacy of filgrastim in improving survival in rhesus macaques with H-ARS. Although a number of human PK studies using filgrastim are available, data on the PK of filgrastim in the rhesus macaque are limited (Table 3-1). Based on the available data, C_{max} and AUC for the indicated filgrastim dose in human and the filgrastim dose used in rhesus macaque PK studies can be compared. C_{max} and AUC for a 10 μg/kg SC dose in rhesus macaques is similar to these values in human administered 5 μg/kg SC (compare Farese (*42*) with Johnston (*43*) and Lubaneu (*44*) ; see Table 3-1 and Table 3-2).

Table 3-1. Filgrastim Pharmacokinetic Parameters in the Rhesus Macaque

Study	AUC (ng·hr/mL)	C_{max} (ng/mL)	CL (mL/hr/kg)	T_{max} (hr)	$T_{1/2}$ (hr)
n=2 Irradiated Rhesus Macaque (TBI of 6.0 Gy, 250kVp x-ray) 10 μg/kg SC, Single Dose Farese (42) **Day 1**	117.36	19.9	5.73	2.16	3.0
n=2 Irradiated Rhesus Macaque (TBI of 6.0 Gy, 250kVp x-ray) 10 μg/kg SC, Single Dose Farese (42) **Day 7**	196.56			3.84	3.6
n=4 Irradiated Rhesus Macaque (TBI of 9.2 Gy, 250kVp x-ray) followed by autologous bone marrow transplantation 300 μg/kg IV, Single dose Farese (45)	6340 ± 940	3570 ± 990	47.9 ± 6.5	0.31 ± 0.13	4.7 ± 2.1

Table 3-2. Filgrastim Pharmacokinetic Parameters in Humans

Study	Conditions	AUC (ng·hr/mL)	C_{max} (ng/mL)	CL (mL/hr/kg)	Vd (mL/kg)	$T_{1/2}$ (hr)
3.45 μg/kg IV Single Package Insert (41)	Normal			30-42		3.85
5.0 μg/kg SC Single n=3 Johnston (43)	Cancer Patients: Pre-chemo-therapy	167	15.4	29.9		2.64
5.0 μg/kg SC Single n=3 Johnston (43)	Cancer Patients: During chemo-therapy	126	10.7	39.6		3.37
20 μg/kg IV Infusion over 24 hours Package Insert (41)	Normal Steady State		48 (mean) 56 (median)			
3.45 μg/kg SC Single Package Insert (41)	Normal		4			3.5
11.5 μg/kg SC Single Package Insert (41)	Normal		49			
5.0 μg/kg SC Single Lubaneu (44)	Normal	159.69± 33.2	21.7±3.9			2.2±0.7
10.0 μg/kg SC Single Lubaneu (44)	Normal	523.8± 107.4	57.6±12.4			2.7±0.7

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Pharmacodynamics

Filgrastim binds to the G-CSF receptor of cells of the neutrophil lineage and leads to increases in ANC; hence, neutrophil levels are a relevant indicator of filgrastim PD.

In rhesus macaques, ionizing radiation causes a drop in circulating neutrophils lasting approximately 15 to 24 days for Grade 3 neutropenia (<500/µL) or 10 to 12 days for Grade 4 neutropenia (<100/µL). (*35*) As shown in Section 4.0 (Table 4-2) and reported by Farese, et al. (*46*), filgrastim administration (10 µg/kg/day) enhanced neutrophil recovery and significantly decreased the duration of neutropenia from 18.6 to 14.3 days (Grade 3) and 12.3 to 10.4 days (Grade 4). Febrile neutropenia was also reduced from 6.2 to 3.8 days.

Filgrastim is used to increase neutrophil levels in patients with neutropenia. In a Phase 3 study conducted in cancer patients (*41*) administration of 4 to 8 µg/kg/day of filgrastim SC, the incidence of febrile neutropenia was reduced from 76% to 40% compared to placebo. For other secondary endpoints, filgrastim treatment reduced the incidence (84% vs. 96% for cycle 1, 57% vs. 77% for all cycles) and duration (median: 6 vs. 2 days for cycle 1, 3 vs. 1 day for all cycles; mean: 5.64 vs. 2.44 days for all cycles) of severe neutropenia. Filgrastim treatment also reduced the severity of neutropenia. An ANC nadir in filgrastim-treated patients during the first cycle was 496/µL compared to 204/µL, and for all cycles the ANC nadir was 403/µL vs.161/µL.

The PK data described above and the PD data provide the basis of the filgrastim dose selected for AXG15 (Section 4.0) corresponds to the human dose of filgrastim recommended by the SNS Radiation Working Group for treatment of H-ARS.

4.0 Summary of the Efficacy of filgrastim in a Rhesus model of H-ARS

**Study AXG15: A Sixty-Day Efficacy Study of Subcutaneous NEUPOGEN®
(Filgrastim) to Treat the Hematopoietic Syndrome of the Acute Radiation Syndrome
(ARS-HS) Following an LD$_{50/60}$ of Total-Body Irradiation (TBI) in Rhesus Macaques**

4.1 Study Design

This study was designed as a GLP, treatment-blinded, randomized, placebo-controlled
study to evaluate the ability of filgrastim to improve survival at 60 days in the rhesus
macaque model of H-ARS characterized in Study AXR01. (*46*) The study (62 planned
animals) was powered to demonstrate a 30% improvement in survival, one-sided, at a 5%
significance level. An interim analysis for efficacy and futility was included in the design
when at least 50% of the rhesus macaques were 60 days past irradiation. In this study,
animals in both cohorts were to be provided medical management based on objective
criteria.

Objectives:
- To assess the efficacy of filgrastim to improve survival following a lethal TBI in
  rhesus macaques receiving medical management.
- To evaluate secondary parameters including studying indices of hematopoietic
  recovery, MST, incidence of FN and infection, number of whole blood
  transfusions, body weights, and incidence and severity of diarrhea in the rhesus
  macaque model of H-ARS.

Endpoints:
- **Primary Endpoint:**
  - overall survival measured at 60 days after radiation exposure
- **Secondary Endpoints**:
  - mean survival time of decedents,
  - neutrophil-related parameters (e.g. ANC nadir, duration of neutropenia
    [ANC<500/µL and <100/µL], day of ANC recovery [ANC≥500/µL and
    ≥1000/µL], recovery from FN [ANC<500/µL, core body temperature ≥103°F
    concurrently]),
  - platelet-related parameters (nadir, duration of platelet count <20,000/µL,
    recovery to platelet count ≥20,000/µL), incidence of infection,
- **Other**
  - antibiotic administration,
  - number of whole blood transfusions,
  - body weights,
  - incidence and severity of diarrhea,
  - cage side observations (e.g. activity, posture, stool consistency, vomit,
    hemorrhage, respiration, alopecia),
  - immunogenicity,

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- number of days of dosing,
- gross necropsy observations,
- microbiological analysis of select organs, and
- histopathological examination of select organs.

Dose of Radiation: Targeted total exposure at 7.50 Gy ± 0.15 Gy

Study Drug Administration: Filgrastim 10 µg/kg/day or Placebo (D5W)
- One injection of either test or control article was administered per day, beginning on Study Day 1 (SD1) between 20 to 26 hours following irradiation and on SD2 and beyond within 24 hours ± 2 hours of the previous day's dosing until the ANC ≥1000/µL for 3 consecutive days or if at any time the ANC was ≥10,000/µL for more than 2 consecutive days within SD1 through SD5 or if at any time the ANC was ≥10,000/µL beginning on SD6.

4.2   Methods

The study was conducted at the University of Maryland, Baltimore, MD. Forty-six (38 male [83%], 8 [18%] nonpregnant female) approximately 3 to 6 years old, rhesus macaques, *Macaca mulatta,* body weight within 4.0 to 6.5 kg were exposed to midline, TBI from the 6 MV LINAC. The LINAC settings were adjusted to deliver irradiation exposure at a dose rate of 0.80 Gy/min ± 0.03 Gy/min, to the midline tissue dose resulting in a targeted total exposure at 7.50 Gy ± 0.15 Gy. The radiation dose selected for this study was based on the dose-response curves established in Study AXR01 (see Section 2.2).

Animals were randomized in cohorts of 6 to10 to the target radiation dose prior to each irradiation into 2 treatment groups (placebo control and 10 µg/kg/day filgrastim). One injection of either test or control article was administered per day, beginning on SD1 between 20 to 26 hours following irradiation and on SD2 and beyond within 24 hours ± 2 hours of the previous day's dosing until the ANC ≥1000/µL for 3 consecutive days or if at any time the ANC was ≥10,000/µL for more than 2 consecutive days within SD1 through SD5 or if at any time the ANC was ≥10,000/µL beginning on SD6.

Less than 1% of doses (5/774) occurred outside of the specified time window (24 hours ± 2 hours of the previous day's dosing). At any point following discontinuation of dosing, if the ANC was <500/µL, daily injections were re-initiated the same day and on subsequent days and continued until the ANC was ≥1000/µL for 3 consecutive days. All animals received medical management in response to clinical signs.

The research staff performed clinical observations daily from SD0 through SD25, then at least on SD28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60, and at termination. Other clinical observations were made to determine medical management. Parameters evaluated included body weight, core body temperature, capillary refill time, skin tent time, petechia,

ecchymosis, and swelling. Medical management (as described in Section 2.2) was provided for the following indications: dehydration, pain, elevated body temperatures, ulcers, diarrhea, emesis, weight loss, depressed appetite, anemia and thrombocytopenia, and positive blood cultures.

Clinical observations were also used to determine whether euthanasia criteria (as performed in Study AXR01, the study that characterized the rhesus macaque model of H-ARS) were met (see Section 2.2).

Blood samples for a complete blood count were obtained at the following time points:
- prior to SD0 (baseline)
- SD0 (before radiation exposure)
- before dosing of study drug
- daily on SD1 through 25; then at least on SD28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60, and at termination, unless clinically indicated to sample more or less frequently.

In addition, whenever the ANC was $\geq 1000/\mu L$, samples were collected daily following that particular SD until count remained $\geq 1000/\mu L$ for 3 consecutive days.

On the first SD that FN (ANC $<500/\mu L$ and body temperature $\geq 103°F$) was observed (or any day on which the body temperature was $\geq 105°F$) a microbiologic culture of the blood was collected aseptically and tested for aerobic and anaerobic bacteria. Additional specimens were collected if protocol-defined criteria were met.

Blood was collected to evaluate the presence of an antibody response to filgrastim prior to irradiation and at termination (SD60 or earlier if euthanasia was performed).

Cage-side observations occurred twice daily by the husbandry staff for mortality, moribundity, and general health. Veterinarians performed cage-side observations twice daily at a minimum of 6 hours apart and were blinded to treatment type and antibiotic treatment. Parameters evaluated during cage-side observations included: activity, posture, stool consistency, evidence of vomit, hemorrhage, respiratory activity, alopecia, seizure activity, and qualitative food consumption.

A full gross necropsy, which included examination of the external surface of the body, palpation for superficial swellings or for enlarged organs or masses within body cavities, was performed on all animals that were euthanized or died during the study. All internal organs were examined for abnormalities including hemorrhage, ulcerations, etc. The kidney, lung, liver, and spleen were obtained aseptically and microbial analysis was performed.

The following tissues from each animal were preserved in 10% neutral buffered formalin: heart, lung, liver, spleen, right and left kidney, large and small intestine, thymus,

mesenteric lymph nodes, skin, decalcified sternum and femur, and any lesions or abnormalities observed during necropsy gross observations.

All preserved tissues (with exception of animal identification) were processed, embedded in paraffin, sectioned and stained with hematoxylin and eosin, and examined microscopically. The study veterinary pathologist was blinded to the treatment until after all slides were examined.

4.3   Results

Primary Endpoint: Survival

This study demonstrated that administration of filgrastim 10 µg/kg/day SC starting on SD1 (20-26 hours) following an $LD_{50/60}$ midline, total-body exposure to 6 MV LINAC photon irradiation and administered to effect based on ANC, significantly *(*one-sided, $P<0.004$) improved overall 60 day survival in rhesus macaques (Table 4-1 and Figure 4-1). The efficacy of filgrastim was demonstrated in accordance with the Lan-Demets version of the O'Brien-Fleming boundary to provide an overall one-sided P=0.05 test.

The criteria of the "per-protocol interim analysis" (for efficacy) were met and the study was terminated for efficacy when 74% of the animals had completed the in-life phase of the study.

Table 4-1. Survival Placebo and Filgrastim-Treated Rhesus Macaques Exposed to 7.5 Gy		
Treatment	**Survival at 60 days** **n/N (%)**	**Mean Day of Death** **Day After Irradiation (Range)**
Control	9/22 (41%)	21 (11-43)
Filgrastim	19/24 (79%)	12 (8-19)
One-sided P value	*<0.004**	*0.029***

*One sided P value based on chi-square test.
** Two-sided P value based on Wilcoxon rank sum test.

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Figure 4-1. Survival of Rhesus Macaques Following Filgrastim Treatment
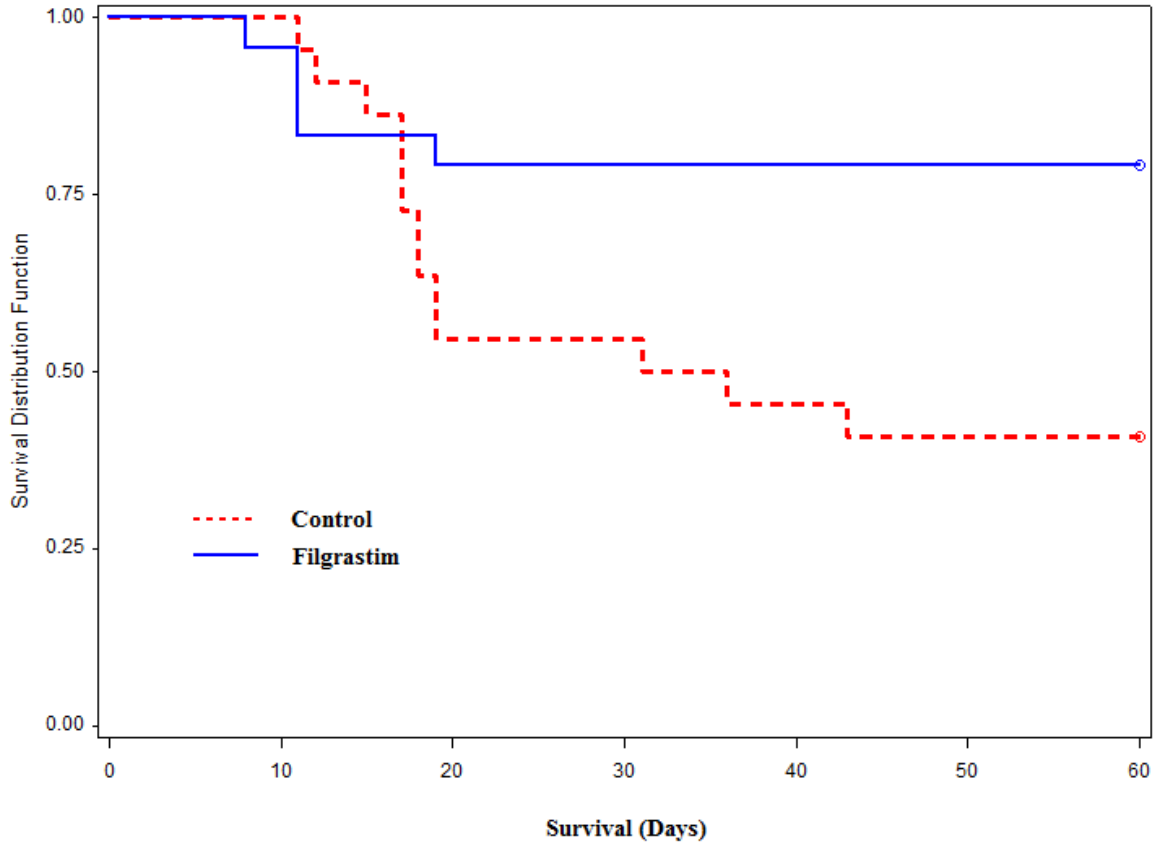
Secondary Endpoints

Secondary objectives that were evaluated included indices of hematopoietic recovery, MST (days) of decedents, incidence of febrile neutropenia and infection, number of whole blood transfusions, body weights and temperatures, and incidence and severity of diarrhea.

Survival Time of Decedents

The MST of the decedents was 9 days earlier in the filgrastim cohort than in the controls.

Absolute Neutrophil Count

The 7.50 Gy exposure reduced the ANC in both cohorts from pre-irradiation values to <500 cells/μL within 5 days after TBI (Figure 4-2). The data in Figure 4-2 represent the mean value of all animals that were alive on each day. It is censored for animals that succumbed during the study. The ANC in both control and filgrastim-treated cohorts decreased further to <100 cells/μL by 7.1 days and 6.5 days, respectively (Table 4-2). Absolute neutropenia was observed in 58% (14/24) of all filgrastim-treated animals and 63% (12/19) of the filgrastim-treated survivors. There was no significant difference in the incidence of febrile neutropenia in either cohort. The incidence of absolute neutropenia in all of the controls was 82% (18/22) and 91% (10/11) in the survivors. Filgrastim significantly decreased the duration of Grade 3 and Grade 4 neutropenia and improved the day of recovery of ANC to either ≥500/μL or ≥1000/μL but did not affect the ANC nadir.

Figure 4-2. Mean Absolute Neutrophil Counts in Rhesus Macaques Following Irradiation and Filgrastim Treatment
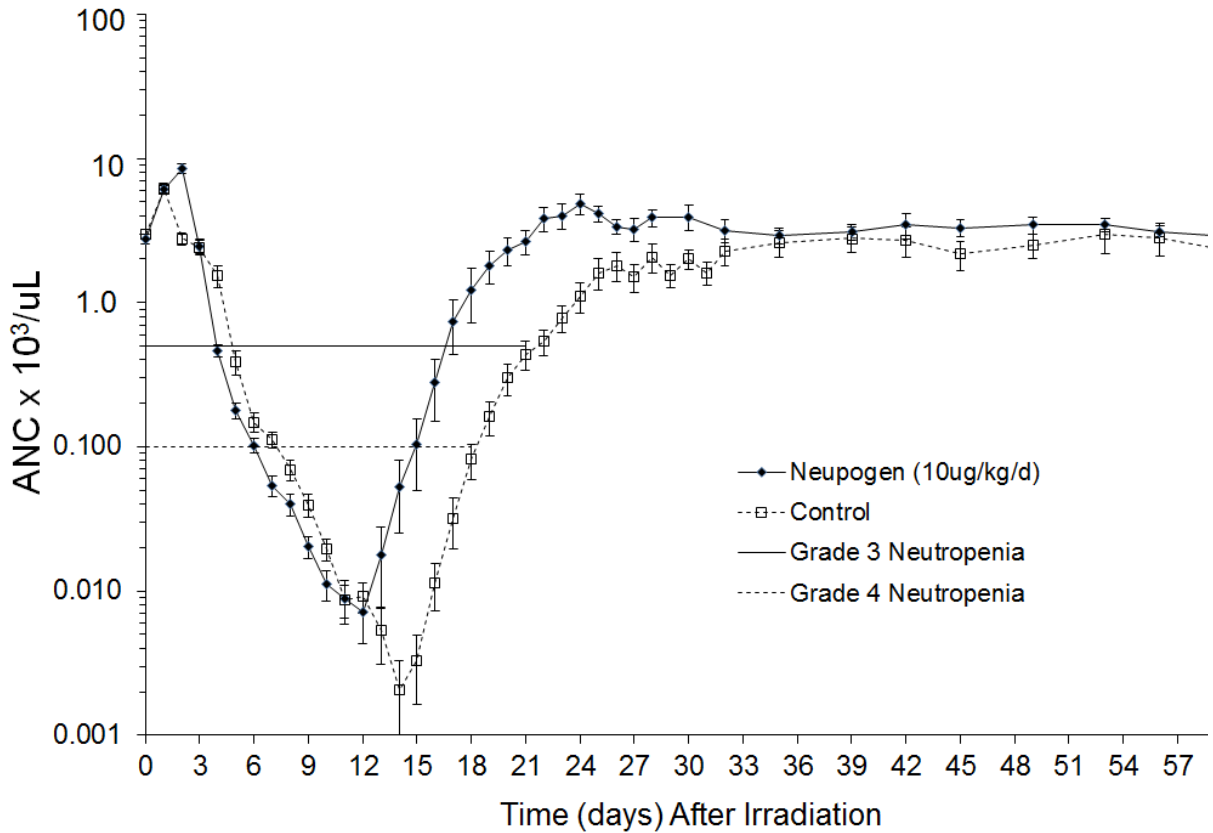
Duration of Neutropenia, ANC Nadir, and Recovery Time

The stimulation of granulopoiesis by filgrastim administration in lethally irradiated rhesus macaques resulted in significant reduction in the duration of neutropenia and the time to recovery of ANC >1000 cells/µL. Filgrastim reduced the duration of Grade 4 neutropenia from 18.6 days to 14.3 days and Grade 3 neutropenia from 12.3 days to 10.4 days (Table 4-2).

