FDA Advisory Committee Briefing Document

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

to be held on May 3, 2013

Safety and Efficacy of Currently Approved Leukocyte Growth Factors (LGFs) as Potential Treatments for Radiation-induced Myelosuppression Associated with a Radiological/Nuclear Incident

This document was prepared on April 4, 2013

Table of Contents

	Page
1. Introduction	1
2. Draft Topics for Discussion	4
3. Regulatory Background	5
4. Brief Summary of the NIAID Study	11
5. FDA Reviews of the NIAID Study:	
Nonclinical	13
Clinical	44
Statistical	83
6. FDA Reviewer Summaries of Published Reports of LGF Use:	
In Animal Studies	118
In Radiological/Nuclear Accidents	134
In Radiation Oncology	155

This document contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought this issue to this advisory committee in order to gain the committee's insights and opinions, and the background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion at the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

1. Introduction

This meeting was prompted by the National Institute of Allergy and Infectious Disease (NIAID) submission to the FDA of the data from a non-human primate study that reported filgrastim, a leukocyte growth factor (LGF), reduced mortality following lethal irradiation of the animals.[1] FDA seeks the committee members' perspective on the sufficiency of these data, in the context of known filgrastim clinical effects, to conclude that the product is reasonably likely to produce clinical benefit in humans exposed to myelosuppressive doses of radiation in a radiological/nuclear incident. If these data are insufficient, then FDA anticipates the discussion will identify the major data deficiencies. If these data are sufficient to support filgrastim clinical benefits in patients exposed to myelosuppressive doses of radiation in a radiological/nuclear incident, then FDA requests a discussion of the extent to which filgrastim efficacy can be extrapolated to other LGFs. The "reasonably likely" threshold for assessing a filgrastim treatment effect is predicated upon potential drug approval under the "Animal Rule" provision of FDA regulations, as discussed below.[2]

LGFs are products that stimulate the proliferation and differentiation of normal white blood cells (leukocytes). Four LGFs are currently FDA-approved, and all are biological products licensed under biological license applications:

1. 103353: Neupogen (filgrastim), Amgen, Inc., licensed 1991
2. 103362: Leukine (sargramostin), Genzyme, Inc., licensed 1991
3. 125031: Neulasta (pegfilgrastim), Amgen, Inc., licensed 2002
4. 125294: Tbo-Filgrastim (tbo-filgrastim), Sicor Biotech, UAB, licensed 2012

Although the specific labeling indication statements differ among the LGFs, all the products share an indication for use among certain patients receiving myelosuppressive chemotherapy. The indications generally pertain to use of the products to decrease the serious infection risk associated with "febrile neutropenia." Labeling further notes that the agents appear to decrease this febrile neutropenia risk by shortening the duration of severe neutropenia following chemotherapy-induced bone marrow injury (also known as "chemotherapy-induced neutropenia" or CIN), as demonstrated in clinical studies. These CIN studies were not designed to demonstrate survival benefits and labeling does not claim a survival benefit in this setting. The febrile neutropenia/neutrophil response from the CIN experience may have implications for helping to assess the potential treatment use of LGFs among patients with radiation-induced bone marrow injury. However, radiation injury doses may vary markedly and injure parts of the body not typically impacted by oncologic chemotherapy. These, and potentially other differences between radiation and chemotherapy injury, are important considerations in weighing the value of the CIN information.

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[1] Farese, AM, et al, Filgrastim improves survival in lethally irradiated nonhuman primates. Radiat Res 2013; 179(1):89-100.

[2] As used in this document, all references to *drugs* include human drugs and therapeutic biological products unless otherwise specified, and the term *approval* refers to approval or licensure.

A multi-federal agency document titled, "Nuclear/Radiological Incident Annex," defines a radiological/nuclear incident. [3] The document states a radiological/nuclear incident is characterized by the release of radioactive material from a deliberate act, an accident, or general mismanagement and may center around different materials or industrial practices, including:

- Commercial nuclear facilities.
- Federal nuclear weapons facilities.
- Radioactive material sources, industrial uses, or technologically enhanced, naturally occurring radioactive material.
- Transportation incidents involving nuclear/radioactive material.
- Domestic nuclear weapons accidents.
- Foreign incidents involving nuclear or radioactive materials.
- Terrorism involving facilities or nuclear/radiological materials.

The annex document further describes how "an expeditious Federal response is required to mitigate the consequences of a nuclear/radiological incident." In line with this concept, the NIAID has submitted source data from a recently published non-human primate study to the FDA in support of filgrastim use in the radiological/nuclear incident setting.

In this briefing document, FDA includes three individual reviewer analyses of the NIAID study (clinical, nonclinical and statistical) as well as individual FDA reviewer analyses of: 1) other published reports of the use of LGFs in animal models of radiation injury, 2) published reports of LGF use in radiation oncology, and 3) published reports of LGF use in radiation accidents. The following are the major points from these reviews:

- Clinical, nonclinical and statistical reviewer analyses support the NIAID study's primary endpoint finding of improved 60-day survival among animals receiving filgrastim, compared to animals receiving a control article (5% dextrose in water).

- The NIAID cites published data to support a proposed initial filgrastim human dose of 5 mcg/kg daily administered subcutaneously (SC). FDA-approved SC filgrastim doses range from 5 to 12 mcg/kg /day administered SC, depending upon the therapeutic setting; the doses are also adjusted based upon blood neutrophil count responses.

- Published reports of studies using a variety of animal models appear to support a survival advantage for the use of LGFs in the radiation injury setting; most reports cite the use of filgrastim.

- Published reports of LGF use in the radiation oncology setting appear to provide few implications for use of LGFs in the radiological/nuclear incident setting since patients were typically receiving or had received chemotherapy for cancer, treatment features that confound the overall experience.

[3] Internet accessed on 03/24/2013 at:
http://www.fema.gov/pdf/emergency/nrf/nrf_nuclearradiologicalincidentannex.pdf

- Published reports of LGF use in the radiation accident setting are too sparse to provide definitive evidence of efficacy for LGFs in the radiological/nuclear incident setting.

The FDA-approval of a product for use in a radiological/nuclear setting will facilitate access to this product in the event of such an emergency. In the absence of an FDA-approved product, under specific criteria the FDA can issue an Emergency Use Authorization (EUA) during a declared emergency or threat justifying emergency authorized use involving a heightened risk of attack on the public or US military forces, potential for a public health emergency with the significant potential to affect national security, or identification of a material threat sufficient to affect national security.[4] There are multiple steps involved to provide access to products under the EUA process. The availability of an FDA-approved product with an indication for treatment of a condition caused by exposure to radiation will expedite access to important medical therapy during a radiological/nuclear emergency event. This advisory committee discussion may facilitate the development and possible FDA approval of LGFs as medical countermeasures (MCMs) for use in the radiological/nuclear setting.

[4] Section 564 of the Federal Food, Drug and Cosmetic Act

2. Draft Topics for Discussion

The FDA review team requests the committee members to consider the data presented by the NIAID from a Good Laboratory Practices (GLP) compliant non-human primate study, as well as the known benefit of LGFs in the CIN setting, in order to address the following items:

1. With respect to the NIAID study, other published reports and the known effects of radiation upon human bone marrow:

    a. Is there a reasonably well-understood pathophysiological mechanism of radiation-induced bone marrow toxicity and its prevention or substantial reduction by filgrastim?

    b. Does the single animal species (i.e., rhesus macaques) represent a sufficiently well-characterized animal model for predicting the response in humans?

    c. Is the animal study primary endpoint (survival) clearly related to the desired benefit in humans?

    d. Is information on the kinetics and pharmacodynamics of filgrastim *or* other information sufficient to allow selection of an effective dose in humans?

2. Considering the known filgrastim effects in the CIN setting, the NIAID study data, and assuming filgrastim would be administered in a clinical dose regimen similar to that evaluated in the NIAID study, is filgrastim therapy reasonably likely to produce clinical benefits in humans exposed to radiation that is likely to induce myelosuppression during or following a radiological/nuclear incident?

    a. If no, what additional data must be obtained to support filgrastim use in the radiological/nuclear incident setting?

    b. If yes, do you support an acceptable risk to benefit profile for filgrastim in the radiological/nuclear incident setting?

3. To what extent, if any, could filgrastim safety and efficacy in the radiological/nuclear incident setting be generalized to the use of other LGFs, in the absence of performing definitive animal efficacy studies for each agent?

3. Regulatory Background

LGFs are examples of potential "medical countermeasure" (MCM) products. MCM products include both pharmaceutical interventions, such as vaccines, antimicrobials, antidotes, and antitoxins, and non-pharmaceutical interventions, such as ventilators, diagnostics, personal protective equipment (PPE), and patient decontamination that may be used to prevent, mitigate, or treat the adverse health effects of an intentional, accidental or naturally occurring public health emergency.[5]

Although this is the first FDA advisory committee to consider a potential MCM product for use in the radiological/nuclear incident setting, other committees have considered anti-infective MCM products (such as levofloxacin for treatment of pneumonic plaque and raxibacumab for treatment of inhalational anthrax). In alignment with the briefing documents for these other committees, we provide the following information to facilitate an understanding of FDA MCM product regulation.

1. What is the Animal Rule?

The Animal Rule refers to a specific FDA regulation that describes the criteria for approval of new drugs and biological products when human efficacy studies are not ethical or feasible.[6]

Most drug and biological products for the treatment or prevention of human disease are studied in adequate and well- controlled clinical trials that enroll patients with the disease, and these clinical trials, along with other investigations and studies, serve as the basis for approving or licensing the product. However, prospective human studies of a product's efficacy in the radiological/nuclear incident setting cannot be conducted because it would not be ethical or feasible to conduct these studies. In situations such as this, when human efficacy studies cannot be conducted, MCM product efficacy needs to be demonstrated in adequate and well-controlled studies conducted in animal models of the disease or condition or interest (i.e., radiation-induced myelosuppression).

2. What are FDA's essential study conduct expectations for definitive Animal Rule efficacy studies?

In January 2009, FDA published a draft *Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule*, which provides information on the development of animal models in which to study efficacy, including the critical characteristics of an animal model that need to be addressed under the Animal Rule. For example, these

[5] US Department of Health and Human Services: 2012 Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) Strategy. Internet accessed at: http://www.phe.gov/Preparedness/mcm/phemce/Documents/2012-PHEMCE-Strategy.pdf

[6] Final Rule published in the Federal Register, Vol 67. No. 105, May 31, 2002, pages 37988-37998; Regulations: 21 CFR § 314.600-650 (New Drugs), 21 CFR § 601.90-95 (Biological Products)

elements include the characteristics of the radiation (dose, extent) that causes the bone marrow injury, the host susceptibility and response to radiation, the natural history of myelosuppressive radiation exposure in humans and its comparability to the animal model, the trigger and timing of the MCM intervention, the characteristics of the medical intervention, and study design considerations.

Studies conducted under the Animal Rule are designed to demonstrate efficacy, not safety. Safety data are derived from clinical (human) studies.

The NIAID incorporated FDA guidance into the design of their non-human primate study, including the choice of 60 day survival as the study's endpoint.

3. Are animal modeling data the only efficacy data or information to be considered when products are approved under the Animal Rule?

No. FDA regulations state, "In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency."

Additionally, in the document titled, "Animal Models—Essential Elements to Address Efficacy Under the Animal Rule," FDA has stated that, "If a candidate product is targeted at a common pathway in the pathophysiologic cascade, information may be available on the candidate product's use for diseases that possess a similar pathway. Information for a product approval for the treatment of neutropenia secondary to chemotherapy in cancer patients may provide useful data to support studying this product for the reduction of mortality in patients with neutropenia secondary to acute radiation syndrome. This information in the related condition, although not required, lends further support to the candidate product's efficacy for the indication to be studied." Hence, the filgrastim CIN data may add supportive mechanism of action data that help assess the extent to which the animal efficacy results are "reasonably likely" to predict filgrastim clinical benefits in the radiological/nuclear incident setting.

To illustrate the use of existing data to help support a product approval under the Animal Rule, we cite the experience with levofloxacin, a drug which was originally approved in 1996, but was also approved in 2012 for the treatment and prophylaxis of plague due to *Yersinia pestis* in certain patients.

The original levofloxacin approval (in 1996) cited the drug's use in certain infectious disease settings (such as community-acquired pneumonia). In 2012, a New Drug Application (NDA) Animal Rule efficacy supplement to the levofloxacin NDA was discussed at an advisory committee; data from a confirmatory animal study was proposed to support the drug's use in the treatment of plague. Following the committee's advice, the Agency used the human efficacy and safety data from the FDA-approved indications of nosocomial pneumonia and community-acquired pneumonia to demonstrate the ability of levofloxacin at the approved dose and regimen to penetrate into the lung and treat pneumonic processes. This information was used to support the efficacy of levofloxacin demonstrated in the African Green Monkey model for the treatment of pneumonic plague. At the advisory committee meeting (April 4, 2012), the results of a single adequate and well-controlled animal efficacy study (26 African green monkeys) were discussed;

all advisors voted in favor of a conclusion that the animal model provided substantial evidence of levofloxacin efficacy in the treatment of human plague.

At the upcoming advisory committee, the Agency has invited the LGF manufacturers to summarize the clinical data from the CIN experience since these data may have both efficacy and safety relevance to the radiation-induced bone marrow injury setting.

The following items may be useful to consider when considering the types of existing information/data that may be leveraged for potential use in the Animal Rule efficacy situation:

Product therapeutic class. Information that is available about other drugs in a class may impact the design of animal efficacy studies as well as assist in interpretation of the animal study findings.

Activity in disease or condition of similar pathophysiology. If a candidate drug acts in a pathophysiologic/therapeutic action pathway similar to approved drug(s), then this information may assist in helping to estimate the candidate drug's activity in a new disease/condition.

Pharmacokinetics or Pharmacodynamics (PK/PD) data in affected animals or humans. If a candidate drug has been approved for use in humans for other indications, then the pharmacokinetic/pharmacodynamics (PK/PD) information for the existing indications may be supportive of the new indication.

4. Does FDA anticipate a detailed reexamination of the LGF data that supported the initial licensure of these products?

No. The only data to be vetted in detail at the advisory committee are the NIAID non-human primate study data with filgrastim. The first LGF was licensed over 20 years ago and post-marketing clinical use of these products continues to support their safety and efficacy for the approved indications, as described in medical professional society guidelines.[7] However, consideration of the applicability of the information from the CIN setting will be an important consideration for the discussion. These considerations are discussed within the clinical reviewer's vetting of the NIAID non-human primate study (attached).

5. What are the criteria for approval of a product under the Animal Rule?

The Animal Rule states that a drug can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the study drug is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, FDA may take into account other data, including human data, available to the Agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

[7]Smith, TJ et. al. Update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol 2006; 24 (19):3187-3205.

- The effect is demonstrated in more than one 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;

- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

Therefore, data from appropriate studies to address each of the above bullet points would need to be provided to support the conclusion that the product is effective.

6. Are there any unique requirements for a product approved under the Animal Rule?

Yes, approval under Animal Rule subjects the marketing applicant (generally the manufacturer) to three requirements:

a. Postmarketing studies. The applicant must conduct postmarketing studies, such as field studies, to verify and describe the drug's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include, as part of their application, a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible. Recognizing the complexities involved in the conduct of postmarket studies during emergencies, the US HHS is working to develop systems to facilitate a sponsor's ability to satisfy these requirements.

b. Approval with restrictions to ensure safe use. If FDA concludes that a drug product shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the drug product, commensurate with the specific safety concerns presented by the drug product such as:

- Distribution restricted to certain facilities or health care practitioners with special training or experience;

- Distribution conditioned on the performance of specified medical procedures, including medical follow-up; and

- Distribution conditioned on specified record-keeping requirements.

c. Information to be provided to patient recipients. For drug products or specific indications approved under the Animal Rule, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the drug's approval was based on efficacy studies conducted in animals

alone and must give the drug's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the drug product for the use approved under the Animal Rule, if possible.

7. What is the "acute radiation syndrome" (ARS) and its relationship to radiation-induced myelosuppression?

ARS is the acronym that has been applied to a variety of clinical syndromes that may result from the exposure of humans to toxic doses of radiation. Radiation-induced myelosuppression is one component of ARS; other components of ARS may ultimately prove fatal to a patient even if the patient survives the bone marrow damage due to radiation-induced myelosuppression.

In the Centers for Disease Control and Prevention (CDC) document titled, "Acute Radiation Syndrome: A Fact Sheet for Physicians," ARS is defined as, "an acute illness caused by irradiation of the entire body (or most of the body) by a high dose of penetrating radiation in a very short period of time (usually a matter of minutes)."[8] The document further describes "three classic ARS Syndromes" as:

- Bone marrow syndrome: "sometimes referred to as hematopoietic syndrome, the full syndrome will usually occur with a dose between 0.7 and 10 Gy (70 – 1000 rads) though mild symptoms may occur with doses as low as 0.3 Gy or 30 rads. The survival rate of patients with this syndrome decreases with increasing dose. The primary cause of death is the destruction of bone marrow, resulting in infection and hemorrhage."

- Gastrointestinal (GI) syndrome: "the full syndrome will usually occur with a dose greater than approximately 10 Gy (1000 rads) although some symptoms may occur with doses as low as 6 Gy or 600 rads. Survival is extremely unlikely with this syndrome. Destructive and irreparable changes in the GI tract and bone marrow usually cause infection, dehydration, and electrolyte imbalance. Death usually occurs within 2 weeks."

- Cardiovascular (CV)/Central Nervous System (CNS) syndrome: "the full syndrome will usually occur with a dose greater than approximately 50 Gy (5000 rads) although some symptoms may occur as low as 20 Gy or 2000 rads. Death occurs within 3 days. Death likely is due to collapse of the circulatory system as well as increased pressure in the confining cranial vault as the result of increased fluid content caused by edema, vasculitis, and meningitis."

8. Is there a marketing application currently under consideration for use of filgrastim in the radiological/nuclear setting?

[8] Internet accessed on 03/24/2013 at: http://www.bt.cdc.gov/radiation/arsphysicianfactsheet.asp

No. The NIAID submitted the non-human primate study data to FDA for maintenance within a "pre-IND" (Investigational New Drug) archival record. No clinical studies have been conducted by the NIAID under this "pre-IND." FDA licensure of filgrastim for use in the radiological/nuclear setting would necessitate the manufacturer to submit a supplementary application to the current filgrastim biological license application.

9. Do the four currently approved LGFs have the same risks and benefits?

The four currently licensed LGFs are each unique biological products, with different molecular structures, pharmacokinetics, preclinical and clinical effects. While the ability to stimulate bone marrow blood leukocyte production is a shared characteristic of the products, the products differ in many ways.

5. A Brief Summary of the NIAID Study

The NIAID study is referred to as study AXG15 and was titled, "A Sixty-Day Efficacy Study of Subcutaneous Filgrastim (Neupogen) 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."

- The study was initiated on October 8, 2007 and terminated on September 21, 2010. The study was conducted at the University of Maryland School of Medicine with all microbiology and laboratory assessments also performed at the University.

- The study protocol described the planned randomization (1:1) of 62 nonhuman primates (NHP, Rhesus Macaques) to either daily vehicle (control) or Neupogen (g-CSF) 10 mcg/kg, subcutaneously.

- The animals received 750 cGy total body irradiation from a linear accelerator and were subsequently cared for using double-blinded design procedures.

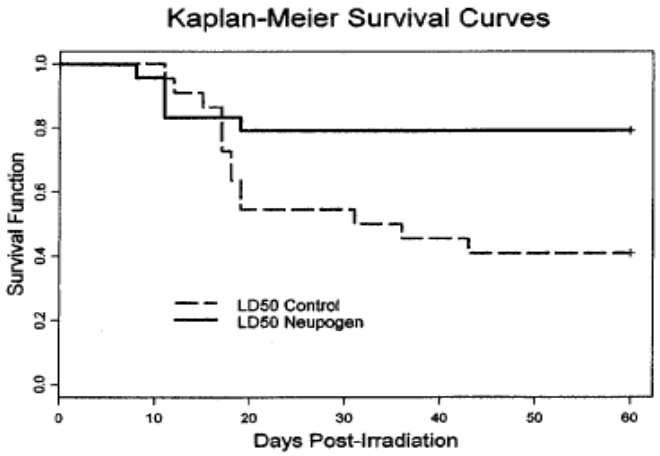
- Supportive care (antibiotics, wound care, nutrition supplementation, blood transfusion, etc.) was implemented with various evaluations over a 60 day follow-up period.

- A pre-specified euthanasia protocol (cage-side observations) was followed by study veterinarians blinded to animal treatment assignment.

- Of the 18 animals that died, 3 (all in control group) were "found dead," and all others were euthanized.

- The sponsor used an early stopping rule and stopped the study with 46 animals because the primary endpoint, a comparison of the number of surviving at day 60, showed 79% (19/24) of Neupogen group survivors versus 41% (9/22) control group survivors (the Chi-square test two sided $p < 0.0079$ and Fisher's exact text two sided $p = 0.0147$).

- The Kaplan-Meier survival curves by treatment are presented below; the logrank test p-value for comparing the equality of the two curves is 0.018.



- Even though the study is a blinded randomized study, because of the small sample size, the baseline information is not quite balanced in this study. In exploratory analyses conducted by FDA, the Kolmogorov-Smirnov (KS) test for testing equality of the Propensity score distributions (Neupogen vs. Control) resulted in a p-value of 0.0034. These propensity scores were obtained from a logistic regression model with treatment as dependent variable, and gender, source, group, and dose variables as covariates. The propensity scoring approach (two-strata method) was used to adjust the imbalance in the baseline information. The 46 animals were grouped into two strata using the propensity scores (cut-off point is 50 percentile). Cox model with treatment as the covariate was conducted for each stratum, and the combined hazard ratio (Neupogen vs. Control) was obtained as 0.31 with 95% confidence interval (0.10, 0.99).

- All additional exploratory analyses of survival indicated the advantage of using Neupogen compared to Vehicle (Control).

- For the animals that had ANC values that "recovered" from the nadir (19 animals in the Neupogen group and 12 animals in the Control group), the duration of severe neutropenia (absolute neutrophil count, ANC < 500/mcL) was less in the Neupogen group—mean of 19 days (95% CI: 17, 20) in the Control group versus 14 days (95% CI: 13, 15) in the Neupogen group.

- Febrile neutropenia was experienced by 91% (20/22) controls and 79% (19/24) of Neupogen-treated animals. The study was not designed to demonstrate an effect upon the febrile neutropenia outcome.

- FDA inspection of the animal care facility disclosed no deficiencies that undermined the integrity of the study data.

IND 100,228

PHARMACOLOGY AND TOXICOLOGY REVIEW
(Supporting Document # 8 and # 10 received July 28, 2011 and July 06, 2012, respectively)

Sponsor and Address: NIAID, NIH
Bethesda, MD

Reviewer: Ronald Honchel, Ph.D.
Toxicologist, HFD-160

Drug: NEUPOGEN® (Filgrastim, human recombinant G-CSF)

Submission Contents:

ARX01: A Pilot Study to Define the Dose-Response Curve in Rhesus Macaques Exposed to Increasing Doses of Total Body Irradiation (TBI) and Receiving Supportive Care.

AXG15: A 60-Day Efficacy Study of Subcutaneous Filgrastim to Treat the Hematopoietic Syndrome of The Acute Radiation Syndrome (ARS-HS) Following an $LD_{50/60}$ of TBI in Rhesus Macaques.

Response to an Information request sent to the Sponsor on November 08, 2011.

EXECUTIVE SUMMARY

The $LD_{30/60}$, $LD_{50/60}$, and $LD_{70/60}$ for ARS-HS in Rhesus macaques provided supportive care following TBI using a 6 MV linear accelerator (LINAC) radiation source was determined by exposing animals (8 males/group) to 720, 755, 785, 805, 840, or 890 cGy TBI at a rate of 80 cGy/min. Level 2 supportive care was provided that included i.v. fluids, prophylactic antibiotics, and blood transfusions. The primary endpoint was survival at 60 days post-TBI. The calculated $LD_{30/60}$, $LD_{50/60}$, and $LD_{70/60}$ radiation doses were 709, 752, and 797 cGy, respectively.

In the efficacy study (AXG15), Rhesus monkeys were exposed to 750 cGy TBI using a 6 MV linear accelerator (LINAC) radiation source. All animals were administered via s.c. injection 10 µg/kg/day filgrastim (20 M and 4 F) or vehicle (18 M and 4 F) at 20-26 hr post TBI with the follow up frequency of daily dosing based on blood ANC levels. Extensive supportive care (the Sponsor termed as Level 2) was provided that included i.v. fluids, prophylactic antibiotics, and blood transfusions. The primary endpoint was overall survival 60 days post irradiation. Secondary endpoints included mean survival time of decedents and effect on hematology parameters. Mortality was significantly decreased in the TBI + filgrastim group (21%) compared to the TBI + vehicle group (59%). Although the mean ANC at nadir was not significantly different, the duration of

days with ANC <500/μL (grade 3 neutropenia), duration of days with ANC <100/μL (grade 4 neutropenia), and days to recovery to ANC ≥ 1000/ μL were significantly improved in the treatment group compared to the control group. Interestingly, the mean survival time for decedents was much lower in the treatment group (12.0 days) compared to the control group (21 days).

From the DMIP nonclinical reviewer's perspective, there were a number of potential deficiencies in the AXG15 study report such as a limited histopathology battery (an example of a typical histopathology battery collected in nonclinical safety studies can be found at www.toxikon.com/userfiles/files/Toxikon's Preclinical...) and the lack of clinical chemistry data (typically evaluated in both nonclinical and clinical safety studies). On the other hand, filgrastim is an approved drug with a well-established safety profile in the oncologic setting. Whether a similar safety profile will hold in the ARS setting could not be inferred from this study. It is the considered opinion of this reviewer that the identified potential deficiencies do not change the overall nonclinical conclusion that filgrastim was radioprotective in study AXG15.

Pivotal animal rule efficacy studies are considered combined nonclinical/clinical studies from review perspective requiring separate nonclinical and clinical reviews. The Sponsor stated that the 10 μg/kg/day dose in monkey was bioequivalent to a human dose of 5 μg/kg/day (the filgrastim dose approved for patients receiving myelosuppressive chemotherapy) based on previous PK and PD studies (the study report stated that the rationale for the dose conversion was summarized in IND 100228 serial # 000). In addition to the dose, the frequency of dosing in this monkey study differed somewhat from than that recommended in the filgrastim labeling for patients receiving myelosuppressive therapy. It is also pertinent to note that both studies were performed using a high level of supportive care that included i.v. fluids, prophylactic antibiotics, and blood transfusions. The nonclinical reviewer will defer to the clinical team in regards to the evaluation of filgrastim dosing (including animal-to-human dose-conversion) and the adequacy, appropriateness, non-bias nature of the supportive care that was provided in AXG15.

There are a number of sources of variability in response to TBI with this type of bio-study and such studies must be designed in a manner to minimize their occurrence. For example, circadian effects are reported to influence TBI-induced mortality. However, the sponsor did not provide the time of day when animals were irradiated, thus it is not clear whether circadian effect on irradiation response was controlled for. In addition, because of the number of animals involved and the complex nature of the irradiation and support process required for these type of studies, animals in a single study are usually irradiated on many different days over a time period that could be months long. To minimize the impact of timing of irradiation on study results, animals irradiated on different days should include a mixture of treatment/control groups. This mixing of groups was accomplished in the TBI dose-response study (ARX01). However, 2 of the 5 irradiation days for AXG15 used 6 animals from one group and only 1 animal from the other group. Additionally, 10 and 17 animals from one vendor were place into control and treated groups, respectively, whereas 12 and 7 animals from the other vendor were place into

control and treated groups, respectively. Ideally, there should have been roughly the same ratio of control:treated for each vendor.