Table 4-2. Mean First Day of Grade 3 (ANC <500 cells/µL) and Grade 4 (ANC <100 cells/µL) Neutropenia, and Median Duration of Grade 3 and Grade 4 Neutropenia in Control and Filgrastim-Treated Rhesus Macaques Exposed to 7.50 Gy				
Treatment	Mean First Day Grade 3 Neutropenia[‡] (± SE, range)	Mean Duration of Grade 3 Neutropenia[*] in Days (± SE, range)	Mean First Day Grade 4 Neutropenia[‡] (± SE, range)	Mean Duration of Grade 4 Neutropenia[*] in Days (± SE, range)
Control	4.9±0.2 (3-6)	18.6±0.8[b] (14-22)	7.1±0.4 (5-10)	12.3±0.6[b] (8-15)
Filgrastim 10 µg/kg, QD	4.3±0.1 (3-5)	14.3±0.5[a] (9-19)	6.5±0.3 (4-9)	10.4±0.6[a] (4-14)
Two-sided P value	*0.0145*	*<0.0001*	*0.2189*	*0.009*
*Note that ANC durations do not include data from decedent animals unless recovery occurred to that level prior to death, [‡] Includes all animals, [a] n = 12, [b] n = 19				

The mean nadir by day after TBI was not significantly different between the filgrastim-treated and control cohort (Table 4-3). The time of recovery to an ANC >1000 cells/µL was significantly reduced in the filgrastim-cohort (Table 4-3, Figure 4-3a and Figure 4-3b).

Table 4-3. Mean Nadir and Recovery from Neutropenia in Placebo and Filgrastim-Treated Rhesus Macaques Exposed to 7.50 Gy			
Treatment	Nadir of ANC[‡] (cells/µL)	Mean Number of Days to Recovery - ANC ≥500/µL* (± SE, range)	Mean Number of Days to Recovery - ANC ≥1000/µL * (± SE, range)
Control	1.5±1.0	23.3±0.8[a] (20-27)	25.8±0.9[a] (22-32)
Filgrastim 10 µg/kg,	5.0±2.0	18.7±0.5[b] (14-23)	19.7±0.6[b] (15-25)
Two-sided P value	*0.115*	*<0.0001*	*<0.0001*

[*]Note that ANC recovery parameters do not include data from decedent animals unless recovery occurred to that level prior to death.
[‡] Includes all animals; [a] n = 12; [b] n = 19

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Log-rank tests were used to compare duration of neutropenia and time to recovery from neutropenia between controls and filgrastim-treated animals. Animals that died were censored at 60 days (i.e. they were considered to have not recovered by Day 60 and their duration continued until day 60). Figure 4-3a and Figure 4-3b show the proportion of animals with duration of neutropenia and time to recovery of ANC ≥1000/µL, respectively.

As shown in these figures, the filgrastim-treated group had a shorter duration of neutropenia and shorter time to recovery of ANC ≥1000/µL.

Figure 4-3a. Duration of Neutropenia – Number of Days That ANC <500/µL
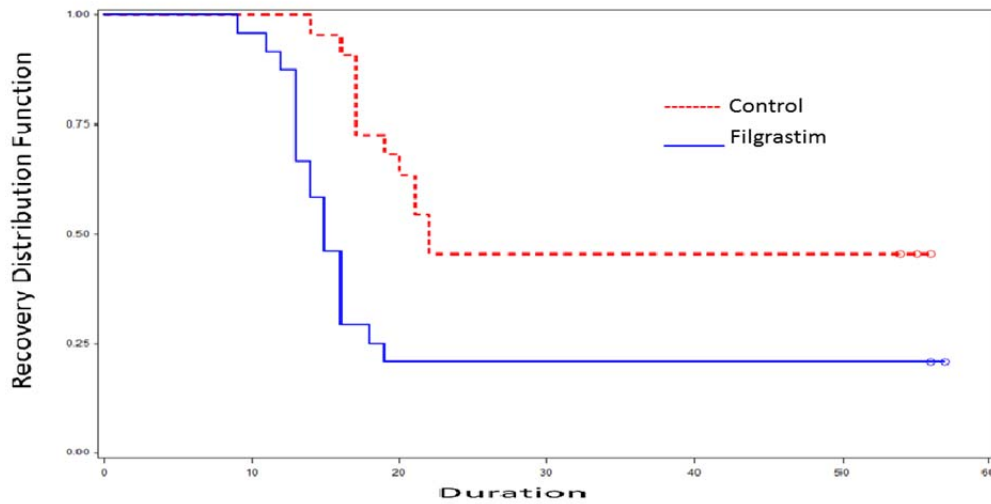
Figure 4-3b. Time to Recovery to ANC ≥ 1000/µL
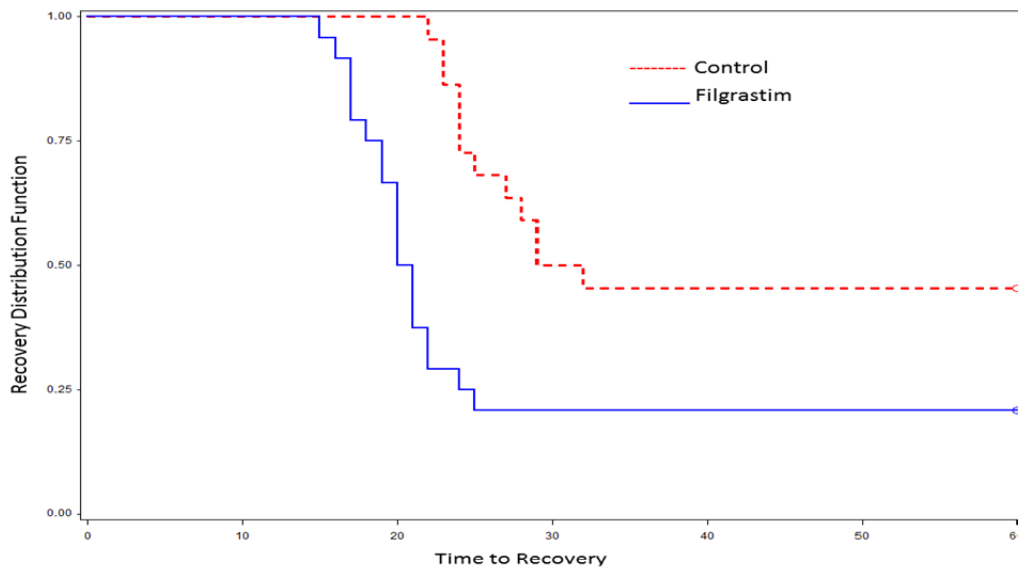
Analysis of duration of Grade 3 neutropenia using time to event (death) analysis methods with animals that die censored at 60 days showed a median of 22.0 days (17.0-NE, 95% confidence limit [CL]) in the controls and 15.0 days (13.0-16.0, 95% CL) in the filgrastim cohort following 7.5 Gy irradiation (log-rank *P*=0.0028). Analysis of time to recovery to ANC <1000/μL) using time to event analysis methods with animals that die censored at 60 days, showed a median of 30.5 days (24-NE, 95% CL) in the controls and 20.5 days (19.0-22, 95% CL) in the filgrastim-treated cohort following 7.5 Gy irradiation (log-rank *P*=0.0017).

Figure 4-4a presents trajectories of neutrophils over time, with treated animals coded in blue and control animals coded in red. Death times are marked by solid circles, with the same color coding. This plot shows that all animals had very low neutrophil counts between Day 7 and Day 11, and deaths occurred in both arms during this time period; however, between Day 12 and Day 20, the control animals generally had substantially lower neutrophils counts than the filgrastim-treated animals, and the deaths during this time period were almost all among the control animals. These data suggest that the increase in neutrophil counts in the filgrastim-treated animals, in the Day 12 through Day 20 time period may have led to reduced risk of death. To better illustrate these treatment group differences and to better visualize death times with overlapping values, an enlarged view for the Day 7 through Day 20 time period is shown in Figure 4-4b.

Figure 4-4a. Neutrophil Recovery: Full Course
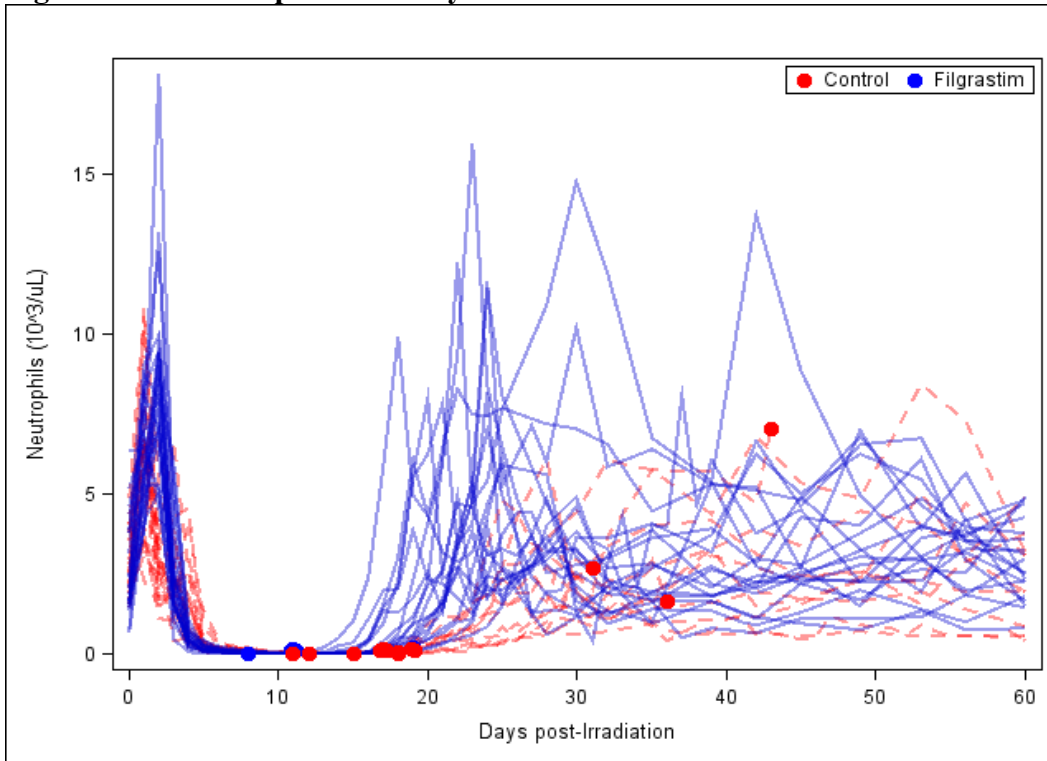
Figure 4-4b. Neutrophil Recovery: Days 7-20

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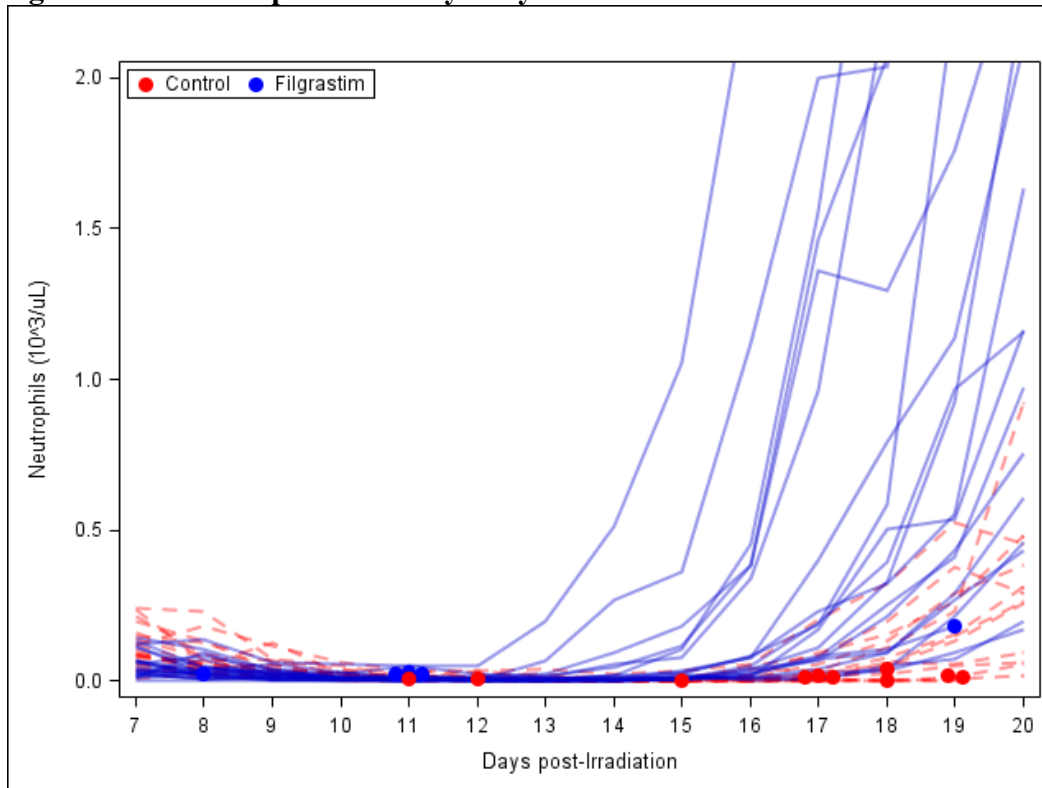
Febrile Neutropenia

Febrile neutropenia is defined as the ANC <500/µL and the core body temperature ≥103.0ºF occurring concurrently. The range of the first day of FN for the filgrastim-treated group was SD5 to15 and for the control-treated group was SD4 to 17 after TBI (Table 4-4). Filgrastim treatment reduced the duration of FN by 2.4 days and antibiotic requirements by 4.8 days.

Table 4-4. Febrile Neutropenia (FN) Following Total Body Irradiation of 7.5 Gy in Rhesus Macaques Administered Filgrastim or Control Article: Incidence (n/N), and Mean (± SE) First Day of Occurrence and Number of Days

Article	Incidence	First Day FN[†]	Number of Days FN*
Control	90.0% (20/22)	11.7±0.8	5.8±1.4
Filgrastim	79.1% (19/24)	10.7±0.7	3.9±0.8
Two-Sided P value	*0.418*	*0.3882*	*0.2206*

[†] Includes all animals
*Includes only survivors

Incidence of Infection: Presence of Bacteria

Details about the presence of bacteria in peripheral blood cultures are tabulated in Table 4-5.

Table 4-5. Summary of Blood Culture Results in Rhesus Macaques Following Exposure to 7.5 Gy Total Body Irradiation Administration of Filgrastim or Control Article

	Peripheral Blood Cultures Performed		Peripheral Blood Cultures Positive for Bacteria		Number of Animals Having at Least 1 Bacteria-Positive Blood Culture	
	N	%	N	%	N	%
Control n=22	71	54	39	55	19	86
Filgrastim n=24	60	46	24	40	14	58
Total n=46	131	100	63	48	33	72
Two-sided P value					*0.035*	

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Platelet Parameters

Although there was a trend for decreased time to thrombocytopenia and earlier recovery of platelet counts, filgrastim did not significantly improve platelet-related parameters compared to controls.

Table 4-6. Mean Values for Platelet-Related Parameters in Rhesus Macaques Following Exposure to 7.5 Gy Total Body Irradiation Administration of Filgrastim or Control Article				
Treatment	**First Day Platelet Count[‡] <20,000/µL (± SE, range)**	**Duration (days)* <20,000/µL (± SE, range)**	**Nadir[‡] (Platelet/µL) (± SE, range)**	**Day of Recovery to Platelet Count ≥20,000/µL* (± SE, range)**
Control	9.7±0.2 (8-11)	17.1±2.1[a] (11-33)	1000±0 (0-4000)	26.9±2.2[a] (20-44)
Filgrastim	9.3±0.2 (8-11)	12.6±1.4[b] (6-33)	1000±0 (0-6000)	22.0±1.4[b] (15-42)
Two-sided P value	*0.115*	*0.077*	*0.134*	*0.062*
[‡] Includes both survivors and nonsurvivors. *Note that durations and day of recovery do not include data from decedent animals unless recovery occurred to that level, e.g. platelet count ≥20,000/µL prior to death. [a] n=11, [b] n=19				

Analysis of time to recovery to platelet count <20,000/µL using time-to-event analysis methods with animals that die censored at 60 days, showed a median of 22 days (18-28, 95% CL) in the filgrastim-treated animals and a nonestimable (NE) median duration (24.0-NE, 95% CL) in the control cohort following 7.5 Gy irradiation (log-rank P=0.0085).

Other Endpoints

Incidence and Duration of Antibiotic Use
All animals required antibiotic support as specified by the study protocol. Filgrastim treatment reduced the antibiotic requirements by 4.8 days.

Transfusion Parameters
Transfusion parameters are presented in Table 4-7.

Table 4-7. Transfusion Parameters for Rhesus Macaques Following Exposure to 7.50 Gy Total Body Irradiation and Administration of Filgrastim or Control Article		
Treatment	**Number of Transfusions (1 unit = 54 mL) (± SE, range)**	**First Day Transfusion Occurred Within Radiation Cohort (± SE, range)**
Control	2.4±0.3 (0-6.5)	11.8±0.6 (10-14)
Filgrastim	1.8±0.3 (0-5)	10.8±0.9 (10-18)
P value	*0.278*	*0.847*
*Wilcoxon rank sum test (nonparametric) Transfusion parameters include both survivors and nonsurvivors.		

Diarrhea Severity
Each animal was evaluated on every study day for the presence and severity of diarrhea. The highest severity score an animal received over their in-life phase of the study is presented in Table 4-8.

Table 4-8. Total Body Irradiation (7.5 Gy) of Rhesus Macaques Administered Filgrastim or Control Article: Occurrence and Severity of Diarrhea						
Severity	**Grade 1 (soft stool)**		**Grade 2 (loose and/or watery stool)**		**Grade 3 (bloody diarrhea)**	
Treatment	**Number of Animals per Total Animals**	**%**	**Number of Animals per Total Animals**	**%**	**Number of Animals per Total Animals**	**%**
Control	0/22	0	17/22	77.3%	5/22	22.7%
Filgrastim	2/24	8.33%	19/24	79.2%	2/24	8.33%

Body Weight
Body weight was obtained for each animal every day that the animal was anesthetized. The number of animals experiencing a 10% loss in body weight from baseline value occurred to a greater degree in the filgrastim-treated animals (Table 4-9).

Table 4-9. Total Body Irradiation (7.5 Gy) of Rhesus Macaques Administered Filgrastim or Control Article: Occurrence and Severity of Body Weight Loss

Treatment	≥10% Body Weight Loss		≥25% Body Weight Loss	
	n	**%**	**n**	**%**
Control	13	59.1%	0	0
Filgrastim	17	70.8%	0	0

Cage Side Observations

Veterinarians, blinded to the treatment assignment, observed the animals and graded the observation twice daily for the following parameters: activity, posture, stool consistency, vomit, hemorrhage, respiration, and alopecia (Table 4-10).

Table 4-10. Grading Scale for Parameters Evaluated During Cage-Side Observations

Activity	Posture	Stool Consistency	Vomit	Hemorrhage	Respiratory	Alopecia
		NA = no stool present				
0 = Normal	0 = Normal	0 = Formed	0 = none	0 = no blood in cage	0 = normal	0 = normal coat
1 = limited	1 = hunched	1 = soft	1= present, evidence of 1 episode	1= individual blood spots in cage (≤10 spots)	1= mildly increased respiration rate or effort or intermittent cough	1= loss of <25% of normal coat
2 = absent	2 = recumbent	2 = loose and/or watery	2 = persistent, evidence of multiple episodes	2 = coalescing blood or >10 spots in cage	2 = respiratory distress or open mouth breathing, persistent cough	2 = loss of >25% but <50% of normal coat
		3 = bloody diarrhea		3 = estimated to be in excess of 20% of blood volume, life-threatening	3 = agonal	3 = loss of >50% of normal coat
						4 = complete loss of coat

The highest grade reported for each animal over the course of the study is presented in Table 4-11. Generally the grading of the observations was similar between the 2 treatment groups.

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Table 4-11. Summary of Grading of Cage-Side Observations: Most Severe Grade Scored for Each Animal Over Total Observation Period

	Activity				Posture	
	Control (n)	Filgrastim (n)			Control (n)	Filgrastim (n)
Grade 0	2	6		Grade 0	4	4
Grade 1	18	18		Grade 1	16	20
Grade 2	2	0		Grade 2	2	0
	Hemorrhage				Respiration	
	Control (n)	Filgrastim (n)			Control (n)	Filgrastim (n)
Grade 0	4	4		Grade 0	20	23
Grade 1	12	10		Grade 1	1	1
Grade 2	6	10		Grade 2	1	0
	Stool Consistency				Alopecia	
	Control (n)	Filgrastim (n)			Control (n)	Filgrastim (n)
Grade 0	0	1		Grade 0	17	18
Grade 1	0	2		Grade 1	3	2
Grade 2	17	19		Grade 2	2	2
Grade 3	5	2		Grade 3	0	2
	Vomit					
	Control (n)	Filgrastim (n)				
Grade 0	8	13				
Grade 1	10	6				
Grade 2	4	5				

Because an equal number of animals were not used in each group (control, n=22; filgrastim, n=24) and all animals did not have the same number of total observations evaluated due to lethality (59% in controls, 21% in filgrastim), observations were also evaluated as the number of times a specific grade was observed versus all observations made for that parameter in a specific treatment group (control or filgrastim). The grading of severity for the parameters observed was similar between the 2 groups when all observations were considered.