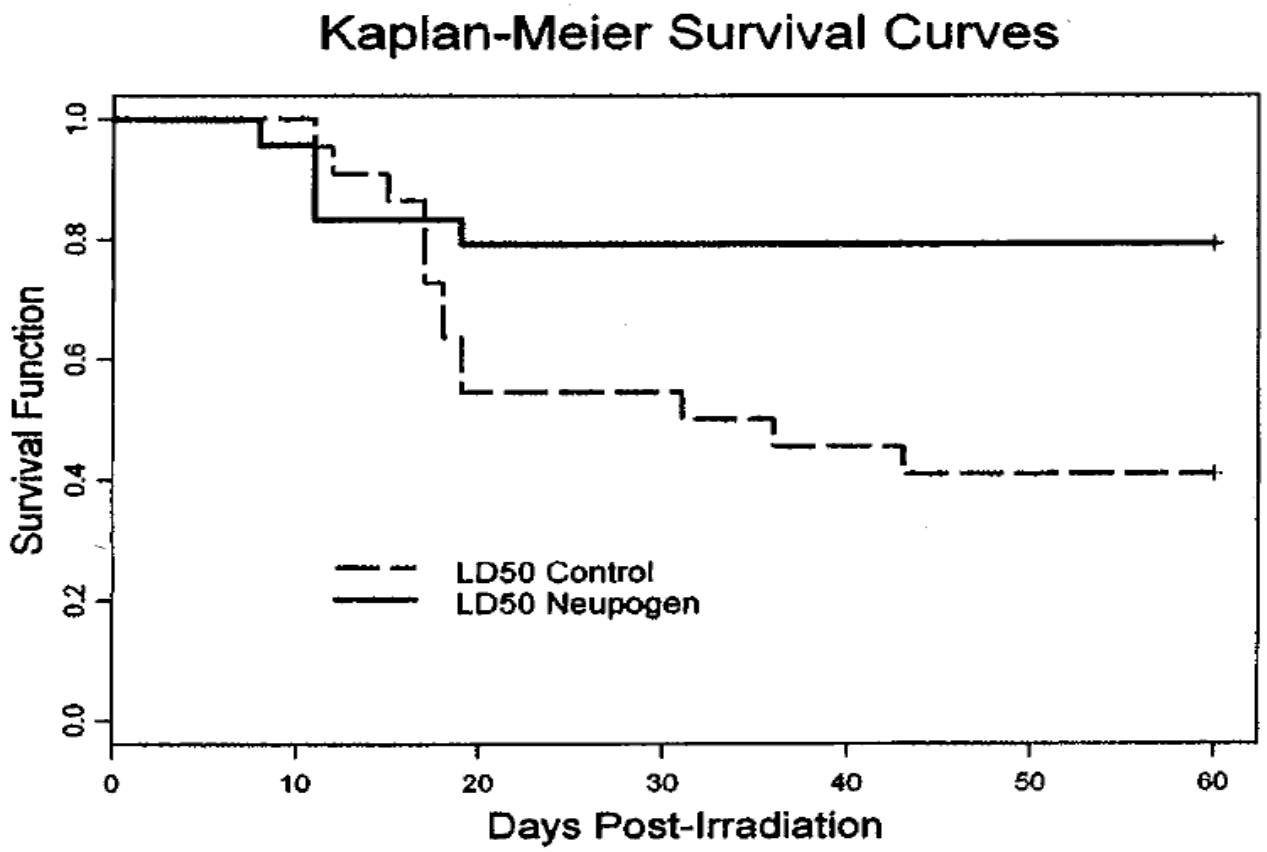
For a GLP study such as this one, the Sponsor should be able to demonstrate that dosing solutions contained the targeted amount of drug, and if not, what was the actual amount of drug administered. That information cannot be reliably obtained in this study due to analyzes being performed after expiration dates. Dosing was performed from October 2007 thru April 2008. Dosing solution analysis was not performed until January 2010 thru April 2010. As a result, many of the dosing solution samples (36 of 48) had expired by the time of LC-MS/MS analysis. The expired samples exhibited chromatographic peaks that were 5-6 times lower that non-expired samples. All dosing solution samples had expired by the time of G-CSF activity analysis. The relative potency (activity on a μg/mL basis) for retention samples range from 0.92 to 2.85 relative to the 1.00 activity assigned to the rhG-CSF standard. Overall, the dosing solutions were not considered homogenous. This was likely due to the analyzes not being performed until after the expiration date. On one hand, filgrastim is an approved drug and the drug used in this study was likely homogenous. On the other hand, this assumption was not proven by dosing analysis.

One concern when conducting studies is bias, especially when a parameter that is being evaluated is not blinded. The following information request was sent to the Sponsor on November 8, 2011 in order to help confirm that the increased survival in treated groups was not due to bias:

"Please provide a mortality summary table that includes: 1) date of euthanasia or found dead; 2) reason for euthanasia; 3) probable cause of death (based on necropsy and histopathological findings). In addition, please provide for each animal (for example, in an Appendix): 1) what supportive care was administered; 2) the study day (Day 0-60) and the reason the supportive care was initiated; and 3) amount and duration when applicable. Please make sure the animal is clearly identified as being in the treatment group or control group."

The Sponsor submitted a response to the above information request on July 6, 2012. There was one control animal (# 040129) that exhibited a staph infection. It was not clear from the study report that the animal was correctly counted as a TBI mortality (i.e. euthanized based on the criteria outlined in the protocol) or that the animal was removed only as a safety precaution to prevent spread of infection (and thus should not have been included as a TBI mortality, but simply excluded from the study). Otherwise, all other animals appear to have been euthanized based on the criteria outlined in the protocol. Submitted clinical observation data was limited to activity, respiration, posture, stool consistency, vomiting, hemorrhage, and alopecia. Other clinical signs were apparently observed (i.e. lesions and abnormal skin conditions) but were not provided in the cageside observation section. There were no apparent differences in the level of supportive care provided to each group.

Despite the deficiencies cited above, the overall nonclinical conclusion is that under AXG15 study conditions, filgrastim significantly increased the 60-day survival rate in NHP exposed to 750 cGy TBI compared to the animals administered vehicle alone. This conclusion assumed that there was no significant difference in radiation dose or bias in the decision to administer supportive care for vehicle control and treatment groups. Interestingly, the mean survival time for decedents was much lower for the treatment group (12.0 ± 4.1 days, mean ± S.D.) than the control group (21 ± 9.6 days). This is an unusual finding in that radioprotective agents are expected to increase both the survival rate and the mean survival time for decedents. Another unexpected finding was that mean absolute neutrophil counts were similar or slightly decreased for the filgrastim group compared to the vehicle control group from Days 3-11 post TBI (see Figure 5 page 25 of this review). If one looks below at the Kaplan-Meyer Survival Curves (provided by the Sponsor), survival is also initially similar or slightly decreased for the filgrastim group compared to control. However, only 1 treated animal dies after Day 12, whereas the majority of the vehicle control animals die after Day 12 (thus the explanation for the decreased mean survival time for decedents results). Filgrastim administration did not initially provide the expected increase in mean ANC following TBI and likewise did not initially provide protection against TBI-induced mortality. Around Day 12, mean ANC levels were increased in the filgrastim group compared to the vehicle control group and greatly increased survival was observed in the filgrastim group compared to the vehicle control group thereafter. A similar finding was observed in a recently published study (Plett et al., *Health Phys.* 103:343-355, 2012) that used C57BL/6 mice suggesting the usual short-term effects (and thus benefits) of filgrastim administration may not be observed following lethal TBI exposure, but that filgrastim is still somehow able to provide radioprotection. Almost all animals died or were euthanized between Days 8-19. If one excludes the control animal with a skin staph infection euthanized to prevent the spread of infection, the only deaths that did not occur between Days 8-19 where 2 control and 1 treated animals that died after Day 19 (i.e., minor, if any, difference in late term mortality between control and treated groups). Comparing death date and individual ANC values (Appendix K submitted with SDN08) all but one monkey that died between Days 8-19 (10/21 for control compared to 4/24 for treated) was experiencing Grade 4 neutropenia at the time of death. The lone exception was a treated animal (# 0311025) with near Grade 4 neutropenia (ANC = 180/µL with Grade 4 less than 100/µL) at time of death but had experienced Grade 4 neutropenia the previous 14 days. Thus, death in the monkey study appears to be strongly correlated with Grade 4 neutropenia. The duration and severity of Grade 4 neutropenia in the treatment group was initially (Days 8-11) similar to that observed in the control group and no apparent treatment-related radioprotective effect was observed during this period. The duration and severity of Grade 4 neutropenia in the treatment group was then improved compared to that observed in the control group from Day 12-19. This was the period with the highest control group mortality rate (8 of the 12 control deaths occurred in this period) whereas only 1 death was observed during this period in the treatment group. These results suggest that the radioprotective effect of filgrastim was due to an overall decrease in the duration and severity of Grade 4 neutropenia from the Day 12-19 post-irradiation period. All the tables and figures in this document are excerpts from the sponsor's study report.



PHARMACOLOGY

Study title: A Pilot Study to Define the Dose-Response Curve in Rhesus Macaques Exposed to Increasing Doses of Total Body Irradiation (TBI) and Receiving Supportive Care.

Study no.:	ARX01
Conducting laboratory and location:	University of Maryland School of Medicine
Date of study initiation:	Not stated
GLP compliance:	No
QA statement:	No

Key Study Findings

The Sponsor stated that based on the literature, the approximate $LD_{50/30}$ for nonhuman primates without supportive care is 665 cGy and 640 cGy for 2 MeV x-ray and ^{60}Co, respectively. The objective of this blinded, randomized study was to determine the $LD_{30/60}$, $LD_{50/60}$, and $LD_{70/60}$ for ARS-HS in Rhesus macaques provided supportive care following TBI generated from a 6 MV linear accelerator (LINAC) radiation source. Animals (8 males/group) were exposed to 720, 755, 785, 805, 840, or 890 cGy TBI at a rate of 80 cGy/min. Level 2 supportive care was provided that included i.v. fluids,

prophylactic antibiotics, and blood transfusions. The primary endpoint was survival at 60 days post-TBI. The calculated $LD_{30/60}$, $LD_{50/60}$, and $LD_{70/60}$ radiation doses were 709, 752, and 797 cGy, respectively.

Methods

Doses:	720, 755, 785, 805, 840, or 890 cGy TBI (Dosimetry performed prior to and during each radiation procedure)
Frequency of dosing:	Once (cohorts of 2-8 animals were irradiated every 2-4 weeks with no more than 2 animals/cohort receiving the same radiation dose)
Radiation source:	6 MV linear accelerator (LINAC)
Radiation procedure:	Animals were acclimated to being placed into a Supine Restraint Device. Fasted animals were: 1) administered 1-2 mg/kg (p.o., i.v., or i.m.) antiemetic Ondansetron 45-90 min prior to TBI; 2) anesthetized with 10 ± 5 mg/kg i.m. ketamine and, if necessary, 10 ± 5 mg/kg i.m. or s.c. xylazine prior to irradiation and were transported to LINAC facility once anesthetized; 3) allowed to recover from anesthesia and then exposed to TBI at a rate of 80 cGy/min (TBI delivered as 50% to the anterior position and 50% to the posterior position); 4) administered a second dose of 1-2 mg/kg (p.o., i.v., or i.m.) Ondansetron 35 to 45 min post TBI; and 5) returned to their cage.
Species/Strain:	Monkey/Rhesus
Number/Sex/Group:	8 males/group
Age:	3-4 years
Weight:	4.7 to 6.2 kg on day of TBI
Satellite groups:	Blood for transfusion was obtained from male Rhesus macaques at least 5 years of age.
Supportive care/Euthanasia:	Listed under “Medical Management” and “Euthanasia” below (as stated verbatim in the submission).
Deviation from study protocol:	None stated.

6.2 Medical Management

Supportive care is provided to all NHPs as indicated by cageside and clinical observations, as per the approved IACUC protocol. Supportive care measures include hydration fluids, antibiotics, analgesics, antidiarrheals, antipyretics, anti-emetics, anti-ulcertives, nutritional support and blood transfusions.

Cageside observations were performed twice daily by the veterinarians at least 6 hours apart. NHP activity, posture, stool consistency, vomit, hemorrhage, respiratory or seizure activity, and alopecia, were graded and recorded.

Clinical Observations: The NHP is anesthetized (Ketamine HCl Inj., 10mg/kg, KetaSet®, Fort Dodge, Fort Dodge, IA) or a combination of ketamine and Xylazine (AnaSed®, Ben Venue Laboratories) at 1mg/kg and clinical parameters such as body weight, body temperature, complete blood count (CBC) (Beckman Coulter Ac·T diff™, including a manual white blood cell (WBC) differential performed on a Wright-Giemsa-stained blood film), dehydration status, presence of mouth ulcers, and observation of blood in the stool are assessed.

Analgesics: Buprenorphine HCl (Hospira, Lake Forest, IL) (IM at 0.01mg/kg up to 0.02mg/kg, BID) was administered whenever mouth ulcers or bloody stools were observed and from study day 5 to 35. Mouth ulcers were cleansed with hydrogen peroxide or Nolvasan solution and rinsed with saline. Bupivacaine gel, a mixture of 0.1ml of 25% Bupivacaine HCl (Marcaine®, Hospira) with a dab of surgical lubricant (Surgilube®, Fougera®, Melville, NY), was applied to the area with a cotton tipped applicator.

Anti-Ulcerative: Sucralfate (Carafate®, Axcan Scandipharm Inc. & Nostrum Laboratories, Inc.) was administered (1g/day BID) from study day 5 to 35 or if bloody stool was observed.

Antidiarrheals: Following the observation of diarrhea, Loperamide Hydrochloride (Imodium, McNeil), (0.1-0.2mg/kg PO BID) was administered. If diarrhea persisted for three (3) successive days during Imodium treatment or if watery stool without any signs of formed stool were observed, diphenoxylate hydrochloride (Lomotil, Pfizer Inc, 0.1mg/kg PO BID for 3 days) was administered. If diarrhea persisted after three (3) days, Imodium treatment was re-administered.

Antibiotics: Antibiotics were initiated when the absolute neutrophil count (ANC) was <500/µL and continued until the animal maintained an ANC >500/µL for 48 hours. The primary antibiotic was enrofloxacin (Baytril®, Bayer HealthCare LLC,Shawnee Mission, KS). Additionally, gentamicin sulfate (GentaMax®, Pheonix Scientific, Inc., 5mg/kg QD IM or IV) was administered in combination with Baytril when the body temperature ≥103°F and was continued for 24hr. Rocephin (Roche Laboratories Inc., Nutley, NJ) or Primaxin (Merck & Co Inc., Whitehouse Station, NJ) was administered when microbial resistance was demonstrated to enrofloxacin or gentamicin.

Antipyretic: Carprofen (Rimadyl®, Pizer Inc. 2.2mg/kg BID or 4.4mg/kg QD, IM, IV, or PO) was administered when a body temperature of ≥104°F was observed. It was continued for 48 hours after the first day the temperature was <104°F.

Nutritional Support: On all days post-irradiation animals received fresh fruit, soft food, and bottles containing diluted fruit juice or oral rehydrator (Prang™, Bio-Serv®). Animals that were observed to have weight loss ≥10% of their baseline body weight received BIO-SERV certified Rhesus Liquidiets at 15ml/kg by oral gastric gavage (OG). Volume was reduced to 7ml/kg if the animal was also receiving OG reverse osmosis (RO) water for hydration
Blood Product Support: Whole blood, anticoagulated with 10% citrate, dextrose phosphate with adenine (CPD-A) (Sigma-Aldrich, St. Louis, MO) was obtained from healthy, male NHPs, bw ≥ 7.0 kg. Blood was filtered through a 70 micron cell strainer (BD Falcon™) and irradiated to 2500 cGy (Gamma Cell Elite 1000) prior to use. Transfusions of whole blood were administered at 7-14ml/kg, IV, using a 18 micron blood filter (Hemonate) following a decrease of ≥5% in HCT resulting in a HCT ≤25% over a 24 hr time period, HCT is <20%, or there was obvious signs of uncontrolled hemorrhage.

Fluid Support: Fluid support was provided based on a grading system delineated as mild, moderate or severe dehydration.
Mild: presence of tacky mucus membranes or a skin tent time (STT) or capillary refill time (CRT) ≥2 but <3sec. Mild animals received a bolus of LRS (10-15mL/kg) by slow IV push and reverse osmosis (RO) water (10-15mL/kg) by oral gastric feeding tube (OG).
Moderate: NHPs displaying any of the mild criteria plus dry mucous, >3% increase in hematocrit (HCT) from the day before (not transfusion related), sunken eyes, or STT or CRT ≥3sec. Moderate animals received a bolus of LRS (20-30mL/kg) over 15-20 min. by slow push and RO water (7-10 mL/kg by OG).
Severe: NHPs displaying any of the mild and or moderate criteria plus had pale mucous membranes, >5% increase in HCT from the day before (not transfusion related), a rapid and weak pulse, cold extremities, lethargy, or rapid breathing. Severely dehydrated animals received fluids as described for moderate dehydration with the addition of a slow IV infusion (15±5 mL/kg/h) administered over a period of 2-4 hours. Animals may have been placed in a restraint device at this time and allowed to awaken. Midazolam HCl (Bedford Laboratories™) (0.2mg/kg) may have been administered to calm the NHPs while in the restraint.

6.3 Euthanasia

A specific set of criteria for euthanasia was applied by all veterinarians. Any NHP exhibiting recumbency in the cage or decreased or absent responsiveness to touch or they experienced hemorrhage from the GI tract to be in excess of 20% of the estimated blood volume in any 24 hour period or they experienced unrelieved pain were euthanized. Any NHP which experienced any combination of the following observations such as respiratory distress, decreased food and water intake, reluctance to move for >24hrs, and severe dehydration classified an animal to be euthanasized. Animals are euthanized by veterinarians using DEA Class III euthanasia solution (Euthasol®, [Virbac AH Inc.] 0.27ml/kg IV). Expiration is confirmed by a lack of heart beat, absent femoral artery pulse, and lack of chest respiration.

Observations and Results

Mortality

Veterinarians were blinded to the radiation dose. Mortality results are shown in the Sponsor's Table 2 below. The Sponsor stated that radiation dose was a significant predictor of mortality (p = 0.01).

Table 2.

Percent Lethality and Mean Survival Time of Decedents Following Radiation in Rhesus Macaques

Radiation Exposure	720 cGy	755 cGy	785 cGy	805 cGy	840 cGy	890 cGy
% Lethality	38%	50%	75%	63%	75%	100%
Decedents/total	3/8	4/8	6/8	5/8	6/8	8/8
Survival time of decedents (days)	**720 cGy**	**755 cGy**	**785 cGy**	**805 cGy**	**840 cGy**	**890 cGy**
Mean±SEM	20.0±5.5	18.3±2.1	22.2±6.0	16.2±3.0	17.5±1.6	21.1±3.6
Median	15.0	18.5	16.5	14.0	17.5	18.0

Table 2. Rhesus macaques were exposed to TBI using average 2 MV LINAC photons at a dose rate of 80 cGy/min. The TBI was delivered as 50% in the anterior (AP), then 50% in the posterior (PA)directions for total dose cohorts (n=8 each) of 720, 755, 785, 805, 840 and 890 cGy. Animals were observed for 60d post TBI for cageside observations and euthanasia under protocol criteria for all-cause mortality by veterinarians that were "blinded" to the radiation dose for each animal.

Clinical Signs

Clinical signs were monitored daily, but a complete summary of results was not provided (not a critical issue since this is a dose-range finding study). The only summary data provided was a Table summarizing the occurrence and severity of diarrhea. All animals experienced diarrhea with no apparent dose-relationship between diarrhea occurrence/severity and radiation dose.

Body Weights

Body weights were recorded daily, but a complete summary of results was not provided (not a critical issue since this is a dose-range finding study). The only summary data provided was a Table showing the occurrence of 10% or more and 25% or more body weight loss compare to the pre-TBI value with no apparent dose-relationship between severe body weight loss and radiation dose.

Hematology

Blood samples for CBC analyzes were collected on almost a daily basis. Neutrophil results are summarized in the Sponsor's Tables 4-6 and Figure 4 below. The Sponsor stated that there were no significant differences between groups.

Table 4.

Neutrophil-related parameters for rhesus macaques following exposure to 6 MeV photon irradiation

TBI Dose (cGy)		First day (d) and range ANC < 500µL or 100µL (n=8)		Duration (days and range) ANC < 500µL or 100µL		Recovery to ANC ≥ 1000µL	ANC Nadir (µL) (n=8)
		< 500/µL	< 100/µL	< 500/µL	< 100/µL	≥ 1000/µL	
720	Mean±SEM	4.6±0.3	7.3±0.3	16.0±0.5‡	11.5±1.3‡‡	23.4±0.8‡	0
	Median	NA	NA	16.0‡	10.5‡‡	24.0‡	0
	Range	d4-6	d6-9	15-18days	9-18days	d21-24	NA
755	Mean±SEM	5.5±0.6	7.1±0.4	24.0±7.3†	9.8±1.0†	26.7±3.7	0
	Median	NA	NA	17.0†	9.5†	23.0	0
	Range	d3-6	d5-8	16-46days	8-12days	d22-23*	NA
785	Mean±SEM	4.6±0.3	6.5±0.4	14.3±1.5	10.3±0.9	21.7±1.2	5±0.5
	Median	NA	NA	14.0	10.0	21.0	0
	Range	d4-6	d6-8	12-17days	9-12days	d20-24	0-4
805	Mean±SEM	5.0±0.0	6.5±0.3	15.0±1.0	10.3±0.9	24.7±2.7	0
	Median	NA	NA	16.0	10.0	22.0	0
	Range	NA	d6-8	13-16 days	9-12 days	d22-23	NA
840	Mean±SEM	5.0±0.3	6.4±0.4	17.0±2.0Δ	12.7±2.3	42.0±18Δ	0
	Median	NA	NA	NA	NA	NA	NA
	Range	d4-6	d5-8	15 or 19 days	8-15days	d24 or d27	NA
890	Mean±SEM	4.5±0.2	6.3±0.3	DNO	DNO	DNO	0
	Median	NA	NA	NA	NA	NA	0
	Range	d4-5	d5-8	DNO	DNO	DNO	NA

The mean, standard error of the mean (SEM), median and range (where applicable) are reported for each radiation cohort. The day of the occurrence of an absolute neutrophil count (ANC) below 500/µL or 100/µL for each irradiation dose is shown. The duration of neutropenia is defined as an ANC below either 500/µL or 100/µL. The durations (d) do not include data from decedent animals unless recovery occurred to that level, e.g., ANC ≥ 500/µL prior to death. The duration of neutropenia was estimated as the number of days that a subject has an observed or an imputed ANC below 500/µL. Any single observed ANC that was ≥ 500 /µL and was immediately preceded and followed by ANC < 500/µL was counted as a day of severe neutropenia. The time to recovery was estimated as the number of days from study day 1 until the first 2 consecutive observed or imputed ANC after the nadir was ≥ 1,000/µL. The time to recovery of ANC >1000/µL ranged from 21.7d to 42.0d post TBI. There are no significant differences in these values relative to radiation dose. There were only two survivors in the 840cGy cohort, responsible for the 42.0d value for this parameter. The average recovery time to an ANC >1000/µL for all survivors is approximately 26.2d. The ANC nadir was the first lowest observed or imputed ANC that occurred at least 2 days after irradiation. One animal (1/48) did not experience absolute neutropenia (ANC = 0/µL) during the course of the study. TBI dose and survivors/total NHPs are: 720cGy, 5/8; 755cGy, 4/8; 785cGy, 2/8; 805cGy, 3/8; 840cGy, 2/8; 890cGy, 0/8.

*One animal survived but the ANC after the nadir did not attain ≥ 1,000/µL at study day 60. Not applicable (NA) indicates all numbers were equal to the mean value or in the case of the median where it is not applicable due to n=2. Did not observe (DNO) indicates that ANC for all animals at 890cGy did not recover to the specified level prior to death. Δ n=2, n=3, † n=4, ‡n=5, ‡‡ n=6

Table 5.

Occurrence of grade 3 or 4 neutropenia: The first and final day that grade 3 or 4 neutropenia occurred in an animal within each radiation cohort					
TBI		**ANC (d) < 100/μL**		**ANC (d) < 500/μL**	
Dose (cGy)		First day (n=8)	Final day	First day (n=8)	Final day
720	Day	6	23[‡‡]	4	22[‡]
	Range	d6-9	d15-23	d4-6	d18-22
755	Day	5	18[‡]	3	46[†]
	Range	d5-8	d15-18	d3-6	d20-46
785	Day	5	17	4	20
	Range	d5-8	d14-17	d4-6	d15-20
805	Day	6	18	5	20
	Range	d6-8	d15-18	NA	d17-20
840	Day	6	21	4	23[Δ]
	Range	d6-8	d15-21	d4-6	d20,23
890	Day	5	DNO	4	DNO
	Range	d5-8	DNO	d4-5	DNO

The first and final day when an ANC below either 500/μL or 100/μL occurred in an animal in each radiation group is shown in Table 5. Additionally, the range of each of these occurrences for each irradiation group is presented. TBI dose and survivors/total NHPs are: 720cGy, 5/8; 755cGy, 4/8; 785cGy, 2/8; 805cGy, 3/8; 840cGy, 2/8; 890cGy, 0/8.

Not applicable (NA) indicates all numbers were equal to the mean value. Did not observe (DNO) indicates that ANC for all animals at 890cGy did not recover to the specified level prior to death.

[Δ] n=2, n=3, [†] n=4, [‡]n=5, [‡‡] n=6

Table 6.

Febrile neutropenia (FN) and antibiotic requirements for rhesus macaques following exposure to 6 MeV photon irradiation: Incidence, duration

TBI Dose (cGy)	First day FN (n=8)	Duration (d) FN	Days on Antibiotics	Days CBT ≥103.0°F (n=8)
720	8.0±0.7	6.4±2.0‡	19.6±1.4‡	11.1±1.8
755	10.0±1.3	8.5±2.1†	27.3±5.9†	12.5±3.7
785	7.1±1.1	7.5±0.5Δ	16.0±0Δ	13.1±3.0
805	7.6±2.1	1.0±0.6	18.0±0	5.5±1.0
840	10.9±0.9	6.5±3.5Δ	19.0±2.0Δ	7.0±1.4
890	8.5±0.8	DNO	DNO	8.9±1.9

Febrile neutropenia (FN) is defined as the ANC <500/μL and the core body temperature (CBT) ≥103.0°F. The mean of the first day of the occurrence of FN for each irradiation dose is shown. The duration for FN and the numbers of days an animal required antibiotic administration was determined for the survivors only. However, all animals in the study did experience FN. The mean numbers of days an animal's CBT ≥103.0°F is shown for all animals regardless of survival. The standard error of the mean (SEM) is reported for each parameter. TBI dose and survivors/total NHPs are: 720cGy, 5/8; 755cGy, 4/8; 785cGy, 2/8; 805cGy, 3/8; 840cGy, 2/8; 890cGy, 0/8.

The duration of FN or antibiotic requirements for the 890cGy cohort was not observed (DNO) due to 100% lethality.

Δ n=2, n=3, † n=4, ‡n=5, ‡‡ n=6

Figure 4. Mean Absolute Neutrophil Counts in rhesus monkeys following irradiation (2 MeV LINAC) and supportive care.

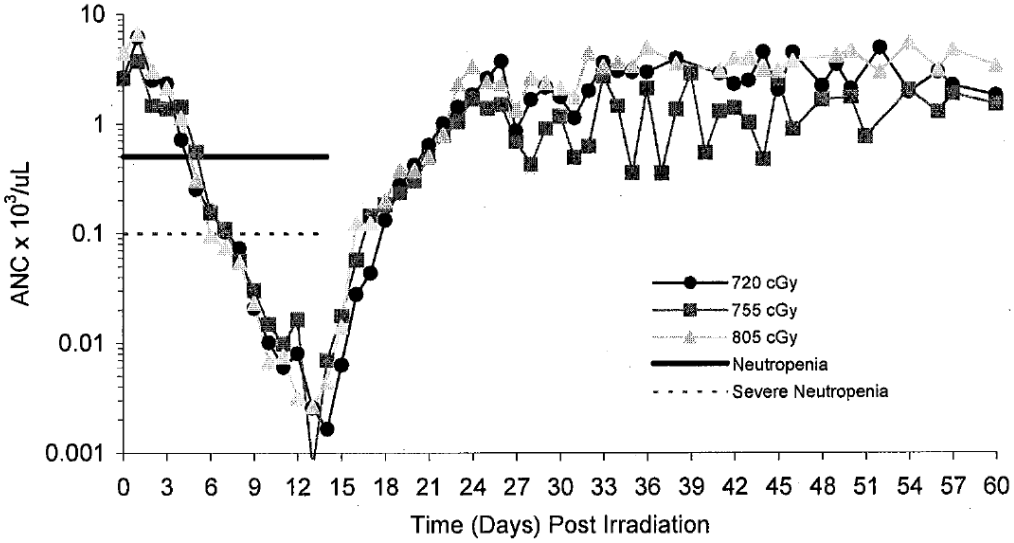


Figure 4 shows the changes in the absolute number of neutrophils (ANC) in the peripheral blood of rhesus macaques (n=8/radiation dose) as a function of time post TBI and dose (cGy). Survivors/total NHPs are: 720cGy 5/8, 755cGy 4/8, 805cGy 3/8. TBI, of 2 MV average energy LINAC-derived photons was administered at 80 cGy/min. The radiation doses shown approximate the LD_{30}, LD_{50}, and $LD_{70/60}$ estimated from the resultant data set.