Finally, the observations were evaluated as total observations per group every 15 days. The majority of animals were scored as "normal" (Grade 0) and Grade 1 or Grade 2 scores were general occurred during the first 30 days of the study in a similar amount observed in controls and filgrastim-treated animals. In summary, cage-side observations were similar between the 2 groups. The majority of animals in both groups (with the exception of vomit in the filgrastim-treated animals) had at least 1 occurrence of a Grade 1 or Grade 2

observation in all parameters except respiration, alopecia. The filgrastim-treated animals did not demonstrate either a less severe or more severe response to 7.5 Gy irradiation based on the parameters scored during cage-side observations.

Cage-Side Observation Analysis by Parameter

Each cage-side observation parameter in Table 4-11 is presented individually. For activity, posture, stool consistency, and hemorrhage, the first figure shows the data over the entire study and the second figure shows the breakdown over 15-day intervals. Figures are followed by tables of the data over the entire study. For the other parameters, conclusions are provided in text only.

Activity

See Figure 4-5 and Figure 4-6, and Table 4-12.

Figure 4-5. Activity Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (Total)

Grade 0 = normal; Grade 1= limited;
Grade 2 = recumbent, no activity
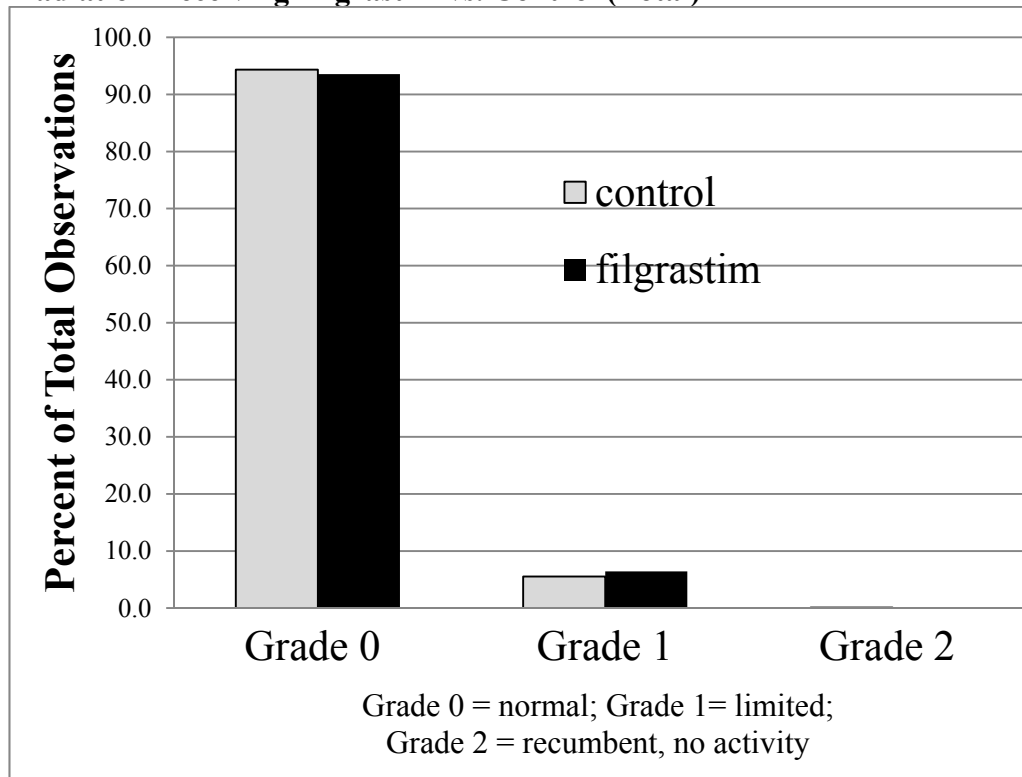
Figure 4-6. Activity Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (by Time Period)

Grade 0 = normal; Grade 1 = limited; Grade 2 = recumbent,or no activity

Table 4-12. Activity Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control				
Activity	**Control**		**Filgrastim**	
	Number of Observations (N=1609)	**Percentage of Total Observations (%)**	**Number of Observations (N=2390)**	**Percentage of Total Observations (%)**
Grade 0 – normal	1518	94.3	2236	93.6
Grade 1 – limited	89	5.5	154	6.4
Grade 2 – absent	2	0.1	0	0.0

The percentage of normal or limited activity (Grade 0 and 1, respectively) between filgrastim-treated and control animals was similar. Absent activity (Grade 2) was observed in only in 2 controls.

Posture

See Figure 4-7 and Figure 4-8, and Table 4-13.

Figure 4-7. Posture Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (Total)

Figure 4-8. Posture Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (by Time Period)
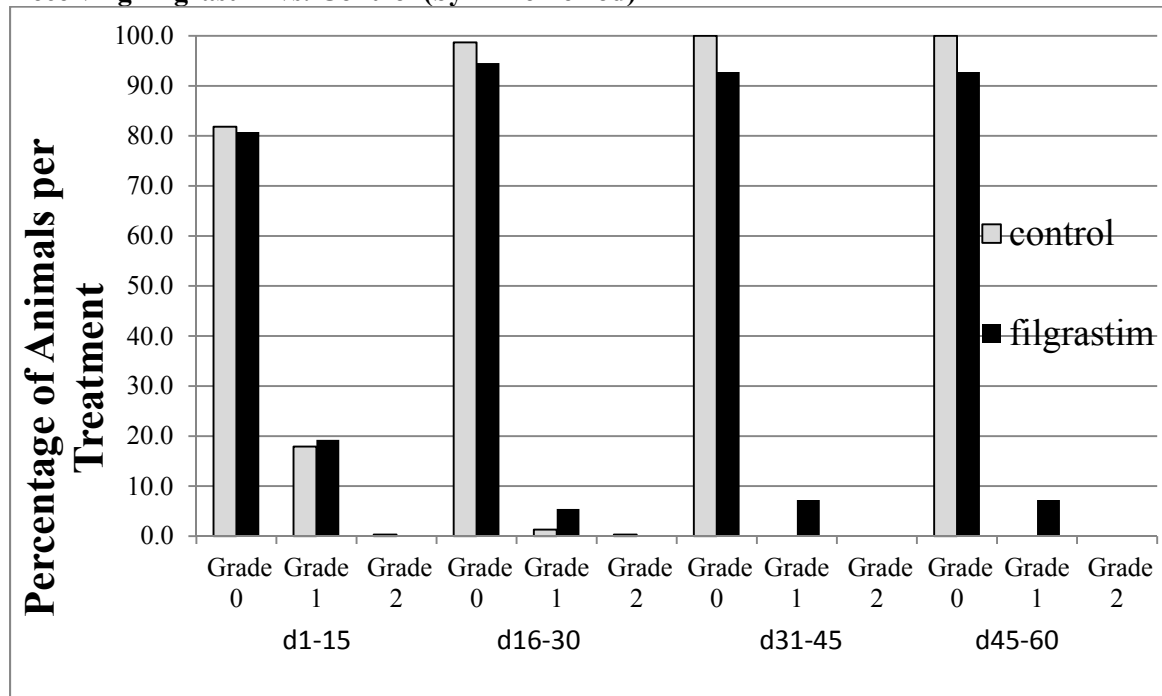
Table 4-13. Posture Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control				
	Control		Filgrastim	
Posture	Number of Observations	Percentage of Total Observations	Number of Observations	Percentage of Total Observations
	1608	-	2391	-
Grade 0 – normal	1496	93.0	2135	89.3
Grade 1 – hunched	109	6.8	256	10.7
Grade 2 – recumbent	3	0.2	0	0.0

The majority of rhesus macaques in both treatment groups were observed to exhibit normal posture (Grade 0) between 89% and 93% of all observations (controls, filgrastim-treated animals, respectively). Hunched posture (Grade 1) was observed in the filgrastim-treated animals slightly more often than in the control animals (10.7% versus 6.8%, respectively). Recumbent posture (Grade 2) was only observed on 3 (0.2%) occasions in the control group.

Stool Consistency
See Figure 4-9, Figure 4-10, and Table 4-14.

Figure 4-9. Stool Consistency Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (Total)
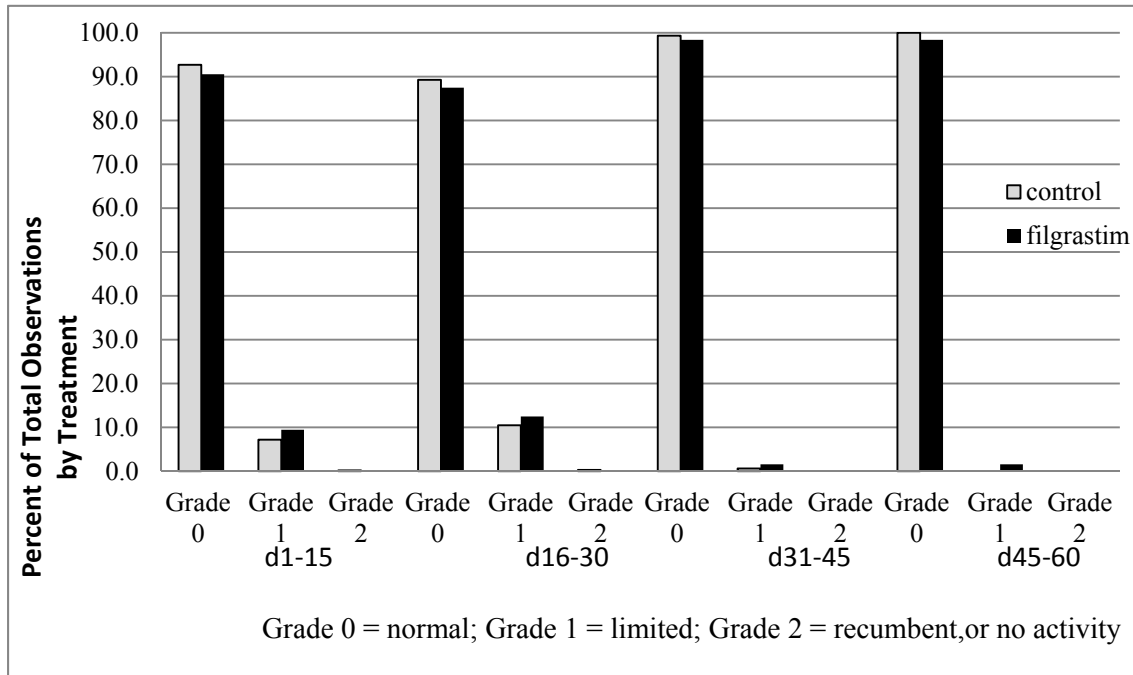

NA = no stool; Grade 0 = formed; Grade 1 = soft;
Grade 2 = loose and/or watery; Grade 3 = bloody diarrhea

Figure 4-10. Stool Consistency Score in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (by Time Period)

NA = no stool; Grade 0 = formed; Grade 1 = soft; Grade 2 = loose and/or watery;
Grade 3 = bloody diarrhea

Table 4-14. Stool Consistency in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control

Stool Consistency	Control		Filgrastim	
	Number of Observations (N=1608)	Percentage of Total Observations (%)	Number of Observations (N=2386)	Percentage of Total Observations (%)
NA – no stool present	325	20.2	486	20.4
Grade 0 – formed	979	60.9	1529	64.1
Grade 1 – soft	101	6.3	183	7.7
Grade 2 – loose and/or watery	175	10.9	185	7.8
Grade 3 – bloody diarrhea	28	1.7	3	0.1

The percentage of no stool (NA) was equal between the 2 treatment groups. The percentage of observations of formed stool (Grade 0) and soft stool (Grade 1) was slightly higher in the filgrastim-treated animals; however, loose/watery stool (Grade 2) observations occurred more often in the control group. Loose and/or watery stool (Grade 3) was observed earlier and more often in the control group than the filgrastim-treated group (28 vs. 3 times, respectively).

Hemorrhage

Figure 4-11. Hemorrhage Score in Rhesus Macaques Following 7.50 Gy Receiving Filgrastim vs. Control (Total)

0 = no blood in cage; 1 = ≤10 blood spots in cage; 2 = coalescing blood or >10 spots in cage; 3= estimated to be >20% blood volume, life threatening
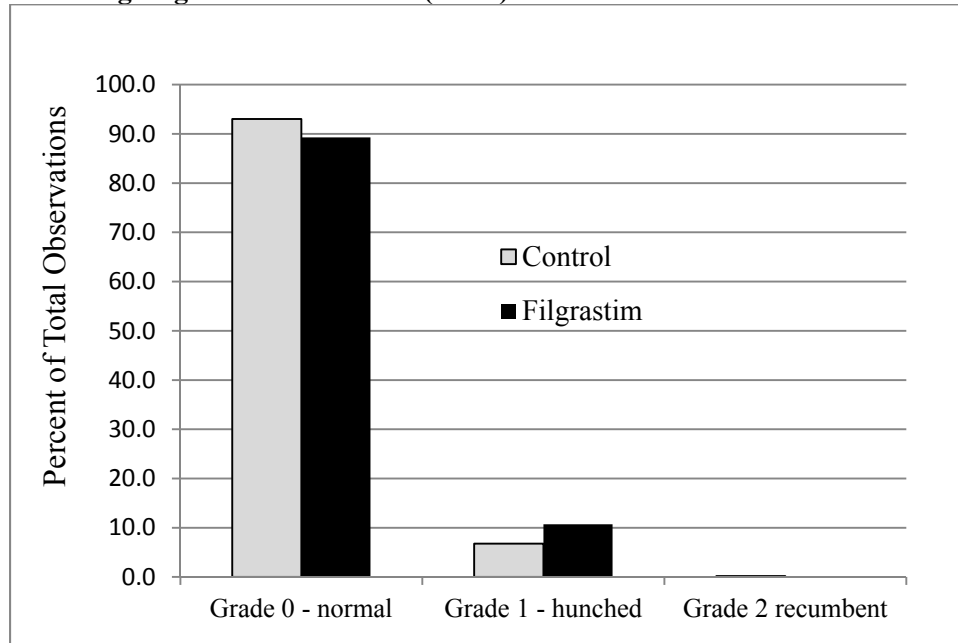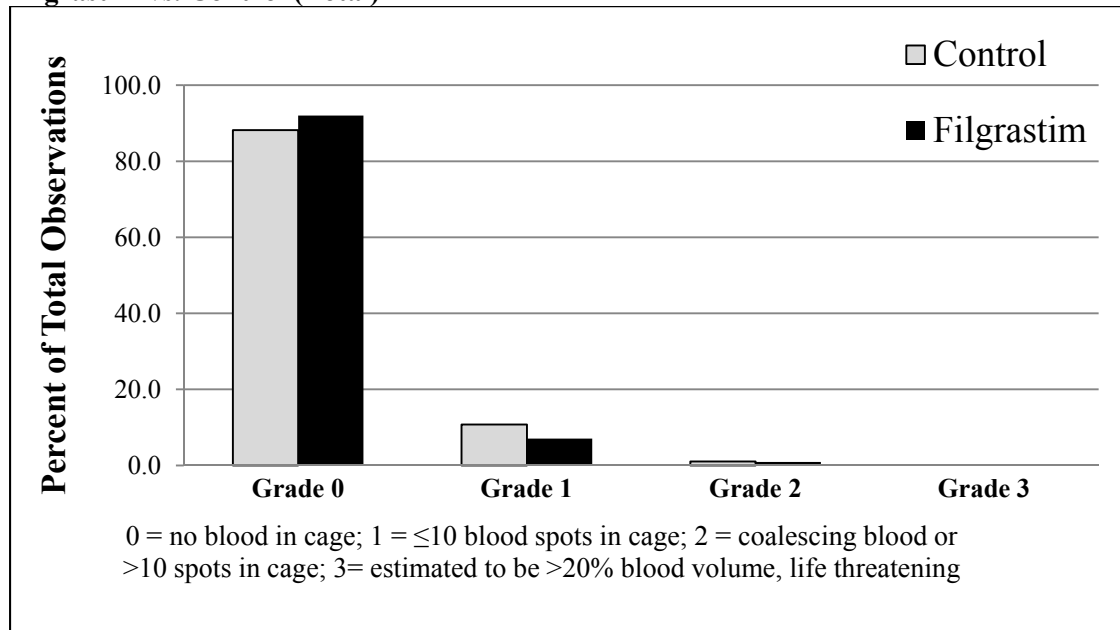
Figure 4-12. Hemorrhage Scores in Rhesus Macaques Following 7.5 Gy Total Body Irradiation Receiving Filgrastim vs. Control (by Time Period)

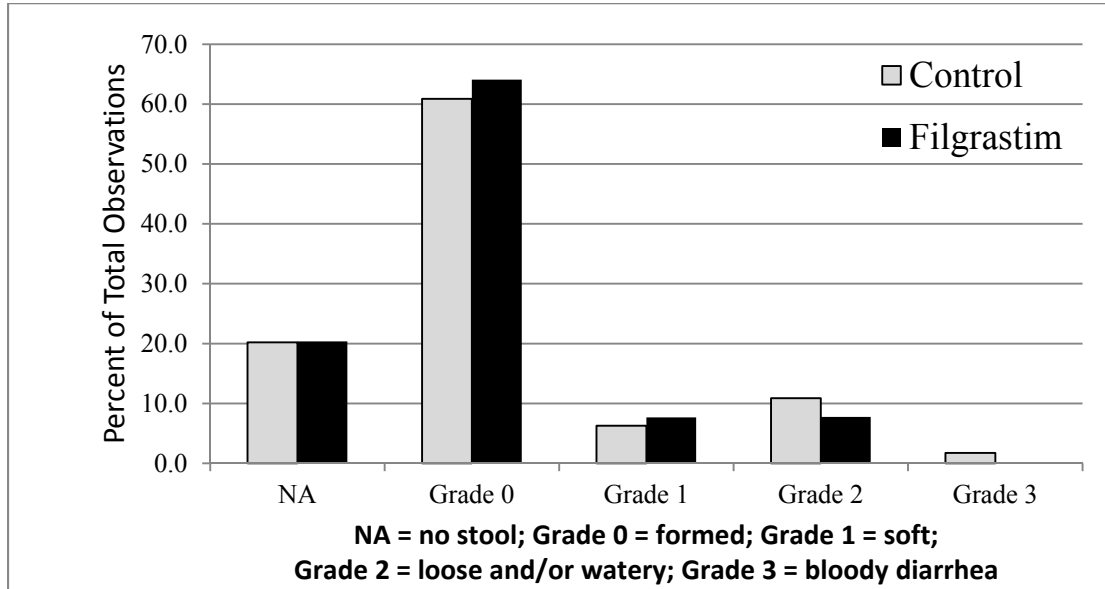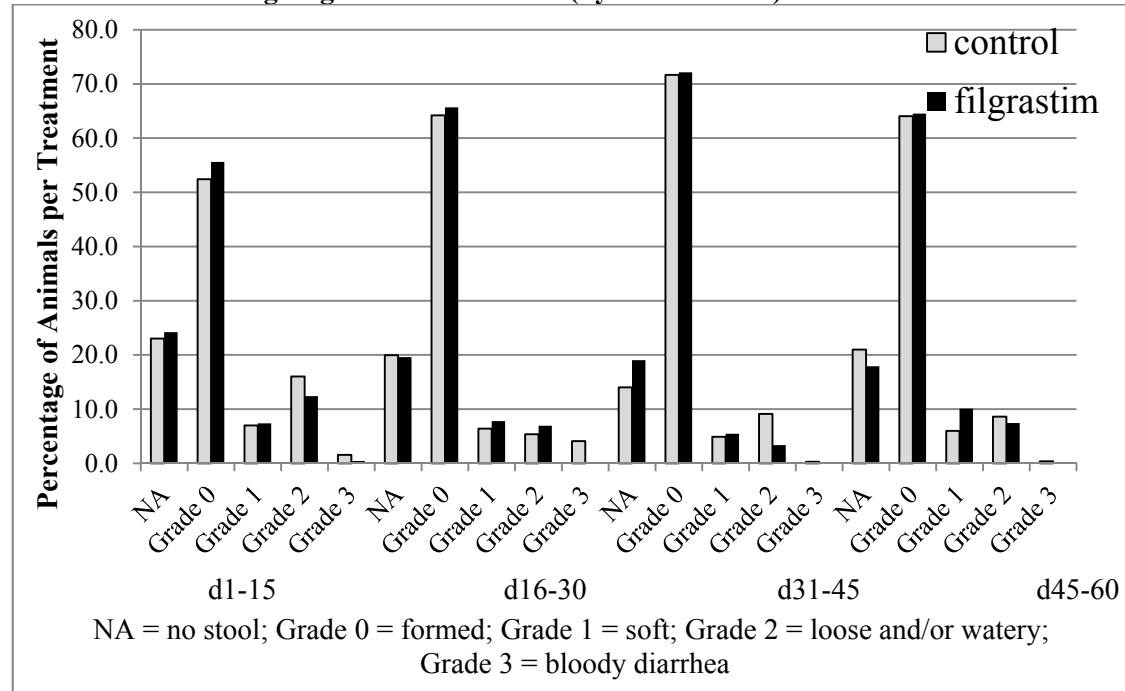Grade 0 = no blood in cage; 1 = ≤10 blood spots in cage; 2 = coalescing blood or >10 spots in cage; 3= estimated to be >20% blood volume, life threatening
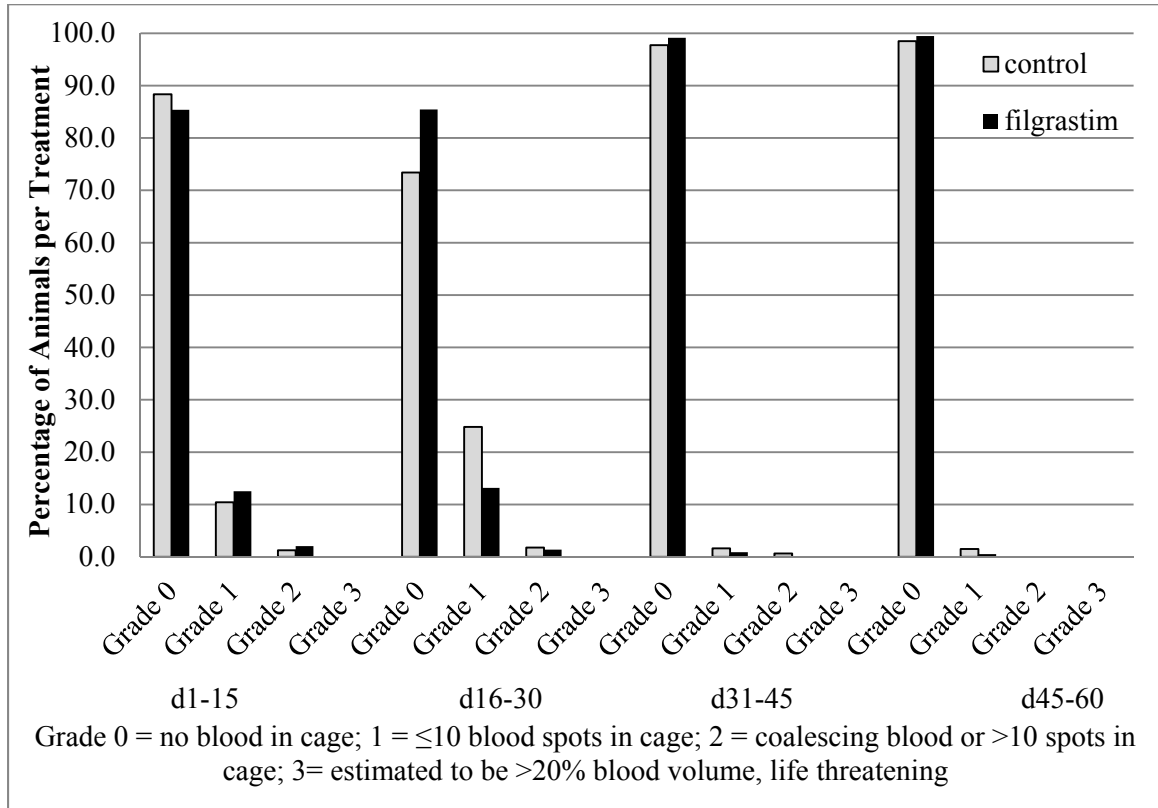
Table 4-15. Hemorrhage Scores in Rhesus Macaques Following 7.50 Gy Total Body Irradiation Receiving Filgrastim vs. Control

Hemorrhage	Control		Filgrastim	
	Number of Observations (N=1608)	Percentage of Total Observations (%)	Number of Observations (N=2392)	Percentage of Total Observations (%)
Grade 0 – no blood in cage	1418	88.2	2201	92.0
Grade 1 – ≤10 blood spots in cage	173	10.8	169	7.1
Grade 2 – coalescing blood or >10 spots in cage	17	1.1	22	0.9
Grade 3 – estimated to be >20% blood volume, life threatening	0	0.0	0	0.0

The majority of animals in both groups were observed to have no blood in the cage (Grade 0). Control animals were observed to have higher frequency of ≤10 blood spots in cage (Grade 1) than filgrastim-treated animals. The observation of coalescing blood or >10 spots in cage (Grade 2) was similar in both groups. Neither group was observed to have blood loss estimated to be >20% blood volume or life-threatening blood loss during cage-side observations (Grade 3) (Figure 4-11 and Table 4-15**).**

Between SD1 and SD15 the number of observations or the percentage of animals in each group that were observed to have individual blood spots in cage (≤10 spots) (Grade 1) was similar. A higher percentage of controls experienced Grade 1 hemorrhage between SD15 and SD30 compared to filgrastim-treated animals. The observation of coalescing blood or >10 spots in cage (Grade 2) was similar between both groups over the course of the study. (Figure 4-12)

Vomiting

No vomit (Grade 0) was the most frequent observation in both the control and filgrastim cohorts. Vomit present, evidence of 1 episode (Grade 1), was observed approximately the same number of times in both groups. Persistent evidence of multiple episodes of vomit (Grade 2), was observed more often in the filgrastim-treated group than in the control group (8 days vs. 2 days, respectively).

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Respiration

There was no difference in the respiratory observation between the controls and filgrastim-treated animals. Normal respiration (Grade 0) was observed in both groups for the duration of the study with 3 exceptions: (i) a filgrastim-treated animal was observed to have mildly increased respiration rate or effort or intermittent cough (Grade 1) at the observation immediately prior to expiration on SD11; (ii) a control animal, that survived to the end of the study, was observed at Grade 1 on SD17; (iii) a second control animal was observed to have respiratory distress or open-mouth breathing, persistent cough (Grade 2) immediately prior to euthanasia on SD17.

Alopecia

Most animals in either cohort had a normal coat (Grade 0) during the study and no animals were observed to have a complete loss of coat (Grade 4). Additionally, no control animals were observed to have a loss of >50% of normal coat (Grade 3). In the control group, observations of loss of <25% of normal coat (Grade 1) (n=2) occurred between SD13 and 17 and SD37 to 60 and loss of >25% but <50% of normal coat (Grade 2) on SD14 and 15 and SD60 in 1 animal. The observations of alopecia in filgrastim-treated animals occurred at Grade 1 (n=3) on SD7, SD11 to 28, and SD31 to 60, Grade 2 (n=2) on SD15 to 60, Grade 3 (n=2) on SD16 to 60.

Immunogenicity (Anti-Filgrastim Antibodies)
One (4%) filgrastim-treated animal developed antifilgrastim antibody with a titer of 1:10. This is comparable to studies in humans where the reported frequency of posttreatment antifilgrastim antibodies is 3% (see APPENDIX for Amgen Package Insert for NEUPOGEN®).

Of the 20 control animals, 1 tested weakly positive for antibody at serum dilutions of 1:25 and 1:50. This observation may be the result of a false-positive assay reaction since this animal was not exposed to filgrastim. The immunogenicity assay was limited by the lack of a positive Reference Control.

Necropsy and Histopathology
Complete necropsies were performed on all animals which were found dead (n=3), euthanized (n=15) or sacrificed at study end (survivors) (SD60). In filgrastim-treated animals, both the gross and histological observations showed a decrease in the whole-body radiation-induced pathology. This was most evident in the appearance of the bone marrow. In the control animals that had bone marrow lesions (11 of 22 animals), all had moderate to severe depletion in both the erythroid and myeloid components. In some of these 11 animals, there was marked depletion with only a few small areas of active marrow with immature erythroid and myeloid cells remaining. In contrast, only 5 of 24 filgrastim-treated animals displayed lesions in the bone marrow.

Of the 5 filgrastim-treated animals with bone marrow lesions, 4 had moderate to severe depletion and 1 had mild to moderate depletion with maintenance of marrow regenerative activity. In evaluating lymphoid tissues (thymus, mesenteric lymph node, and spleen), a similar pattern was observed. The control group had a higher percent of animals with some level of lymphocytic depletion in all 3 tissues as compared to the filgrastim-treated group. It is evident from the histological data that filgrastim had a protective effect on the radiation induced alterations in the thymus, spleen, and lymph nodes.

A number of the other tissues examined during this study also demonstrated an apparent protective effect related to the filgrastim treatment. Most evident was a decrease in hemorrhage and bacterial emboli (sepsis) in the filgrastim-treated animals compared to the control animals. This finding was observed in the lungs, kidneys, hearts, skin, and livers.

The small intestine exhibited little difference in the type or extent of pathological lesions or in the percent of animals affected when comparing the filgrastim-treated to control groups. Radiation injury of the small intestine (mucosa and probably endothelium) was similar in the control and filgrastim-treated animals. In the large intestine, there was an increase in total lesions in the filgrastim-treated animals compared to the control animals. Most prominent was the increase in inflammation. There were few differences in other lesions.

Bacterial embolization, as seen in this study, was interpreted as originating in the radiation-damaged intestine with peracute or acute vascular involvement of multiple organs. These emboli usually had no related inflammatory changes.

These data demonstrate that filgrastim enhanced the survival of irradiated animals but in animals in which it did not, the lesions observed were similar in extent, distribution, and severity to those seen in the untreated, control animals. Alternatively, in any animal that survived the 60-day test period, the pathologic alterations, especially in the bone marrow, resolved and there were few differences between the untreated control and filgrastim-treated animals. Death (and survival) in both groups was related to bone marrow and lymphoid cell (thymus, mesenteric lymph node, and spleen) recovery which prevented or reduced the bacterial embolization and probable endotoxemia.

Microbial Analysis of Tissues and Blood at Necropsy
Samples of blood, spleen, liver, lung and kidney were collected from all animals at necropsy for microbial analysis (Table 4-16).
- 2 of the 5 filgrastim-treated animals euthanized during the study were positive for bacteria in blood and organs
- 3 control animals found dead were positive for bacteria in blood and organs
- 10 control animals euthanized during the study and 2 were negative for bacteria, 4 were positive for bacteria in organs and blood, and 4 were positive for bacteria in blood only

Table 4-16. Number and Percentage of Animals with Positive Bacteriology Results in Organs and Blood at Necropsy for Rhesus Macaques Euthanized before End of Study

	Organs	Percentage of Microbial Positive Organs	Blood	Percentage of Microbial Positive Blood
Control (n=10)	4	40%	8	80%
Filgrastim (n=5)	2	40%	2	40%

4.4   Analysis of Decedent Animals

Veterinarians (n=6) who were blinded to the animal's treatment performed cage-side observations and assessed the animal's health on a daily basis. Generally, the 5 veterinarians were assigned a day during the work week to perform morning and evening cage-side observations. A sixth veterinarian was routinely assigned for all morning and evening observations occurring over the weekends during the study. A decision to euthanize an animal was based on the criteria outlined in Section 2.2. A total of 18 animals died prior to the end of study: 3 were found dead and 15 were euthanized. Five of 6 veterinarians made all euthanasia decisions with 1exception as indicated in Table 4-17. The summary indicates an even distribution of euthanasia decisions.

Table 4-17. Euthanasia Decisions by Veterinarian

Veterinarian	Control Euthanized		Filgrastim Euthanized		Total Number of Euthanasia Authorizations per Veterinarian	Percentage of Euthanasia Authorizations per Veterinarian
	n	Study Day	n	Study Day	n	n
1	2	12, 15			2	13%
2	1	36	1	8	2	13%
3	2	18,19	2	11, 11	4	27%
4	3	18, 19, 43			3	20%
5	2	17, 19	1	19	3	20%
Study Director*			1	11	1	7%
*The animal's condition deteriorated rapidly and a veterinarian was not immediately available to evaluate and authorize euthanasia, therefore the study director authorized the euthanasia. (See Table 4-18, Animal ID R03018.)						

Table 4-18. List of Decedent Animals

Animal ID	Treatment	Final ANC/µL	Final Body Temp (ºF)	Day of Death	Euthanasia Criteria (if applicable)	Bacteriology on Necropsy Samples	
						Blood	Organ(s)
030613	Filgrastim	24	104.2	8	Severe Condition	negative	negative
03026	Filgrastim	0	103.9	11	Abnormal Activity, Abnormal Appearance	negative	negative
0401153	Filgrastim	10	104.2	11	Abnormal Activity, Abnormal Appearance	positive	positive
03018	Filgrastim	0	92.0	11	Inactivity	positive	positive
0311025	Filgrastim	180	102.5	19	Severe Condition	negative	negative
04063	Control	8	102.0	11	Found Dead	positive	positive
04057	Control	4	102.3	12	Inactivity	positive	negative
03R0225	Control	0	103.0	15	Inactivity	positive	positive
050247	Control	0	103.0	17	Abnormal Activity, Abnormal Appearance	positive	positive
04021	Control	0	102.5	17	Found Dead	positive	positive
03R0716	Control	0	101.0	17	Inactivity	positive	negative
040605	Control	8	101.0	18	Found Dead	positive	positive
03697	Control	40	95.6	18	Inactivity	positive	positive
040159	Control	0	103.8	19	Abnormal Activity, Abnormal Appearance	positive	positive
050103	Control	0	103.8	19	Severe Condition	negative	negative
R03038	Control	2688	102.0	31	Severe Condition	positive	negative
03010	Control	1638	103.6	36	Severe Condition	negative	negative
040129	Control	1062	101.2	43	Severe Condition	positive	negative

- 60% (3/5) of filgrastim-treated animals that died at or before SD19 had negative bacterial cultures at necropsy in both the blood and the organs.
- 90% of controls (9/10) that died or were euthanized in the same time frame had at least a microbial positive blood culture at necropsy.
- Necropsy of filgrastim-treated animals euthanized prior to SD60 showed that the bone marrow lesions observed were similar in extent, distribution, and severity to those seen in the untreated, control animals.
- 40% (2/5) filgrastim-treated animals were neutropenic on the day of death (SD11, SD11).
- 60% (6/10) of the control animals were absolutely neutropenic when death occurred at or before SD19.

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4.5   Analysis of Medical Management Parameters

The various elements of medical management provided to the animals on study are summarized on Table 4-19.

Table 4-19. Supportive Care: Number of Control or Filgrastim-treated Animals Provided Specific Supportive Care Measures

Antibiotics: General and by Specific Antibiotic

Treatment	Antibiotics	Baytril	Gentamicin	Rocephin	Primaxin IM
Control (n=22)	22	22	20	11	3
Filgrastim (n=24)	24	24	19	10	3

Anti-Fungal, Antibiotics

Treatment	Fluconazole	Metronidazole*	Erythromycin	Ophthalmic Ointment*
Control (n=22)	6	3	1	0
Filgrastim (n=24)	3	0	0	1

Hydration: Based on Severity of Dehydration

Treatment	Hydration IV push (Mild)	Hydration oral gastric tube & IV push (Moderate)	Hydration gavage & IV drip (Severe)
Control (n=22)	22	22	1
Filgrastim (n=24)	24	24	1

Analgesics, Ulcer Treatments, Antiseptics, Anti-Inflammatory Agents

Treatment	Bupren-orphine	Carafate	Bupivacaine, Nolvasan, and/or H_2O_2	Nolvasan, H_2O_2	Rimadyl	Pepcid*
Control (n=22)	22	19	16	1	18	1
Filgrastim (n=24)	24	18	13	0	15	0

*Administered by veterinarian directive

Blood Products

Treatment	Transfusion
Control (n=22)	21
Filgrastim (n=24)	21

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Review of the Medical Management provided to the study animals indicated that the 2 groups were similar with regards to the type of treatments received. Two differences observed were: twice as many control animals (n=6) were administered fluconazole (persistent fever of >5 days despite additional or alternative antibiotic therapy) than filgrastim-treated animals (n=3) and only control animals (n=3) met the criteria for metronidazole treatment (gram-negative anaerobe identified in blood culture).

4.6   Study AXG15 Summary

Study AXG15 was designed as a proof-of-concept GLP efficacy study conducted to demonstrate the efficacy of filgrastim in the rhesus macaque model of H-ARS (defined and characterized in Study AXR01). Study AXG15 was terminated early for efficacy after the per protocol interim analysis showed a statistically significant improvement in survival of filgrastim-treated animals compared to controls. The analysis of the primary endpoint showed significantly higher 60-day survival in the filgrastim-treated animals compared to the control group (79% versus 41%).

The occurrence and duration of neutropenia was significantly less among animals in the filgrastim-treated group. Similarly, control animals had earlier first day of neutropenia and later days to recovery than filgrastim-treated animals. Deaths in both groups occurred in animals with very low neutrophil counts in the days just prior to death. Absolute neutropenia was observed in 58% of the filgrastim-treated animals and 82% of the controls. While control animals had a greater incidence of febrile neutropenia, the difference was not statistically significant. Nonetheless, control animals had statistically significantly higher incidence of bacteria positive blood culture than filgrastim-treated animals (86% versus 58%).

There were no major differences between treatment groups for parameters scored during cage-side observations or for transfusions. There were some trends suggesting worse outcomes for controls with respect to stool consistency. The type and frequency of medical management measures per treatment group were similar.

Necropsies indicated that the lesions noted in animals that died before SD 60 were similar with the exception that bone marrow aplasia was less severe in the filgrastim-treated animal that was euthanized at SD19. Bone marrow lesions were more frequently noted in controls compared to filgrastim-treated animals. This observation directly correlated to the number of the controls that succumbed before SD60. Animals who survived the 60-day study were similar irrespective of treatment arm.

5.0 Summary

Currently, there are no FDA-approved drug products for the treatment of individuals following accidental exposure to radiation leading to H-ARS. Treatment in prior events has been based on clinical experience managing the symptoms arising from radiation exposure and included both the administration of hematopoietic growth factors (such as filgrastim) as well as medical management (antibiotics, pain management, antipyretics, antiemetics, etc.) in response to clinical symptoms.

Treatment strategies for personnel exposed to potentially lethal doses of radiation have been the subject of several international conferences and working groups and one of the drug products recommended for treatment of H-ARS is filgrastim. Filgrastim is not currently indicated for the treatment of H-ARS. This lack of a label indication would limit access to filgrastim for administration to individuals with H-ARS in radiation accidents. The NIAID, on being delegated the authority to develop radiation nuclear countermeasures, evaluated filgrastim for use in the treatment of H-ARS.

Since clinical trials for products for use in the treatment of ARS resulting from unintentional radiation exposure cannot be ethically conducted, such products would have to be developed via the FDA Animal Rule pathway. This drug development pathway requires the conduct of efficacy studies in well-characterized animal models in relevant species. To meet the requirements of this development pathway, the NIAID conducted Study AXR01 to characterize a rhesus macaque model of H-ARS. Subsequently, the NIAID conducted Study AXG15 to test the efficacy of filgrastim in the well-characterized animal model defined in Study AXR01.

The results from Study AXG15 demonstrated that treatment with filgrastim (10 µg/kg/d, SC) significantly improved survival in a rhesus macaque model of H-ARS. The filgrastim dose used in this study was representative of the currently approved dose of NEUPOGEN® (filgrastim, Amgen, Inc.) Filgrastim treatment also led to an improvement of neutrophil-related parameters and incidence of infection. Therefore, the data from Study AXG15 support the use of filgrastim for the treatment of H-ARS.

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National Institute of
Allergy and
Infectious Diseases

APPENDIX

Amgen's US Package Insert for NEUPOGEN® (filgrastim)

NEUPOGEN® (filgrastim)

DESCRIPTION

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. NEUPOGEN® is the Amgen Inc. trademark for filgrastim, which has been selected as the name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF).

NEUPOGEN® is a 175 amino acid protein manufactured by recombinant DNA technology.[1] NEUPOGEN® is produced by *Escherichia coli* (*E coli*) bacteria into which has been inserted the human granulocyte colony-stimulating factor gene. NEUPOGEN® has a molecular weight of 18,800 daltons. The protein has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine necessary for expression in *E coli*. Because NEUPOGEN® is produced in *E coli*, the product is nonglycosylated and thus differs from G-CSF isolated from a human cell.

NEUPOGEN® is a sterile, clear, colorless, preservative-free liquid for parenteral administration containing filgrastim at a specific activity of $1.0 \pm 0.6 \times 10^8$ U/mg (as measured by a cell mitogenesis assay). The product is available in single-use vials and prefilled syringes. The single-use vials contain either 300 mcg or 480 mcg filgrastim at a fill volume of 1.0 mL or 1.6 mL, respectively. The single-use prefilled syringes contain either 300 mcg or 480 mcg filgrastim at a fill volume of 0.5 mL or 0.8 mL, respectively. See table below for product composition of each single-use vial or prefilled syringe.

	300 mcg/ 1.0 mL Vial	480 mcg/ 1.6 mL Vial	300 mcg/ 0.5 mL Syringe	480 mcg/ 0.8 mL Syringe
filgrastim	300 mcg	480 mcg	300 mcg	480 mcg
Acetate	0.59 mg	0.94 mg	0.295 mg	0.472 mg
Sorbitol	50.0 mg	80.0 mg	25.0 mg	40.0 mg
Polysorbate 80	0.04 mg	0.064 mg	0.02 mg	0.032 mg
Sodium	0.035 mg	0.056 mg	0.0175 mg	0.028 mg
Water for Injection USP q.s. ad	1.0 mL	1.6 mL	0.5 mL	0.8 mL

CLINICAL PHARMACOLOGY

Colony-Stimulating Factors

Colony-stimulating factors are glycoproteins which act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation commitment, and some end-cell functional activation.

Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation,[2,3] differentiation,[2,4] and selected end-cell functional activation (including enhanced phagocytic ability,[5] priming of the cellular metabolism associated with respiratory burst,[6] antibody dependent killing,[7] and the increased expression of some functions associated with cell surface antigens[8]). G-CSF is not species-specific and has been shown to have minimal direct in vivo or in vitro effects on the production of hematopoietic cell types other than the neutrophil lineage.