Platelet count and transfusion results are summarized in the Sponsor's Table 7 and Figure 5 below. The Sponsor stated that there were no significant differences between groups.

Table 7.

Duration of cytopenia: Platelet-related parameters

TBI Dose (cGy)		First day platelet count (d) < 20,000/μL (n=8)	Duration (d)* < 20,000/μL	Nadir‡ (PLT/μL) (n=8)	Recovery to platelet count ≥ 20,000/μL	# Trans-fusions‡ (54mL) (n=8)	First day (d) transfusion occurred within radiation cohort (n=8)
720	Mean±SEM	8	12.6±1.8‡	0.5±0.4	24.3±1.4‡	2.3±0.6	9
	Range	d8-10	d8-12	0-3	d18-27	0.5-5.5	d9-15
755	Mean±SEM	8	19.8±9.5†	0.4±0.2	29.5±10.2†	2.0±0.7	11
	Range	d8-11	d8-48	0-1	d18-60	0.5-6.5	d11-15
785	Mean±SEM	7	13.7±3.2	0±0	22.7±3.2	1.8±0.3	11
	Range	d7-9	d11-20	NA	d19-29	0.5-3	d11-13
805	Mean±SEM	9	8.7±0.3	0.4±0.2	19.0±0.8	1.5±0.5	10
	Range	d9-11	d8-9	0-1	d18-20	0-4	d10-14
840	Mean±SEM	8	20.5±1.5Δ	0.1±0.1	30.0±2.0Δ	2.3±0.6	11
	Range	d8-10	19,22	0-1	28,32	0.5-5.5	d11-13
890	Mean±SEM	9	DNO	0.1±0.1	DNO	3.2±0.9	11
	Range	d9-10	DNO	0-1	DNO	1-9	d11-15

The mean, standard error of the mean (SEM) and range of the first day thrombocytopenia, defined as a platelet (PLT) count < 20,000/μL, is observed in an animal in each radiation cohort, duration of thrombocytopenia, platelet nadir, day recovery from thrombocytopenia occurs, number of whole blood transfusions administered (54mL) and first day a transfusion is observed in an animal in each radiation cohort are displayed. The standard error of the mean (SEM) is also shown. *Note that durations (d) do not include data from decedent animals unless recovery occurred to that level, e.g., PLT ≥ 20,000/μL prior to death. ‡ The platelet nadir and number of transfusions includes both survivors and nonsurvivors. All but one animal (1/48) received a transfusion. TBI dose and survivors/total NHPs are: 720cGy, 5/8; 755cGy, 4/8; 785cGy, 2/8; 805cGy, 3/8; 840cGy, 2/8; 890cGy, 0/8. The duration of thrombocytopenia and platelet recovery was not observed (DNO) due to 100% lethality in the 890cGy cohort.

Δ n=2, n=3, † n=4, ‡n=5, ‡‡ n=6

Figure 5. Mean platelet counts in rhesus macaques following irradiation (2 MeV LINAC) and Supportive Care

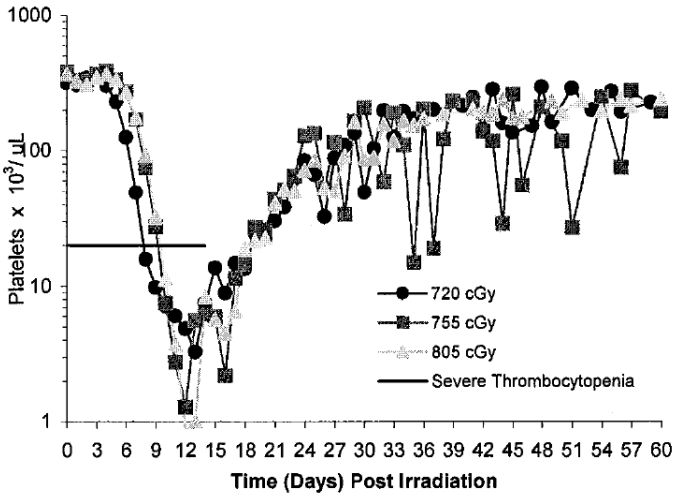


Figure 5 shows the changes in the platelet count x 10^3/µL in the peripheral blood of rhesus macaques (n=8/radiation dose) as a function of time post TBI and dose (cGy). Survivors/total NHPs are: 720cGy 5/8, 755cGy 4/8, 805cGy 3/8. TBI, of 2 MV average energy LINAC-derived photons was administered at 80 cGy/min. The radiation doses shown approximate the LD_{30},

Absolute lymphocyte count (ALC) results for Days 1-4 are summarized in the Sponsor's Table 8 below. The Sponsor stated that there were no significant differences between groups.

Table 8.

Mean ALC x 10^3/uL
Following Radiation in Rhesus Macaques

Study Day	Radiation Dose (cGy)					
	720	755	785	805	840	890
0	4.736	4.281	4.956	4.501	3.975	4.149
1	0.293	0.182	0.229	0.221	0.284	0.221
2	0.266	0.204	0.138	0.235	0.189	0.225
3	0.279	0.164	0.174	0.278	0.125	0.190
4	0.221	0.196	0.155	0.231	0.203	0.154
Each data point represents an n=8 with the exception of n=7 for day 1 and day 2 in the 785 cGy cohort.						

Clinical Chemistry

Not performed.

Blood Cultures

Blood culture results are summarized in the Sponsor's Table 13 below.

Blood cultures were obtained when febrile neutropenia (FN) (ANC <500/μL and body temperature ≥103°F) was observed or any day the body temperature was ≥105°F. Additional blood cultures were collected if FN persisted for 5 consecutive days after a previous blood culture collection. The treatment regimen was altered if an organism was isolated from a blood culture that demonstrated resistance to the current treatment antibiotic. Table 13 enumerates the number of animals by radiation dose from which organisms were isolated from their blood cultures which were resistant to at least one of the following antibiotics, baytril, gentamicin, rocephin or claforan. There were 30 animals from the 48 study animals (62.5%) which had at least one organism that was resistant to at least 1 of the above mentioned antibiotics.

Table 13.

Total Body Irradiation of Rhesus Macaques in the Hematopoietic Syndrome: Antibiotic Resistance observed in blood cultures

	Antibiotics					Number and Percentage	
Radiation Dose (cGy)	**Baytril** Enrofloxacin	**Gentamicin**	**Rocephin** Ceftriaxone	**Claforan** Cefotaxime	**Primaxin** Imipenem	Total # Animals	Percent
720	3	1	0	0	0	3	37.5%
755	6	4	2	0	0	6	75.0%
785	2	2	1	0	0	3	37.5%
805	4	2	3	1	0	4	50.0%
840	6	3	2	0	0	7	87.5%
890	7	2	4	1	0	7	87.5%

Animals (n=48) were total body irradiated across the hematopoietic syndrome (720cGy, 755cGy, 785cGy, 805cGy, 840cGy or 890cGy, n=8 per dose). Blood cultures were obtained per protocol guidelines. The number of animals in each radiation cohort from which an organism was obtained by blood culture which was resistant to an antibiotic is enumerated. Some organisms were resistant to multiple antibiotics.

Gross Pathology and Histopathology

Necropsy was performed only on animals that were euthanized or expired. Tissues from heart, lung, spleen, liver, kidney, mesenteric lymph nodes, thymus, small intestine and colon as well as bone marrow were collected in order to determine the cause of death. Sections 7.9.1 and 7.92 from the Sponsor's submission were cut and pasted below.

7.9.1 700 cGy Series (720, 755, 785 cGy)

Gross necropsy summary:

Gastrointestinal (GI) tissue: Numerous areas of hemorrhage and petechiae are evident with increasing dose of radiation in both the small and large intestine. Hemorrhagic areas are noted on both serosal and mucosal surfaces. These are most prominent in the highest dose in the 700 series, 785 cGy. Areas of mucosal edema are also noteworthy. Minimal food content was noted in several of the animals.

Other organs: The reports all noted that the major organs, liver, spleen, kidneys, lungs and heart, were all relatively within normal limits (WNL). The spleen may be enlarged within two of the NHPs, although we do not have "normal" spleens for comparison. Petechiae and ecchymosis were noted on the body such as arms, legs, chest and "trunk". The hyrdation status was noted as WNL for all except one of the NHPs. Several NHPs at 785cGy were noted to have oral ulceration.

Histopathological assessment:

Bone Marrow:
Bone marrow isolated from femoral bone and sternum show evidence of significant radiation effects on hematopoietic system. Marked, diffuse areas of hypocellularity with noted myeloid and megakaryocyte depletion. Severity of myeloid and erythroid hypocellularity increases with radiation dose. Some tissue preps show no evidence of regeneration where others at lower radiation doses have "scattered" areas of myeloid regeneration. Myeloid regeneration exceeds that noted for erythroid recovery. Animals surviving the duration of the study show evidence of normal marrow cellularity with predominance of myeloid recovery.

Lymphoid tissue:
Severe effects are noted on all lymphoid tissue in lymph nodes (LN), spleen (SPL) and thymus (T). Higher radiation doses may result in no observable thymic tissue. The revealing comment "diffuse lymphoid depletion", is noted for all LN, SPL, and T in all NHPs euthanized during the in-life phase. At 785cGy exposure several NHPs were noted as being "lymphocyte depleted" with no T tissue observable.

GI tissue:
Histological analysis/observations of the small and large intestinal regions vary to a degree with the observations noted during the gross necropsies. The necropsy results suggested marked areas of hemorrhage on both the serosal and mucosal surfaces. The histological reports for the 785cGy cohort euthanized during the in-life phase are recorded as "few small

areas of mucosal hemorrhage" and "scattered mucosal hemorrhages" in both the small and large intestines. Re-examination of the GI tissue regions will be required to reconcile these observations between the gross necropsy and histological sections. The gross pathology suggests a larger presence of hemorrhage and petechiae than noted from the tissue sections.

The cellular structure of the GI regions are not well analyzed/assessed by the histologist relative to the presence of crypts and villus height and integrity as a unit of regeneration or pathology. Few remarks are offered that refer to the basic villus and crypt relationship. It is noted in only a few NHPs that there is "modest blunting" of villi. At 785cGy this is associated with "noted crypt regeneration and mucosal crypt hyperplasia". The histology of the villus/crypt regions at 720 and 755cGy are recorded primarily as WNL or "no significant lesions noted".

Other major organs:
The histological picture of the other major organs is not demonstrative of significant radiation-induced damage. The remarks pertain to the kidney and suggest frequent of areas of "tubular necrosis and interstitial inflammation". The most predominant observations for all organs noted are "rare" and "scattered" hemorrhage. (See notes on Bacteriology) There are consistent observations of bacterial "emboli" in all organs and that these "emboli" can be associated with areas of hemorrhage and/or necrosis.

Bacteriology:
The presence and severity of bacterial "emboli" in critical organs increases with radiation dose and time, during the 2nd and 3rd weeks, post TBI. Bacterial emboli are observed in liver, lung, spleen, heart, kidneys, thymus, stomach and small and large intestine. Many bacterial emboli are associated with "scattered" areas of hemorrhage and petechiae. The lethality and morbidity are deemed due to terminal sepsis, diffuse bacterial colonization, "widespread bacterial embolization" and possible septic shock.

Conclusion: Morbidity and mortality.
The combination of bacterial colonization in major organs and presence of multiple areas of hemorrhage/petechiae in intestine, kidney, lung and liver in concert with severe neutropenia and thrombocytopenia present a confluence of lethal events.

Note that all major sequelae including cytopenia, bacteremia/sepsis and hemorrhage are radiation dose and time post TBI dependent.

7.9.2. 800 cGy Series (805, 840, 890 cGy)

Gross Necropsy Summary.
The observations noted upon necropsy of NHPs euthanized during the in-life phase of the 800 cGy series are similar to those noted for the 700 cGy radiation series, although increased in incidence and severity. Especially noteworthy are the incidence of hemorrhagic areas on GI surfaces.

GI tissue. Hemorrhagic areas are observed on mucosal surfaces of most NHPs examined. The occurrence does vary and would require a more thorough examination to compare GI tissue of all NHPs euthanized. The variable nature of the extent of hemorrhagic areas is found primarily at the two lowest dose cohorts (805 and 840cGy) in this series. The 890cGy cohort is noted to have the highest incidence of mucosal and serosal hemorrhagic areas

that predominate in the large intestine. Ecchymosis is noted as well as numerous mucosal areas with "frank hemorrhage" and "petechiae".

Other organs. Several notes refer to the spleen being enlarged in several NHPs although as noted previously, we do not have "normal" spleen weights for comparison. Other NHPs have spleens noted as WNL. The bodies of all NHPs have numerous petechiae to include the face, arms, legs and trunk. All NHPs euthanized during the in-life phase presented with a lean body mass and marked loss of body weight.

Histopathological assessment.

Bone Marrow (BM):
The BM sections from the femur and sternum are markedly depleted of myeloid and erythroid cells in all NHPs euthanized during the in-life phase of the study. The consistent observation is described as "severe depletion of all marrow elements". There is no evidence of any significant BM regeneration. BM cellular content is described as containing scattered "histiocytes" and macrophages. It is noted that the NHPs euthanized at the end of the study have active myeloid and erythroid cells with moderate to normal cellularity and evidence of mitotic activity. Mild "hyperplasia" is observed in various samples of both BM sites.

Lymphoid tissue.
Severe effects on all lymphoid tissue is noted. The extent of lymphopenia appears to be dose dependent as well as somewhat orga dependent within the lymph nodes (LN), spleen and thymic tissue. Splenic lymphocyte depletion is noted as "moderate" while LN depletion is noted as "diffuse". Diffuse lymphocyte depletion is also noted for the thymus which in some NHPs is noted as "non-observable" and "not present".

GI tissue.
The GI tissue of both small and large intestine for the majority of NHPs are noted to have several areas of "acute hemorrhage" in mucosal tissue. The mucosa also has a background of inflammation. In the few NHPs where the crypt and/or villus structure are mentioned; the comments note "villus blunting" and areas of epithelial hyperplasia in the crypt region. There is moderate to "acute enteritis" in addition to "necrotic" crypts in the highest dose, 890cGy cohort.

Lung.
The 800 cGy radiation series shows more pathology in lung tissue. More scattered areas of hemorrhage and mixed inflammatory sites. All hemorrhagic sites appear to be associated with bacterial infiltration and suggest bronchogenic pneumonia. There are also noted infiltration of histocytes and macrophages.

Bacteriology.
Bacterial colonization and emboli noted in organs of all nonsurvivors; those NHPs euthanized during the in-life phase. The presence of bacteria and bacterial emboli are noted in all major organs, liver, spleen, lung, kidney and intestinal tissue. There are also many bacterial emboli are associated with the scattered areas of hemorrhage and petechiae within the organs.

Conclusion: Morbidity and mortality
Death was likely due to terminal sepsis consequent to many areas of bacterial emboli and hemorrhage in major organs to include large and small intestine, lung, liver, kidneys and lymph nodes. The pattern of inflammation, hemorrhage and fibrin deposition suggested that the observed bacteria extended from the intestine to the mesenteric LNs and portal vasculature of the liver, and possibly the lung.

Special Evaluation

The liver, spleen, lung, and kidney of animals that were euthanized, expired, and necropsied animals (not all survivors were necropsied) were assayed for the presence of gram positive or gram negative bacteria at necropsy. Gram negative and/or positive bacteria were observed in at least one of the organs in 30 of the 39 animals evaluated.

Study title: A 60-Day Efficacy Study of Subcutaneous Filgrastim to Treat the Hematopoietic Syndrome of The Acute Radiation Syndrome (ARS-HS) Following an $LD_{50/60}$ of TBI in Rhesus Macaques.

Study no.:	AXG15
Conducting laboratory and location:	University of Maryland School of Medicine
Date of study initiation:	Not stated.
GLP compliance:	Yes except for determination of concentration and stability of dosing solutions.
QA statement:	Yes
Drug, lot #, and % purity:	Neupogen (manufactured by Amgen); Lot #s 072817, 082184, 097918, and 107093; standard commercially available stock with a labeled concentration of 300 µg/mL

Key Study Findings

Rhesus monkeys were exposed to 750 cGy TBI using a 6 MV linear accelerator (LINAC) radiation source on Day 0. Animals were administered via single daily s.c. injection 10 µg/kg/day filgrastim (20 M and 4 F) or vehicle (18 M and 4 F) at 20-26 hr post TBI (see the methods section below for frequency of dosing). The primary endpoint was overall survival 60 days post irradiation. Secondary endpoints included mean survival time of decedents and effect on hematology parameters. Mortality was significantly decreased in the TBI + filgrastim group (21%) compared to the TBI + vehicle group (59%). Although the mean ANC at nadir was not significantly different, the duration of days with ANC <500/µL (grade 3 neutropenia), duration of days with ANC <100/µL (grade 4 neutropenia), and days to recovery to ANC ≥ 1000/ µL were significantly improved in the treatment group compared to the control group. Interestingly, the mean survival time for decedents was much lower for the treatment group (12.0 ± 4.1 days, mean ± S.D.) than the control group (21 ± 9.6 days). Overall, the results suggested that administration of filgrastim at 20-26 hr post TBI was radioprotective in this study.

Methods

Neupogen Doses:	Neupogen (10 μg/kg/day) or vehicle first administered 20-26 hr after TBI (Day 1).
Frequency of dosing:	Neupogen (or vehicle for the control group) was administered daily until ANC ≥ 1000/μL for 3 consecutive days or ANC ≥ 10,000/μL for 2 consecutive days between Days 1-5 and anytime ANC ≥ 10,000/μL after Day 6. Daily dosing was then resumed at ANC < 500/μL and discontinued again at ANC ≥ 1000/μL for 3 consecutive days.
Route of administration:	Subcutaneous injection
Dose volume:	0.154 mL/kg
Formulation/Vehicle:	Solution/5% dextrose in water
Radiation Dose:	750 cGy TBI administered at a rate of 80 ± 3 cGy/min to 6-10 animals/irradiation day (Day 0) (The measured TBI to chest was 738 ± 15 cGy)
Radiation source:	6 MV linear accelerator (LINAC)
Radiation procedure:	Animals were acclimated to the Supine Restraint Device. Fasted animals were: 1) administered 1-2 mg/kg (i.v. or i.m.) Ondansetron 45-90 min prior to TBI; 2) the anesthesia method was not stated in this study, but in ARX01 monkeys were anesthetized with 10 ± 5 mg/kg i.m. ketamine and, if necessary, 10 ± 5 mg/kg i.m. or s.c. xylazine prior to irradiation and transported to LINAC facility; 3) allowed to recover from anesthesia and then exposed to TBI at a rate of 80 cGy/min (TBI delivered as 50% to the anterior position and 50% to the posterior position); 4) the animals were re-anesthetized for transport back to housing area; 5) administered a second dose of 1-2 mg/kg (i.v. or i.m.) Ondansetron 35 to 45 min post TBI; and 6) returned to their cage.
Species/Strain:	Monkey/Rhesus obtained from 2 different vendors
Number/Sex/Group:	N = 22 for TBI + vehicle group (18 M and 4 F) and 24 for TBI + Neupogen group (20 M and 4 F)
Age:	3-6 years old
Weight:	4.0-6.5 kg on Day 0
Satellite groups:	None
Supportive care/Euthanasia:	Listed under "Medical Management" and "Euthanasia" below (as stated verbatim in the submission).
Deviation from study protocol:	There were numerous protocol deviations. However, there was no deviation severe enough that it would have been expected to impact study results.

6.2.8. Medical Management

Medical management is provided to all NHPs as indicated by cageside and clinical observations, as per the approved IACUC protocol. Medical management measures include hydration fluids, antibiotics, analgesics, antidiarrheals, antipyretics, anti-emetics, anti-ulceratives, nutritional support and blood transfusions.

Cageside observations were performed twice daily by the veterinarians at least 6 hours apart. NHP activity, posture, stool consistency, vomit, hemorrhage, respiratory or seizure activity, and alopecia, were graded and recorded.

Clinical Observations: The NHP was anesthetized (Ketamine HCl Inj., 10mg/kg) or a combination of ketamine and Xylazine at 1mg/kg and clinical parameters such as body weight, body temperature, complete blood count (CBC) (Beckman Coulter Ac·T diff™, including a manual white blood cell (WBC) differential performed on a Wright-Giemsa-stained blood film), dehydration status, presence of mouth ulcers, and observation of blood in the stool were assessed.

Analgesics: Buprenorphine HCl (IM at 0.01mg/kg up to 0.02mg/kg, BID) was administered whenever mouth ulcers or bloody stools were observed and from study day 5 to 35. Mouth ulcers were cleansed with hydrogen peroxide or Nolvasan solution and rinsed with saline. Bupivacaine gel, a mixture of 0.1ml of 25% Bupivacaine HCl (Marcaine®, Hospira) with a dab of surgical lubricant (Surgilube®, Fougera®, Melville, NY), was applied to the area with a cotton tipped applicator.

Anti-Ulcerative: Sucralfate (Carafate®, Axcan Scandipharm Inc. & Nostrum Laboratories, Inc.) was administered (1g/day BID) from study day 5 to 30 or if bloody stool was observed.

Antidiarrheals: Following the observation of diarrhea, Loperamide Hydrochloride (Imodium®, 0.1-0.2mg/kg PO, BID) was administered. If diarrhea persisted for three (3) successive days during Imodium treatment or if watery stool without any signs of formed stool were observed, diphenoxylate hydrochloride (Lomotil®, 0.1mg/kg PO, BID for 3 days) was administered. If diarrhea persisted after three (3) days, Imodium treatment was re-established.

Antibiotics: Antibiotics were initiated when the absolute neutrophil count (ANC) was <500/µL and continued until the animal maintained an ANC >500/µL for 48 hours. The primary antibiotic was enrofloxacin (Baytril®, Bayer HealthCare LLC). Additionally, gentamicin sulfate (5 ± 1.0 mg/kg QD IM or IV) was administered in combination with Baytril when the body temperature ≥103°F and was continued for 24 hours. Ceftriaxone (Rocephin®, Roche Laboratories Inc., Nutley, NJ), or imipenem and cilastatin (Primaxin IM®, Merck & Co Inc., Whitehouse Station, NJ) was administered when microbial resistance to enrofloxacin or gentamicin was encountered.

Antipyretic: Carprofen (Rimadyl®, Pfizer Inc. 2.2mg/kg BID or 4.4mg/kg QD, IM, IV, or PO) was administered when a body temperature of ≥104°F was observed. It was continued for 48 hours after the first day the temperature was <104°F.

Nutritional Support: On all days post-irradiation animals received fresh fruit, soft food, and bottles containing diluted fruit juice or oral rehydrator (Prang™, Bio-Serv®). Animals that were observed to have weight loss ≥10% of their baseline body weight received BIO-SERV certified Rhesus Liquidiets at 15ml/kg by oral gastric gavage (OG). Volume was reduced to 7ml/kg if the animal was also receiving OG reverse osmosis (RO) water for hydration.

Blood Product Support: Whole blood, anticoagulated with 10% citrate, dextrose phosphate with adenine (CPD-A) was obtained from healthy, male NHPs, bw ≥ 7.0 kg. Blood was filtered through a 70 micron cell strainer and irradiated to 2500 cGy (Gamma Cell Elite 1000) prior to use. Transfusions of whole blood were administered at 7-14ml/kg, IV, using a 18 micron blood filter following a decrease of ≥5% in HCT resulting in a HCT ≤25% over a 24 hour time period, HCT is <20%, or there was obvious signs of uncontrolled hemorrhage.

Fluid Support: Fluid support was provided based on a grading system delineated as mild, moderate or severe dehydration.
Mild: presence of tacky mucus membranes or a skin tent time (STT) or capillary refill time (CRT) ≥2 but <3sec. Mild animals received a bolus of LRS (10-15mL/kg) by slow IV push and reverse osmosis (RO) water (10-15mL/kg) by oral gastric feeding tube (OG).
Moderate: NHPs displaying any of the mild criteria plus dry mucous, >3% increase in hematocrit (HCT) from the day before (not transfusion related), sunken eyes, or STT or CRT ≥3sec. Moderate animals received a bolus of LRS (20-30mL/kg) over 15-20 min. by slow push and RO water (7-10 mL/kg by OG).
Severe: NHPs displaying any of the mild and or moderate criteria plus had pale mucous membranes, >5% increase in HCT from the day before (not transfusion related), a rapid and weak pulse, cold extremities, lethargy, or rapid breathing. Severely dehydrated animals received fluids as described for moderate dehydration with the addition of a slow IV infusion (15±5 mL/kg/h) administered over a period of 2-4 hours. Animals may have been placed in a restraint device at this time and allowed to awaken. Midazolam HCl (0.2mg/kg) may have been administered to calm the NHPs while in the restraint.

6.2.9. Euthanasia

A specific set of criteria for euthanasia was applied by all veterinarians. Any NHP which was recumbent in the cage or had decreased or absent responsiveness to touch or experienced hemorrhage from the GI tract to be in excess of 20% of the estimated blood volume in any 24 hour period or they experienced unrelieved pain was euthanized. Any NHP which experienced any combination of the following observations such as respiratory distress, decreased food and water intake, reluctance to move for >24 hours, and severe dehydration classified an animal to be euthanized. Animals were euthanized by veterinarians using DEA Class III euthanasia solution (Euthanasia III®, [Med-Pharmex] 0.27ml/kg IV). Expiration was confirmed by a lack of heart beat, absent femoral artery pulse, and lack of chest respiration.

Observations and Results

Mortality

Mortality was observed in 59% (13/22) of the TBI + vehicle group compared to 21% of (5/24) of TBI + Neupogen group ($p<0.004$ using a chi square test of a one-tailed null hypothesis). Interestingly, the mean survival time for decedents was much lower for the treatment group (12.0 ± 4.1 days, mean ± S.D.) than the control group (21 ± 9.6 days).

Clinical Signs

Cage-side observations were performed twice daily. More detailed clinical evaluation was performed once daily on Days 0-25, 28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60 and termination. Clinical evaluation included recording body weight, core body temperature, capillary refill time, skin tent time, petechia, eccymosis, and swelling. The Sponsor did not provide a tabulated summary of clinical sign incidence/severity, just written statements with the most notable findings being: 1) the number of observations for limited activity or hunched posture was twice as much in the treatment group compared to the control group and multiple episodes of vomiting were observed more frequently in the treatment group than the control group (i.e. possible drug-related negative effects); and 2) there were only 3 observations of watery stool in the treatment group compared to 26 observations in the control group and hemorrhage on Days 15-30 was seen in a higher percentage of control animals than treatment animals (i.e. possible drug-related beneficial effect).

Body Weights

Body weights were recorded on Days 0-25, 28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60 and termination. The only tabulated summary data provided was a Table showing the occurrences of 10% or more (71% for the treatment group compared to 59% for the control group) and 25% or more (0% for both groups) body weight loss compare to the pre-TBI value.

Feed Consumption

Not evaluated.

Hematology

Blood samples for hematology analyzes were collect prior to Day 0 and on Days 0-25, 28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60 and termination. Absolute neutrophil count (ANC) results are summarized in Table 9 and Figure 5 below (provided by the Sponsor). Although the mean ANC at nadir was not significantly different, the duration of days with ANC <500/μL (grade 3 neutropenia), duration of days with ANC <100/μL (grade 4 neutropenia), and days to recovery to ANC ≥ 1000/ μL were significantly improved in the treatment group compared to the control group.

Table 9.