Preclinical Experience

Filgrastim was administered to monkeys, dogs, hamsters, rats, and mice as part of a preclinical toxicology program which included single-dose acute, repeated-dose subacute, subchronic, and chronic studies. Single-dose administration of filgrastim by the oral, intravenous (IV), subcutaneous (SC), or intraperitoneal (IP) routes resulted in no significant toxicity in mice, rats, hamsters, or monkeys. Although no deaths were observed in mice, rats, or monkeys at dose levels up to 3450 mcg/kg or in hamsters using single doses up to approximately 860 mcg/kg, deaths were observed in a subchronic (13-week) study in monkeys. In this study, evidence of neurological symptoms was seen in monkeys treated with doses of filgrastim greater than 1150 mcg/kg/day for up to 18 days. Deaths were seen in 5 of the 8 treated animals and were associated with 15- to 28-fold increases in peripheral leukocyte counts, and neutrophil-infiltrated hemorrhagic foci were seen in both the cerebrum and cerebellum. In contrast, no monkeys died following 13 weeks of daily IV administration of filgrastim at a dose level of 115 mcg/kg. In an ensuing 52-week study, one 115 mcg/kg dosed female monkey died after 18 weeks of daily IV administration of filgrastim. Death was attributed to cardiopulmonary insufficiency.

In subacute, repeated-dose studies, changes observed were attributable to the expected pharmacological actions of filgrastim (ie, dose-dependent increases in white cell counts, increased circulating segmented neutrophils, and increased myeloid:erythroid ratio in bone marrow). In all species, histopathologic examination of the liver and spleen revealed evidence of ongoing extramedullary granulopoiesis; increased spleen weights were seen in all species and appeared to be dose-related. A dose-dependent increase in serum alkaline phosphatase was observed in rats, and may reflect increased activity of osteoblasts and osteoclasts. Changes in serum chemistry values were reversible following discontinuation of treatment.

In rats treated at doses of 1150 mcg/kg/day for 4 weeks (5 of 32 animals) and for 13 weeks at doses of 100 mcg/kg/day (4 of 32 animals) and 500 mcg/kg/day (6 of 32 animals), articular swelling of the hind legs was observed. Some degree of hind leg dysfunction was also observed; however, symptoms reversed following cessation of dosing. In rats, osteoclasis and osteoanagenesis were found in the femur, humerus, coccyx, and hind legs (where they were accompanied by synovitis) after IV treatment for 4 weeks (115 to 1150 mcg/kg/day), and in the sternum after IV treatment for 13 weeks (115 to 575 mcg/kg/day). These effects reversed to normal within 4 to 5 weeks following cessation of treatment.

In the 52-week chronic, repeated-dose studies performed in rats (IP injection up to 57.5 mcg/kg/day), and cynomolgus monkeys (IV injection of up to 115 mcg/kg/day), changes observed were similar to those noted in the subacute studies. Expected pharmacological actions of filgrastim included dose-dependent increases in white cell counts, increased circulating segmented neutrophils and alkaline phosphatase levels, and increased myeloid:erythroid ratios in the bone marrow. Decreases in platelet counts were also noted in primates. In no animals tested were hemorrhagic complications observed. Rats displayed dose-related swelling of the hind limb, accompanied by some degree of hind limb dysfunction; osteopathy was noted microscopically. Enlarged spleens (both species) and livers (monkeys), reflective of ongoing extramedullary granulopoiesis, as well as myeloid hyperplasia of the bone marrow, were observed in a dose-dependent manner.

Pharmacologic Effects of NEUPOGEN®

In phase 1 studies involving 96 patients with various nonmyeloid malignancies, NEUPOGEN® administration resulted in a dose-dependent increase in circulating neutrophil counts over the dose range of 1 to 70 mcg/kg/day.[9-11] This increase in neutrophil counts was observed whether NEUPOGEN® was administered IV (1 to 70 mcg/kg twice daily),[9] SC (1 to 3 mcg/kg once daily),[11] or by continuous SC infusion (3 to 11 mcg/kg/day).[10] With discontinuation of NEUPOGEN® therapy, neutrophil counts returned to baseline in most cases within 4 days. Isolated neutrophils displayed normal phagocytic (measured by zymosan-stimulated chemoluminescence) and chemotactic (measured by migration under agarose using N-formyl-methionyl-leucyl-phenylalanine [fMLP] as the chemotaxin) activity in vitro.

The absolute monocyte count was reported to increase in a dose-dependent manner in most patients receiving NEUPOGEN®; however, the percentage of monocytes in the differential count remained within the normal range. In all studies to date, absolute counts of both eosinophils and basophils did not change and were within the normal range following administration of NEUPOGEN®. Increases in lymphocyte counts following NEUPOGEN® administration have been reported in some normal subjects and cancer patients.

White blood cell (WBC) differentials obtained during clinical trials have demonstrated a shift towards earlier granulocyte progenitor cells (left shift), including the appearance of promyelocytes and myeloblasts, usually during neutrophil recovery following the chemotherapy-induced nadir. In addition, Dohle bodies, increased granulocyte granulation, and hypersegmented neutrophils have been observed. Such changes were transient and were not associated with clinical sequelae, nor were they necessarily associated with infection.

Pharmacokinetics

Absorption and clearance of NEUPOGEN® follows first-order pharmacokinetic modeling without apparent concentration dependence. A positive linear correlation occurred between the parenteral dose and both the serum concentration and area-under-the-concentration-time curves. Continuous IV infusion of 20 mcg/kg of NEUPOGEN® over 24 hours resulted in mean and median serum concentrations of approximately 48 and 56 ng/mL, respectively. Subcutaneous administration of 3.45 mcg/kg and 11.5 mcg/kg resulted in maximum serum concentrations of 4 and 49 ng/mL, respectively, within 2 to 8 hours. The volume of distribution averaged 150 mL/kg in both normal subjects and cancer patients. The elimination half-life, in both normal subjects and cancer patients, was approximately 3.5 hours. Clearance rates of NEUPOGEN® were approximately 0.5 to 0.7 mL/minute/kg. Single parenteral doses or daily IV doses, over a 14-day period, resulted in comparable half-lives. The half-lives were similar for IV administration (231 minutes, following doses of 34.5 mcg/kg) and for SC administration (210 minutes, following NEUPOGEN® doses of 3.45 mcg/kg). Continuous 24-hour IV infusions of 20 mcg/kg over an 11- to 20-day period produced steady-state serum concentrations of NEUPOGEN® with no evidence of drug accumulation over the time period investigated.

Pharmacokinetic data in geriatric patients (≥ 65 years) are not available.

CLINICAL EXPERIENCE

Cancer Patients Receiving Myelosuppressive Chemotherapy

NEUPOGEN® has been shown to be safe and effective in accelerating the recovery of neutrophil counts following a variety of chemotherapy regimens. In a phase 3 clinical trial in small cell lung cancer, patients received SC administration of NEUPOGEN® (4 to 8 mcg/kg/day, days 4 to 17) or placebo. In this study, the benefits of NEUPOGEN® therapy were shown to be prevention of infection as manifested by febrile neutropenia, decreased hospitalization, and decreased IV antibiotic usage. No difference in survival or disease progression was demonstrated.

In the phase 3, randomized, double-blind, placebo-controlled trial conducted in patients with small cell lung cancer, patients were randomized to receive NEUPOGEN® (n = 99) or placebo (n = 111) starting on day 4, after receiving standard dose chemotherapy with cyclophosphamide, doxorubicin, and etoposide. A total of 210 patients were evaluated for efficacy and 207 evaluated for safety. Treatment with NEUPOGEN® resulted in a clinically and statistically significant reduction in the incidence of infection, as manifested by febrile neutropenia; the incidence of at least one infection over all cycles of chemotherapy was 76% (84/111) for placebo-treated patients versus 40% (40/99) for NEUPOGEN®-treated patients (p < 0.001). The following secondary analyses were also performed. The requirements for in-patient hospitalization and antibiotic use were also significantly decreased during the first cycle of chemotherapy; incidence of hospitalization was 69% (77/111) for placebo-treated patients in cycle 1 versus 52% (51/99) for NEUPOGEN®-treated patients (p = 0.032). The incidence of IV antibiotic usage was 60% (67/111) for placebo-treated patients in cycle 1 versus 38% (38/99) for NEUPOGEN®-treated patients (p = 0.003). The incidence, severity, and duration of severe neutropenia (absolute neutrophil count [ANC] < 500/mm^3) following chemotherapy were all significantly reduced. The incidence of severe neutropenia in cycle 1 was 84% (83/99) for patients receiving NEUPOGEN® versus 96% (106/110) for patients receiving placebo (p = 0.004). Over all cycles, patients randomized to NEUPOGEN® had a 57% (286/500 cycles) rate of severe neutropenia versus 77% (416/543 cycles) for patients randomized to placebo. The median duration of severe neutropenia in cycle 1 was reduced from 6 days (range 0 to 10 days) for patients receiving placebo to 2 days (range 0 to 9 days) for patients receiving NEUPOGEN® (p < 0.001). The mean duration of neutropenia in cycle 1 was 5.64 ± 2.27 days for patients receiving placebo versus 2.44 ± 1.90 days for patients receiving NEUPOGEN®. Over all cycles, the median duration of neutropenia was 3 days for patients randomized to placebo versus 1 day for patients randomized to NEUPOGEN®. The median severity of neutropenia (as measured by ANC nadir) was 72/mm^3 (range 0/mm^3 to 7912/mm^3) in cycle 1 for patients receiving NEUPOGEN® versus 38/mm^3 (range 0/mm^3 to 9520/mm^3) for patients receiving placebo (p = 0.012). The mean severity of neutropenia in cycle 1 was 496/mm^3 ± 1382/mm^3 for patients receiving NEUPOGEN® versus 204/mm^3 ± 953/mm^3 for patients receiving placebo. Over all cycles, the ANC nadir for patients randomized to NEUPOGEN® was 403/mm^3 versus 161/mm^3 for patients randomized to placebo. Administration of NEUPOGEN® resulted in an earlier ANC nadir following chemotherapy than was experienced by patients receiving placebo (day 10 vs day 12). NEUPOGEN® was well tolerated when given SC daily at doses of 4 to 8 mcg/kg for up to 14 consecutive days following each cycle of chemotherapy (see ADVERSE REACTIONS).

Several other phase 1/2 studies, which did not directly measure the incidence of infection, but which did measure increases in neutrophils, support the efficacy of NEUPOGEN®. The regimens are presented to provide some background on the clinical experience with

NEUPOGEN®. No claim regarding the safety or efficacy of the chemotherapy regimens is made. The effects of NEUPOGEN® on tumor growth or on the anti-tumor activity of the chemotherapy were not assessed. The doses of NEUPOGEN® used in these studies are considerably greater than those found to be effective in the phase 3 study described above. Such phase 1/2 studies are summarized in the following table.

Type of Malignancy	Regimen	Chemotherapy Dose	No. Pts.	Trial Phase	NEUPOGEN® Daily Dosage[a]
Small Cell Lung Cancer	Cyclophosphamide Doxorubicin Etoposide	1 g/m^2/day 50 mg/m^2/day 120 mg/m^2/day x 3 q 21 days	210	3	4 − 8 mcg/kg SC days 4 − 17
Small Cell Lung Cancer[11]	Ifosfamide Doxorubicin Etoposide Mesna	5 g/m^2/day 50 mg/m^2/day 120 mg/m^2/day x 3 8 g/m^2/day q 21 days	12	1/2	5.75 − 46 mcg/kg IV days 4 − 17
Urothelial Cancer[12]	Methotrexate Vinblastine Doxorubicin Cisplatin	30 mg/m^2/day x 2 3 mg/m^2/day x 2 30 mg/m^2/day 70 mg/m^2/day q 28 days	40	1/2	3.45 − 69 mcg/kg IV days 4 − 11
Various Nonmyeloid Malignancies[13]	Cyclophosphamide Etoposide Cisplatin	2.5 g/m^2/day x 2 500 mg/m^2/day x 3 50 mg/m^2/day x 3 q 28 days	18	1/2	23 − 69 mcg/kg[b] IV days 8 − 28
Breast/Ovarian Cancer[14]	Doxorubicin[c]	75 mg/m^2 100 mg/m^2 125 mg/m^2 150 mg/m^2 q 14 days	21	2	11.5 mcg/kg IV days 2 − 9 5.75 mcg/kg IV days 10 − 12
Neuroblastoma	Cyclophosphamide Doxorubicin Cisplatin	150 mg/m^2 x 7 35 mg/m^2 90 mg/m^2 q 28 days (cycles 1,3,5) [d]	12	2	5.45 − 17.25 mcg/kg SC days 6 − 19

[a]   NEUPOGEN® doses were those that accelerated neutrophil production. Doses which provided no additional acceleration beyond that achieved at the next lower dose are not reported.

[b]   Lowest dose(s) tested in the study.

[c]   Patients received doxorubicin at either 75, 100, 125, or 150 mg/m^2.

[d]   Cycles 2,6 = cyclophosphamide 150 mg/m^2 x 7 and etoposide 280 mg/m^2 x 3.
    Cycle 4 = cisplatin 90 mg/m^2 x 1 and etoposide 280 mg/m^2 x 3.

Patients With Acute Myeloid Leukemia Receiving Induction or Consolidation Chemotherapy

In a randomized, double-blind, placebo-controlled, multi-center, phase 3 clinical trial, 521 patients (median age 54, range 16 to 89 years) were treated for de novo acute myeloid leukemia (AML). Following a standard induction chemotherapy regimen comprising daunorubicin, cytosine arabinoside, and etoposide[15] (DAV 3+7+5), patients received either NEUPOGEN® at 5 mcg/kg/day or placebo, SC, from 24 hours after the last dose of chemotherapy until neutrophil recovery (ANC 1000/mm^3 for 3 consecutive days or 10,000/mm^3 for 1 day) or for a maximum of 35 days.

Treatment with NEUPOGEN® significantly reduced the median time to ANC recovery and the median duration of fever, antibiotic use, and hospitalization following induction chemotherapy. In the NEUPOGEN®-treated group, the median time from initiation of chemotherapy to ANC recovery (ANC $\geq 500/mm^3$) was 20 days (vs 25 days in the control group, p = 0.0001), the median duration of fever was reduced by 1.5 days (p = 0.009), and there were statistically significant reductions in the durations of IV antibiotic use and hospitalization. During consolidation therapy (DAV 2+5+5), patients treated with NEUPOGEN® also experienced significant reductions in the incidence of severe neutropenia, time to neutrophil recovery, the incidence and duration of fever, and the durations of IV antibiotic use and hospitalization. Patients treated with a further course of standard (DAV 2+5+5) or high-dose cytosine arabinoside consolidation also experienced significant reductions in the duration of neutropenia.

There were no statistically significant differences between NEUPOGEN® and placebo groups in complete remission rate (69% NEUPOGEN® vs 68% placebo, p = 0.77), disease-free survival (median 342 days NEUPOGEN® [n = 178], 322 days placebo [n = 177], p = 0.99), time to progression of all randomized patients (median 165 days NEUPOGEN®, 186 days placebo, p = 0.87), or overall survival (median 380 days NEUPOGEN®, 425 days placebo, p = 0.83).

Cancer Patients Receiving Bone Marrow Transplant

In two separate randomized, controlled trials, patients with Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) were treated with myeloablative chemotherapy and autologous bone marrow transplantation (ABMT). In one study (n = 54), NEUPOGEN® was administered at doses of 10 or 30 mcg/kg/day; a third treatment group in this study received no NEUPOGEN®. A statistically significant reduction in the median number of days of severe neutropenia (ANC < $500/mm^3$) occurred in the NEUPOGEN®-treated groups versus the control group (23 days in the control group, 11 days in the 10 mcg/kg/day group, and 14 days in the 30 mcg/kg/day group [11 days in the combined treatment groups, p = 0.004]). In the second study (n = 44, 43 patients evaluable), NEUPOGEN® was administered at doses of 10 or 20 mcg/kg/day; a third treatment group in this study received no NEUPOGEN®. A statistically significant reduction in the median number of days of severe neutropenia occurred in the NEUPOGEN®-treated groups versus the control group (21.5 days in the control group and 10 days in both treatment groups, p < 0.001). The number of days of febrile neutropenia was also reduced significantly in this study (13.5 days in the control group, 5 days in the 10 mcg/kg/day group, and 5.5 days in the 20 mcg/kg/day group [5 days in the combined treatment groups, p < 0.0001]). Reductions in the number of days of hospitalization and antibiotic use were also seen, although these reductions were not statistically significant. There were no effects on red blood cell or platelet levels.

In a randomized, placebo-controlled trial, 70 patients with myeloid and nonmyeloid malignancies were treated with myeloablative therapy and allogeneic bone marrow transplant followed by 300 mcg/m^2/day of a filgrastim product. A statistically significant reduction in the median number of days of severe neutropenia occurred in the treated group versus the control group (19 days in the control group and 15 days in the treatment group, p < 0.001) and time to recovery of ANC to $\geq 500/mm^3$ (21 days in the control group and 16 days in the treatment group, p < 0.001).

In three nonrandomized studies (n = 119), patients received ABMT and treatment with NEUPOGEN®. One study (n = 45) involved patients with breast cancer and malignant melanoma. A second study (n = 39) involved patients with HD. The third study (n = 35)

involved patients with NHL, acute lymphoblastic leukemia (ALL), and germ cell tumor. In these studies, the recovery of the ANC to $\geq 500/mm^3$ ranged from a median of 11.5 to 13 days.

None of the conditioning regimens used in the ABMT studies included radiation therapy.

While these studies were not designed to compare survival, this information was collected and evaluated. The overall survival and disease progression of patients receiving NEUPOGEN® in these studies were similar to those observed in the respective control groups and to historical data.

Peripheral Blood Progenitor Cell Collection and Therapy in Cancer Patients

All patients in the Amgen-sponsored trials received a similar mobilization/collection regimen: NEUPOGEN® was administered for 6 to 7 days, with an apheresis procedure on days 5, 6, and 7 (except for a limited number of patients receiving apheresis on days 4, 6, and 8). In a non-Amgen-sponsored study, patients underwent mobilization to a target number of mononuclear cells (MNC), with apheresis starting on day 5. There are no data on the mobilization of peripheral blood progenitor cells (PBPC) after days 4 to 5 that are not confounded by leukapheresis.

Mobilization: Mobilization of PBPC was studied in 50 heavily pretreated patients (median number of prior cycles = 9.5) with NHL, HD, or ALL (Amgen study 1). CFU-GM was used as the marker for engraftable PBPC. The median CFU-GM level on each day of mobilization was determined from the data available (CFU-GM assays were not obtained on all patients on each day of mobilization). These data are presented below.

The data from Amgen study 1 were supported by data from Amgen study 2 in which 22 pretreated breast cancer patients (median number of prior cycles = 3) were studied. Both the CFU-GM and CD34^{+} cells reached a maximum on day 5 at >10-fold over baseline and then remained elevated with leukapheresis.

Progenitor Cell Levels in Peripheral Blood by Mobilization Day					
	Overall Study 1 CFU-GM/mL		Study 2 CFU-GM/mL		Study 2 CD34$^+$ (x 10^4/mL)
	No. Samples	Median (25% − 75%)	No. Samples	Median (25% − 75%)	No. Samples	Median (25% − 75%)
Day 1	11	18 (13 − 62)	20	42 (15 − 151)	20	0.13 (0.02 − 0.66)
Day 2	7	22 (3 − 61)	n/a	n/a	n/a	n/a
Day 3	10	138 (39 − 364)	n/a	n/a	n/a	n/a
Day 4	18	365 (158 − 864)	18	576 (108 − 1819)	17	2.11 (0.58 − 3.93)
Day 5	36	781 (391 − 1608)	21	960 (72 − 1677)	22	3.16 (1.08 − 6.11)
Day 6	46	505 (199 − 1397)	22	756 (70 − 3486)	22	2.67 (1.09 − 4.40)
Day 7	37	333 (111 − 938)	22	597 (118 − 2009)	21	2.64 (0.78 − 4.22)
Day 8	15	383 (94 − 815)	12	51 (10 − 746)	12	1.61 (0.38 − 4.31)

n/a = not available

In three studies of patients with prior exposure to chemotherapy, the median CFU-GM yield in the leukapheresis product ranged from 20.9 to 32.7 x 10^4/kg body weight (n = 105). In two of these studies where CD34$^+$ yields in the leukapheresis product were also determined, the median CD34$^+$ yields were 3.11 and 2.80 x 10^6/kg, respectively (n = 56). In an additional study of 18 chemotherapy-naive patients, the median CFU-GM yield was 123.4 x 10^4/kg.

Engraftment: Engraftment following NEUPOGEN®-mobilized PBPC is summarized for 101 patients in the following table. In all studies, a Cox regression model showed that the total number of CFU-GM and/or CD34$^+$ cells collected was a significant predictor of time to platelet recovery.

In a randomized, unblinded study of patients with HD or NHL undergoing myeloablative chemotherapy (Amgen study 3), 27 patients received NEUPOGEN®-mobilized PBPC followed by NEUPOGEN® and 31 patients received ABMT followed by NEUPOGEN®. Patients randomized to the NEUPOGEN®-mobilized PBPC group compared to the ABMT group had significantly fewer days of platelet transfusions (median 6 vs 10 days), a significantly shorter time to a sustained platelet count > 20,000/mm^3 (median 16 vs 23 days), a significantly shorter time to recovery of a sustained ANC $\geq$ 500/mm^3 (median 11 vs 14 days), significantly fewer days of red blood cell transfusions (median 2 vs 3 days) and a significantly shorter duration of posttransplant hospitalization.