Neutrophil-related parameters for rhesus macaques following exposure to 750 cGy total-body irradiation by 2 MV (average) photons and administration of Test Article (Neupogen®) or Control Article (5% Dextrose in Water)

Tx		First day and range ANC < 500/μL or 100/μL		Duration (days and range) ANC < 500/μL or 100/μL		Recovery to ANC ≥ /1000μL	ANC Nadir (/μL)
		< 500/μL	< 100/μL	< 500/μL	< 100/μL	≥ 1000/μL	
Test Article	Mean ±SEM	4.3±0.1	6.5±0.3	14.3±0.5	10.4±0.6	19.7±0.6	5±2
	P value	Not done	Not done	<0.0001	0.009	<0.0001	0.115
	Median	NA	NA	14.0	10.0	20	0
	Range	SD3-5	SD4-9	9-19 days	4-14 days	15-25 days	NA
Control Article	Mean ±SEM	4.9±0.2	7.1±0.3	18.6±0.8	12.3±0.6	25.8±0.9	1±1
	Median	NA	NA	18.0	12.0	24.5	0
	Range	SD3-6	SD5-10	14-22 days	8-15 days	22-32 days	NA

Table 9. The mean, standard error of the mean (SEM), median and range (where applicable) are reported for each radiation cohort. The day of the occurrence of an absolute neutrophil count (ANC) below 500/μL or 100/μL following exposure to 750 cGy TBI is shown. The duration of neutropenia is defined as an ANC below either 500/μL or 100/μL. The durations do not include data from decedent animals unless recovery occurred to that level, e.g., ANC ≥ 500/μL prior to death. The duration of neutropenia was estimated as the number of days that a subject has an observed or an imputed ANC below 500/μL. Any single observed ANC that was ≥ 500 /μL and was immediately preceded and followed by ANC < 500/μL was counted as a day of severe neutropenia. The time to recovery was estimated as the number of days from SD1 until the first 2 consecutive observed or imputed ANC after the nadir was ≥ 1,000/μL. The ANC nadir was the first lowest observed or imputed ANC that occurred at least 2 days after irradiation.

Figure 5.

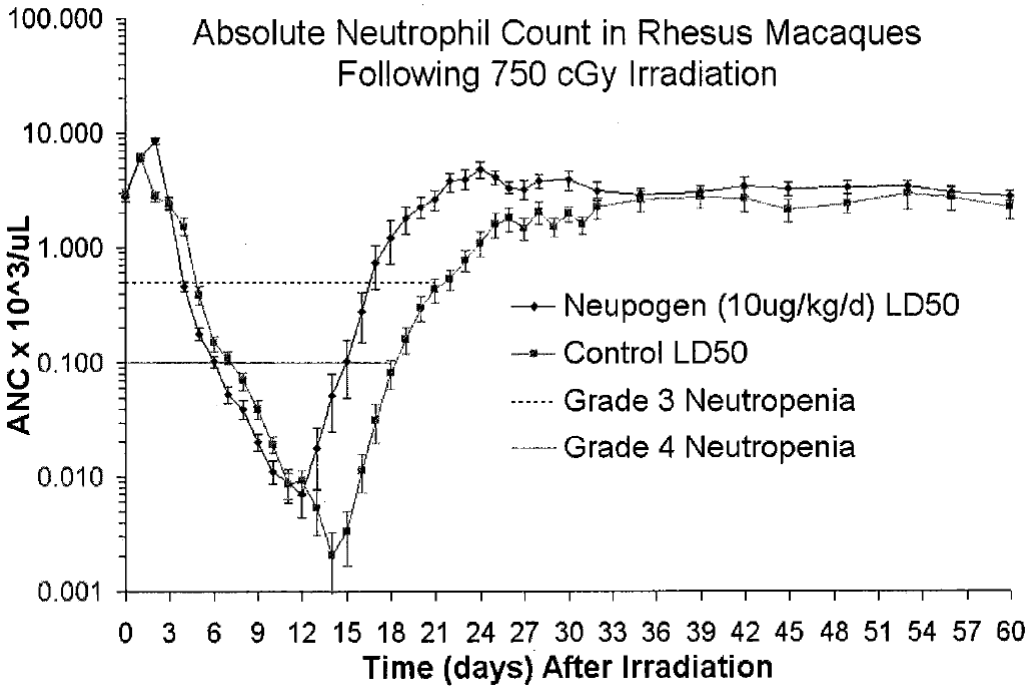


Figure 5. Mean (±SEM) neutrophil count x 10^3/µL in the peripheral blood of rhesus macaques administered Neupogen® (n=24) or 5% Dextrose in Water (n=22) as a function of time post TBI. The blood sample from 1 Neupogen®-treated animal was not analyzed on SD 7. TBI at 750 cGy, of 2 MV average energy LINAC-derived photons was administered at 80 cGy/min. The radiation dose shown approximates the $LD_{50/60}$.

Platelet (PLT) results are summarized in Table 10 and Figure 6 below (provided by the Sponsor). There was a near-significant improvement in duration of days with PLT <20,000/µL and days to recovery to PLT ≥ 20,000/ µL. The control animals received on average 2.4 ± 0.3 blood transfusions during the PLT <20,000/µL period compared to 1.8 ± 0.3 blood transfusions for treatment animals (transfusions required special PLT count rules to be applied). Overall, the results are suggestive of improved PLT counts in the treatment group compared to control group; however, the PLT data was probably affected by the blood transfusions. Therefore, it is difficult to determine the exact drug-related effect on PLT. There were no other drug-related changes in hematology parameters observed in this study.

Table 10.

Platelet-related parameters for rhesus macaques following exposure to 750 cGy total-body irradiation by 2 MV (average) photons and administration of Test Article (Neupogen®) or Control Article (5% Dextrose in Water)

Article		First day platelet count < 20,000/μL	Duration (days)* < 20,000/μL	Nadir‡ (PLT/μL)	Recovery to platelet count ≥ 20,000/μL	No. Trans-fusions‡ (54mL)	First day transfusion occurred within radiation cohort
TA	Mean ± SEM	9.3 ±0.2	12.6 ±1.4‡	1 ±0	22.0 ±1.4‡	1.8 ±0.3	10.8 ±0.9
	P value	Not done	0.077	0.134	0.062	Not done	Not done
	Median	9.0	10	1	21	1.3	12
	Range	SD8-11	SD6-33	0-6	SD15-42	0-5	SD10-18
CA	Mean ± SEM	9.7 ±0.2	17.1 ±2.1†	1 ±0	26.9 ±2.2†	2.4 ±0.3	11.8 ±0.6
	Median	10	14	0	24	2.0	12
	Range	SD8-11	SD11-33	0-4	SD20-44	0-6.5	SD10-14

Table 10. The mean, standard error of the mean (SEM) and range of the first day thrombocytopenia, defined as a platelet (PLT) count < 20,000/μL, is observed in an animal in each radiation cohort, duration of thrombocytopenia, platelet nadir, day recovery from thrombocytopenia occurs, number of whole blood transfusions administered (54mL) and first day a transfusion is observed in an animal in each radiation cohort is displayed. The standard error of the mean (SEM) is also shown. *Note that durations do not include data from decedent animals unless recovery occurred to that level, e.g., PLT ≥ 20,000/μL prior to death. ‡ The platelet nadir and number of transfusions includes both survivors and nonsurvivors.
‡n=19, † n=11

Figure 6.

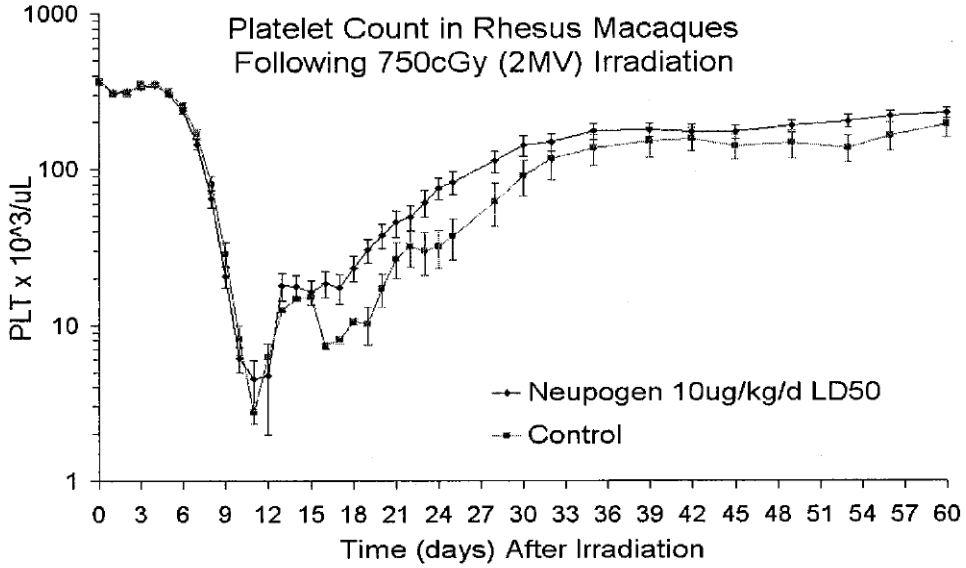


Figure 6. Mean (±SEM) platelet count x 10^3/µL in the peripheral blood of rhesus macaques administered Neupogen® (n=24) or 5% Dextrose in Water (n=22) as a function of time post TBI. The blood sample from 1 Neupogen®-treated animal was not analyzed on SD 7. TBI at 750 cGy, of 2 MV average energy LINAC-derived photons was administered at 80 cGy/min. The radiation dose shown approximates the $LD_{50/60}$. Note the effect of platelet transfusions has elevated both the platelet nadir and recovery displayed.

Clinical Chemistry

Clinical chemistry analyzes were not performed.

Gross Pathology and Histopathology

Full gross necropsies were performed on animals at termination. The tissues/organs collected and preserved in 10% neutral-buffered formalin are listed below.

TISSUE	NUMBER OF SAMPLES
1. Heart	2 (from 2 different areas)
2. Lung	3 (from 3 different areas)
3. Liver	3 (from 3 different areas)
4. Spleen	2 (from 2 different areas)
5. Kidney (left and right)	4 (2 from different areas of each kidney)
6. Large Intestine	3 (from 3 different areas)
7. Small Intestine	3 (from 3 different areas)
8. Thymus	2 (from 2 different areas)
9. Mesenteric lymph nodes	3 (from 3 different areas)
10. Skin	1 sample
11. Sternum[A]	1 (2 cm transverse section)
12. Femur[A]	1 (2-3 cm cross section)
13. Any Lesions or abnormalities	1 or 2 samples from a representative area

[A] Decalcified prior to section

Adequate Battery

No. The tissue collection list is minimal and appears to target only the primary tissues affected by radiation exposure.

Peer Review

Yes

Findings

Hemorrhaging was observed in 33% of treated animals compared to 80% of controls at necropsy with the incidence and severity of bleeding much less (when present) in treated compared to control animals. Ecchymosis was observed in 8% of treated animals compared to 32% of control animals. Abnormal heart (mild flaccid and/or displaying ventricular dilation/hypertrophy) was also observed in 8% of treated animals compared to 32% of control animals. Enlarged lymph nodes were observed in 13% of treated animals over 50% of control animals. These increased incidences in macroscopic findings did not correlated with any specific microscopic findings.

In general, there were no apparent differences in severity of microscopic findings for treatment group compared to control group. Incidence of microscopic findings are summarized in Table 22 below (provided by the Sponsor). The incidence of microscopic findings tended to be lower in the treatment group compared to control group in all tissues/organs except small and large intestine. The study report noted that the incidence of depletion of erythroid and myeloid components of the bone marrow and depletion of lymphocytes in the thymus were more than 50% less in the treatment group compared to control. Depletion of lymphocytes in the spleen was 40% less in the treatment group compared to control. The above lesions were the only lesions noted in bone marrow, thymus and spleen. The difference in liver microscopic findings was due to 6 incidences of bacteria emboli noted in controls compared to 2 such incidences in the treatment group. Similar bacteria emboli results were observed in lung (7 incidences in control compared to 2 incidences in treated) and kidney (8 incidences in control compared to 2 incidences in treated), but there were a number of other microscopic finding identified in these organs.

Table 22.

Percent of Animals With Microscopic Lesions

Organ Group	Liver	Heart	Lung	Thymus	Spleen	MLN	Skin	Kidney	Small Intestine	Large Intestine	Bone Marrow
Control	27	41	50	55	45	77	27	50	86	32	50
Neupogen®	8	21	29	21	25	63	17	29	91*	54	21
* n=23, unable to evaluate slides on 1 NHP due to the poor quality of tissue processing.											

Special Evaluation

Aerobic and anaerobic blood cultures were collected to evaluate for the presence of bacteremia/fungemia when febrile neutropenia (FN; when ANC less than 500/μL and body temperature greater than 103°F). Necropsy samples from kidney, lung, spleen, and liver were collected for quantitative microbial analysis. Blood culture results are summarized in Table 12 below (provided by the Sponsor). Overall, the percent of animals having at least one bacteria-positive blood culture was slightly decreased for the treatment group (19/24 or 79%) compared to the control group (20/22 or 91%). The Sponsor stated that tissues samples were negative for microbial analysis in 58% of treated animals compared to 41% of control animals. The Sponsor also stated that this data supports that treatment group animals had fewer systemic infections than control group animals.

Table 12.

Summary of Blood Culture Results in rhesus macaques following exposure to 750 cGy total-body irradiation by 2 MV (average) photons and administration of Test Article (Neupogen®) or Control Article (5% Dextrose in Water)

	Peripheral Blood Cultures Performed		Peripheral Blood Cultures Negative for Bacteria		Number of NHPs Having at Least One Bacteria-Positive Blood Culture		Cultures positive for Gram - Bacteria		Cultures positive for Gram + Bacteria		Cultures positive for Gram – and Gram + Bacteria	
	No.	**%**	**No./ total**	**%**	**No./ total**	**%**	**No./ total**	**%**	**No./ total**	**%**	**No./ total**	**%**
Total	131		68/131	52	33/46	72	7/63	11	59/63	94	3/63	5
Article												
Test n=24	60/131	46	36/60	60	14/24	58	1/7	14	23/63	37	3/3	100
Control n=22	71/131	54	32/71	45	19/22	86	6/7	86	36/63	57	0/3	0

Duration of FN, days on antibiotic, and days body temperature ≥ 103°F were all decreased (although not significantly) in the treatment group compared to controls.

Toxicokinetics

Pharmacokinetics/Toxicokinetics were not performed. Blood samples were collected on Day 60 (or earlier for moribund or expired animals) and the serum analyzed using an ELISA assay for the presence of anti-G-CSF antibodies. ADAs were detected in one treated and one control animal.

Dosing Solution Analysis

Drug concentration was determined using LC-MS/MS (GLP). G-CSF activity was also determined by measuring G-CSF dependent proliferation of M-NFS-60 cells (GLP). Analysis was performed on retention samples from dosing solutions prepared on the day

of injection and collected before the first animal was dosed and after the last animal was dosed. Dosing was performed from October 2007 thru April 2008. Dosing solution analysis was not performed until January 2010 thru April 2010. As a result, many of the dosing solution samples (36 of 48) had expired by the time of LC-MS/MS analysis. The expired samples exhibited chromatographic peaks that were 5-6 times lower that non-expired samples. All dosing solution samples had expired by the time of G-CSF activity analysis. The relative potency (activity on a µg/mL basis) for retention samples range from 0.92 to 2.85 relative to the 1.00 activity assigned to the rhG-CSF standard. Overall, the dosing solutions were not considered homogenous, likely due to so much of the analyzes was performed after the expiration date. On one hand, Neupogen is an approved drug and the drug used in this study was likely homogenous. On the other hand, in a GLP study such as this one, from a nonclinical standpoint the Sponsor should be able to demonstrate that dosing solutions contained the targeted amount of drug, and if not what was the actual amount of drug administered. That information cannot be reliably obtained in this study due to analyzes being performed after expiration dates.

OVERALL CONCLUSIONS

Filgrastim significantly increased the 60-day survival rate in NHP exposed to 750 cGy TBI compared to the animals administered vehicle alone in study AXG15.

RECOMMENDATIONS

None.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RONALD HONCHEL
11/09/2012

ADEBAYO A LANIYONU
11/10/2012

Medical Officer Review of Nonhuman Primate Study

preIND:	100228, submission of July 22, 2010
Product:	Filgrastim (Neupogen)
Sponsor:	National Institute of Allergy and Infectious Diseases
Reviewer:	Dwaine Rieves/Director/DMIP
Today:	October 31, 2012

1. Reviewer's summary observations: *The sponsor supplied the results of a non-human primate (NHP) study that was terminated early due to statistical success upon a primary endpoint that showed filgrastim (g-CSF, 10/mcg/kg daily per leukocyte count results) therapy almost doubled the survival rate in comparison to placebo following exposure of animals to approximately 7.5 Gy radiation. The primary endpoint results are consistent with other results from the study that generally indicate g-CSF group animals experienced fewer infections and less overall morbidity compared to control group animals as evidenced by:*

-most categories of "cage-side observations" such as posture, activity, stool consistency, vomiting, etc. showed less intense severity scores in the g-CSF group than in the control group;

-the occurrence of febrile neutropenia (while not statistically significant between the two groups) trended lower in the g-CSF group (79%) than in the control group (91%) even though the study was not designed to demonstrate a difference in febrile neutropenia rates;

-the rate of g-CSF group animals with positive blood cultures (58%) similarly trended lower than that for control group animals (86%);

-the rate of g-CSF group animals with positive blood and positive organ cultures at autopsy (17%) also trended lower than that for control group animals (32%); similarly, g-CSF group animals tended to have a higher incidence of completely negative blood/organ cultures at autopsy (58% versus 41%).

Overall, deaths occurred in 13 of 22 control group animals and 5 of 24 g-CSF group animals. All but three deaths were related to euthanasia; three control group animals were "found dead." Pre-specified criteria were used by study staff in order to determine when an animal should be euthanized. I can find no signals to suggest that investigators biased the study results in terms of misappropriation of euthanasia. For example, the maximum temperature and weight loss of the decedent animals was largely the same between the study groups.

Blood counts (leukocytes, platelets, hemoglobin) were closely monitored throughout the study and all animals experienced severe neutropenia (absolute neutrophil count < 500/mcL) after irradiation. The g-CSF group had acceleration of neutrophil recovery compared to the control group; in general, g-CSF therapy decreased the period of severe neutropenia by four days. This observation is generally similar to that observed in

clinical studies of leukocyte growth factors where the duration of neutropenia in the clinical studies appeared to vary with the intensity of chemotherapy (for example, in the study of sargramostim following induction chemotherapy for leukemia, the growth factor accelerated the neutrophil recovery by 4 days; however, the clinical studies among patients receiving chemotherapy for lung and breast cancer generally show acceleration of neutrophil recovery by two or three days).

Overall, the study results are in alignment with clinical data that have demonstrated favorable treatment effects for leukocyte growth factors in the setting of chemotherapy-induced neutropenia; this animal study supports a therapeutic role of g-CSF (and perhaps other leukocyte growth factors) as a mitigant of hematologic injury following myelosuppressive radiation.

The major study limitations pertain to the following:

-caretakers appear to have been aware of leukocyte count results since study drug dosing was determined by absolute neutrophil count results; this knowledge may have resulted in some bias in animal care that is not captured in the study documents;

-the study did not obtain g-CSF pharmacokinetic data; this deficiency somewhat complicates inference of dosing from the non-human primates to humans. However, the confirmatory clinical studies for g-CSF (chemotherapy-induced neutropenia) used doses that ranged between 4 and 8 mcg/kg (although some supportive studies used higher doses) and clinical studies of leukocyte growth factors have consistently shown decreased risk for febrile neutropenia (serious infection) in association with the use of factor doses that accelerated neutrophil recovery after chemotherapy, effects shown across a range of doses and different drugs. The similarity of this accelerated neutrophil recovery pharmacodynamic outcome between the animals in this study and the human experience suggests that the filgrastim dose used in confirmatory clinical studies is likely reasonable also for use in the radiological/nuclear incident setting.

-the supportive care of the monkeys included the use of Baytril (an antibiotic) which was administered once animals experienced an absolute neutrophil count of < 500/mcL, even without fever; this practice is not consistent with the care of humans. Nevertheless, the therapy was applied uniformly to both study groups so it likely made little if any difference in the study's primary endpoint outcome. Other aspects of supportive care were intended to mimic the clinical situation (such as the use of blood transfusion, intravenous hydration); however, the extent to which these supportive care measures may have altered animal responses is unclear since, to my researching, standard "supportive care" of irradiated monkeys has not been defined in veterinary practice. For example, blood transfusions may have actually decreased survival since the animals were

not cross-matched. Transfusions were given to 42 of the 46 animals; only one control group animal and three g-CSF group animals did not receive transfusions. Nevertheless, the randomization procedures likely controlled for any adverse effects of supportive care upon survival.

-the chromatography/spectroscopy studies used to analyze the g-CSF retention samples were performed for some samples after the date of the manufacturer's expiry and the results showed degradation of the g-CSF. However, expired drug was not administered to animals and, because the drug was clinical grade, the chromatography/spectroscopy findings likely have little relevance to the study.

-the immunoassay for antibody formation did not include a positive control sample and the determination of a positive appears to relate to the assay kit manufacturer's recommendation (which is directed to samples of human blood). Considering the robust pharmacodynamic outcomes (accelerated neutrophil recovery) it appears highly unlikely that antibody formation occurred in a manner that might neutralize g-CSF activity.

The only outstanding issues relate to a request for datasets for our statisticians to duplicate the primary endpoint and neutrophil results and a few clarifying requests (below). An inspection of the facility is pending and these findings are important to further help assess whether or not uncontrolled bias entered the survival results.

We will request clarification of the following:

a. *Provide a copy of SOP AP405, "NHP Euthanasia Criteria."*

b. *The study report appears to indicate that animal caretakers were aware of blood leukocyte results. Please confirm.*

c. *One of the FDA-NIAID meeting minutes records appears to indicate that NIAID may have previously obtained pharmacokinetic data from non-human primates that received filgrastim. Please clarify and supply a copy of the study report(s), if available. Please provide any additional information that helps support the contention that the dose of filgrastim used in the animal study (10 mcg/kg/day) equates to a human dose of 5 mcg/kg/day.*

2. Background: On July 22, 2010, the sponsor submitted a complete study report for two studies:

1) Study AXR01 titled, "A Pilot Study to Define the Dose Response Curve in Rhesus Macaques Exposed to Increasing Doses of Total Body Ionizing Radiation and Receiving Supportive Care."

2) Study AXG15 titled, "A Sixty-Day Efficacy Study of Subcutaneous Filgrastim (Neupogen) 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."

Reviewer's comment:

This study report was submitted by the NIAID to preIND 100228. Overall, the most relevant INDs for hematologic-related acute radiation syndrome (ARS) are:

IND 100228 sponsored by NIAID
IND 011510 sponsored by CDC, an IND for treatment use of filgrastim
IND 012704 from the CDC for a preEUA

IND 125031 sponsored by Amgen for pegylated filgrastim (no ARS proposals)
IND 007110 sponsored by Amgen for pegylated filgrastim (original IND)
IND 002482 sponsored by Amgen for filgrastim (original IND)
IND 116259 sponsored by Genzyme for Sargramostim
BLA 125294 sponsored by Sicor Biotech for tbo-filgrastim/

The sponsor identifies Study AXG15 as a GLP-compliant study. I am reviewing this study from a clinical perspective because the study report was performed in order to help support a finding of safety and efficacy under the "Animal Rule" and several aspects of the animal study were intended to model the clinical setting. I will not focus upon details of GLP compliance; the pharm-toxicology experts are also currently reviewing this study for these items. My goal is to place the study design and results in somewhat of a clinical perspective, building upon the experience with the clinical data that supported filgrastim licensure. This review is confined to Study AXG15 since study AXR01 was an exploratory study performed to determine the proper radiation dose for study AXG15.

2. Overview: The supplied study report identifies the study initiation date as October 8, 2007 and the study completion date as September 21, 2010. The study was conducted at the University of Maryland School of Medicine with all microbiology/laboratory assessments also performed at the University. Statistical expertise was provided by Dr. Barry Katz from Indiana University School of Medicine.

In the study, 46 nonhuman primates (NHP, Rhesus Macaques) received 750 cGy total body irradiation from a linear accelerator. The animals were randomized to either daily placebo injections or Neupogen (g-CSF) 10 mcg/kg subcutaneously and subsequently cared for using double-blinded design procedures. Supportive care was implemented with various evaluations over a 60 day follow-up period. The primary endpoint, a comparison of the number of surviving NHP at day 60, showed 79% (19/24) of g-CSF group survivors versus 41% (9/22) control group survivors ($p < 0.004$). Febrile neutropenia was experienced by 91% (20/22) controls and 79% (19/24) of g-CSF-treated animals ($p = 0.418$). The median survival time of the decedents in the g-CSF cohort was 12 days versus 21 days in the controls. Of the 18 animals that died, 3 (all control) were "found dead" and all others were euthanized.

Reviewer's comment: From the top level results, one sees that, overall, 18 animals died (5 in g-CSF group—21% and 13 in the control group—59%). The mortality rate (59%) in the control group suggests that the chosen radiation dose was consistent with the targeted lethal radiation dose (LD 50/60). This 60 day mortality rate is far higher than the mortality rate one would expect for patients entering clinical trials of leukocyte growth factors (LGFs) following chemotherapy for cancer and suggests that the systemic injury from the administered radiation far exceeded the injury patients typically experience during cancer chemotherapy.

Since the study only examined 60 day survival and animals were euthanized at the end of 60 days, it was not designed to determine whether or not filgrastim provided an overall-survival advantage for an extended period of time.

The survival treatment effect is not solidly paralleling the febrile neutropenia effect in that a relatively striking survival treatment effect was reported while the difference in febrile neutropenia did not achieve statistical success (although the numerical rate difference favored g-CSF). This observation suggests that, in the experimental setting of radiation injury to monkeys, febrile neutropenia may not be a solid surrogate for a filgrastim treatment effect. The nature of the supportive care in the monkey study may have also impacted the febrile neutropenia outcome—for example, monkeys received prophylactic antibiotics once severe neutropenia appeared (ANC < 500/mm3). In clinical studies, prophylactic antibiotics were not administered once severe neutropenia appeared.

In clinical studies of cancer chemotherapy, leukocyte growth factors have been shown to decrease the incidence of febrile neutropenia but have not shown overall survival advantage effects (the studies were not powered to assess survival effects since follow-up was not over a prolonged period of time and survival among patients is probably determined by the response to the chemotherapy and the cancer progression—rather than bone marrow suppression induced by chemotherapy).

3. Study AXG15 Protocol Review:

Dates: The final study protocol was signed by Ms. Ann Farese (Study Director) on October 9, 2007.

Primary Objective: "to determine whether the test article, Neupogen (filgrastim), administered at 10 mcg/kg/day SC starting on day 1 (20-24 hours) following an LD 50/60 exposure to 6 MV Linear Accelerator (LINAC) photon irradiation and administered to effect based on absolute neutrophil count (ANC), will significantly improve overall survival 60 days after radiation exposure of rhesus macaques receiving medical management [IV fluids, blood products, nutrition and antibiotics] compared to animals receiving Control Article and the same medical management."

Secondary Objectives: The protocol notes that, "this study was not designed to have sufficient power to detect differences in secondary endpoints. However, a comparison of the secondary endpoints will be performed between the treatment groups (test article versus control). Secondary objectives include studying indices of hematopoietic recovery, mean survival time, incidence of febrile neutropenia and infection, number of whole blood transfusions, and incidence and severity of diarrhea in the rhesus macaque model of ARS-HS. In addition, filgrastim immunogenicity, body weights and significant organ pathology will also be examined."

Interim analysis: The protocol notes that, "This study may be terminated early when the cohort associated with at least 50% of the monkeys are 60 days past irradiation and statistical analysis shows futility for efficacy."

Test article: Neupogen (g-CSF) or placebo (5% dextrose in water).

Randomization: Animals were to be stratified by gender and then assigned to treatment group using computer generated random numbers by the study statistician. The protocol example cites 62 animals/31 in each group; Rhesus macaques approximately 3 to 6 years of age and weighing 4 to 6.5 kg. The study planned to have 31 animals in each study group.

Irradiation: a 6 MV LINAC photon source "will be used to irradiate all animals" to a midline tissue dose of 750 cGy/min. "The radiation physicist will be blinded to treatment type."

Dosing: "Personnel performing test article administration are to be blinded to treatment type." Injections were to begin on study day 1 and continue daily until the ANC ≥ 1,000/mcL for 3 consecutive days or if at any time the ANC is ≥ 10,000/mcL. "At any point following discontinuation of dosing, if the ANC is < 500/mcL, daily injections will be re-initiated and continued until the ANC is ≥ 1,000/mcL for 3 consecutive days. The Neupogen (g-CSF) dose was to be 10 mcg/kg/day.

Reviewer's comment: The study personnel were not blinded to hematological test results since dosing was contingent upon these results. Additionally, a protocol amendment changed the dosing paradigm to:

-begining dosing on day 1
-continue daily SC dosing until:
- the ANC was ≥ 1,000 for 3 consecutive days, or
-the ANC was ≥ 10,000/mcL for 3 consecutive days within SD 1-5, or
-anytime the ANC ≥ 10,000/mcL beginning on SD 6.

Having access to hematologic results conceivably could have resulted in some unblinding however, the observed difference in the hematologic recovery was only a few days which, although statistically and physiologically significant, seems unlikely to have culminated in unblinding bias in the care of the animals. The animals were irradiated on different days. Specifically, irradiation was provided among six groups where the dates were

generally separated by one to two months. The study report indicates that all animals were housed in individual cages with controlled care conditions.

Observations: "Veterinarians will perform cage-side observations in accordance with SOP AP406, twice daily. Personnel performing cage-side observations are to be blinded to treatment type and antibiotic treatment." Cage-side observations were to consist of:

-activity
-posture
-stool consistency
-evidence of vomit
-hemorrhage
-respiratory activity
-seizure activity
-presence of food in hopper

Clinical observations were to be performed daily through day 25, then at least on study days 28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60 and at termination. Parameters to be evaluated were:

-weight
-core body temperature
-capillary refill time
-skin tent time
-Petechia
-ecchymosis
-swelling

Supportive care/medical management was to be performed daily ("as needed") for the following reasons:

-dehydration
-pain
-elevated body temperature
-ulcers
-diarrhea
-emesis
-weight loss, depressed appetite
-anemia and thrombocytopenia
-positive blood cultures

SOPs described the use of antibiotics, blood transfusion, intravenous fluids and other support.

Blood/laboratory evaluations consisted of CBCs (including differential and platelet counts) obtained during quarantine at pre-specified times, then daily from day 0 (pre-irradiation) through day 25. After day 25, CBCs were obtained at days 28, 30, 32, 35, 39, 42, 45, 49, 53, 56 and 60 (or if moribund euthanasia or animal is found dead). Whenever the study day ANC was ≥ 1,000/mcL, samples were to be collected daily following that

particular study day until the count was shown to be maintained at that level for 3 consecutive days.

Blood culture was performed on day 60. Blood culture was also performed any study day when febrile neutropenia (ANC < 500/mcL and body temperature ≥ 103° F) was observed or any day in which body temperature was ≥ 105 degrees F. An additional blood specimen was to be collected 24 hours after the first blood culture if febrile neutropenia persisted and the preliminary bacteriology report of the first culture was either negative or a gram positive organism was identified. An additional blood culture was to be obtained if febrile neutropenia persisted for five consecutive days after a previous blood culture collection. Blood was to be cultured for both aerobic and anaerobic organism using BACTEC culture vials.

Filgrastim immunogenicity was performed pre-irradiation then on day 60 (or earlier if moribund euthanasia).

Termination: "Only a veterinarian blinded to treatment type may authorize an unscheduled euthanasia, using the criteria specified in SOP AP405, NHP Euthanasia Criteria." Necropsies were to be performed on all animals euthanized or that died during the study. Moribund animals were to be sedated by ketamine and euthanized by an overdose of barbiturate.

Animals surviving to day 60 were to be euthanized and necropsied. Heart, lung, liver and spleen tissues were to be cultured for microbes.

Statistical tests:

Interim analysis: A single interim analysis was described; this was to be performed after the cohort associated with at least 50% of the monkeys are 60 days post irradiation. Formal efficacy analyses were to be based on the Lan-Demets version of the O'Brien-Fleming boundary to provide an overall P = 0.05 test. "Futility will be assessed informally based on conditional power. For example, termination due to futility may be considered if conditional power is very low (e.g., less than 0.10), under the assumption that the hypothesized treatment difference is correct." The interim analysis was to be performed on unblinded data.

Primary endpoint: "Overall survival measured at 60 days post randomization is the primary endpoint." Furthermore, "the primary analysis will be conducted on the ITT population using a chi square test of a one-tailed null hypothesis using a 5% significance level."

Summary descriptive statistics were to be performed for the secondary endpoints of:

-survival time
-ANC nadir (lowest ANC any time after irradiation)
-duration of neutropenia (ANC < 500/mcL and ANC < 100/mcL)
-time to recovery to ANC ≥ 500/mcL, 1,000/mcL

-platelet nadir
-time to recover to platelet count ≥ 20,000/mcL
-number of days of fever (temperature ≥ 103 degrees F)
-incidence of infection (number of animals with any documented infection/positive blood cultures or tissue or evidence of sepsis at necropsy)
-incidence of febrile neutropenia

The analytical plan included comparison between groups using Fisher's exact or chi-square tests as well as comparisons of survival time using the Kaplan-Meier product limit method.

"Analyses of laboratory values over time will attempt to account for the missing data and in those cases where it is not addressed, the analyses will be interpreted cautiously Trajectories of the laboratory values by treatment type will be examined graphically and a variety of statistical models will be considered based on the distribution, pattern and missing data configuration of the observed data."

Amendments:

A number of protocol amendments were performed (most during 2007). Among the notable amendments:

-dosing was changed to state that dosing would continue "until the ANC ≥ 1,000/mcL for 3 consecutive days or **if at any time the ANC is ≥ 10,000/mcL for more than 2 consecutive days within study days 1 through 5 or if at any time the ANC is ≥ 10,000/mcL beginning on study day 6.**" This was added on January 23, 2008.

-the interim analysis was clarified, "An interim analysis for efficacy or futility to determine **if the study may be terminated prior to completion** will be conducted once." Furthermore, "AXG15 is terminated. The criteria of the interim analysis have been met. The efficacy of Neupogen in accordance with the Lan-Demets version of the O'Brien-Fleming boundary to provide an overall one-sided P = 0.05 test was demonstrated." These two items were added on June 16, 2008 and July 18, 2008, respectively.

4. Study Report Summary:

The following aspects of the study conduct were summarized within the study report.

BACKGROUND:

Study drug: filgrastim was supplied from Besse Medical, a supplier that shipped the product directly to the site. Liquid chromatography/mass spectroscopy and a bioassay was used to verify activity/stability of the filgrastim.

Supportive care: Cageside observations were performed twice daily by the veterinarians at least six hours apart. Supportive procedures consisted of:

- buprenorphine (IM at 0.01 mg/kg up to 0.02 mg/kg, BID) for analgesia whenever mouth ulcers or bloody stools were observed and from study day 5 to 35.

- sucralfate administered at 1 g/day BID from study day 5 to 30 or if bloody stool was observed.

- loperamide (Imodium 0.1 to 0.2 mg/kg PO BID) was administered if diarrhea observed. If diarrhea persisted for three successive days during Imodium therapy or if watery stool without any signs of formed stool were observed, diphenoxylate (Lomotil, 0.1 mg/kg PO, BID for 3 days) was administered. If diarrhea persisted after 3 days, Imodium treatment was restarted.

- antibiotics were initiated when the ANC was < 500/mcL and continued until the animal maintained an ANC > 500/mcL for 48 hours. The primary antibiotic was enrofloxacin (Baytril, Bayer). Additionally, gentamicin (approximately 5 mg/kg daily, IM or IV) was administered in combination with enrofloxacin when the body temperature ≥ 103° F and was continued for 24 hours. Ceftriaxone (Rocephrin, Roche) or imipenem and cilastatin (Primaxin IM, Merck) was administered when microbial resistance to enrofloxacin or gentamicin was encountered.

- carprofen (Rimadyl, Pfizer, 2.2 mg/kg BID or 4.4 mg/kg QD, IM, IV or PO) was administered when a body temperature of ≥ 104° F was observed. It was continued for 48 hours after the first day the temperature was < 104° F.

- Animals that had a weight loss ≥ 10% of their baseline body weight received BIO-SERV certified Rhesus Liquidiets at 15 mL/kg by oral gastric gavage.

- whole blood was obtained from healthy non-human primates (NHP) and irradiated. Transfusions were administered at 7 to 14 mL/kg IV following a decrease of ≥ 5% in hematocrit resulting in a hematocrit ≤ 25% over a 24 hour time period or hematocrit < 20% or signs of uncontrolled hemorrhage.

- fluid support (lactated Ringer's Solution, LRS) was provided based on a grading system of mild, moderate or severe dehydration.
  - -mild…bolus of LRS (10 to 15 mL/kg) by slow IV push plus water (10 to 15 mL/kg) by feeding tube
  - -moderate…bolus of LRS (20 to 30 mL/kg) and water (7 to 10 mL/kg) by feeding tube
  - -severe…same as moderate plus a slow IV infusion (15 mL/kg/hour) over two to four hours.

Euthanasia:

SOP AP405 (NHP Euthanasia Criteria) outlined the clinical observations that prompted euthanasia. The protocol stated that only a veterinarian blinded to treatment could authorize euthanasia/animals were observed twice daily for cage side evaluations. The study report notes that, "Any NHP which was recumbent in the cage or had decreased or absent responsiveness to touch or experienced hemorrhage from the GI tract to be in excess of 20% of the estimated blood volume in any 24 hour period or they experienced unrelieved pain was euthanized. Any NHP which experienced any combination of the following observations such as respiratory distress, decreased food and water intake, reluctance to move for > 24 hours, and severe dehydration classified an animal to be euthanized." Animals were euthanized using Euthasia III solution 0.27 mL/kg IV.

Enrollment and randomization:

Monkeys were irradiated in cohorts of 6 to 10. The randomization code (active/placebo) within each cohort was provided to the unblended member of the radiation physics team. However, the research assistants actually responsible for irradiation were blinded to treatment assignment.

Animals weighed between 4 and 6.5 kg on the day of irradiation. Of the 46, 38 were males and 8 were females. No animals had previously been irradiated or participated in another study.

Study equipment:

Prior to irradiation, a phantom was used to verify the correct irradiation. The phantom was used to verify the irradiation dose for each monkey.

Study drugs:

The study drug doses (10 mcg/kg filgrastim and 0.154 mL/kg dextrose) were prepared daily based on the previous day's body weight. The filgrastim was diluted with the control agent dextrose to a volume of 0.154 mL/kg (approximately 0.9 mL total volume for a 6 kg monkey/concentration approximately 65 mcg/mL). The study drugs were administered 20 to 26 hours post irradiation/then daily. At any point following discontinuation of dosing, if the ANC was < 500/mcL, daily injections were re-initiated and continued until the ANC was ≥ 1,000/mcL for three consecutive days.

Clinical observations:

Animals were sedated for clinical observations (ketamine) which were performed daily from SD 0 through SD25 then at least on SD 28, 30, 32, 35, 39, 42, 45, 49, 53, 56, 60 and at termination. Animals were evaluated for weight, core temperature, capillary refill time, skin tent time, petechial, ecchymosis and swelling. Laboratory evaluations were performed then. Medical management (rehydration/ulcer therapy, etc) were also provided then.

Febrile neutropenia was defined as ANC < 500/mcL and body temperature ≥ 105° F. On the first day of febrile neutropenia, blood was cultured (aerobic and anaerobic). An additional blood culture was collected 24 hours after the first if the febrile neutropenia persisted and the preliminary bacteriology report of the first culture was either negative or a gram positive organism was identified. An additional blood specimen was obtained if febrile neutropenia persisted for 5 consecutive days after a previous blood culture collection.

Antibody formation to filgrastim was assessed based on blood samples obtained pre-treatment and at day 60 (or prior to euthanasia) for 44 monkeys.

Disposition of monkeys:

A full necropsy was performed on each monkey. Tissue from kidney, lung, liver and spleen were cultured; multiple other tissues were fixed in formalin. All fixed tissue was examined for histopathology.

RESULTS:

Primary endpoint:

Table 1. Primary Endpoint: Survival Rate Comparison

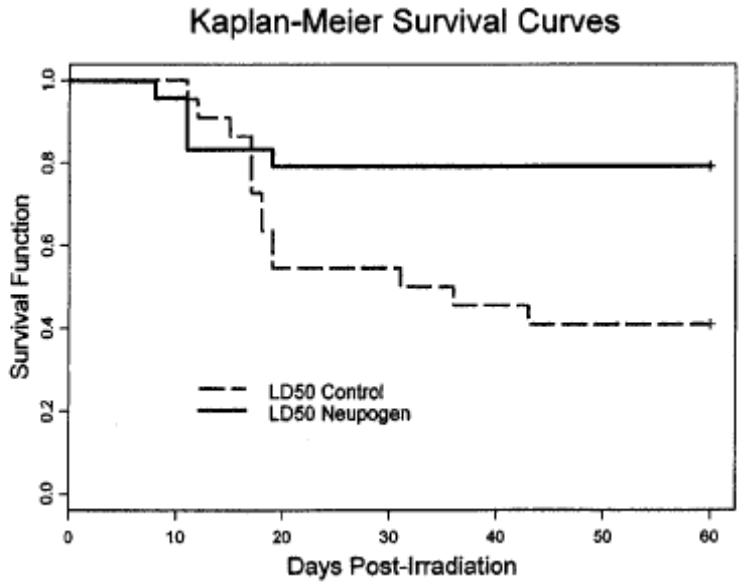
Outcome	Placebo N = 22	Filgrastim N = 24	Total	Statistic
Alive	9	19	28	P < 0.004
Dead	13	5	18	
Euthanized	10	5	15	
Survival rate	**41%**	**79%**	n/a	

Reviewer's comment: The data show that the g-CSF group had a survival rate nearly twice that of the placebo group.

The labeling for approved leukocyte growth factors (LGFs) largely examines the human response to sublethal doses of cancer chemotherapy and shows that the LGFs generally decrease the risk for febrile neutropenia and accelerate neutrophil recovery following the drug-induced myelosuppression. This study in monkeys examined a dose of radiation that resulted in nearly 60% lethality by day 60 among the control group animals with a fairly striking decrease in 60 day mortality among monkeys that received filgrastim.

The Kaplan-Meier survival curve is shown below and shows that most of the deaths occurred between day 10 and 30 (in both study groups). The first death (a g-CSF group animal) occurred on day 8. Three g-CSF group animals died on day 11—which results in a shorter duration of survival among the g-CSF decedents than among the control group decedents. This finding is likely of little or no meaningfulness since the overall

difference in survival is substantial and only five g-CSF group animals died (compared to 13 control group animals). The following figure is from the sponsor's study report.



Overall, the median survival time for the decedents was 11 days for the five monkeys in the g-CSF group but 18 days for the 13 monkeys in the placebo group. The imbalance appears related to the relatively small number of deaths among the g-CSF group, where one monkey died on day 8 and 3 died on day 11. Deaths were scatted throughout the first six weeks for the placebo group animals.

The causes for the deaths were predominantly related to either being “found dead” (3 control group animals) or the cage-side observations disclosing animals with signs that triggered euthanasia (necrotic skin, recombinant posture-unresponsiveness criteria). The study report notes that most cage side observations were normal. Cage-side observations were performed twice daily on a daily schedule for the first month of observation then twice daily on an intermittent (non-daily) schedule for the second month. The observations were graded on a pre-specified scale with 0 equating to normal and higher grades relating to increasing abnormality, as shown below. Shaded cells indicate numerically higher rates of abnormalities.

The following scale is excerpted from the sponsor's study report.

Grading Scale Used During Cageside Observations.

Parameter Observed	Grade				
	0	1	2	3	4
Activity	Normal	Limited	Absent		
Posture	Normal	Hunched	Recumbent		
Stool Consistency*	Formed	Soft	Loose and/or watery	Bloody diarrhea	
Vomit	None	Present, evidence of 1 episode	Persistent, evidence of multiple episodes		
Hemorrhage	No blood in cage	Individual blood spots in cage (≤ 10 spots)	Coalescing blood or > 10 spots in cage	Estimated to be in excess of 20% of blood volume, life-threatening	
Respiratory	Normal	Mildly increased respiration rate or effort or intermittent cough	Respiratory distress or open mouth breathing, persistent cough	Agonal	
Alopecia	Normal coat	Loss of < 25% of normal coat	Loss of > 25% but < 50% of normal coat	Loss of > 50% of normal coat	Complete loss of coat
*NA = The absence of stool at the time of observation was recorded					

Table 2. Cage-side Observations: Maximum Abnormality for Each Animal

Observation	Placebo, n = 22	g-CSF, n = 24
Activity		
0	3 (14%)	9 (38%)
1	17 (77%)	15 (63%)
2	2 (9%)	0
Posture		
0	4 (18%)	5 (21%)
1	16 (73%)	19 (79%)
2	2 (9%)	0
Stool		
0	0	1 (4%)
1	1 (5%)	2 (8%)
2	18 (82%)	19 (79%)
3	3 (14%)	2 (8%)
Vomiting		
0	8 (36%)	12 (50%)
1	11 (50%)	7 (29%)
2	3 (14%)	5 (21%)

Hemorrhage		
0	4 (18%)	4 (17%)
1	12 (55%)	9 (38%)
2	6 (27%)	11 (46%)
Respiratory		
0	20 (91%)	23 (96%)
1	1 (5%)	1 (4%)
2	1 (5%)	0
Alopecia		
0	17 (77%)	18 (75%)
1	3 (14%)	2 (8%)
2	2 (9%)	2 (8%)
3	0	2 (8%)

Table 3. Cage-side Observation Category Rates for Living NHPs

Category	Placebo Rate Higher	g-CSF Rate Higher	Total Categories
Normal	2	5	7
Any Abnormality	11	5	16
Maximum Abnormality	4	3	7

Reviewer's comment: Overall, it appears that the cage-side observations generally suggest that placebo animals displayed more signs of illness; this observation is particularly of note since the observation times are not balanced between the study groups—observation terminated with animal deaths. Hence, the g-CSF group had a greater potential for detection of abnormalities.

Clinical observations were performed daily for the first 25 days then at scheduled time intervals for the remainder of the study. Blood for laboratory evaluations were performed at these times and the animals were weighed and had temperatures recorded as well as medications/fluids/transfusions administered. Below are the major findings from the maximum temperature, weight change and transfusion history for each animal.

Table 4. Maximum Temperature, Weight Loss (% of baseline) and Transfusion Use

Observation	Placebo, n = 22	g-CSF, n = 24
Maximum temperature		
> 105°	4 (18%)	1 (4%)
> 104° to ≤ 105°	13 (59%)	15 (63%)
> 103° to ≤ 104°	4 (18%)	7 (29%)
>102° to ≤ 103°	1 (5%)	1 (4%)
Maximum weight loss (% of baseline)		
> 20	0	1
> 15 to ≤ 20	7 (32%)	7 (29%)
> 10 to ≤ 15	3 (14%)	7 (29%)
> 5 to ≤ 10	11 (50%)	8 (32%)

> 0 to ≤ 5	1 (5%)	1 (4%)
No transfusion	1 (5%)	3 (13%)

*percent of baseline

Reviewer's comment: In general, the weight change and maximum temperature for each animal were similar between the two study groups. Of course, the imbalance in the survival results in a longer observation period for the g-CSF group animals such that these animals have more observations than the control group animals (i.e., the observation periods are not balanced). In general, one would expect the features of the euthanized animals in each group to be similar and the following table examines this consideration.

Table 5. Cage-side Observations: Maximum Abnormality for Each Decedent

Observation	Placebo, n = 13	g-CSF, n = 5
Activity		
0	2	0
1	9	5
2	2	0
Posture		
0	2	0
1	9	5
2	2	0
Stool		
0	0	1
1	1	1
2	12	2
3	0	1
Vomiting		
0	6	2
1	4	1
2	3	2
Hemorrhage		
0	4	3
1	6	1
2	3	1
Respiratory		
0	12	4
1	0	1
2	1	0
Alopecia		
0	10	5
1	2	0
2	1	0

Table 6. Summary of Cage-side Observations of Decedents (mean score/range)

Observation	Placebo, n = 13	g-CSF, n = 5
Activity	1 (0 through 2)	1 (all 1)
Posture	1 (0 through 2)	1 (all 1)
Stool	2 (1 through 2)	2 (0 through 3)
Vomiting	1 (0 through 2)	1 (0 through 2)
Hemorrhage	1 (0 through 2)	1 (0 through 2)
Respiratory	0 (0 through 2)	0 (O through 1)

Table 7. Summary of Clinical Observations of Decedents (mean/range)

Observation	Placebo, n = 13	g-CSF, n = 5
Maximum Temperature	104.5° (102.8 through 105.4)	104.2° (102.8 through 105.2)
Maximum Weight Loss*	-9.3 (-6.3 through -20)	-9.8 (-6.8 through -16.4)

*percent of baseline

Reviewer's comment: The comparison of cage-side and clinical observations do not reveal any obvious differences between the decedents. Because the overall observations suggested the placebo animals had greater severity of illness, these observations support the meaningfulness of the survival results.

The sponsor has also submitted a summary of the use of supportive care (such as antibiotics and anti-diarrheals). These tables are not duplicated here but show similar use of these measures between the study groups. Blood transfusions were administered to 42 of the animals (four did not receive transfusions—one in the control group and three in the g-CSF group).

A large number of secondary endpoints were explored and here I summarize the ones that may be particularly interesting in the context of the clinical experience with Neupogen.

a. Febrile neutropenia (FN)

FN was diagnosed in largely the same manner in the animals as it was in humans in clinical studies (ANC < 500/mcL and body temperature ≥ 103° F)

Table 8. Febrile Neutropenia Occurrence

FN	Placebo n = 22	g-CSF n = 24	Statistic
Yes	20 (91%)	19 (79%)	P = 0.42

FN = febrile neutropenia

Reviewer's comment: The lack of a difference in the occurrence of FN is different from the clinical study observations (as described in LGF labeling); in placebo-controlled clinical studies, LGF therapy importantly lowered the occurrence of FN. The lack of a difference in the NHP study may related to many factors—such as the extent of the

radiation injury (it was administered to result in lethality—unlike the chemotherapy dose used in clinical studies) and the use of prophylactic antibiotics in the NHP study once severe neutropenia developed (prophylactic antibiotics are not typically used in clinical practice in the absence of signs/symptoms of infection).

In general, the labeling for LGFs indicate that FN correlates with the occurrence of clinically important infection (or infection risks). Blood cultures were obtained with the occurrence of febrile neutropenia and at the time of death.

Table 9. Blood Culture Results

Outcome	Placebo, n = 22	g-CSF, n = 24
Any positive culture	19 (86%)	14 (58%)
Total cultures	71 cultures from 20 animals	60 cultures From 20 animals

Table 10. Blood Culture Results by Gram Status of Each Sample

Outcome	Placebo n = 71 cultures	g-CSF n = 60 cultures
Any bacteria	39 (55%)	24 (40%)
Any Gram negative bacteria	6 (8%)	1 (2%)
Any Gram positive bacteria	36 (51%)	23 (38%)
Gram positive and negative bacteria	3 (4%)	0

Reviewer's comment: Although more cultures were obtained from the control group, the occurrence of positive cultures trended higher in the control group (p = 0.05, Fisher's exact test). The small imbalance in Gram negative bacteria result is also notable (suggesting more infection among the control group).

Tissue cultures (as well as blood cultures) were obtained at autopsy. Table 11 summarizes the culture findings at autopsy. Four organs were cultured (liver, spleen, lung and kidney) as well as blood.

Table 11. Terminal Blood and Organ Microbiological Results

Outcome	Placebo n = 22	g-CSF n = 24
Positive blood & positive organs*	7 (32%)	4 (17%)
Positive blood & negative organs	6 (27%)	6 (25%)
Negative blood & positive organs	0	0
Negative blood & negative organs	9 (41%)	14 (58%)

*any positive organ

Reviewer's comment: The microbiological data suggest that control group animals experienced more infections than the g-CSF group animals since the general pattern of blood and necropsy tissue microbiology showed more control group animals had infections. The following table summarizes the findings in the decedents and, perhaps due to the small numbers of animals, suggests that more of the g-CSF decedents may have experienced illness not directly related to culture-positive infection (since there is a suggestion of an imbalance in the distribution of culture negative blood and tissue). However, the numbers are too small to form conclusions.

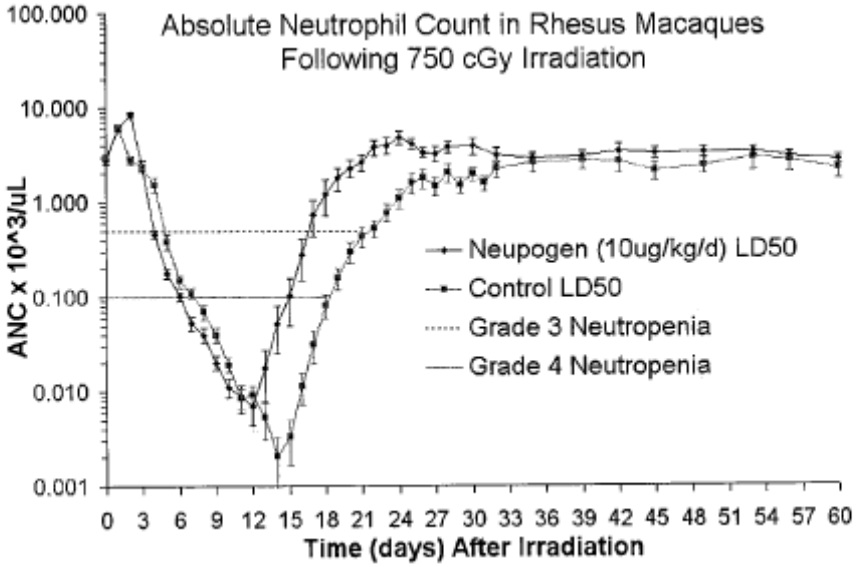
Table 12. Terminal Blood and Organ Microbiological Results in Decedents

Outcome	Placebo n = 13	g-CSF n = 5
Positive blood & positive organs*	7 (54%)	2 (40%)
Positive blood & negative organs	4 (31%)	0
Negative blood & positive organs	0	0
Negative blood & negative organs	2 (15%)	3 (60%)

*any positive organ

b. Incidence of severe neutropenia (ANC < 500/mcL)

All animals experienced severe neutropenia as shown in the following figure where grade 3 neutropenia is an ANC < 500/mcL. Shown are results for all animals, excerpted from the sponsor's study report.



The figure (mean/standard error) shows that severe neutropenia generally appeared by day 4 to 6 in the animals and resolved by day 17 for the g-CSF group animals but day 21 for the control group animals. Data from all animals are summarized in the curves.

c. Duration of severe neutropenia--SN (ANC < 500/mcL)

Table 13. Duration of Severe Neutropenia (ANC < 500/mcL)

Outcome	Placebo n = 12	g-CSF n = 19
Median	18 days	14 days
Mean	19 days	14 days
Range	14 – 22 days	9 – 19 days

The duration of SN includes data from animals that recovered their ANC values (n = 12 control group animals and 19 g-CSF group animals). The sponsor reports P < 0.0001 for the comparison of the duration.

Reviewer's comment: the results for the duration of severe neutropenia are relatively long in comparison to the experience cited in labeling for 3 of the 4 LGFs. One of the LGFs (Sargramostin) cited a longer duration of SN, perhaps related to the intensity of the induction chemotherapy for leukemia. These observations underscore the importance of the marrow damage intensity in estimating treatment effects upon the duration of SN.

	Duration of SN
Filgrastim study (lung cancer, median)	*3 days for placebo, 1 day for active*
Pegfilgrastim study (breast cancer, mean)	*2 days for active, 2 days for g-CSF control*
Sargramostin (Leukemia, median)	*17days for placebo, 13 days for active*
Tbo-filgrastim (breast cancer, mean)	*4 days for placebo, 1 day for active*

The importance of the underlying marrow toxicity (radiation versus chemotherapy) is also illustrated in a cross study comparison of the lung cancer results described in the Neupogen label and the Study ABX15 results, as follows.