		Amgen-sponsored Study 1 N = 13	Amgen-sponsored Study 2 N = 22	Amgen-sponsored Study 3 N = 27	Non-Amgen-sponsored Study N = 39
Median PBPC/kg Collected	MNC	9.5×10^8	9.5×10^8	8.1×10^8	10.3×10^8
	CD34$^+$	n/a	3.1×10^6	2.8×10^6	6.2×10^6
	CFU-GM	63.9×10^4	25.3×10^4	32.6×10^4	n/a
Days to ANC $\geq 500/mm^3$	Median	9	10	11	10
	Range	8 – 10	8 – 15	9 – 38	7 – 40
Days to Plt. $\geq 20,000/mm^3$	Median	10	12.5	16	15.5
	Range	7 – 16	10 – 30	8 – 52	7 – 63

n/a = not available

Three of the 101 patients (3%) did not achieve the criteria for engraftment as defined by a platelet count $\geq 20,000/mm^3$ by day 28. In clinical trials of NEUPOGEN® for the mobilization of PBPC, NEUPOGEN® was administered to patients at 5 to 24 mcg/kg/day after reinfusion of the collected cells until a sustainable ANC ($\geq 500/mm^3$) was reached. The rate of engraftment of these cells in the absence of NEUPOGEN® posttransplantation has not been studied.

Patients With Severe Chronic Neutropenia

Severe chronic neutropenia (SCN) (idiopathic, cyclic, and congenital) is characterized by a selective decrease in the number of circulating neutrophils and an enhanced susceptibility to bacterial infections.

The daily administration of NEUPOGEN® has been shown to be safe and effective in causing a sustained increase in the neutrophil count and a decrease in infectious morbidity in children and adults with the clinical syndrome of SCN.[16] In the phase 3 trial, summarized in the following table, daily treatment with NEUPOGEN® resulted in significant beneficial changes in the incidence and duration of infection, fever, antibiotic use, and oropharyngeal ulcers. In this trial, 120 patients with a median age of 12 years (range 1 to 76 years) were treated.

Overall Significant Changes in Clinical Endpoints Median Incidence[a] (events) or Duration (days) per 28-day Period			
	Control Patients[b]	NEUPOGEN® -treated Patients	p-value
Incidence of Infection	0.50	0.20	< 0.001
Incidence of Fever	0.25	0.20	< 0.001
Duration of Fever	0.63	0.20	0.005
Incidence of Oropharyngeal Ulcers	0.26	0.00	< 0.001
Incidence of Antibiotic Use	0.49	0.20	< 0.001

   Incidence values were calculated for each patient, and are defined as the total number of events experienced divided by the number of 28-day periods of exposure (on-study). Median incidence values were then reported for each patient group.

b   Control patients were observed for a 4-month period.

The incidence for each of these 5 clinical parameters was lower in the NEUPOGEN® arm compared to the control arm for cohorts in each of the 3 major diagnostic categories. All 3 diagnostic groups showed favorable trends in favor of treatment. An analysis of variance showed no significant interaction between treatment and diagnosis, suggesting that efficacy did not differ substantially in the different diseases. Although NEUPOGEN® substantially reduced neutropenia in all patient groups, in patients with cyclic neutropenia, cycling persisted but the period of neutropenia was shortened to 1 day.

As a result of the lower incidence and duration of infections, there was also a lower number of episodes of hospitalization (28 hospitalizations in 62 patients in the treated group vs 44 hospitalizations in 60 patients in the control group over a 4-month period [p = 0.0034]). Patients treated with NEUPOGEN® also reported a lower number of episodes of diarrhea, nausea, fatigue, and sore throat.

In the phase 3 trial, untreated patients had a median ANC of 210/mm^3 (range 0 to 1550/mm^3). NEUPOGEN® therapy was adjusted to maintain the median ANC between 1500 and 10,000/mm^3. Overall, the response to NEUPOGEN® was observed in 1 to 2 weeks. The median ANC after 5 months of NEUPOGEN® therapy for all patients was 7460/mm^3 (range 30 to 30,880/mm^3). NEUPOGEN® dosing requirements were generally higher for patients with congenital neutropenia (2.3 to 40 mcg/kg/day) than for patients with idiopathic (0.6 to 11.5 mcg/kg/day) or cyclic (0.5 to 6 mcg/kg/day) neutropenia.

INDICATIONS AND USAGE

Cancer Patients Receiving Myelosuppressive Chemotherapy

NEUPOGEN® is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever (see CLINICAL EXPERIENCE). A complete blood count (CBC) and platelet count should be obtained prior to chemotherapy, and twice per week (see LABORATORY MONITORING) during NEUPOGEN® therapy to avoid leukocytosis and to monitor the neutrophil count. In phase 3 clinical studies, NEUPOGEN® therapy was discontinued when the ANC was ≥ 10,000/mm^3 after the expected chemotherapy-induced nadir.

Patients With Acute Myeloid Leukemia Receiving Induction or Consolidation Chemotherapy

NEUPOGEN® is indicated for reducing the time to neutrophil recovery and the duration of fever, following induction or consolidation chemotherapy treatment of adults with AML.

Cancer Patients Receiving Bone Marrow Transplant

NEUPOGEN® is indicated to reduce the duration of neutropenia and neutropenia-related clinical sequelae, e.g., febrile neutropenia in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by marrow transplantation (see CLINICAL EXPERIENCE). It is recommended that CBCs and platelet counts be obtained at a minimum of 3 times per week (see LABORATORY MONITORING) following marrow infusion to monitor the recovery of marrow reconstitution.

Patients Undergoing Peripheral Blood Progenitor Cell Collection and Therapy

NEUPOGEN® is indicated for the mobilization of hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis. Mobilization allows for the collection of increased numbers of progenitor cells capable of engraftment compared with collection by leukapheresis without mobilization or bone marrow harvest. 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 (see CLINICAL EXPERIENCE).

Patients With Severe Chronic Neutropenia

NEUPOGEN® is indicated for chronic administration to reduce the incidence and duration of sequelae of neutropenia (eg, fever, infections, oropharyngeal ulcers) in symptomatic patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia (see CLINICAL EXPERIENCE). It is essential that serial CBCs with differential and platelet counts, and an evaluation of bone marrow morphology and karyotype be performed prior to initiation of NEUPOGEN® therapy (see WARNINGS). The use of NEUPOGEN® prior to confirmation of SCN may impair diagnostic efforts and may thus impair or delay evaluation and treatment of an underlying condition, other than SCN, causing the neutropenia.

CONTRAINDICATIONS

NEUPOGEN® is contraindicated in patients with known hypersensitivity to *E coli*-derived proteins, filgrastim, or any component of the product.

WARNINGS

Allergic Reactions

Allergic-type reactions occurring on initial or subsequent treatment have been reported in < 1 in 4000 patients treated with NEUPOGEN®. These have generally been characterized by systemic symptoms involving at least two body systems, most often skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea), and cardiovascular (hypotension, tachycardia). Some reactions occurred on initial exposure. Reactions tended to occur within the first 30 minutes after administration and appeared to occur more frequently in patients receiving NEUPOGEN® IV. Rapid resolution of symptoms occurred in most cases after administration of antihistamines, steroids, bronchodilators, and/or epinephrine. Symptoms recurred in more than half the patients who were rechallenged.

SPLENIC RUPTURE

SPLENIC RUPTURE, INCLUDING FATAL CASES, HAS BEEN REPORTED FOLLOWING THE ADMINISTRATION OF NEUPOGEN®. INDIVIDUALS RECEIVING NEUPOGEN® WHO REPORT LEFT UPPER ABDOMINAL AND/OR SHOULDER TIP PAIN SHOULD BE EVALUATED FOR AN ENLARGED SPLEEN OR SPLENIC RUPTURE.

Acute Respiratory Distress Syndrome (ARDS)

Acute respiratory distress syndrome (ARDS) has been reported in patients receiving NEUPOGEN®, and is postulated to be secondary to an influx of neutrophils to sites of inflammation in the lungs. Patients receiving NEUPOGEN® who develop fever, lung infiltrates, or respiratory distress should be evaluated for the possibility of ARDS. In the event that ARDS occurs, NEUPOGEN® should be withheld until resolution of ARDS or discontinued. Patients should receive appropriate medical management for this condition.

Alveolar Hemorrhage and Hemoptysis

Alveolar hemorrhage manifesting as pulmonary infiltrates and hemoptysis requiring hospitalization has been reported in healthy donors undergoing PBPC mobilization. Hemoptysis resolved with discontinuation of NEUPOGEN®. The use of NEUPOGEN® for PBPC mobilization in healthy donors is not an approved indication.

Sickle Cell Disorders

Severe sickle cell crises, in some cases resulting in death, have been associated with the use of NEUPOGEN® in patients with sickle cell disorders. Only physicians qualified by specialized training or experience in the treatment of patients with sickle cell disorders should prescribe NEUPOGEN® for such patients, and only after careful consideration of the potential risks and benefits.

Thrombocytopenia

Thrombocytopenia has been reported commonly in patients receiving NEUPOGEN®. Platelet counts should be monitored closely.

Patients With Severe Chronic Neutropenia

The safety and efficacy of NEUPOGEN® in the treatment of neutropenia due to other hematopoietic disorders (eg, myelodysplastic syndrome [MDS]) have not been established. Care should be taken to confirm the diagnosis of SCN before initiating NEUPOGEN® therapy.

MDS and AML have been reported to occur in the natural history of congenital neutropenia without cytokine therapy.[17] Cytogenetic abnormalities, transformation to MDS, and AML have also been observed in patients treated with NEUPOGEN® for SCN. Based on available data including a postmarketing surveillance study, the risk of developing MDS and AML appears to be confined to the subset of patients with congenital neutropenia (see ADVERSE REACTIONS). Abnormal cytogenetics and MDS have been associated with the eventual development of myeloid leukemia. The effect of NEUPOGEN® on the development of abnormal cytogenetics and the effect of continued NEUPOGEN® administration in patients with abnormal cytogenetics or MDS are unknown. If a patient with SCN develops abnormal cytogenetics or myelodysplasia, the risks and benefits of continuing NEUPOGEN® should be carefully considered.

PRECAUTIONS

General

Simultaneous Use With Chemotherapy and Radiation Therapy

The safety and efficacy of NEUPOGEN® given simultaneously with cytotoxic chemotherapy have not been established. Because of the potential sensitivity of rapidly dividing myeloid cells to cytotoxic chemotherapy, do not use NEUPOGEN® in the period

24 hours before through 24 hours after the administration of cytotoxic chemotherapy (see DOSAGE AND ADMINISTRATION).

The efficacy of NEUPOGEN® has not been evaluated in patients receiving chemotherapy associated with delayed myelosuppression (e.g., nitrosoureas), with mitomycin C, or with myelosuppressive doses of antimetabolites such as 5-fluorouracil.

The safety and efficacy of NEUPOGEN® have not been evaluated in patients receiving concurrent radiation therapy. Simultaneous use of NEUPOGEN® with chemotherapy and radiation therapy should be avoided.

Potential Effect on Malignant Cells

NEUPOGEN® is a growth factor that primarily stimulates neutrophils. However, the possibility that NEUPOGEN® can act as a growth factor for any tumor type cannot be excluded. In a randomized study evaluating the effects of NEUPOGEN® versus placebo in patients undergoing remission induction for AML, there was no significant difference in remission rate, disease-free, or overall survival (see CLINICAL EXPERIENCE).

The safety of NEUPOGEN® in chronic myeloid leukemia (CML) and myelodysplasia has not been established.

When NEUPOGEN® is used to mobilize PBPC, tumor cells may be released from the marrow and subsequently collected in the leukapheresis product. The effect of reinfusion of tumor cells has not been well studied, and the limited data available are inconclusive.

Leukocytosis

Cancer Patients Receiving Myelosuppressive Chemotherapy

White blood cell counts of 100,000/mm^3 or greater were observed in approximately 2% of patients receiving NEUPOGEN® at doses above 5 mcg/kg/day. There were no reports of adverse events associated with this degree of leukocytosis. In order to avoid the potential complications of excessive leukocytosis, a CBC is recommended twice per week during NEUPOGEN® therapy (see LABORATORY MONITORING).

Premature Discontinuation of NEUPOGEN® Therapy

Cancer Patients Receiving Myelosuppressive Chemotherapy

A transient increase in neutrophil counts is typically seen 1 to 2 days after initiation of NEUPOGEN® therapy. However, for a sustained therapeutic response, NEUPOGEN® therapy should be continued following chemotherapy until the post-nadir ANC reaches 10,000/mm^3. Therefore, the premature discontinuation of NEUPOGEN® therapy, prior to the time of recovery from the expected neutrophil nadir, is generally not recommended (see DOSAGE AND ADMINISTRATION).

Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The incidence of antibody development in patients receiving NEUPOGEN® has not been adequately determined. While available data suggest that a small proportion of patients developed binding antibodies to filgrastim, the nature and specificity of these antibodies has not been adequately studied. In clinical studies comparing NEUPOGEN® and Neulasta®, the

incidence of antibodies binding to NEUPOGEN® was 3% (11/333). In these 11 patients, no evidence of a neutralizing response was observed using a cell-based bioassay. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay, and the observed incidence of antibody positivity in an assay may be influenced by several factors including timing of sampling, sample handling, concomitant medications, and underlying disease. Therefore, comparison of the incidence of antibodies to NEUPOGEN® with the incidence of antibodies to other products may be misleading.

Cytopenias resulting from an antibody response to exogenous growth factors have been reported on rare occasions in patients treated with other recombinant growth factors. There is a theoretical possibility that an antibody directed against filgrastim may cross-react with endogenous G-CSF, resulting in immune-mediated neutropenia; however, this has not been reported in clinical studies or in post-marketing experience. Patients who develop hypersensitivity to filgrastim (NEUPOGEN®) may have allergic or hypersensitivity reactions to other *E coli*-derived proteins.

Cutaneous Vasculitis

Cutaneous vasculitis has been reported in patients treated with NEUPOGEN®. In most cases, the severity of cutaneous vasculitis was moderate or severe. Most of the reports involved patients with SCN receiving long-term NEUPOGEN® therapy. Symptoms of vasculitis generally developed simultaneously with an increase in the ANC and abated when the ANC decreased. Many patients were able to continue NEUPOGEN® at a reduced dose.

Information for Patients and Caregivers

Patients should be referred to the "Information for Patients and Caregivers" labeling included with the package insert in each dispensing pack of NEUPOGEN® vials or NEUPOGEN® prefilled syringes. The "Information for Patients and Caregivers" labeling provides information about neutrophils and neutropenia and the safety and efficacy of NEUPOGEN®. It is not intended to be a disclosure of all known or possible effects.

Laboratory Monitoring

Cancer Patients Receiving Myelosuppressive Chemotherapy

A CBC and platelet count should be obtained prior to chemotherapy, and at regular intervals (twice per week) during NEUPOGEN® therapy. Following cytotoxic chemotherapy, the neutrophil nadir occurred earlier during cycles when NEUPOGEN® was administered, and WBC differentials demonstrated a left shift, including the appearance of promyelocytes and myeloblasts. In addition, the duration of severe neutropenia was reduced and was followed by an accelerated recovery in the neutrophil counts.

Cancer Patients Receiving Bone Marrow Transplant

Frequent CBCs and platelet counts are recommended (at least 3 times per week) following marrow transplantation.

Patients With Severe Chronic Neutropenia

During the initial 4 weeks of NEUPOGEN® therapy and during the 2 weeks following any dose adjustment, a CBC with differential and platelet count should be performed twice

weekly. Once a patient is clinically stable, a CBC with differential and platelet count should be performed monthly during the first year of treatment. Thereafter, if clinically stable, routine monitoring with regular CBCs (i.e., as clinically indicated but at least quarterly) is recommended. Additionally, for those patients with congenital neutropenia, annual bone marrow and cytogenetic evaluations should be performed throughout the duration of treatment (see WARNINGS and ADVERSE REACTIONS).

In clinical trials, the following laboratory results were observed:

- Cyclic fluctuations in the neutrophil counts were frequently observed in patients with congenital or idiopathic neutropenia after initiation of NEUPOGEN® therapy.
- Platelet counts were generally at the upper limits of normal prior to NEUPOGEN® therapy. With NEUPOGEN® therapy, platelet counts decreased but usually remained within normal limits (see ADVERSE REACTIONS).
- Early myeloid forms were noted in peripheral blood in most patients, including the appearance of metamyelocytes and myelocytes. Promyelocytes and myeloblasts were noted in some patients.
- Relative increases were occasionally noted in the number of circulating eosinophils and basophils. No consistent increases were observed with NEUPOGEN® therapy.
- As in other trials, increases were observed in serum uric acid, lactic dehydrogenase, and serum alkaline phosphatase.

Drug Interaction

Drug interactions between NEUPOGEN® and other drugs have not been fully evaluated. Drugs which may potentiate the release of neutrophils, such as lithium, should be used with caution.

Increased hematopoietic activity of the bone marrow in response to growth factor therapy has been associated with transient positive bone-imaging changes. This should be considered when interpreting bone-imaging results.

Carcinogenesis, Mutagenesis, Impairment of Fertility

The carcinogenic potential of NEUPOGEN® has not been studied. NEUPOGEN® failed to induce bacterial gene mutations in either the presence or absence of a drug metabolizing enzyme system. NEUPOGEN® had no observed effect on the fertility of male or female rats, or on gestation at doses up to 500 mcg/kg.

Pregnancy Category C

NEUPOGEN® has been shown to have adverse effects in pregnant rabbits when given in doses 2 to 10 times the human dose. Since there are no adequate and well-controlled studies in pregnant women, the effect, if any, of NEUPOGEN® on the developing fetus or the reproductive capacity of the mother is unknown. However, the scientific literature describes transplacental passage of NEUPOGEN® when administered to pregnant rats during the latter part of gestation[18] and apparent transplacental passage of NEUPOGEN® when administered to pregnant humans by ≤ 30 hours prior to preterm delivery (≤ 30 weeks gestation).[19] NEUPOGEN® should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In rabbits, increased abortion and embryolethality were observed in animals treated with NEUPOGEN® at 80 mcg/kg/day. NEUPOGEN® administered to pregnant rabbits at doses of 80 mcg/kg/day during the period of organogenesis was associated with increased fetal resorption, genitourinary bleeding, developmental abnormalities, decreased body weight, live births, and food consumption. External abnormalities were not observed in the fetuses of dams treated at 80 mcg/kg/day. Reproductive studies in pregnant rats have shown that NEUPOGEN® was not associated with lethal, teratogenic, or behavioral effects on fetuses when administered by daily IV injection during the period of organogenesis at dose levels up to 575 mcg/kg/day.

In Segment III studies in rats, offspring of dams treated at > 20 mcg/kg/day exhibited a delay in external differentiation (detachment of auricles and descent of testes) and slight growth retardation, possibly due to lower body weight of females during rearing and nursing. Offspring of dams treated at 100 mcg/kg/day exhibited decreased body weights at birth, and a slightly reduced 4-day survival rate.

Women who become pregnant during NEUPOGEN® treatment are encouraged to enroll in Amgen's Pregnancy Surveillance Program. Patients or their physicians should call 1-800-77-AMGEN (1-800-772-6436) to enroll.

Nursing Mothers

It is not known whether NEUPOGEN® is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised if NEUPOGEN® is administered to a nursing woman.

Women who are nursing during NEUPOGEN® treatment are encouraged to enroll in Amgen's Lactation Surveillance Program. Patients or their physicians should call 1-800-77-AMGEN (1-800-772-6436) to enroll.

Pediatric Use

In a phase 3 study to assess the safety and efficacy of NEUPOGEN® in the treatment of SCN, 120 patients with a median age of 12 years were studied. Of the 120 patients, 12 were infants (1 month to 2 years of age), 47 were children (2 to 12 years of age), and 9 were adolescents (12 to 16 years of age). Additional information is available from a SCN postmarketing surveillance study, which includes long-term follow-up of patients in the clinical studies and information from additional patients who entered directly into the postmarketing surveillance study. Of the 531 patients in the surveillance study as of 31 December 1997, 32 were infants, 200 were children, and 68 were adolescents (see CLINICAL EXPERIENCE, INDICATIONS AND USAGE, LABORATORY MONITORING, and DOSAGE AND ADMINISTRATION).

Pediatric patients with congenital types of neutropenia (Kostmann's syndrome, congenital agranulocytosis, or Schwachman-Diamond syndrome) have developed cytogenetic abnormalities and have undergone transformation to MDS and AML while receiving chronic NEUPOGEN® treatment. The relationship of these events to NEUPOGEN® administration is unknown (see WARNINGS and ADVERSE REACTIONS).

Long-term follow-up data from the postmarketing surveillance study suggest that height and weight are not adversely affected in patients who received up to 5 years of NEUPOGEN® treatment. Limited data from patients who were followed in the phase 3 study for 1.5 years did not suggest alterations in sexual maturation or endocrine function.

The safety and efficacy in neonates and patients with autoimmune neutropenia of infancy have not been established.

In the cancer setting, 12 pediatric patients with neuroblastoma have received up to 6 cycles of cyclophosphamide, cisplatin, doxorubicin, and etoposide chemotherapy concurrently with NEUPOGEN®; in this population, NEUPOGEN® was well tolerated. There was one report of palpable splenomegaly associated with NEUPOGEN® therapy; however, the only consistently reported adverse event was musculoskeletal pain, which is no different from the experience in the adult population.

Geriatric Use

Among 855 subjects enrolled in 3 randomized, placebo-controlled trials of NEUPOGEN® use following myelosuppressive chemotherapy, there were 232 subjects age 65 or older, and 22 subjects age 75 or older. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other clinical experience has not identified differences in the responses between elderly and younger patients.