Table 14. Cross Study Comparisons: Neupogen Lung Cancer/Study AGX15

Outcome	g-CSF or placebo among patients receiving chemotherapy for lung cancer (n = 210)		g-CSF or placebo among NHP post- 7.5 Gy radiation (n = 46)	
	Placebo	g-CSF	Placebo	g-CSF
FN rate	76%	40%	91%	79%
SN rate	77%	57%	100%	100%
SN duration, median	3 days	1 day	18 days	14 days

FN = febrile neutropenia; SN = severe neutropenia (ANC < 500/mcL). The SN is shown across all chemotherapy cycles in the g-CSF study; the duration of SN includes data from animals who had a recovery of ANC to ≥ 500/mcL. The dose of Neupogen in the clinical study was 4 to 8 mcg/kg subcutaneously daily.

d. Time to recovery to ANC ≥ 500/mcL

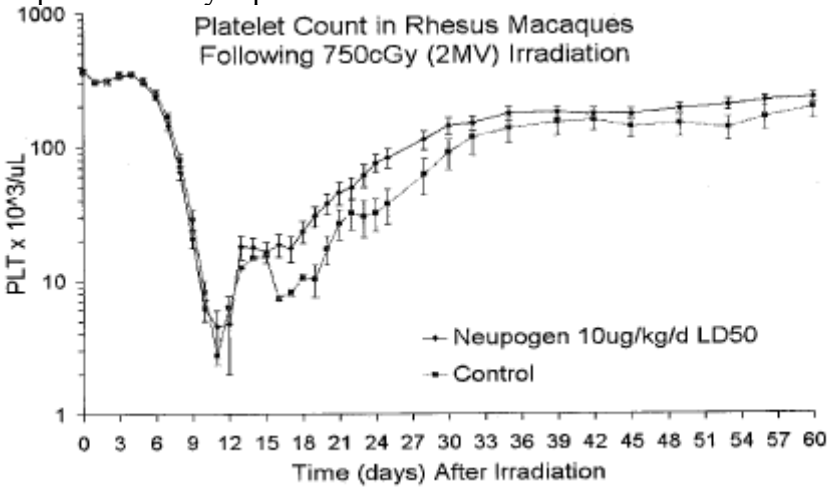
Table 15. Time to Recovery to ANC ≥ *500*/mcL

Outcome	Placebo n = 12	g-CSF n = 19
Median	23 days	19 days
Mean	23 days	19 days
Range	20 – 27 days	14 – 23 days

P < 0.0001 for the comparison of duration among animals that recovered their ANC values to values that were no longer SN.

e. Platelet outcomes

All animals experienced severe thrombocytopenia (platelet count < 20,000/mcL) and recovery of platelet counts appeared somewhat more rapid in g-CSF-treated animals although the difference was not statistically significant (when computed among the animals that had recovery of platelet counts). The following figure is excerpted from the sponsor's study report.



f. Transfusions

Overall, 42 of 46 animals received a transfusion of whole blood (3 g-CSF animals did not receive transfusions and one control group animal did not receive a transfusion).

g. Immunogenicity

"An anti-Neupogen antibody was detected in 2 of 44 animals." Specifically, one control animal and one g-CSF animal appeared to have positive end of study antibody results. However, the sponsor notes that a positive control for the immunoassay has not been developed such that the assay results

The sponsor used a standard operating procedure (SOP) based upon a Quantikine human-g-CSF kit from R & D Systems. The SOP noted that, "If the mean value of any of the

post-Neupogen dilutions is greater than or equal to the sum of the mean value of the baseline dilutions plus 0.100, then the post-Neupogen sample is positive for anti-g-CSF antibody."

Reviewer's comment: The basis for immunoassay positivity is not clear but it appears that the criteria are based upon those used for human samples.

h. Drug dosing:

Study drugs were administered, in part, contingent upon the blood counts. Study drug was administered an average of 22 days (± 1) for g-CSF group animals and an average of 28 days (± 1) for control group animals. Study drug could have been reinitiated in situations where the ANC returned to less than 500/mcL—one animal in each group had study drug reinitiated.

i. Pathology results:

Autopsies (gross and tissue histopathology) were performed on all animals. The study report largely provides results in terms of comparison of one study group to the other; however, the report notes that the imbalance in survival importantly confounds this comparison. The following are important observations:

- "There were few differences between the untreated control and the neupogen-treated animals that survived to 60 days or more."

- "In general, in both experimental groups, animals that survived until the end of the experimental period had the least number of pathological alterations."

The following table summarizes the distribution of microscopic lesions between the two study groups.

Table 16. Percent of Animals with Microscopic Lesions, by Organ

Organ/Tissue	Placebo, n = 22	g-CSF, n = 24
Liver	27	8
Heart	41	21
Lung	50	29
Spleen	45	25
Mediastinal lymph nodes	77	63
Skin	27	17
Kidney	50	29
Small intestine*	86	91
Large intestine*	32	54
Bone marrow	50	21

*the imbalance is related to more inflammatory changes in the g-CSF group

Reviewer's comment: It is difficult to infer much from the pathology findings since the main observation is that the earlier an animal is autopsied post-radiation, the more notable were pathology changes (regardless of treatment group assignment). The sponsor postulates that the increased inflammation in the g-CSF group (intestine findings) was related to more neutrophil recovery in these animals (since more than half the control animals had been sacrificed, most (10 of 13) before day 21 (the time point where SN was beginning to resolve in this group).

Appendix A. Summary of Decedents

The following is a summary of information provided by the sponsor as a supplement to the final study report. The control group decedents are summarized first.

Control Group:

1. NHP number R03038: this animal was euthanized on study day 31 based upon its clinical condition that met criteria for euthanasia. The information identifies generalized erythema, skin scaling and cracking, conjunctivitis with purulent exudates. The review of the supportive care for the animal shows that the animal had experienced FN and had received Baytril, gentamicin and Rocephrin. Transfusions and oral as well as intravenous hydration had been administered. Bloody stool was reported and mouth ulcers also noted. At autopsy, blood cultures were positive but organ cultures were negative.

2. NHP number 04021: this animal was found dead on day 17. The information from supportive care indicates that the animal had experienced FN and had received Baytril, gentamicin. Oral as well as intravenous hydration was also administered as were blood transfusions. The supportive care notes identify bloody stool and mouth ulcers. At autopsy, both blood and organ cultures were positive for bacteria.

3. NHP number 03R0225: this animal was euthanized on day 15 based on recumbent status and decreased response to touch that met the euthanasia criteria. The supportive care information indicates the animal had FN and received Baytril, genatmicin as well as oral and intravenous hydration. Transfusions were also administered. Loose stool was reported as was vomiting and mouth ulcers. At autopsy, both blood and organ cultures were positive for bacteria.

4. NHP number 040605: this animal was found dead on day 18. The supportive care information indicates the animal had FN and received Baytril and gentamicin as well as oral and intravenous hydration. Transfusions were also administered. The information also indicates the animal had loose stool and bloody gums. At autopsy, both blood and organ cultures were positive for bacteria.

5. NHP number 03R0716: this animal was euthanized on day 17 because of inactivity and recumbent posture that met the euthanasia criteria. The supportive care information indicates the animal had FN and received Baytril and gentamicin. Oral as well as intravenous hydration was administered as well as transfusions. Loose stools were reported as were mouth ulcers. At autopsy, blood was positive for bacteria but organ cultures were negative.

6. NHP number 03697: this animal was euthanized on day 18 because of recumbent posture, inactivity and decreased responsiveness that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration were administered as were transfusions.

Loose stools were reported as were mouth ulcers. At autopsy, both blood and organ cultures were positive for bacteria.

7. NHP number 050103: this animal was euthanized on day 19 because of necrotic skin and mouth lesions that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril, gentamicin and Rocephrin as well as Primaxin. Oral and intravenous hydration were administered as were transfusions. Loose stools were observed as were mouth ulcers. At autopsy, both blood and organ cultures were negative.

8. NHP number 03010: this animal was euthanized on day 36 because of "progressive tissue necrosis" that met the euthanasia criteria. The supportive care information indicates that the animal experienced FN and received Baytril, gentamicin, Rocephrin and Primaxin and fluconazole. Oral and intravenous hydration was administered along with transfusions. Loose stools were observed along with mouth ulcers. At autopsy, both blood and organ cultures were negative.

9. NHP number 050247: this animal was euthanized on day 17 because of bloody vomitus, limited responsiveness, pale skin and increased pulse that met the euthanasia criteria. The supportive care information indicates that the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration was administered along with transfusions. Loose stools were reported. At autopsy, both blood and organ cultures were positive for bacteria.

10. NHP number 040129: this animal was euthanized on day 43 because of a "zoonotic coagulase positive staph infection" that prompted the euthanasia based on "veterinarian recommendation and potential risk to other animals." The supportive care information indicates the animal experienced FN and received Baytril, gentamicin, Rocephrin, Primaxin and fluconazole. Oral and intravenous hydration was administered as were transfusions. Bloody, loose stools were reported. At autopsy, blood culture was positive but organ cultures were negative.

11. NHP number 04057: this animal was euthanized on day 12 because of recumbent posture, decreased activity and shallow respiration that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration was administered as were transfusions. Blood in loose stools was reported. At autopsy, blood was positive for bacteria but organ cultures were negative.

12. NHP number 04063: this animal was found dead on day 11. The supportive care information indicates the animal never experienced FN but did received Baytril because of neutropenia. The animal received oral and intravenous hydration but did not receive transfusions. Bloody diarrhea was reported as was vomiting. At autopsy, both blood and organ cultures were positive for bacteria.

13. NHP number 040159: this animal was euthanized on day 19 because of decreased food and water intake, rough coat, abnormal activity and appearance that met euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril, gentamicin and Rocephrin. Oral and intravenous hydration was administered as were transfusions. Loose stools (bloody) and mouth ulcers were reported. At autopsy, both blood and organ cultures were positive for bacteria.

g-CSF Group:

1. NHP number 03026: this animal was euthanized on day 11 because of abnormal appearance and activity that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration was administered as were transfusions. Bloody loose stools were reported as was vomiting. At autopsy, both blood and organ cultures were negative.

2. NHP number 0311025: this animal was euthanized on day 19 because of "progressive tissue necrosis" that met euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril, gentamicin, Rocephrin and Primaxin. Oral and intravenous hydration was administered as were transfusions. Bloody, loose stools were reported. At autopsy, both blood and organ cultures were negative.

3. NHP number 0401153: this animal was euthanized on day 11 because of "abnormal activity and appearance, head down, rough coat, edema and difficulty with movement" that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration were administered but transfusions were not. A mouth sore was reported. At autopsy, both blood and organ cultures were positive.

4. NHP number 03018: this animal was euthanized on day 11 because of "inactivity, recumbent in cage and extremely low body temperature." The supportive care information indicates the animal did not experience FN but did receive Baytril because of neutropenia. Bleeding from the mouth was reported. Oral and intravenous hydration were administered as were transfusions. At autopsy, both blood and organ cultures were positive.

5. NHP number 030613: this animal was euthanized on day 8 because of "laceration on penis, generalized redness on body and necrotic areas on abdomen, umbilicus, chest and right thigh" that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration were administered but no transfusions. Loose stool was reported. At autopsy, both blood and organ cultures were negative.

Appendix B. Approved Leukocyte Growth Factors: Indication by Dose

Product/approval year	Dose	Indication
Filgrastim (Neupogen); 1991	5 mcg/kg/day as SC injection or IV infusion	Patients receiving myelosuppressive chemotherapy for cancer
	10 mcg/kg/day as IV or SC infusion	Patients receiving BMT following myeloablative cancer chemotherapy
	10 mcg/kg/day as SC injection or infusion	Patients undergoing PBPC mobilization prior to cancer chemotherapy
	5 or 6 mcg/kg/day BID SC or QD SC	Severe chronic neutropenia*
Sargramostim (Leukine); 1991	0.25 mg/m^2/day as IV infusion	Patients receiving chemotherapy for AML
	0.25 mg/m^2/day as IV infusion or SC injection	Mobilization of PBPC
	0.25 mg/m^2/day as IV infusion or SC injection	Myeloid reconstitution after autologous PBPC transplant
	0.25 mg/m^2/day as IV infusion	Myeloid reconstitution after autologous or allogeneic BMT
	0.25 mg/m^2/day as IV infusion	Myeloid reconstitution after autologous or allogeneic BMT failure or delay
Pegfilgrastim (Neulasta); 2002	6 mg SC once per chemotherapy cycle	To decrease the incidence of febrile neutropenia in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia
Tbo-filgrastim; 2012	5 mcg/kg/day as SC injection	To reduce the duration of severe neutropenia in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia

*the specific dose depends on whether the patient has congenital or idiopathic or cyclic neutropenia; AML = acute myeloogenous leukemia; SC = subcutaneous; IV = intravenous; PBPC = peripheral blood progenitor cell; BMT = bone marrow transplant; Information regarding potential pediatric use is available only in the filgrastim and sargrastim labeling. The pegfilgrastim label states that, "the 6 mg fixed-dose formulation should not be used in infants, children and smaller adolescents weighing less than 45 kg."

Appendix C: Leukocyte Growth Factor: Major Study Results from Labeling

Product	Setting	Study Results
Filgrastim	Myelosuppressive chemotherapy for cancer	A randomized study of placebo vs filgrastim during lung cancer chemotherapy cycles: • febrile neutropenia in 76% (84/111) placebo vs 40% (40/99) filgrastim • severe neutropenia incidence over all cycles occurred in 77% (416/543 cycles) placebo vs 57% (286/500 cycles) filgrastim • filgrastim group also had decreased hospitalization and decreased neutropenia duration and severity • no notable study group difference in survival A randomized study of placebo vs filgrastim during AML induction chemotherapy: • filgrastim decreased median time to ANC recovery (20 days vs 25 days) • filgrastim decreased duration of fever, antibiotic use and hospitalization • no notable study group difference in survival
	Autologous or allogeneic BMT following myeloablative chemotherapy	In three placebo-controlled studies, filgrastim decreased the median number of days of severe neutropenia with no notable study group difference in survival
	PBPC mobilization and engraftment	Within a subset from a randomized study of placebo/filgrastim among patients who received myeloablative therapy for lymphoma, patients who received filgrastim-mobilized PBPC (vs autologous BMT) had a shortened time to ANC recover and a shorter duration of post-transplant hospitalization
	Severe chronic neutropenia	In a controlled study, filgrastim therapy: • decreased the episodes of hospitalization (28 hospitalizations in 62 filgrastim group patients vs 44 hospitalizations in 60 control group patients) • also decreased the incidence of fever, infection and need for antibiotics
Sargramostim	AML chemotherapy	In a placebo-controlled study of sargramostim among patients receiving chemotherapy: Sargramostim group patients had: • shortened median duration of severe neutropenia • shorted median time to neutrophil recovery

		• decreased incidence of severe infections and deaths due to infection (3 vs 11) with no notable difference in overall survival
	Mobilization and engraftment of PBPC	Two retrospective reviews compared sargramostim-mobilized PBPC and non-sargramostim mobilized PBPC; patients who received sargramostim had: • More granulocyte-marcophage colony forming units (CFU-GM) in the PBPC • Shorter time to engraftment
	Autologous BMT	Three single-center, randomized, placebo-controlled studies among patients receiving autologous BMT post-chemotherapy showed that patients receiving sargramostim had improved time to engraftment in two studies and a trend toward improvement in the third
	Allogeneic BMT	A placebo-controlled study of sargramostim among patients receiving allogeneic BMT post-chemotherapy showed that sargramostim: • shortened time to engraftment • decreased number of patients with bacteremia and overall incidence of infection
	Bone marrow transplant failure or engraftment delay	A historically-controlled study indicated that sargramostim improved median survival after either autologous or allogeneic BMT failure
Pegfilgrastim	Metastatic breast cancer chemotherapy	Two randomized, active control studies showed that filgrastim and pegfilgrastim had similar: mean days of severe neutropenia rates of febrile neutropenia (~ 10 to 20%) A placebo-controlled study showed that sargrastim-treated patients had: • decreased febrile neutropenia (1% vs 17%) • decreased hospitalization (1% vs 14%) • decreased IV anti-infective use (2% vs 10%)
Tbo-filgrastim	Stage II – IV breast cancer chemotherapy	In a randomized study, tbo-filgrastim decreased the duration of severe neutropenia in comparison to placebo (1.1 days vs 3.8 days)

ANC = absolute neutrophil count; AML = acute myelogenous leukemia; severe neutropenia = ANC < 500/mm^3

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/s/

RAFEL D RIEVES
11/02/2012

Medical Officer's Addendum Review of Nonhuman Primate (NHP) Study

preIND:	100228, submission of December 3, 2012
Product:	Filgrastim (Neupogen)
Sponsor:	National Institute of Allergy and Infectious Diseases (NIAID)
Reviewer:	Dwaine Rieves/Director/DMIP
Today:	March 21, 2013

1. **Reviewer's summary observations:**

This addendum is to follow-up on items identified in my October 31, 2012 review which provided a clinical perspective on NHP Study AXG15. The NIAID provided information in a December 2, 2012 submission that addressed certain requests, as follows:

a. *Requested a copy of SOP AP405: "NHP Euthanasia Criteria;"*

b. *Clarification of whether animal caretakers were aware of blood leukocyte results;*

c. *Information that helps support the contention that the dose of filgrastim used in the animal study (10 mcg/kg/day) equates to a human dose of 5 mcg/kg/day.*

Also, during the review over the last few months, we re-examined the autopsy data to try to decipher any important difference in the findings between the early decedents—filgrastim verus placebo. This was re-examined because the initial few deaths occurred in the filgrastim group.

The following are the major points from this addendum:

The supplied euthanasia SOP appears reasonable. Further, FDA conducted an inspection of the Animal Care Facility. This inspection revealed no deficiencies that compromised the study data integrity; consequently, FDA has observed no study conduct issues that undermine the study data credibility.

NIAID confirms that veterinarians performing cage-side observations were not aware of blood leukocyte results; caretakers providing supportive care were aware of the results.

NIAID has provided a published summary of Study AXG15 that references other publications to support the contention that a monkey filgrastim dose of 10 mcg/kg/day approximates the exposure one anticipates for humans receiving 5 mcg/kg/day. This contention appears reasonable, based on the limited published data, and also reasonable based upon the general similarity in pattern of neutrophil response to irradiation (monkeys) relative to chemotherapy (humans). Additionally, filgrastim is approved for human use at 10 mcg/kg/day subcutaneously for patients undergoing peripheral blood cell mobilization; hence, the difference in 5 versus 10 mcg/kg concern appears minor given the known biological variability of humans/monkeys and the AXG15 survival data.

Close examination of the autopsy findings from early decedents suggests similar pathological findings between the filgrastim and control group animals—findings suggesting that sepsis likely

played an important role in the death of the animals in each group; the autopsy data do not signal unblinding effects that could have contributed to euthanasia.

2. Request for a copy of SOP AP405: "NHP Euthanasia Criteria;"

NIAID supplied a copy of the requested SOP. This SOP was dated effective April 4, 2007 and states, "It is the responsibility of the veterinarian to make the decision to euthanize a NHP in accordance with the criteria described in this SOP." The SOP further describes these criteria for euthanasia (as excerpted):

5.2. A decision to euthanize a NHP prior to the end of the study will be made:

5.2.1. After all reasonable therapeutic options have been discussed between the veterinarian and the study director and have failed.

5.2.2. It has been determined by the veterinarian that the death of the animal is inevitable.

5.2.3. Unrelievable pain or distress is present.

5.2.4. In the event that the study director is unavailable, the disposition of the NHP is the decision of the veterinarian.

5.3. Single Criteria

5.3.1. An observation of any one of the following symptoms is justification for euthanasia:

5.3.1.1. Inactivity: Recumbent in the cage with decreased or absent responsiveness to touch.

5.3.1.2. Self-mutilation (full thickness skin damage): If not responsive to increased "enrichment efforts" over a 2 week period or if repeated surgical intervention is required from self trauma.

5.3.1.3. Seizure activity that is either not responsive to medication or that continues despite medication, as determined by a veterinarian.

5.3.1.4. Hemorrhage from the GI tract or other orifice estimated to be in excess of 20% of estimated blood volume in any 24 hour period.

5.3.1.5. Hyperthermia (rectal temperature ≥ 106°F).

5.3.1.6. Loss of body weight >25% of baseline for 72 hours.

5.3.1.7. Hypothermia (rectal body temp. <96°F) for 6 hours.

5.4. Combination Criteria

5.4.1. Observations of a combination of two of the following symptoms are justification for euthanasia:

5.4.1.1. Respiratory distress: Labored breathing (pulse of < 90 and > 40 breath/min.)

5.4.1.2. Abnormal activity: difficulty with ambulation, decreased food and water intake, self mutilation, reluctance to move for > 24 hours.

5.4.1.3. Clinical condition: severe dehydration, shallow respiration or hyperthermia (rectal temperature > 105.5˚F) which is unresponsive to antipyretic therapy for > 72 hours.

5.4.1.4. Loss of body weight >20% of baseline body weight for greater than 72 hours.

5.4.1.5. Abnormal appearance: rough coat, head down, tucked abdomen, pallor, exudates around eyes and/or nose.

5.5. Additional Criteria

5.5.1. 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.

Reviewer's Comment: The study protocol required veterinarians to be blinded to treatment assignment and to perform cage-side observations twice daily. In the response, NIAID also notes that personnel providing supportive care were aware of blood leukocyte responses—this did not include veterinarians. Overall, the euthanasia criteria and implementation plan appear reasonable. Additionally, FDA inspection of the animal care facility did not reveal any findings that undermined the data integrity—including application of the euthanasia criteria.

3. Clarification of whether animal caretakers were aware of blood leukocyte results;

NIAID reports that, "Husbandry staff was not aware of the blood leukocyte results. Research personnel providing supportive care were aware of results. This knowledge is essential in order to provide appropriate care based on signs and clinical parameters."

4. Information that helps support the contention that the dose of filgrastim used in the animal study (10 mcg/kg/day) equates to a human dose of 5 mcg/kg/day.

NIAID noted that their prior comments about pharmacokinetics in the preIND submission archive occurred at time when Amgen was partnering with the institute. Once this partnership was terminated, NIAID did not obtain the anticipated pharmacokinetic data.

In the original Summary Basis for Approval of filgrastim (1991), FDA noted, "Filgrastim is not species restricted and has efficiently stimulated the generation of neutrophilic granulocyte

colonies from the marrow cells of all mammalian species so far examined. The similarity of the biological response to filgrastim in rabbits, mice, rats, hamsters, dogs and cynomolgus monkeys and the binding of iodinated human G-CSF to murine tissues indicates species cross reactivity of the human material."

Further, the document noted, "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 of filgrastim is 3.5 hours for both normal subjects and cancer patients, with clearance rates of approximately 0.5 – 0.7 mL/min/kg."

In the publication that summarized the AGX15 study, the following comment was provided regarding the choice of the filgrastim dose:

"The route and schedule of Neupogen administration was based on the currently licensed indication of Neupogen for the treatment of cancer patients receiving myelosuppressive chemotherapy or bone marrow transplant. The dose used herein reflects previous pharmacokinetic (PK) and pharmacodynamics (PD) analysis of filgrastim in rhesus monkey and was considered to be bioequivalent to the dose proposed for humans (5 μg/kg/d) (39)."

Reference 39 is the publication titled, "Peg-filgrastim, administered in an abbreviated schedule, significantly improved neutrophil recovery after high-dose, radiation-induced myelosuppression in rhesus macaques" (Radiat Res 2012; 178:403-14). In this report, monkeys were exposed to both peg-filgrastim and filgrastim and PK samples were obtained up to seven days following the initiation of dosing. Specifically, filgrastim PK datapoints were obtained on day 1 and day 7 post total body irradiation (with filgrastim 10 mcg/kg being administered daily).

Reference 39 notes, "The PK and PD profile of filgrastim differs in a predictable way between NHP and humans. In general, the NHP will clear filgrastim about twice as fast as a human, therefore the T1/2 of filgrastim in NHP (1.63 h) is about half the duration of T1/2 in humans (3.5 h) (44-46). The area under the exposure curve (AUC) for the NHP for a given dose of filgrastim is approximately half that for the human, which is consistent with the T1/2 and clearance of filgrastim. The PK and PD data support a dose of 10 μg/kg/day in the NHP as being equivalent to 5 μg/kg/day in the human."

Reference 39 was a study that compared the PK and PD of subcutaneous (SC) filgrastim to SC Peg-filgrastim in rhesus monkeys. Among irradiated monkeys, the PK values for filgrastim varied on day 1 versus day 7, consistent with greater exposure to filgrastim on day 7. The following are PK values that represent the average from two irradiated monkeys where blood was sampled on day 1 (irradiation day):

Average from Two Animals

Drug/species/dose	C_{max} ng/mL	T_{max} (h)	$T^{1/2}$ (h)	AUC ng*day/mL
Filgrastim/monkey/10 mcg/kg	19.9	2.2	3.0	4.9

*Reviewer's comment: Multiple reports cite two pathways for filgrastim elimination: 1) renal and 2) endocytosis within myeloid cells. Consequently, it is not surprising that filgrastim exposure increases during a period of neutropenia. Reference 39 contains a figure that illustrates the greater exposure on day 7 versus day 1. Specifically, the report cites a day 1 filgrastim AUC of 4.9 ng*day/mL and a day 7 filgrastim AUC of 8.2 ng*day/mL.*

The cited references (44-46) are notable on the following points:

Reference 44: Randomized dose-escalation study of SC/01 compared with daily filgrastim in patients receiving chemotherapy. JCO 2000; 18:2522-8.

Reference 44 was a study that compared PK and PD of a SC pegylated filgrastim to SC filgrastim in patients with cancer where blood was sampled on cycles 0 (no chemotherapy) and 1 of chemotherapy. The following PK datapoints were obtained from three patients on cycle 0 (no chemotherapy) during the first 48 hours and then daily through day 15, where filgrastim was administered at 5 mcg/kg/day for five days.

Median and Range from Three Patients

Drug/species/dose	C_{max} ng/mL	T_{max} (h)	$T^{1/2}$ (h)	AUC ng*hr/mL
Filgrastim/human/5 mcg/kg	15.4 (12.5 – 37.2)	4.0 (2.0 – 8.0)	2.6 (2.4 – 3.3)	167 (96.6 – 346)

Reference 45: Three types of recombinant human granulocyte-colony stimulating factor have equivalent biological activities in monkeys. Cytokine 1997;5:2522-8.

Reference 45 was a study that compared PK and PD of SC as well as intravenously (IV) administered filgrastim obtained from three manufacturers (one was Amgen). In the study, cynomolgus monkeys (no irradiation) were given either 1.5 mcg/kg or 5.0 mcg/kg IV or SC daily for five days. No marked differences in PK were observed among the three different commercial filgrastim products. NR = not reported.

Average and SE from Six Monkeys Receiving Amgen Filgrastim

Drug/species/dose	C_{max} ng/mL	T_{max} (h)	$T^{1/2}$ (h)	AUC ng*hr/mL
Filgrastim/monkey/5 mcg/kg	12.8 ± 2.9	NR	NR	67.0 ± 14.8

Reference 46: Effect of escalating doses of recombinant human granulocyte colony-stimulating factor (filgrastim) on circulating neutrophils in healthy subjects. Clin Thera 1998; 20(4):722-36.

Reference 46 was a study that compared the PK and PD of various filgrastim doses administered to healthy humans. The study used fixed (not weight adjusted) doses of SC daily injections at 75 mcg/ 150 mcg, 300 mcg and 600 mcg. Four subjects received the 300 mcg dose and it was administered daily for 10 days. The following PK data are from the four subjects who received a dose approximating 5 mcg/kg/day (fixed at 300 mcg); shown are results from day 1 exposure.

Average and Standard Deviation from Four Healthy Volunteers

Drug/species/dose	C_{max} ng/mL	T_{max} (h)	$T^{1/2}$ (h)	AUC ng*hr/mL

Filgrastim/human/~5 mcg/kg	14.8 (6.6)	4.0 (0)	3.4 (2.2)	119.0 (41.7)

Reviewer's comment: The following is a summary table that uses the mean/median information from the preceding references. NR = not reported. Note that the AUCs are reported in different units.

Summary Table (mean and/or median values, per reference)

Filgrastim by species/dose	C_{max}ng/mL	T_{max} (h)	$T^{1/2}$ (h)	AUC	
human/5 mcg/kg	15.4	4.0	2.6	167.0	ng*hr/mL
human/~5 mcg/kg	14.8	4.0	3.4	119.0	
monkey/5 mcg/kg	12.8	NR	NR	67.0	
monkey/10 mcg/kg	19.9	2.2	3.0	4.9	ng***day**/mL

*Reviewer's comment: If one converts the AUC in the last row of the Summary Table to the same units used in the other lines, then the value is 117.6 ng*hr/mL. The table shows the data as excerpted from the published reports.*

These reports generally support the sponsor's estimate that the monkey probably needs a modestly higher dose of filgrastim to equate to human doses; hence, it appears reasonable to contend that a monkey dose of 10 mcg/kg/day approximates a human dose of 5 mcg/kg/day. Reinforcing this estimate are the neutrophil count responses in irradiated monkeys (given 10 mcg/kg/day) that generally approximate the pattern one might expect given the experience in humans receiving myelosuppressive chemotherapy.