Clinical studies of NEUPOGEN® in other approved indications (ie, bone marrow transplant (BMT) recipients, PBPC mobilization, and SCN) did not include sufficient numbers of subjects aged 65 and older to determine whether elderly subjects respond differently from younger subjects.

ADVERSE REACTIONS

Clinical Trial Experience

Cancer Patients Receiving Myelosuppressive Chemotherapy

In clinical trials involving over 350 patients receiving NEUPOGEN® following nonmyeloablative cytotoxic chemotherapy, most adverse experiences were the sequelae of the underlying malignancy or cytotoxic chemotherapy. In all phase 2 and 3 trials, medullary bone pain, reported in 24% of patients, was the only consistently observed adverse reaction attributed to NEUPOGEN® therapy. This bone pain was generally reported to be of mild-to-moderate severity, and could be controlled in most patients with non-narcotic analgesics; infrequently, bone pain was severe enough to require narcotic analgesics. Bone pain was reported more frequently in patients treated with higher doses (20 to 100 mcg/kg/day) administered IV, and less frequently in patients treated with lower SC doses of NEUPOGEN® (3 to 10 mcg/kg/day).

In the randomized, double-blind, placebo-controlled trial of NEUPOGEN® therapy following combination chemotherapy in patients (n = 207) with small cell lung cancer, the following

adverse events were reported during blinded cycles of study medication (placebo or NEUPOGEN® at 4 to 8 mcg/kg/day). Events are reported as exposure-adjusted since patients remained on double-blind NEUPOGEN® a median of 3 cycles versus 1 cycle for placebo.

	% of Blinded Cycles With Events	
Event	NEUPOGEN® N = 384 Patient Cycles	Placebo N = 257 Patient Cycles
Nausea/Vomiting	57	64
Skeletal Pain	22	11
Alopecia	18	27
Diarrhea	14	23
Neutropenic Fever	13	35
Mucositis	12	20
Fever	12	11
Fatigue	11	16
Anorexia	9	11
Dyspnea	9	11
Headache	7	9
Cough	6	8
Skin Rash	6	9
Chest Pain	5	6
Generalized Weakness	4	7
Sore Throat	4	9
Stomatitis	5	10
Constipation	5	10
Pain (Unspecified)	2	7

In this study, there were no serious, life-threatening, or fatal adverse reactions attributed to NEUPOGEN® therapy. Specifically, there were no reports of flu-like symptoms, pleuritis, pericarditis, or other major systemic reactions to NEUPOGEN®.

Spontaneously reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase occurred in 27% to 58% of 98 patients receiving blinded NEUPOGEN® therapy following cytotoxic chemotherapy; increases were generally mild-to-moderate. Transient decreases in blood pressure (< 90/60 mmHg), which did not require clinical treatment, were reported in 7 of 176 patients in phase 3 clinical studies following administration of NEUPOGEN®. Cardiac events (myocardial infarctions, arrhythmias) have been reported in 11 of 375 cancer patients receiving NEUPOGEN® in clinical studies; the relationship to NEUPOGEN® therapy is unknown. No evidence of interaction of NEUPOGEN® with other drugs was observed in the course of clinical trials (see PRECAUTIONS). There has been no evidence for the development of antibodies or of a blunted or diminished response to NEUPOGEN® in treated patients, including those receiving NEUPOGEN® daily for almost 2 years.

Patients With Acute Myeloid Leukemia

In a randomized phase 3 clinical trial, 259 patients received NEUPOGEN® and 262 patients received placebo postchemotherapy. Overall, the frequency of all reported adverse events was similar in both the NEUPOGEN® and placebo groups (83% vs 82% in Induction 1; 61% vs 64% in Consolidation 1). Adverse events reported more frequently in the NEUPOGEN®-treated group included: petechiae (17% vs 14%), epistaxis (9% vs

5%), and transfusion reactions (10% vs 5%). There were no significant differences in the frequency of these events.

There were a similar number of deaths in each treatment group during induction (25 NEUPOGEN® vs 27 placebo). The primary causes of death included infection (9 vs 18), persistent leukemia (7 vs 5), and hemorrhage (6 vs 3). Of the hemorrhagic deaths, 5 cerebral hemorrhages were reported in the NEUPOGEN® group and 1 in the placebo group. Other serious nonfatal hemorrhagic events were reported in the respiratory tract (4 vs 1), skin (4 vs 4), gastrointestinal tract (2 vs 2), urinary tract (1 vs 1), ocular (1 vs 0), and other nonspecific sites (2 vs 1). While 19 (7%) patients in the NEUPOGEN® group and 5 (2%) patients in the placebo group experienced severe or fatal hemorrhagic events, overall, hemorrhagic adverse events were reported at a similar frequency in both groups (40% vs 38%). The time to transfusion-independent platelet recovery and the number of days of platelet transfusions were similar in both groups.

Cancer Patients Receiving Bone Marrow Transplant

In clinical trials, the reported adverse effects were those typically seen in patients receiving intensive chemotherapy followed by BMT. The most common events reported in both control and treatment groups included stomatitis, nausea, and vomiting, generally of mild-to-moderate severity and were considered unrelated to NEUPOGEN®. In the randomized studies of BMT involving 167 patients who received study drug, the following events occurred more frequently in patients treated with filgrastim than in controls: nausea (10% vs 4%), vomiting (7% vs 3%), hypertension (4% vs 0%), rash (12% vs 10%), and peritonitis (2% vs 0%). None of these events were reported by the investigator to be related to NEUPOGEN®. One event of erythema nodosum was reported moderate in severity and possibly related to NEUPOGEN®.

Generally, adverse events observed in nonrandomized studies were similar to those seen in randomized studies, occurred in a minority of patients and were of mild-to-moderate severity. In one study (n = 45), 3 serious adverse events reported by the investigator were considered possibly related to NEUPOGEN®. These included 2 events of renal insufficiency and 1 event of capillary leak syndrome. The relationship of these events to NEUPOGEN® remains unclear since they occurred in patients with culture-proven infection with clinical sepsis who were receiving potentially nephrotoxic antibacterial and antifungal therapy.

Cancer Patients Undergoing Peripheral Blood Progenitor Cell Collection and Therapy

In clinical trials, 126 patients received NEUPOGEN® for PBPC mobilization. In this setting, NEUPOGEN® was generally well tolerated. Adverse events related to NEUPOGEN® consisted primarily of mild-to-moderate musculoskeletal symptoms, reported in 44% of patients. These symptoms were predominantly events of medullary bone pain (33%). Headache was reported related to NEUPOGEN® in 7% of patients.

Transient increases in alkaline phosphatase related to NEUPOGEN® were reported in 21% of the patients who had serum chemistries measured; most were mild-to-moderate.

All patients had increases in neutrophil counts during mobilization, consistent with the biological effects of NEUPOGEN®. Two patients had a WBC count > 100,000/mm^3. No sequelae were associated with any grade of leukocytosis.

Sixty-five percent of patients had mild-to-moderate anemia and 97% of patients had decreases in platelet counts; 5 patients (out of 126) had decreased platelet counts to < 50,000/mm^3. Anemia and thrombocytopenia have been reported to be related to leukapheresis; however, the possibility that NEUPOGEN® mobilization may contribute to anemia or thrombocytopenia has not been ruled out.

Patients With Severe Chronic Neutropenia

Mild-to-moderate bone pain was reported in approximately 33% of patients in clinical trials. This symptom was readily controlled with non-narcotic analgesics. Generalized musculoskeletal pain was also noted in higher frequency in patients treated with NEUPOGEN®. Palpable splenomegaly was observed in approximately 30% of patients. Abdominal or flank pain was seen infrequently, and thrombocytopenia (< 50,000/mm^3) was noted in 12% of patients with palpable spleens. Fewer than 3% of all patients underwent splenectomy, and most of these had a prestudy history of splenomegaly. Fewer than 6% of patients had thrombocytopenia (< 50,000/mm^3) during NEUPOGEN® therapy, most of whom had a pre-existing history of thrombocytopenia. In most cases, thrombocytopenia was managed by NEUPOGEN® dose reduction or interruption. An additional 5% of patients had platelet counts between 50,000 and 100,000/mm^3. There were no associated serious hemorrhagic sequelae in these patients. Epistaxis was noted in 15% of patients treated with NEUPOGEN®, but was associated with thrombocytopenia in 2% of patients. Anemia was reported in approximately 10% of patients, but in most cases appeared to be related to frequent diagnostic phlebotomy, chronic illness, or concomitant medications. Other adverse events infrequently observed and possibly related to NEUPOGEN® therapy were: injection site reaction, rash, hepatomegaly, arthralgia, osteoporosis, cutaneous vasculitis, hematuria/proteinuria, alopecia, and exacerbation of some pre-existing skin disorders (e.g., psoriasis).

Cytogenetic abnormalities, transformation to MDS, and AML have been observed in patients treated with NEUPOGEN® for SCN (see WARNINGS, PRECAUTIONS: Pediatric Use). As of 31 December 1997, data were available from a postmarketing surveillance study of 531 SCN patients with an average follow-up of 4.0 years. Based on analysis of these data, the risk of developing MDS and AML appears to be confined to the subset of patients with congenital neutropenia. A life-table analysis of these data revealed that the cumulative risk of developing leukemia or MDS by the end of the 8th year of NEUPOGEN® treatment in a patient with congenital neutropenia was 16.5% (95% C.I. = 9.8%, 23.3%); this represents an annual rate of approximately 2%. Cytogenetic abnormalities, most commonly involving chromosome 7, have been reported in patients treated with NEUPOGEN® who had previously documented normal cytogenetics. It is unknown whether the development of cytogenetic abnormalities, MDS, or AML is related to chronic daily NEUPOGEN® administration or to the natural history of congenital neutropenia. It is also unknown if the rate of conversion in patients who have not received NEUPOGEN® is different from that of patients who have received NEUPOGEN®. Routine monitoring through regular CBCs is recommended for all SCN patients. Additionally, annual bone marrow and cytogenetic evaluations are recommended in all patients with congenital neutropenia (see LABORATORY MONITORING).

Postmarketing Experience

The following adverse reactions have been identified during postapproval of NEUPOGEN®. Because these reactions are reported voluntarily from a population of

uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

- splenomegaly (enlarged spleen) and splenic rupture (see WARNINGS: Splenic Rupture)

- acute respiratory distress syndrome (ARDS) (see WARNINGS: Acute Respiratory Distress Syndrome)

- alveolar hemorrhage and hemoptysis (see WARNINGS: Alveolar Hemorrhage and Hemoptysis)

- sickle cell crisis (see WARNINGS: Sickle Cell Disorders)

- cutaneous vasculitis (see PRECAUTIONS: Cutaneous Vasculitis)

- Sweet's syndrome (acute febrile neutrophilic dermatosis)

- decreased bone density and osteoporosis in pediatric SCN patients receiving chronic treatment with NEUPOGEN®

OVERDOSAGE

In cancer patients receiving NEUPOGEN® as an adjunct to myelosuppressive chemotherapy, it is recommended to avoid the potential risks of excessive leukocytosis, that NEUPOGEN® therapy be discontinued if the ANC surpasses 10,000/mm³ after the chemotherapy-induced ANC nadir has occurred. Doses of NEUPOGEN® that increase the ANC beyond 10,000/mm³ may not result in any additional clinical benefit.

The maximum tolerated dose of NEUPOGEN® has not been determined. Efficacy was demonstrated at doses of 4 to 8 mcg/kg/day in the phase 3 study of nonmyeloablative chemotherapy. Patients in the BMT studies received up to 138 mcg/kg/day without toxic effects, although there was a flattening of the dose response curve above daily doses of greater than 10 mcg/kg/day.

In NEUPOGEN® clinical trials of cancer patients receiving myelosuppressive chemotherapy, WBC counts > 100,000/mm³ have been reported in less than 5% of patients, but were not associated with any reported adverse clinical effects.

In cancer patients receiving myelosuppressive chemotherapy, discontinuation of NEUPOGEN® therapy usually results in a 50% decrease in circulating neutrophils within 1 to 2 days, with a return to pretreatment levels in 1 to 7 days.

DOSAGE AND ADMINISTRATION

NEUPOGEN® is supplied in either vials or in prefilled syringes with UltraSafe® Needle Guards. Following administration of NEUPOGEN® from the prefilled syringe, the UltraSafe® Needle Guard should be activated to prevent accidental needle sticks. To activate the UltraSafe® Needle Guard, place your hands behind the needle, grasp the guard with one hand, and slide the guard forward until the needle is completely covered and the guard clicks into place. **NOTE: If an audible click is not heard, the needle guard may not be completely activated.** The prefilled syringe should be disposed of by

placing the entire prefilled syringe with guard activated into an approved puncture-proof container.

Cancer Patients Receiving Myelosuppressive Chemotherapy

The recommended starting dose of NEUPOGEN® is 5 mcg/kg/day, administered as a single daily injection by SC bolus injection, by short IV infusion (15 to 30 minutes), or by continuous SC or continuous IV infusion. A CBC and platelet count should be obtained before instituting NEUPOGEN® therapy and monitored twice weekly during therapy. Doses may be increased in increments of 5 mcg/kg for each chemotherapy cycle, according to the duration and severity of the ANC nadir.

NEUPOGEN® should be administered no earlier than 24 hours after the administration of cytotoxic chemotherapy. NEUPOGEN® should not be administered in the period 24 hours before the administration of chemotherapy (see PRECAUTIONS). NEUPOGEN® should be administered daily for up to 2 weeks, until the ANC has reached 10,000/mm^3 following the expected chemotherapy-induced neutrophil nadir. The duration of NEUPOGEN® therapy needed to attenuate chemotherapy-induced neutropenia may be dependent on the myelosuppressive potential of the chemotherapy regimen employed. NEUPOGEN® therapy should be discontinued if the ANC surpasses 10,000/mm^3 after the expected chemotherapy-induced neutrophil nadir (see PRECAUTIONS). In phase 3 trials, efficacy was observed at doses of 4 to 8 mcg/kg/day.

Cancer Patients Receiving Bone Marrow Transplant

The recommended dose of NEUPOGEN® following BMT is 10 mcg/kg/day given as an IV infusion of 4 or 24 hours, or as a continuous 24-hour SC infusion. For patients receiving BMT, the first dose of NEUPOGEN® should be administered at least 24 hours after cytotoxic chemotherapy and at least 24 hours after bone marrow infusion.

During the period of neutrophil recovery, the daily dose of NEUPOGEN® should be titrated against the neutrophil response as follows:

Absolute Neutrophil Count	NEUPOGEN® Dose Adjustment
When ANC > 1000/mm^3 for 3 consecutive days	Reduce to 5 mcg/kg/day[a]
then: If ANC remains > 1000/mm^3 for 3 more consecutive days	Discontinue NEUPOGEN®
then: If ANC decreases to < 1000/mm^3	Resume at 5 mcg/kg/day

[a] If ANC decreases to < 1000/mm^3 at any time during the 5 mcg/kg/day administration, NEUPOGEN® should be increased to 10 mcg/kg/day, and the above steps should then be followed.

Peripheral Blood Progenitor Cell Collection and Therapy in Cancer Patients

The recommended dose of NEUPOGEN® for the mobilization of PBPC is 10 mcg/kg/day SC, either as a bolus or a continuous infusion. It is recommended that NEUPOGEN® be given for at least 4 days before the first leukapheresis procedure and continued until the last leukapheresis. Although the optimal duration of NEUPOGEN® administration and leukapheresis schedule have not been established, administration of NEUPOGEN® for 6 to 7 days with leukaphereses on days 5, 6, and 7 was found to be safe and effective (see CLINICAL EXPERIENCE for schedules used in clinical trials). Neutrophil counts

should be monitored after 4 days of NEUPOGEN®, and NEUPOGEN® dose modification should be considered for those patients who develop a WBC count > 100,000/mm^3.

In all clinical trials of NEUPOGEN® for the mobilization of PBPC, NEUPOGEN® was also administered after reinfusion of the collected cells (see CLINICAL EXPERIENCE).

Patients With Severe Chronic Neutropenia

NEUPOGEN® should be administered to those patients in whom a diagnosis of congenital, cyclic, or idiopathic neutropenia has been definitively confirmed. Other diseases associated with neutropenia should be ruled out.

Starting Dose:
Congenital Neutropenia: The recommended daily starting dose is 6 mcg/kg BID SC every day.

Idiopathic or Cyclic Neutropenia: The recommended daily starting dose is 5 mcg/kg as a single injection SC every day.

Dose Adjustments:
Chronic daily administration is required to maintain clinical benefit. Absolute neutrophil count should not be used as the sole indication of efficacy. The dose should be individually adjusted based on the patient's clinical course as well as ANC. In the SCN postmarketing surveillance study, the reported median daily doses of NEUPOGEN® were: 6.0 mcg/kg (congenital neutropenia), 2.1 mcg/kg (cyclic neutropenia), and 1.2 mcg/kg (idiopathic neutropenia). In rare instances, patients with congenital neutropenia have required doses of NEUPOGEN® ≥ 100 mcg/kg/day.

Dilution

If required, NEUPOGEN® may be diluted in 5% dextrose. NEUPOGEN® diluted to concentrations between 5 and 15 mcg/mL should be protected from adsorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2 mg/mL. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), NEUPOGEN® is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes.

Dilution of NEUPOGEN® to a final concentration of less than 5 mcg/mL is not recommended at any time. **Do not dilute with saline at any time; product may precipitate.**

Storage

NEUPOGEN® should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking. Prior to injection, NEUPOGEN® may be allowed to reach room temperature for a maximum of 24 hours. Any vial or prefilled syringe left at room temperature for greater than 24 hours should be discarded. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit; if particulates or discoloration are observed, the container should not be used.

HOW SUPPLIED

Use only one dose per vial; do not re-enter the vial. Discard unused portions. Do not save unused drug for later administration.

Use only one dose per prefilled syringe. Discard unused portions. Do not save unused drug for later administration.

<u>Vials</u>

Single-dose, preservative-free vials containing 300 mcg (1 mL) of filgrastim (300 mcg/mL). Dispensing packs of 10 (NDC 55513-530-10).

Single-dose, preservative-free vials containing 480 mcg (1.6 mL) of filgrastim (300 mcg/mL). Dispensing packs of 10 (NDC 55513-546-10).

<u>Prefilled Syringes (SingleJect®)</u>

Single-dose, preservative-free, prefilled syringes with 27 gauge, ½ inch needles with an UltraSafe® Needle Guard, containing 300 mcg (0.5 mL) of filgrastim (600 mcg/mL). Dispensing packs of 10 (NDC 55513-924-10).

Single-dose, preservative-free, prefilled syringes with 27 gauge, ½ inch needles with an UltraSafe® Needle Guard, containing 480 mcg (0.8 mL) of filgrastim (600 mcg/mL). Dispensing packs of 10 (NDC 55513-209-10).

The needle cover of the prefilled syringe contains dry natural rubber (a derivative of latex).

NEUPOGEN® should be stored at 2° to 8°C (36° to 46°F). Avoid shaking.

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Patent: http://pat.amgen.com/neupogen/

AMGEN®

Manufactured by:

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Thousand Oaks, California 91320-1799

Revised: 03/2013

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NEUPOGEN®

(filgrastim)

INFORMATION FOR PATIENTS AND CAREGIVERS

This patient package insert provides information and instructions for people who will be receiving NEUPOGEN® and their caregivers. This patient package insert does not tell you everything about NEUPOGEN®. You should discuss any questions you have about treatment with NEUPOGEN® with your doctor.

What is NEUPOGEN®?

NEUPOGEN® is a man-made form of granulocyte colony-stimulating factor (G-CSF), which is made using the bacteria *Escherichia coli*. G-CSF is a substance naturally produced by the body. It stimulates the growth of neutrophils (**nu**-tro-fils), a type of white blood cell important in the body's fight against infection.

What is NEUPOGEN® used for?

NEUPOGEN® is used to treat neutropenia (nu-tro-**peen**-ee-ah), a condition where the body makes too few neutrophils. Neutropenia may be a long-standing condition where your body does not make enough neutrophils or it may be caused by drugs used to treat cancer. In some cases, your body may make enough neutrophils but as part of your treatment for cancer your doctor may want to increase the number of certain blood cells (CD34 cells) and collect them. The cells are collected using a process called apheresis (ay-fer-**ree**-sis). These collected cells are given back to you after you receive very high doses of treatment for cancer to make your blood counts get back to normal more quickly.

How does NEUPOGEN® work?

NEUPOGEN® works by helping your body make more neutrophils. To make sure NEUPOGEN® is working, your doctor will ask that you have regular blood tests to count the number of neutrophils you have. It is important that you follow your doctor's instructions about getting these tests.

Who should not take NEUPOGEN®?

Do not take NEUPOGEN® if you are:

- Allergic to NEUPOGEN® (filgrastim) or any of its ingredients. See the end of this leaflet for a list of ingredients in NEUPOGEN®.

- Allergic to other medicines made using the bacteria *E coli*. Ask your doctor if you are not sure.

What important information do I need to know about taking NEUPOGEN®?

NEUPOGEN® may reduce your chance of getting an infection, but does not prevent all infections. An infection can still happen during the short time when your/your child's neutrophil levels are low. You must be alert and look for some of the common signs or symptoms of infection, such as fever, chills, rash, sore throat, diarrhea, redness, swelling, or pain around a cut or sore. If you/your child has any of these signs or symptoms during treatment with NEUPOGEN®, tell your doctor or nurse immediately.

There is a possibility that you/your child could have a reaction at an injection site. If there is a lump, swelling, or bruising at an injection site that does not go away, call your doctor.