5. Additional Consideration: Autopsy Findings of Early Filgrastim Group Decedents

One of the observations from the AGX15 study is that a few filgrastim animals died relatively earlier (euthanasia) than placebo group animals. Below is a summary of the histopathology diagnoses for these early filgrastim group decedents:

Animal 030613 was euthanized on day 8 because of "laceration on penis, generalized redness on body and necrotic areas on abdomen, umbilicus, chest and right thigh" that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration were administered but no transfusions. Loose stool was reported. At autopsy, both blood and organ cultures were negative. The autopsy diagnosis was:

-severe myeloid and erythroid depletion, sternal and femoral bone marrow
-mild acute mucosal hemorrhage, small intestine, slide number 1
-mild epidermal hyperplasia, skin
-moderate acute hemorrhage, skin
-mild lymphoid depletion, thymus.

Animal 03026 was euthanized on day 11 because of abnormal appearance and activity that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration was administered as were

transfusions. Bloody loose stools were reported as was vomiting. At autopsy, both blood and organ cultures were negative. The autopsy diagnosis was:

-severe myeloid and erythroid depletion, bone marrow
-mild to moderate lymphoid depletion, mesenteric lymph nodes, thymus and spleen
-mild chronic enteritis with mild lymphangiectasia, small intestine
-mild chronic enteritis, large intestine
-mild hyperkeratosis, skin
-mild to moderate atrophy, fat of thymus and skin
-mild acute hemorrhage, large intestine

Animal 0401153 was euthanized on day 11 because of "abnormal activity and appearance, head down, rough coat, edema and difficulty with movement" that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration were administered but transfusions were not. A mouth sore was reported. At autopsy, both blood and organ cultures were positive. The autopsy diagnosis was:

-multiple "peracute" bacterial emboli, mucosa of small intestine, large intestine, liver, spleen, lung, kidneys and heart
-severe myeloid and erythroid depletion, sternal bone marrow
-moderate to severe acute hemorrhage, large intestine, small intestine, kidney and heart
-mild to moderate lymphoid depletion, spleen, thymus and mesenteric lymph nodes

Animal 03018 was euthanized on day 11 because of "inactivity, recumbent in cage and extremely low body temperature." The supportive care information indicates the animal did not experience FN but did receive Baytril because of neutropenia. Bleeding from the mouth was reported. Oral and intravenous hydration were administered as were transfusions. At autopsy, both blood and organ cultures were positive. The autopsy diagnosis was:

-multiple bacterial emboli, moderate to severe, mucosa of intestine, spleen, kidneys, heart, liver, lung and stomach
-mild to moderate acute hemorrhage, associated with bacterial emboli, lung, heart, stomach, small and large intestine, spleen and liver
-mild to moderate lymphoid depletion, spleen, thymus and mesenteric lymph nodes
-severe diffuse myeloid and erythroid depletion, bone marrow of femur and sternum

Reviewer's comment: This reported autopsy diagnoses for the early filgrastim decedents appear consistent with processes that could have caused the manifest cage-side observations—particularly sepsis related to gastrointestinal tract inflammation and hemorrhage. Similar findings were observed in the Control group early decedents, as shown below.

Animal 04063 was found dead on day 11. The supportive care information indicates the animal never experienced FN but did received Baytril because of neutropenia. The animal received oral and intravenous hydration but did not receive transfusions. Bloody diarrhea was reported as was vomiting. At autopsy, both blood and organ cultures were positive for bacteria. The autopsy diagnosis was:

-multiple acute bacterial emboli, small intestine, lung, thymus, right kidney and liver
-moderate to severe myeloid and erythroid depletion, sternum and femur

-mild multifocal acute hemorrhage, large intestine, small intestine, spleen, lung and mesenteric lymph node number 1
-mild acute necrosis, mucosa of small intestine
-mild lymphoid depletion, spleen

Animal 04057 was euthanized on day 12 because of recumbent posture, decreased activity and shallow respiration that met the euthanasia criteria. The supportive care information indicates the animal experienced FN and received Baytril and gentamicin. Oral and intravenous hydration was administered as were transfusions. Blood in loose stools was reported. At autopsy, blood was positive for bacteria but organ cultures were negative. The autopsy diagnosis was:

-multiple bacterial emboli, lung, kidneys and heart
-severe myeloid and erythroid depletion, bone marrow of sternum and femur
-moderate chronic lymphoplasmacytic gastritis, first gastric slide
-mild multifocal acute hemorrhage, mucosa of large intestine and small intestine
-mild lymphoid depletion, spleen
-nematode parasite, lumen of small intestine.

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/s/

RAFEL D RIEVES
04/01/2013

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA #:	Non-Human Primate (NHP) Studies Submitted in Support of IND 100228
Supplement #:	
Drug Name:	Neupogen
Indication(s):	Treatment of Hematological Syndrome of **Acute Radiation Syndrome** (ARS-HS) under the Animal Rule
Applicant:	NIH/NIAID
Date(s):	Submitted date: Dec, 2012
	Advisory Committee (AC) date: May 3, 2013
Review Priority:	
Biometrics Division:	Division of Biometrics V
Statistical Reviewer:	Lan Huang, Ph.D.
Concurring Reviewers:	Jyoti Zalkikar, Ph.D., Team Leader
	Rajeshwari Sridhara, Ph.D., Director, Division of Biometrics V
Medical Division:	Division of Medical Imaging Products
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Keywords:

Animal study, randomized study two arm study, irradiation, survival (KM and Cox), propensity score, t-test, Fisher's exact test, Chi-square test

Table of Contents

1 EXECUTIVE SUMMARY 5

2 INTRODUCTION 7

2.1. OVERVIEW 7
2.1.1. INDICATION 7
2.1.2. HISTORY OF PROGRAM DEVELOPMENT 7
2.1.3. SPECIFIC STUDIES REVIEWED 8
2.1.4. MAJOR STATISTICAL ISSUES 8
2.2. DATA SOURCES 9

3 STATISTICAL EVALUATION 10

3.1 DATA AND ANALYSIS QUALITY 10
3.2 EVALUATION OF EFFICACY 10
3.2.1 Study Design and Endpoints 10
3.2.2 Statistical Methodologies 12
3.2.3 Subject Disposition, Demographic and Baseline Characteristics 14
3.2.4 Results and Conclusions 17
3.3 EVALUATION OF SAFETY 26

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS 29

5 SUMMARY AND CONCLUSIONS 29

5.1 STATISTICAL ISSUES 29
5.2 COLLECTIVE EVIDENCE 31
5.3 CONCLUSIONS AND RECOMMENDATIONS 32

6 APPENDICES 33

LIST OF TABLES

Table 1: Summary of the key studies in the review 8
Table 2: number of animals by gender, source, and "Group" in Neupogen group and Control group. 14
Table 3: Summary of measures taken at baseline (day of irradiation) 15
Table 4: Summary of count by treatment and outcome (Death or not) 17
Table 5: Hazard ratio (HR) and 95% confidence intervals (CIs) for treatment effect from different Cox survival models 20
Table 6: Start date of event and 95% confidence interval (CI) by treatment 21
Table 7: Duration of recovery and 95% confidence interval (CI) from ANC and PLT drops by treatment for recovered animals 22
Table 8: KM curves and nominal pvalues from logrank test for the two curves. Consider informative censoring and the censoring time is imputed by 60 days – the start date of the related event 23
Table 9: Cox models with and without adjustment of baseline information. The Hazard ratio (HR) is relative rate of recovery for this case. Censoring time is imputed with 60 days- the start date of the related event. In addition to treatment, baseline covariates considered are gender, source, group, doses (chestsum and legsum). 23
Table 10: Summary of animals with Febrile Neutropenia with and without imputation 25
Table 11: Summary of transfusion by treatment 26
Table 12: Safety evaluation from Histopathology on selected organ (p-value is obtained from fisher exact test) 29

LIST OF FIGURES

Figure 1: Distributions of doses (chest and leg) by treatment 16
Figure 2: KM survival curves by treatment 18
Figure 3: Histogram of propensity score (PS) by treatment with normal and kernel distribution curves (for the 46 animals) 19
Figure 4: Histogram of propensity score (PS) by treatment with normal and kernel distribution curves (for the 28 matching animals with PS value difference less than 20% of the logit of the 46 PS values)..... 20
Figure 5: percent of animals with AM/PM abnormal activities (limited or recumbent or no activity) over time (days)..... 27
Figure 6: percent of animals with AM/PM abnormal hemorrhage over time (days)..... 28

1 EXECUTIVE SUMMARY

Granulocyte colony-stimulating factor (G-CSF, 175 amino acids, 18.8 Kd) stimulates bone marrow neutrophil production and neutrophil progenitor proliferation. Neupogen® (Filgrastim) is a recombinant human G-CSF. Neupogen has been shown to be safe and effective in accelerating the recovery of neutrophil counts following a variety of chemotherapy regimens.

In this submission, NIH/NIAID submitted the results from a randomized animal study for evaluating the efficacy of Neupogen in the treatment of Hematological Syndrome of **Acute Radiation Syndrome** (ARS-HS) under the Animal Rule.

The study was designed to determine whether Neupogen will improve mortality rate at 60 days after radiation exposure of Rhesus macaques receiving medical management (intravenous (iV) fluids, blood products, nutrition, and antibiotics) compared to control animals receiving vehicle and the same medical management. Forty six animals (Rhesus macaques from two vendors) were randomized into two arms (Neupogen vs. Control). All animals were exposed to a target of 750 cGy TBI (irradiation). Neupogen or Vehicle was administered daily until:

- 1) Absolute neutrophil count (ANC) $\geq$ 1000/μL for 3 consecutive days; OR
- 2) ANC $\geq$ 10,000/μL for 2 consecutive days between Days 1-5; OR
- 3) anytime ANC $\geq$ 10,000/μL after Day 6

Daily dosing was resumed at ANC < 500/μL and discontinued at ANC $\geq$ 1000/μL for 3 consecutive days.

The primary endpoint specified by the sponsor is overall survival measured at 60-days post randomization. However, the primary endpoint in reality was mortality rate at 60 days evaluated using the chi-square test. Secondary endpoints included survival time of decedents and effect on hematology parameters (such as ANC and Platelets (PLT)). Animals were euthanized based on a specific set of criteria outlined in the protocol. Personnel performing cage-side observations were blinded to whether an animal had received treatment or control and antibiotic treatment.

After Total Body Irradiation, mortality (natural death or euthanized cases) was significantly decreased in the Neupogen group (5/24=21%) compared to the vehicle control group (13/22=59%). The p-value from Chi-square test for evaluating the association between treatment and mortality is 0.0079. A fisher exact test (more proper for data with small sample size) for this evaluation provided a p-value of 0.0147. The sponsor used an early stopping rule and stopped the study with the 46 animals because the p-value (one-sided p-value 0.0079/2=0.0004) was less than 0.0229 (alpha allocated for interim analysis).

In addition to the primary analysis, supportive analyses were conducted to evaluate the survival over time during the 60-day study period (overall survival). The Logrank test for the two Kaplan Meier (KM) survival curves (Neupogen vs. Control) had a nominal p-value of 0.018, in favor of Neupogen. The Neupogen group had Hazard ratio (HR) 0.31 (95% CI as [0.11, 0.88]) compared with Control group, from Cox model with only treatment as the covariate.

Even though the study is a blinded randomized study, because of small sample size, the baseline information may not be quite balanced in this study. Exploratory analyses with adjustment of baseline information were also conducted. A propensity scoring approach (two-strata method) was used to adjust the baseline information. The propensity scores were obtained from a logistic regression model with treatment as dependent variable, and gender, source, group, and dose variables (chestsum and legsum) as covariates. The kolmogorov-smirnov (KS) test for equality of the Propensity score distributions (Neupogen vs. Control) resulted in a nominal p-value of 0.0034. The 46 animals were grouped into two strata using the propensity scores (cut-off point is 50 percentile). Cox model with treatment as the covariate was conducted for each stratum, and the combined hazard ratio (HR) was obtained as (Neupogen versus Control) 0.31 (0.10, 0.99). Similar results for the HR is obtained using Cox model with treatment and Propensity score as the covariates (propensity approach---linear method).

All the exploratory analyses of survival indicated the advantage of using Neupogen instead of Vehicle (Control), with a small sample of 46 animals, with and without adjusting the baseline information.

In addition to exploratory survival analyses, ANC, platelets (PLT), transfusion, supportive care, and safety were explored.

- For all the 46 animals, Neupogen group had faster recovery from events ANC<500/uL, ANC<1000/uL, and PLT<20000/uL, compared with Control group. The relative rate of recovery for Neupogen vs. Control is 3 (95% CI as (1.3, 6.7)) for ANC<500/uL, 3 (95% CI as (1.4, 6.7)) for ANC<1000/uL, 2.4 (95% CI as (1.1, 5.4)) for PLT<20000/uL.

- The Neupogen group had shorter duration to recover from ANC<500/uL and ANC<1000/uL for the 31 recovered animals. The recovery duration is 14 days (95% CI as (13, 15)) in Neupogen group vs. 19 days (95% CI as (17, 20) in Control group for ANC<500/uL; and 16 days (95% CI as (14, 17) in Neupogen group vs. 21 days (95% CI as (19, 23) in Control group for ANC<1000/uL.

- Neupogen group had similar transfusion in terms of both volume and times compared with Control group.

- Safety endpoints include: activity over time, hemorrhage over time, histopatholoy results on bone marrow, lung, liver, small intestine, and large intestine. There is no significant finding on safety.

There is no data on supportive care within each subject in terms of degree of the care. This is one of the limitations of the study.

In conclusion, Neupogen is effective to improve the survival of the animals with total body irradiation (TBI). Neupogen is effective to improve the time to recovery from events ANC<500/uL, ANC<1000/uL, and PLT<20000/uL.

2 INTRODUCTION

2.1. Overview

2.1.1. Indication

Filgrastim (Neupogen) can be used for treatment of Hematological Syndrome of **Acute Radiation Syndrome** (ARS-HS) following a Total-body Irradiation (TBI), under the Animal Rule.

2.1.2. History of Program Development

The treatment of individuals exposed to lethal TBI in the event of a nuclear terrorist attack is of paramount concern to health professionals. Currently, there is no drug approved by the FDA for the treatment of the lethal hematopoietic syndrome of the acute radiation syndrome (AR8-HS).

Granulocyte colony-stimulating factor (G-CSF, 175 amino acids, 18.8 Kd) stimulates bone marrow neutrophil production and neutrophil progenitor proliferation. Neupogen® (Filgrastim) is a recombinant human G-CSF, which has the potential to increase the survival in the case of TBI.

On 5/23/05, there was a preIND meeting between FDA/Division of Biologic Products (DBOP) & NIAID-Amgen-U of MD. In that meeting,

- *NIAID outlined plan for a "pivotal" NHP & murine study*
- *Studies are intended to support g-CSF licensure under Animal Rule*

On 7/11/12, NIAID submitted the final study reports for:

- AXR01: "A pilot study to define the dose response curve in Rhesus macaque exposed to increasing doses of total body ionizing radiation and receiving supportive care."
- AXG15: "A sixty-day efficacy study of subcutaneous filgrastim (Neupogen) to treat the hematopoietic syndrome of the acute radiation syndrome (ARS-HS) following an Lethal Dose (LD) 50/60 of total body irradiation (TBI) in rhesus macaques."

On 12/14/2012, NIAID submitted the data for AXG 15 according to FDA's request.

NIAID (the sponsor) evaluated the efficacy of filgrastim (Neupogen) plus medical management in a lethal, NHP model of ARS-HS in a blinded study (AXG 15). The degree of lethality in the control cohort was established as an LD50/60 at 750 cGy, TBI. Neupogen had been approved for the treatment of cancer patients receiving myelosuppressive chemotherapy. The dose that was used herein reflects previous PK and PD analysis of filgrastim in rhesus macaques (summarized in pre-IND submission plND #100,228 serial 000) and was considered to be bioequivalent to the dose proposed for humans (5i.g/kg/d).

2.1.3. Specific Studies Reviewed

The key study is the non-human primate (NHP) study (AXG 15). All animals are exposed to a target of 750 cGy TBI (Total Body Irradiation) on Day 0. Beginning on Day 1, Neupogen or Vehicle (as Control) was administered daily to the animals until

1) Absolute neutrophil count (ANC) ≥ 1000/μL for 3 consecutive days;
or 2) ANC ≥ 10,000/μL for 2 consecutive days between Days 1-5;
or 3) anytime ANC ≥ 10,000/μL beginning on Day 6

Daily dosing was resumed at ANC < 500/μL and discontinued at ANC ≥ 1000/μL for 3 consecutive days

A summary of the study is shown in Table 1.

Table 1: Summary of the key studies in the review

Study number	Phase and Design	Treatment Period	Follow-up Period	# of Subjects per Arm	Study Population
AXG15	Animal study, Double blinded, randomized two arm study	Up to 60 days	60 days	24 for Neupogen arm and 22 for Control arm	Subjects with Total Body Irradiation (TBI)

2.1.4. Major Statistical issues

The chi-square test for the mortality counts in the primary analysis evaluates the difference in mortality rates and does not evaluate the survival pattern over time within 60 days. With the rejection of the chi-square test for the 2×2 table (treatment as row and mortality as column), we can only claim that the proportion of animals died during the 60-day period is associated with (not independent) treatment (Neupogen vs. Control). Therefore, approaches for evaluating survival patterns over time, such as logrank test for comparing the two survival curves by treatment, and Cox models with treatment as covariate for evaluating the effect of treatment and estimating the Hazard ratio of Neupogen vs. Control will provide more insight of the change of survival over time. However, this study is an atypical survival study in that animals that were seriously sick as defined by a pre-specified criteria were euthanized, and all animals alive at 60 days were also euthanized immediately there after.

Moreover, the assumption is that the double blind randomization process can avoid baseline imbalance in the treatment (Neupogen) group and the control group. But, with a small sample size, some baseline factors (such as gender, age, source, etc) may not be balanced and the possible effect of those baseline factors on survival pattern and other important parameters such as ANC, PLT, etc, in the study can not be evaluated.

There was no apparent difference in the distributions of the baseline variables by treatment. However, the distributions of the propensity scores (Probability of using Neupogen given baseline covariates gender, source, Group, and dose variables from a logistic model) by treatment were observed to be different by kolmogorov-smirnov test (non-parametric method). a Improvement in survival with Neupogen treatment was still observed with further exploration of this potential imbalance of the baseline information with various exploratory analyses including, Cox models with treatment and baseline variables as covariates, a propensity score approach (Cox model with only treatment by stratum, determined by propensity scores) with adjustment of baseline information.

The difference in safety for Neupogen compared with Control was not observed, however this evaluation is limited by small sample size.

There are no missing values in the major efficacy data (survival and baseline variables). However, there are missing values for some secondary measures (such as activity, hemorrhage, grading scales, posture, stool, etc), taken over time during the study. The sponsor imputed the missing values if the missing values are not missing at random and ignored the missing values if the missing values are treated as missing as random. Therefore, the analyses on the secondary endpoints such as activity over time, hemorrhage over time, and others may have bias because of the missing values.

2.2. Data Sources

The study reports are provided by scanned pdf files of the hard copy submission under IND 100228.

The sponsor submitted sas data sets and key programs (according to FDA request) at
\\cdsesub4\NONECTD\IND100228\5197897\pIND 100228 SN 0010
A statistical analysis plan is included in the AXG 15.pdf (version/date: version 1.00 october 8, 2007)

The sponsor submitted information on dosimetry for individual animals (according to FDA's request) at \\cdsesub4\NONECTD\IND100228\5226140

The sponsor published a paper based on the AXG 15 study results. The paper "**Filgrastim Improves Survival in Lethally Irradiated Nonhuman Primates** ", published by Radiation Research Society, can be found at URL: http://www.bioone.org/doi/full/10.1667/RR3049.1

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

The sponsor submitted the data for the animal study AXG 15 according the request from the Agency. The reviewer can reproduce the primary analysis using the data. Only partial variables for efficacy evaluation (death indicator, time to death, ANC, time of ANC, duration of neutropenia, day of ANC recovery, Febrile neutropenia, number of transfusions, etc) and baseline information (including Group, id, gender, etc) are included in the submitted electronic data sets.

The data sets for supportive care, safety, and partial baseline information (including source, dose, reason for death, etc) are in pdf files.

Data including information on supportive care within each subject (degree of support) is not available.

The reviewer entered the data in pdf files into sas data sets.

3.2 Evaluation of Efficacy

The reviewer's comments are in italics in this section.

3.2.1 Study Design and Endpoints

This study was designed to determine whether Neupogen (Amgen, Thousand Oaks, CA) administered as 10 ~g/kg/day subcutaneous (SC) injections starting on day 1 following a lethal total-body irradiation (TBI) exposure to a 6 megavolt (MV) computerized linear accelerator (L1NAC) photon source (at 750 cGy which represent approximate LD50) and administered to effect based on absolute neutrophil count, will improve survival in Rhesus macaques receiving medical management (intravenous (iV) fluids, blood products, nutrition, and antibiotics) compared to control animals receiving vehicle and medical management.

The test article is Neupogen (filgrastim) and the control article is sterile Dextrose 5% in Water (D5W).

Prior to irradiation, animals were randomized to either a control (n=22) or treated (n=24) cohort. On study day (SD) 0, rhesus macaques (n=6 to 10 per "Group", and each "Group" received the irradiation in one day) were exposed to a TBI of 750 ± 15 cGy delivered at 80 ± 3.0 cGy/min, using a 2 MV (average) photon beam from a clinical linear accelerator at 153 cm source to surface distance.

Beginning on day 1, animals were administered daily, 8C injections of either the control article (5% dextrose in water (D5W) (0. 154mLlkg/d)) or filgrastim (Neupogen) (10 ~g/kg/d), until

- 1) Absolute neutrophil count (ANC) ≥ 1000/μL for 3 consecutive days; OR

- 2) ANC ≥ 10,000/μL for 2 consecutive days between Days 1-5; OR
- 3) anytime ANC ≥ 10,000/μL after Day 6

Daily dosing was resumed at ANC < 500/μL and discontinued at ANC ≥ 1000/μL for 3 consecutive days. We defer to the clinical review on the adequacy of dosing.

Following TBI, all animals were monitored for complete blood counts, body weight and temperature, and hydration status for 60 days. Animals received medical management consisting of intravenous fluids, antibiotics, blood transfusions, and other support as required.

A specific set of criteria for **euthanasia** was applied by all veterinarians. Any NHP which was recumbent in the cage or had decreased or absent responsiveness to touch or experienced hemorrhage from the GI tract to be in excess of 20% of the estimated blood volume in any 24 hour period or it experienced unrelieved pain **was euthanized**.

Any NHP which experienced any combination of the following observations such as respiratory distress, decreased food and water intake, reluctance to move for >24 hours, and severe dehydration classified an animal to **be euthanized**.

Animals survived to the end of the experiment (>=60 days) had necropsy (sacrificed for a pathologic examination).

Primary Endpoint:

Overall survival measured at 60-days post randomization (Specified in study protocol)..

Secondary endpoints:

- Survival time of the decedents
- Cage side observations per day (morning and afternoon): activity, posture, stool consistency, vomit, hemorrhage, respiratory, alopecia.
- Hematopoietic recovery parameters:
    - ANC nadir
    - Duration of neutropenia (ANC<500/uL and < 100/uL),
    - Day of ANC recovery (ANC>500/uL and 1000/uL)
- Incidence of Febrile Neutropenia (FN) (ANC<500/uL and core body temperature >= 103 F)
- Platelet-related parameters (day of PLT recovery)
- Number of transfusions
- Body weights and temperatures
- Incidence and severity of diarrhea
- Significant organ pathology:
- Filgrastim immunogenicity

In addition to the primary endpoint, the following secondary endpoints are explored in this review:

- *Survival over time with and without adjustment for baseline information*
- *Mortality counts and rates*
- *Time to recovery from events ANC <100/uL (ANC 100), <500/uL (ANC 500), and <1000/uL (ANC 1000), PLT <20000 (PLT 20000) for all 46 animals*
- *Incidence of Febrile Neutropenia (FN)*
- *Number of transfusions and volume of transfusions*
- *Cage side observations on Activity and Hemorrhage*
- *Pathology on bone marrow, lung, liver, small intestine, and large intestine*

3.2.2 Statistical Methodologies

Primary analysis:

According to the sponsor, the primary analysis will be conducted on the ITT population using a chi square test of a one-tailed null hypothesis using an overall 5% significance level, to compare the overall survival measured at 60-days post randomization, i.e., mortality rate at 60 days between the treatment and the control groups

*Because of the small sample size, fisher exact test will also be used for evaluating the independence assumption in the 2*2 table in addition to chi-square test.*

Interim analysis:

An interim analysis for efficacy and futility will be conducted once: after the cohort associated with at least 50% of the monkeys are 60 days past irradiation. Formal efficacy analyses is based on the Lan-Demets version 1 of the O'Brien-Fleming boundary to provide an overall one-sided alpha = 0.05 test. Futility is assessed informally based on conditional power. For example, termination due to futility may be considered if conditional power is very low (e.g. less than 0.10), under the assumption that the hypothesized treatment difference is correct. In addition, if the first interim analysis indicates that the current study design needs significant modification, the study may be discontinued, and a new study will be initiated. The efficacy interim analysis is performed on unblinded data.

The sponsor conducted an interim analysis with 46 animals, and claimed that for an interim analysis with a fraction of 0.7419 (a cohort of 46/62 animals) information, the spending alpha is 0.0229. Thus, analyses of the primary outcome are considered significant if the resulting p-value is less than 0.0229. The power for the interim analysis is 0.876.

The alpha (two-sided) used in the interim analysis by O'Brien-Fleming is defined as alpha(t)=2-2 Φ(Z(0.05/2)/sqrt(t*)), where t* is the faction of information used (46/62=0.74 in this study), Z(0.05/2) is 1.96, and Φ denotes the standard normal cumulative distribution function (probnorm() in SAS). Alpha(0.75)=0.0229.*

Secondary analyses:

Summary descriptive statistics for secondary endpoints will be provided for all randomized animals by treatment group and by radiation dose. Continuous data (e.g., neutrophil count nadir, duration of ANC, etc.) will be summarized descriptively by mean, standard deviation, median, and range. Categorical data (e.g., incidence of infection, incidence of FN) will be presented as enumeratiöns and percentages.

Dichotomous outcomes will be examined using Fisher's exact or Chi-square tests. Continuous outcomes will be compared between treatment groups using Student's t-tests and those measured over time will be analyzed using mixed linear models. Transformations will be used when necessary to meet the underlying assumptions of the test or model.

Survival time will be estimated for each treatment using the Kaplan-Meier product limit method (KM). Survival curves will be compared using the Cox proportional hazards regression model.

Incidence of significant pathology in bone marrow, heart, lung, liver, spleen, kidney, thymus, mesenteric lymph node, and small and large intestine after necropsy will be summarized with descriptive statistics.

In addition to the Chi-square test for mortality, the sponsor conducted survival analyses using KM method and Cox model without adjustment of baseline information. Additional exploratory analyses were conducted by this reviewer: Cox survival models with adjustment for the baseline information and propensity score approach for adjusting baseline information. Two-sided 0.05 alpha was used in the review.

The propensity score approach includes

1. *two-strata method (with 50 percentile of PS as cut-off point and two strata) which allows the checking of the baseline balance by strata, and uses a non-parametric method (K-M method) to obtain the final results incorporating the baseline information*
2. *Cox model with treatment plus PS as covariates(linear method) is a semi-parametric approach to incorporate the baseline information*
3. *Selection of animals using PS matching process, then comparing the survival patterns of Neupogen group vs. Control group for the selected animals.This leads to sample size reduction, which may reduce the power for efficacy evaluation in the study*

Missing data:

Laboratory values will be measured over time but missing data will occur for animals that die. This is informative missing data rather than data that are missing at random. Analyses of laboratory values over time will attempt to account for the missing data and in those cases where

it is not addressed, the analyses will be interpreted cautiously. Trajectories of the laboratory values by treatment type will be examined graphically and a variety of statistical models will be considered based on the distribution, pattern and missing data configuration of the observed data.