If you have a sickle cell disorder, make sure that you tell your doctor before you start taking NEUPOGEN®. If you have a sickle cell crisis after getting NEUPOGEN®, tell your doctor right away.

Talk to your doctor if you experience unusual bleeding or bruising while taking NEUPOGEN®, as this could mean a decrease of platelets which reduces the ability of blood to clot.

Make sure your doctor knows about all medicines, and herbal or vitamin supplements you are taking before starting NEUPOGEN®. If you are taking lithium you may need more frequent blood tests.

If you/your child are receiving NEUPOGEN® because you are also receiving chemotherapy, the last dose of NEUPOGEN® should be injected at least 24 hours before your next dose of chemotherapy.

There is more information about NEUPOGEN® in the Physician Package Insert. If you have any questions, you should talk to your doctor.

What are possible serious side effects of NEUPOGEN®?

- **Spleen Rupture.** Your spleen may become enlarged and can rupture while taking NEUPOGEN®. A ruptured spleen can cause death. The spleen is located in the upper left section of your stomach area. Call your doctor right away if you/your child has pain in the left upper stomach area or left shoulder tip area. This pain could mean your/your child's spleen is enlarged or ruptured.

- **Serious Allergic Reactions.** NEUPOGEN® can cause serious allergic reactions. These reactions can cause a rash over the whole body, shortness of breath, wheezing, dizziness, swelling around the mouth or eyes, fast pulse, and sweating. If you or your child starts to have any of these symptoms, stop using NEUPOGEN and call your doctor or seek emergency care right away. If you/your child has an allergic reaction during the injection of NEUPOGEN®, stop the injection right away.

- **A serious lung problem called acute respiratory distress syndrome (ARDS).** Call your doctor or seek emergency care right away if you/your child has shortness of breath, trouble breathing or a fast rate of breathing.

What are the most common side effects of NEUPOGEN®?

The most common side effect you/your child may experience is aching in the bones and muscles. This aching can usually be relieved by taking a non-aspirin pain reliever such as acetaminophen.

Some people experience redness, swelling, or itching at the site of injection. This may be an allergy to the ingredients in NEUPOGEN® or it may be a local reaction. If you are giving an injection to a child, look for signs of redness, swelling, or itching at the site of injection because they may not be able to tell you they are experiencing a reaction. If you notice any signs of a local reaction, call your doctor.

WHAT ABOUT PREGNANCY OR BREASTFEEDING?

NEUPOGEN®has not been studied in pregnant women, and its effects on unborn babies are not known. If you take NEUPOGEN® while you are pregnant, it is possible that small amounts of it may get into your baby's blood. It is not known if NEUPOGEN®can get into human breast milk.

If you are pregnant, plan to become pregnant, think you may be pregnant, or are breast feeding, you should tell your doctor before using NEUPOGEN®. If you become pregnant during NEUPOGEN® treatment, you are encouraged to enroll in Amgen's Pregnancy Surveillance Program. You should call 1-800-77-AMGEN (1-800-772-6436) to enroll.

If you breastfeed during NEUPOGEN® treatment, you are encouraged to enroll in Amgen's Lactation Surveillance Program. You should call 1-800-77-AMGEN (1-800-772-6436) to enroll.

How to prepare and give a NEUPOGEN® injection?

NEUPOGEN® should be injected at the same time each day. If you miss a dose contact your doctor or nurse.

You must always use the correct dose of NEUPOGEN®. Too little NEUPOGEN® may not protect you against infections, and too much NEUPOGEN® may cause too many neutrophils to be in your blood. Your doctor will determine your/your child's correct dose based on your/your child's body weight.

If you are giving someone else NEUPOGEN® injections, it is important that you know how to inject NEUPOGEN®, how much to inject, and how often to inject NEUPOGEN®.

NEUPOGEN® is available as a liquid in vials or in prefilled syringes. When you receive your NEUPOGEN®, always check to see that:

- The name NEUPOGEN® appears on the package and vial or prefilled syringe label.

- The expiration date on the vial or prefilled syringe label has not passed. **You should not use a vial or prefilled syringe after the date on the label.**

- The strength of the NEUPOGEN® (number of micrograms in the colored dot on the package containing the vial or prefilled syringe) is the same as your doctor prescribed.

- The NEUPOGEN® liquid in the vial or in the prefilled syringe is clear and colorless. **Do not use NEUPOGEN®** if the contents of the vial or prefilled syringe appear discolored or cloudy, or if the vial or prefilled syringe appears to contain lumps, flakes, or particles.

If you are using vials of NEUPOGEN® only use the syringe that your doctor prescribes.

Your doctor or nurse will give you instructions on how to measure the correct dose of NEUPOGEN®. This dose will be measured in milliliters. You should only use a syringe that is marked in tenths of milliliters, or mL (for example, 0.2 mL). The doctor or nurse may refer to an mL as a cc (1 mL = 1 cc). If you do not use the correct syringe, you/your child could receive too much or too little NEUPOGEN®.

Only use disposable syringes and needles. Use the syringes only once and dispose of them as instructed by your doctor or nurse.

IMPORTANT: TO HELP AVOID POSSIBLE INFECTION, YOU SHOULD FOLLOW THESE INSTRUCTIONS.

Setting up for an injection

1. Find a clean flat working surface, such as a table.

2. Remove the vial or prefilled syringe of NEUPOGEN® from the refrigerator. Allow NEUPOGEN® to reach room temperature (this takes about 30 minutes). Vials or prefilled syringes should be used only once. DO NOT SHAKE THE VIAL OR PREFILLED SYRINGE. Shaking may damage the NEUPOGEN®. If the vial or prefilled syringe has been shaken vigorously, the solution may appear foamy and it should not be used.

3. Assemble the supplies you will need for an injection:

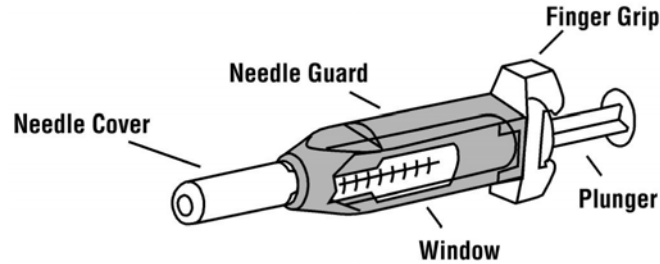   - NEUPOGEN® vial <u>and</u> disposable syringe and needle

Vial

NEUPOGEN®
(Filgrastim)

Disposable Syringe

Plunger

Needle Cover

Syringe Barrel with Markings
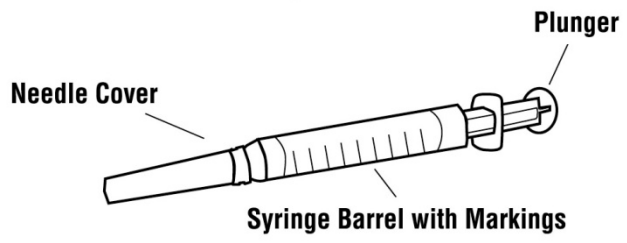
- Or <u>NEUPOGEN</u>® prefilled syringe with transparent (clear) plastic orange needle guard attached

Prefilled Syringe

Finger Grip

Needle Guard

Needle Cover

Plunger

Window

- Two alcohol swabs and one cotton ball or gauze pad

Alcohol Swabs

Alcohol Swab

Cotton Ball

- Puncture-proof disposal container

4.   Wash your hands with soap and warm water.

HOW TO PREPARE THE DOSE OF NEUPOGEN® IN VIALS OR PREFILLED SYRINGES

If you are using NEUPOGEN® in a vial, follow the instructions in Section A. If you are using NEUPOGEN® in a prefilled syringe, go to Section B.

Section A. Preparing the dose using NEUPOGEN® in a vial

1.   Take the cap off the vial. Clean the rubber stopper with one alcohol swab.

2.   Check the package containing the syringe. If the package has been opened or damaged, do not use that syringe. Dispose of that syringe in the puncture-proof disposal container. If the syringe package is undamaged, open the package and remove the syringe.

3.   Pull the needle cover straight off the syringe. Then, pull back the plunger and draw air into the syringe. The amount of air drawn into the syringe should be the same amount (mL or cc) as the dose of NEUPOGEN® that your doctor prescribed.
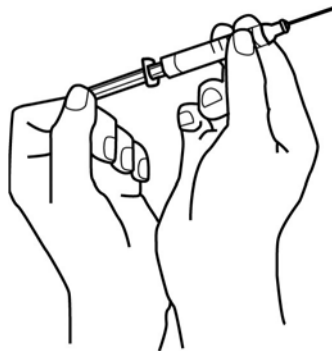
4.   Keep the vial on the flat working surface and insert the needle straight down through the rubber stopper. Do not insert the needle through the rubber stopper more than once.

5.   Push the plunger of the syringe down and inject the air from the syringe into the vial of NEUPOGEN®.
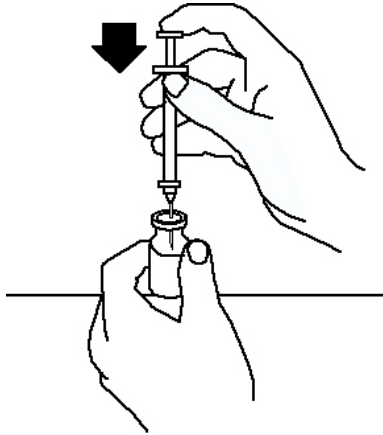
6.   Keeping the needle in the vial, turn the vial upside down. Make sure that the NEUPOGEN® liquid is covering the tip of the needle.
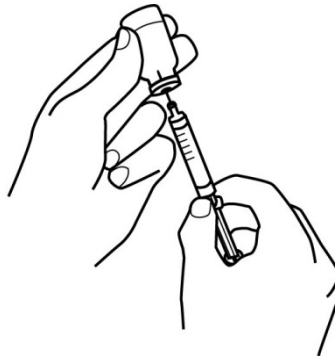
7.   Keeping the vial upside down, slowly pull back on the plunger to fill the syringe with NEUPOGEN® liquid to the number (mL or cc) that matches the dose your doctor prescribed.

8.   Keeping the needle in the vial, check for air bubbles in the syringe. If there are air bubbles, gently tap the syringe with your fingers until the air bubbles rise to the top of the syringe. Then slowly push the plunger up to force the air bubbles out of the syringe.

9.   Keeping the tip of the needle in the liquid, once again pull the plunger back to the number on the syringe that matches your dose. Check again for air bubbles. The air in the syringe will not hurt you, but too large an air bubble can reduce your dose of NEUPOGEN®. If there are still air bubbles, repeat the steps above to remove them.

10.   Check again to make sure that you have the correct dose in the syringe. It is important that you use the exact dose prescribed by your doctor. Remove the syringe from the vial but **do not lay it down** or let the needle touch anything. (Go to "Selecting and preparing the injection site").

Section B. Preparing the dose using NEUPOGEN® in a prefilled syringe

1. Remove the syringe from the package and the tray. Check to see that the plastic orange needle guard is covering the barrel of the glass syringe. **DO NOT push the orange needle guard over the needle cover before injection**. This may activate or lock the needle guard. If the orange needle guard is covering the needle that means it has been activated. DO NOT use that syringe. Dispose of that syringe in the puncture-proof disposal container. Use a new syringe from the package**.**

2. Hold the syringe barrel through the needle guard windows with the needle pointing up. Holding the syringe with the needle pointing up helps to prevent medicine from leaking out of the needle. Carefully pull the needle cover straight off.

3. Check the syringe for air bubbles. If there are air bubbles, gently tap the syringe with your fingers until the air bubbles rise to the top of the syringe. Slowly push the plunger up to force the air bubbles out of the syringe.

4. Push the plunger up to the number (mL) on the syringe that matches the dose of NEUPOGEN® that your doctor prescribed.

5. Check again to make sure the correct dose of NEUPOGEN® is in the syringe.

6. Gently place the prefilled syringe with the window flat on your clean working surface so that the needle does not touch anything.

Selecting and preparing the injection site (for vials and prefilled syringes)

1. Choose an injection site. Four recommended injection sites for NEUPOGEN® are:

   - The outer area of your upper arms

   - The abdomen, except for the two inch area around your navel

   - The front of your middle thighs

   - The upper outer areas of your buttocks

Front   **Back**
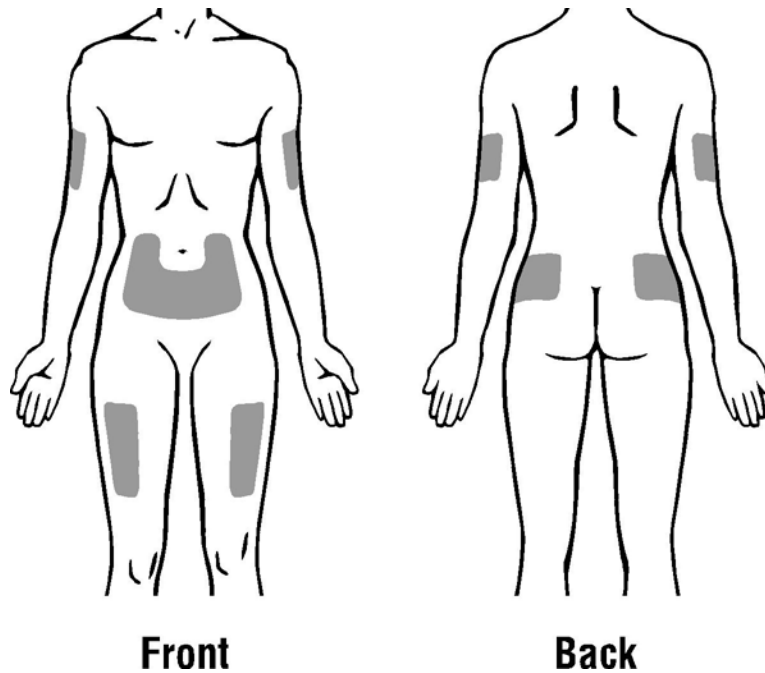
Choose a new site each time you inject NEUPOGEN®. Choosing a new site can help avoid soreness at any one site. Do not inject NEUPOGEN® into an area that is tender, red, bruised, or hard or that has scars or stretch marks.

2.   Clean the injection site with a new alcohol swab.
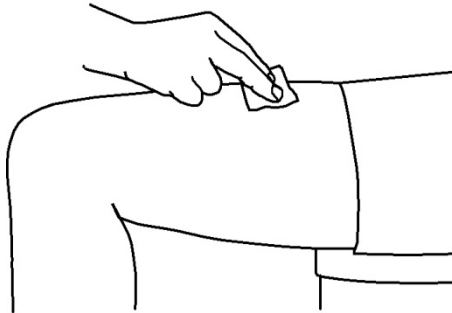
**Injecting the dose of NEUPOGEN® (for vials and prefilled syringes)**

1.   For injecting the dose of NEUPOGEN® from a vial, remove the syringe and needle from the vial. For injecting the dose of NEUPOGEN® from a prefilled syringe, pick up the prefilled syringe from the clean flat working surface by grabbing the sides of the needle guard with your thumb and forefinger.

2.   Hold the syringe in the hand you will use to inject NEUPOGEN®. Use the other hand to pinch a fold of skin at the cleaned injection site. <u>Note: If using a prefilled syringe with a needle guard, hold the syringe barrel through the needle guard windows when giving the injection.</u>

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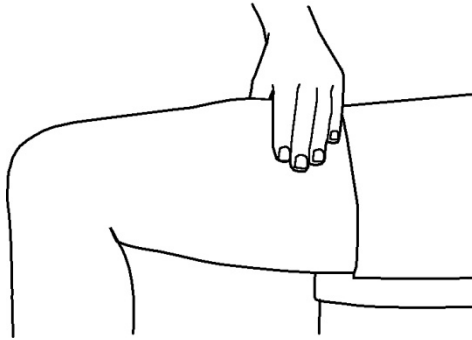
3.   Holding the syringe like a pencil, use a quick "dart-like" motion to insert the needle either straight up and down (90 degree angle) or at a slight angle (45 degrees) into the skin.
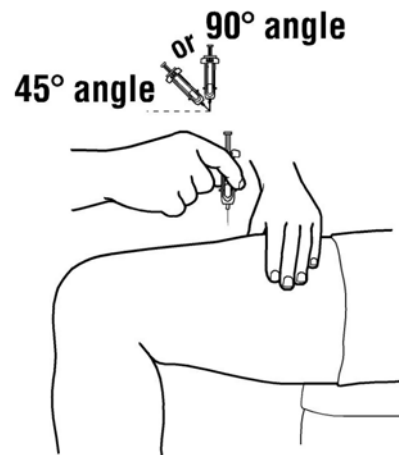
4.   Inject the prescribed dose subcutaneously as directed by your doctor, nurse, or pharmacist.
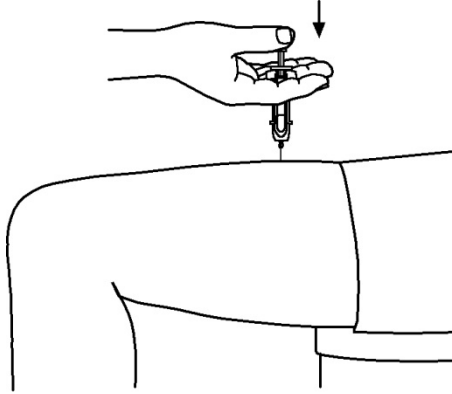

5.   When the syringe is empty, pull the needle out of the skin and place a cotton ball or gauze over the injection site and press for several seconds.
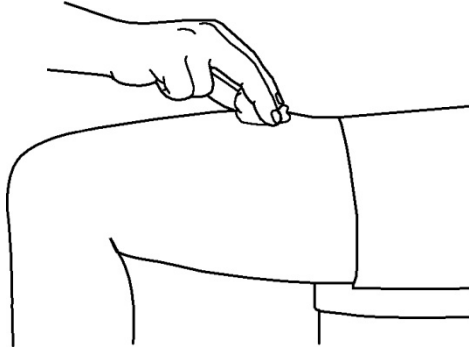
6.   Use the prefilled syringe with the needle guard or a syringe and vial only once. If you are using a syringe, DO NOT put the needle cover (the cap) back on the needle. Discard the vial with any remaining NEUPOGEN® liquid.

Activating the Needle Guard for the prefilled syringe after the injection has been given

1. After injecting NEUPOGEN® from the prefilled syringe, do not recap the needle. Keep your hands behind the needle at all times. While holding the clear plastic finger grip of the syringe with one hand, grasp the orange needle guard with your free hand and slide the orange needle guard over the needle until the needle is completely covered and the needle guard clicks into place. **NOTE: If an audible click is not heard, the needle guard may not be completely activated.**
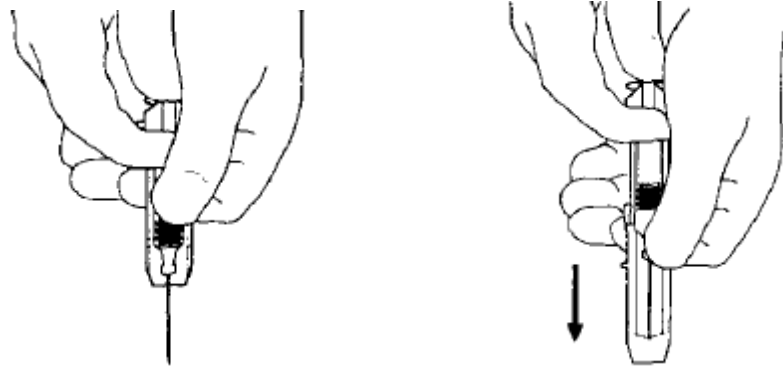
2. Place the prefilled syringe with the activated needle guard into a puncture-proof container for proper disposal as described below.

Disposal of syringes, needles, vials and needle guards

You should always follow the instructions given by your doctor, nurse, or pharmacist on how to properly dispose of containers with used syringes, needles, vials and needle guards. There may be special state and local laws for disposal of used needles and syringes.

*   Place all used needles, needle covers, syringes, and vials (empty or unused contents) into a "Sharps" container given to you by your doctor or pharmacist, or in a hard-plastic container with a screw-on cap or a metal container with a plastic lid such as a coffee can labeled "used syringes." If a metal container is used, cut a small hole in the plastic lid and tape the lid to the metal container. If a hard-plastic container is used, always screw the cap on tightly after each use.

*   Do not use glass or clear plastic containers.

*   When the container is full, tape around the cap or lid to make sure the cap or lid does not come off**. Do not throw the container in the household trash. Do not recycle.**

*   **Always** keep the container out of the reach of children.

How should NEUPOGEN® be stored?

NEUPOGEN® should be stored in the refrigerator at 2° to 8°C (36° to 46°F), but not in the freezer. Avoid shaking NEUPOGEN®. If NEUPOGEN® is accidentally frozen, allow it to thaw in the refrigerator before giving the next dose. However, if it is frozen a second time, do not use it and contact your doctor or nurse for further instructions.

NEUPOGEN® can be left out at room temperature for up to 24 hours. Do not leave NEUPOGEN® in direct sunlight. If you have any questions about storage or how to carry NEUPOGEN® when you travel, contact your doctor, nurse, or pharmacist.

What are the ingredients in NEUPOGEN®?

Each prefilled syringe and vial contains filgrastim in a sterile, clear, colorless, preservative-free solution containing acetate, sorbitol, polysorbate 80, and sodium.

The needle cover on the single-use prefilled syringe contains dry natural rubber (latex), which should not be handled by persons sensitive to this substance.

AMGEN®

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