Additional missing data may occur due to other causes (e.g., non-analyzable sample; inability to obtain sample) but these are more likely to be non-informative. The pattern of the missing data will be examined to determine if it can be considered to be missing completely at random (MCAR), missing at random (MAR), or non-ignorable. Non-analyzable samples are likely to be MCAR and unobtainable samples may be related to available data and thus considered to be MAR.

The information on baseline variables and survival is complete. The missing values in the secondary analyses may be imputed or removed according to the sponsor's pre-specified criteria.

3.2.3 Subject Disposition, Demographic and Baseline Characteristics

No missing values are observed for the baseline variables.

As shown in Table 2, in this study, there are more males than females (all eight females are from one source: Three Springs Science), more animals from Rhenollc in the Neupogen group, varying number of animals in Neupogen and Control groups by "Group". Animals in "Group" one received the TBI first, and in one day. A total of six days were used.

Table 2: number of animals by gender, source, and "Group" in Neupogen group and Control group.

	Gender		Source		total
	Female	Male	Rhenollc	Three Springs Sci	
Control	4(ThreeS)	18	10(M)	12	22
Neupogen	4(ThreeS)	20	17(M)	7	24

	Group						
	1	2	3	4	5	6	total
Control	4	6	1	5	4	2	22
Neupogen	6	1	5	4	4	4	24

There are some medical measures taken on Day 0 (the day of irradiation) for each animal. The summary is presented in Table 3. It seems that the mean values for Lymphoctyes and Platelets are slightly different (Neupogen higher), but the variances are also large.

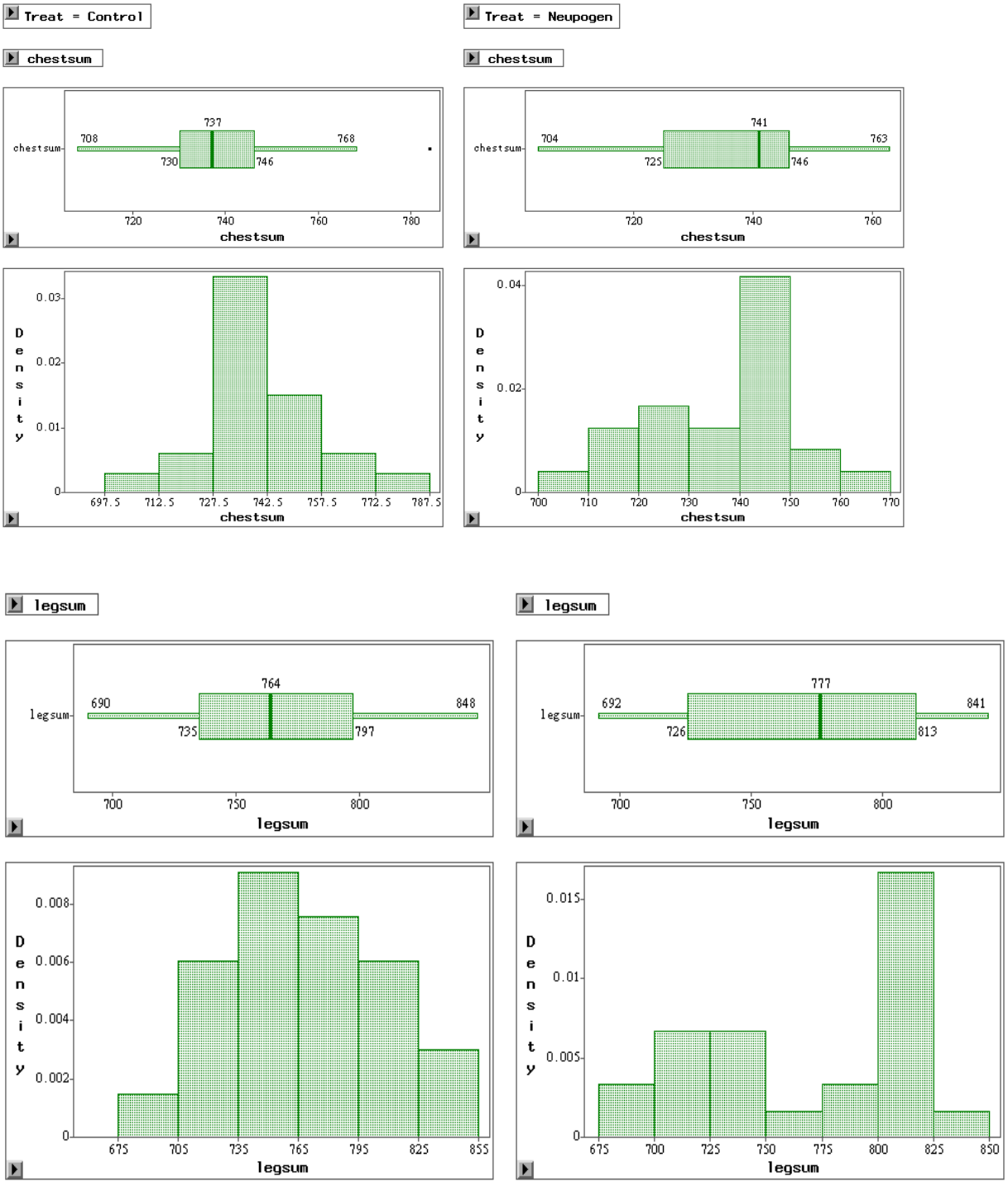
Table 3: Summary of measures taken at baseline (day of irradiation)

variable	description	Neupogen mean	std	Control mean	std
ALC	Absolute lymphocyte count	4.9	1.6	4.7	2.2
ANC	Absolute neutrophil count	2.5	1.5	2.8	1
BAND	Band neutrophils	0	0	0	0
BASO	Basophils	0.5	0.8	0.6	0.7
cells	Number of cells on which differential was based	100	0	100	0
HCT	Hematocrit	39.6	2.6	39.5	2.7
HGB	Hemoglobin	13.3	0.9	13.2	0.9
IMMAT	Immature white blood cells	0	0	0	0
LYMPH	**Lymphoctyes**	64.3	14.5	58.4	12
MNC	Mononuclear cells	5.1	1.6	4.9	2.3
MONO	Monocytes	2.2	1.2	2.8	1.8
NRBC	Nucleated red clood cells	0	0	0	0
PLT	**Platelets**	372.6	69.5	360	65
RBC	Red blood cells	5.3	0.5	5.3	0.4
SEG	Segmented neutrophils	32.1	14.9	36.4	10.8
Temp	Animal's temperature on date of blood draw in degress F	101.9	0.7	102	0.7
WBC	White blood cells	7.7	2.1	7.9	2.7
weight	Animal's weight in Kg on Dya 0 (irradiation day)	5.5	0.6	5.8	0.6

Chestsum is the total irradiation dose around the chest area, and the legsum is the total irradiation dose for legs. The mean(std) for chestsum is 736 cGy (15) for Neupogen group, and 740 cGy (16) for Control group. The mean(std) for legsum is 769 cGy(48.3) for Neupogen group, and 765 cGy(43) for Control group.

We notice there are some differences in the histograms and box plots (Figure 1).

Figure 1: Distributions of doses (chest and leg) by treatment



3.2.4 Results and Conclusions

Mortality comparison

The sponsor used Chi-square test for evaluating the overall survival measured at 60-days post randomization as the primary analysis. At the interim analysis, a Chi-square test indicates there is a significant association between mortality (survival) and treatment (one-sided p-value = 0.0079/2 = 0.0040). Therefore, the sponsor terminated the study with 46 animals instead of 62 animals.

In the report, the sponsor stated that this study determined that Neupogen significantly (p=0.004) improved overall survival (i.e. mortality rate) 60 days after radiation exposure of rhesus macaques receiving medical management (intravenous (iV) fluids, blood products, nutrition, and antibiotics) compared to animals receiving Control Article and the same medical management.

The reviewer obtained consistent results on the Chi-square test (two-sided p=0.0079), and the test is for testing the independence of treatment and outcome (death or not during 60-day period).

A fisher exact test (non-parametric) is more conservative for evaluating the independence in the 2*2 table. And a two-sided p-value of 0.0147 is obtained from the exact test.

There are 59% of death in Control group and 21% death in Neupogen group. The expected count for death (E) is 8.6 for control group, and the observed death is 13 (>8.6). Neupopgen group have observed death as 5 (< expected count 9.4).

Table 4: Summary of count by treatment and outcome (Death or not)

	Not death	Death	Total
Control	9	13 (E=8.6): 59%	22
Neupogen	19	5 (E=9.4): 21%	24
Total	28	18	46

The following analyses are exploratory analyses. The results are consistent with sponsor's findings.

Euthanized animals

Three animal died during the study period (days<60) are found dead (natural death with survival time as 11 days, 17days, and 18 days), and are all in Control group.

During the 60 day study period, fifteen animals are euthanized (n=10 control + 5 Neupogen).

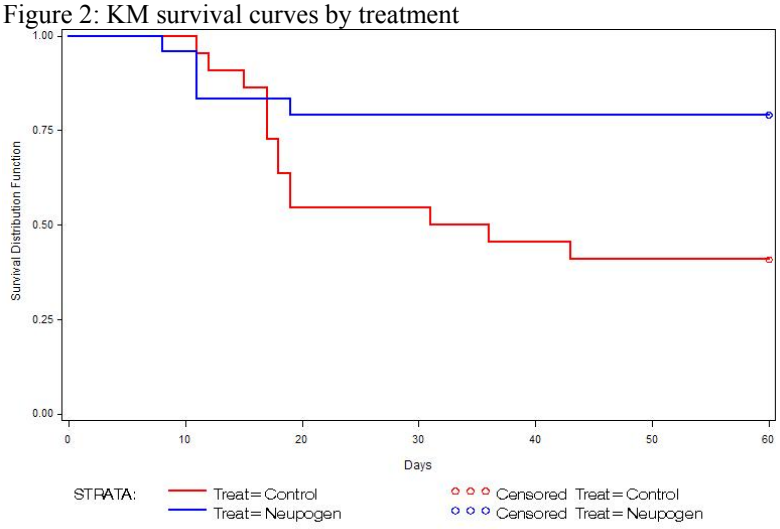
The other animals (46-15-3=28) survived to the end of the experiment (>=60 days) and was sacrificed for a pathologic examination (Necropsy).

All 3 found dead animals had bone marrow, lung, Small intestine, liver abnormal. 2 found dead animals had large intestine abnormal, from histopathology.

Survival analyses

First, we explored the survival pattern in the following figure (Figure 2). Kaplan Meier method (KM) is used to estimate the survival rates by treatment over time. Note that there is a crossing of the two curves in the early period. A few animals with Neupogen died relatively earlier (euthaniasia) than animals with control (4 in Neupogen vs. 1 in Control died within 11 days). After Day 11, only one Neupogen animal died on Day 19, and twelve Control animals died after Day 11. Log-rank test for comparing the two survival curves resulted in a nominal p-value of 0.018 in favor of Neupogen group. The following figure is excerpted from the study report.

Figure 2: KM survival curves by treatment



Cox models with and without adjustment baseline information

Without adjusting for any baseline information, Cox model with only treatment has Hazard Ratio (HR) (Neupogen vs. Control) as 0.31 (95% CI as [0.11, 0.88]).

In Cox model with treatment, gender, source, group, chest dose, and leg dose, the HR (Neupogen vs. Control) is 0.25 with 95% CI as (0.08, 0.77).

Propensity score approach is considered for adjusting the baseline information in the Cox survival models. We fist obtain the estimated probability of (treatment as 1, using Neupogen) by

the logistic regression model (Logit(treatment)=gender+source+group+chest dose+leg dose+error), which is the propensity score (PS).

The histograms of the PS by treatment are presented in Figure 3 with smoothed normal and kernel distribution curves. The distributions of Neupogen group and Control group are different, and KS test (non-parametric) for testing the equality of two distributions had nominal p-value as 0.0034. Therefore, it is necessary to adjust the imbalance of the baseline information by treatment.

The results from several propensity score approaches (PS two-strata method (main propensity score analysis), PS linear method, and Cox model with PS matching animals), are presented in Table 5. The PS distributions by treatment in each stratum (50% as the cut-off point) are similar with KS nominal p-value >0.05. Also note that the 28 PS matching animals have very similar PS distribution (Figure 4) with KS test nominal p-value as 0.9.

Most of the methods presented in Table 5 have the 95% CIs of HR (Neupogen vs. Control) below 1. The method with 28 PS matching animals has HR=0.27 (95% CI as (0.06, 1.29)). The baseline information is balanced very well for the 28 PS matching animals (KS test nominal pvalue as 0.9), but the 95% confidence interval for HR include 1, which may be due to the small sample size.

Figure 3: Histogram of propensity score (PS) by treatment with normal and kernel distribution curves (for the 46 animals)

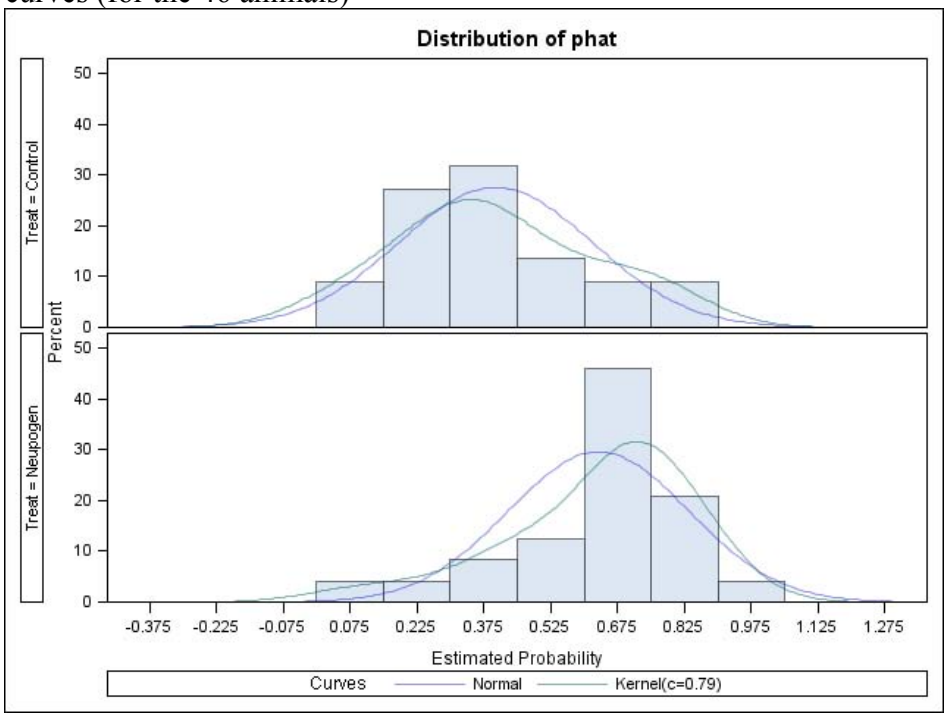


Figure 4: Histogram of propensity score (PS) by treatment with normal and kernel distribution curves (for the 28 matching animals with PS value difference less than 20% of the logit of the 46 PS values)

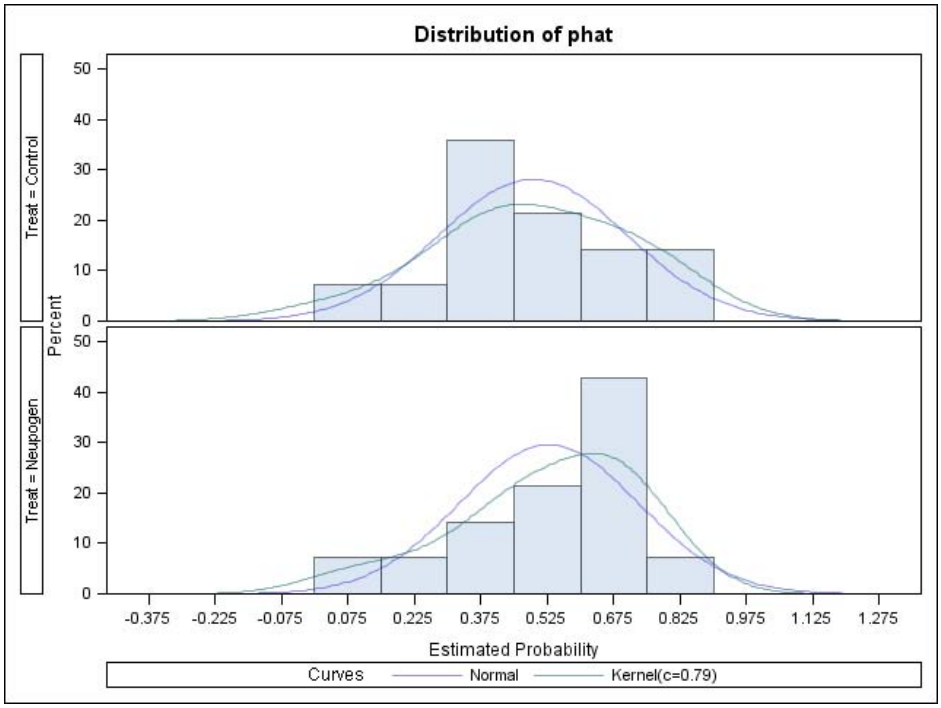


Table 5: Hazard ratio (HR) and 95% confidence intervals (CIs) for treatment effect from different Cox survival models

	HR (Neupogen vs. Control)	95% CI
Cox model with only treatment	0.31	(0.11, 0.88)
Cox model with treatment, 2 strata (PS two-strata method – main analysis)	0.31	(0.10, 0.99)
Cox model with treatment + PS from logistic model (PS linear method – supportive analysis)	0.26	(0.08, 0.80)
Cox model with treatment plus gender, source, group, chest dose and leg dose (supportive analysis)	0.25	(0.08, 0.77)
PS matching animals with Cox model with treatment (tertiary analyses)		
Match with <35% std of logit of PS, with 36 matching subjects (KS test pvalue=0.13)	0.34	(0.11, 1.06)
Match with <20% std of logit of PS, with 28 matching subjects (KS test pvalue=0.90)	0.27	(0.06, 1.29)

Absolute Neutrophil Count (ANC) and Platelets (PLT)

ANC and PLT counts are recorded every day (60 values per animal per variable). The duration of ANC recovery is defined to be the time from events ANC <100/uL, or <500/uL, or <1000/uL, to ANC ≥100/uL, or ≥500/uL, or ≥1000/uL for three consecutive days. The duration of recovery for PLT is defined in a similar way in this review. The sponsor had very complicated definition for recovery from event PLT <20000/uL by considering the possible effect of transfusion on PLT level (not used in this review).

The start dates of the events and the duration of recovery for the animal recovered (31/46 animals) were compared. Survival analyses were used to evaluate the time to recovery from the events. 15/46 animals were dead before a recovery from the events, the observed time for those animals from the start date of the event to death is considered as censoring time (assuming random censoring). We also consider that animals died will not recover from events, so we impute the censoring time by the study duration 60 days – start date of the event,

The following results on start date and duration of recovery, obtained by the reviewer are consistent with the sponsor's finding.

Comparison of the start date of the events:

First, the start times of the events were compared and the results are shown in Table 6. The start date for animals with Neupogen had the events usually earlier than those with Control (half day difference in mean), especially for ANC<500/uL event and ANC<1000/uL event.

Table 6: Start date of event and 95% confidence interval (CI) by treatment

event	Comparison of start date of event	
	Neupogen mean (95% CI) in days (n=24)	Control mean (95% CI) in days (n=22)
ANC <100/uL	6.5 (6.0, 7.1)	7.1 (6.5, 7.7)
ANC<500/uL	**4.3 (4.1, 4.6)**	**4.9 (4.5, 5.2)**
ANC<1000/uL	**4.0(3.8, 4.1)**	**4.6 (4.3, 5.0)**
PLT<20000/uL	9.3 (9.0, 9.6)	9.7 (9.3, 10.1)

Comparison of duration of recovery for recovered animals

Even with earlier onset of the ANC and PLT events, the 31 recovered animals with Neupogen had shorter duration for recovery, especially for ANC<500/uL and ANC<1000/uL (Table 7). The recovery duration is 14 days (95% CI as (13, 15)) in Neupogen group vs. 19 days (95% CI as (17, 20) in Control group for ANC<500/uL; and 16 days (95% CI as (14, 17) in Neupogen group vs. 21 days (95% CI as (19, 23) in Control group for ANC<1000/uL.

Table 7: Duration of recovery and 95% confidence interval (CI) from ANC and PLT drops by treatment for recovered animals

event	Duration from onset of event to recovery (95% CI)	
	Neupogen mean (n=19)	Control mean (n=12)
ANC <100/uL	10.4 (9.1, 11.6)	12.3 (10.9, 13.6)
ANC<500/uL	**14.3 (13.1, 15.4)**	**18.6 (16.9, 20.2)**
ANC<1000/uL	**15.7 (14.4, 16.9)**	**21.4 (19.4, 23.4)**
PLT<20000/uL	10.5 (6.8, 14.2)	15.3 (10.7, 19.8)

Survival analyses on the time to recovery of the events, with and without imputation on the censoring time

The results on time to recovery by treatment is presented with imputation (censoring time is imputed by 60 days minus the start date of the related event). As shown in Table 8, the survival curves (or recovery curves) with x-axis as the days, y-axis as the survival rate (1-recovery rate) for Control group are always higher than the ones for Neupogen group with , for all the events (ANC<100/uL, ANC<500/uL, ANC<1000/uL, and PLT<20000/uL). This indicates that Control group had lower recovery rates over time compared with Neupogen group.

The results from Cox models with and without adjustment for the baseline information are presented in Table 9. The censoring time is imputed by 60 days minus the start date of the related event. Note that the HR here is the relative rate of recovery. For ANC<500/uL and ANC<1000/uL, the relative rate of recovery (HR) values for Neupogen vs. Control are ranged from 2 to 5 (with 95% CIs above 1), which indicates that the chance of recovery for Neupogen group is 2-5 times of the Control group. The difference between Neupogen and Control groups are getting bigger from ANC<100/uL to ANC<1000/uL.

For event PLT<20000/uL, the HR (Neupogen vs. Control) values ranged from 2.5 to 5.5 with all 95% CIs above 1. Note that PLT level is affected the blood transfusions and we have found that the Control group received similar transfusion without adjusting for survival time, and more transfusion with adjusting for survival time, compared with Neupogen group. Even with possible more transfusion, Control group still needs more time to recovery from PLT <20000/uL event.

Results without imputed censoring time assuming random censoring in the Cox survival models are presented in Appendix Tables A1 and A2. The results with and without imputation for the censoring time are consistent.

Table 8: KM curves and nominal pvalues from logrank test for the two curves. Consider informative censoring and the censoring time is imputed by 60 days – the start date of the related event

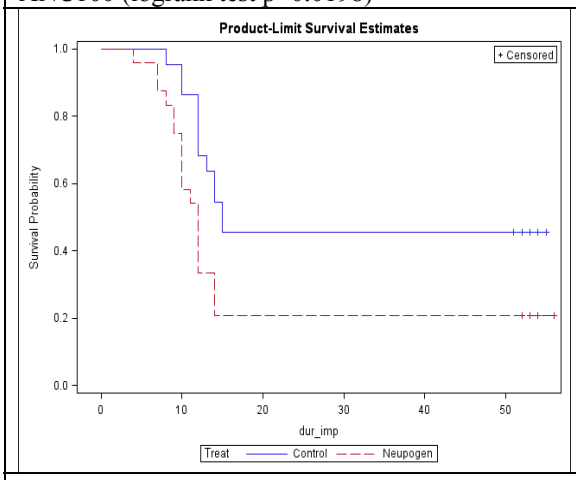
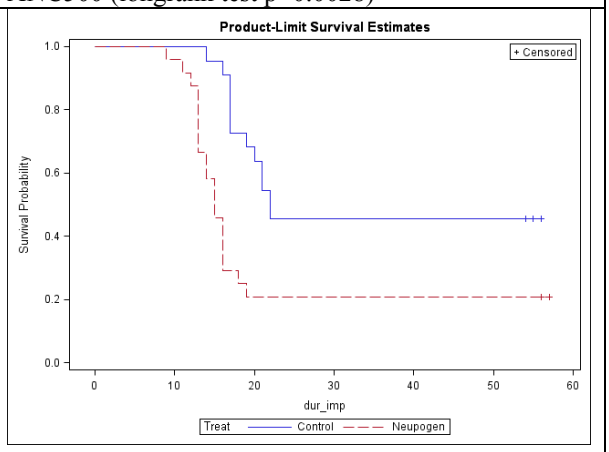
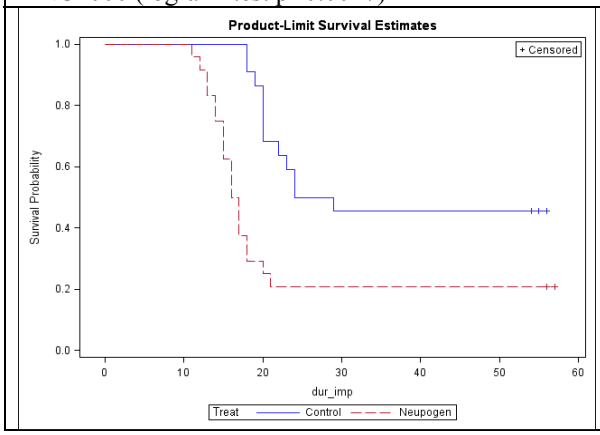
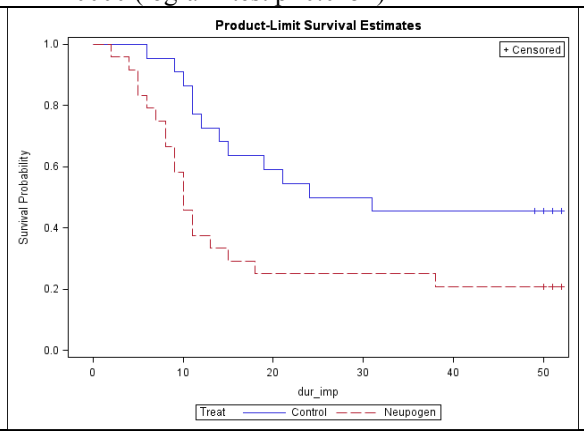
ANC100 (logrank test p=0.0198)	ANC500 (longrank test p=0.0028)
	
ANC1000 (logrank test p=0.0017)	**PLT 20000 (logrank test p=0.0151)**
	

Table 9: Cox models with and without adjustment of baseline information. The Hazard ratio (HR) is relative rate of recovery for this case. Censoring time is imputed with 60 days- the start date of the related event. In addition to treatment, baseline covariates considered are gender, source, group, doses (chestsum and legsum).

Models	HR (Neupogen vs. Control)	95% CI
Event as ANC<100/uL		
Cox model with treatment and covariates	2.34	(0.99,5.58)
Cox model with only treatment	**2.19**	**(1.06,4.53)**
Cox model with treatment + PS from logistic model (PS-linear method)	2.35	(1.03,5.37)
PS-2 strata method (Cox model)	**1.97**	**(0.88,4.42)**
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with	2.20	(0.97,4.98)

36 matching subjects (KSp=0.13)		
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	2.44	(1.01,5.89)
Event as ANC<500/uL		
Cox model with treatment and covariates	4.52	(1.82,11.25)
Cox model with only treatment	**2.83**	**(1.36,5.91)**
Cox model with treatment + PS from logistic model (PS-linear method)	3.51	(1.46,8.49)
PS-2 strata method (Cox model)	**2.97**	**(1.31,6.71)**
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	2.97	(1.29,6.81)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	3.49	(1.42,8.58)
Event as ANC<1000/uL		
Cox model with treatment and covariates	5.26	(2.03,13.62)
Cox model with only treatment	**2.98**	**(1.43,6.22)**
Cox model with treatment + PS from logistic model (PS-linear method)	3.50	(1.46,8.41)
PS-2 strata method (Cox model)	**3.05**	**(1.35,6.89)**
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	2.88	(1.26,6.59)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	3.37	(1.38,8.24)
Event as PLT < 20000/uL		
Cox model with treatment and covariates	4.51	(1.79,11.37)
Cox model with only treatment	**2.36**	**(1.14,4.90)**
Cox model with treatment + PS from logistic model (PS-linear method)	2.72	(1.17,6.33)
PS-2 strata method (Cox model)	**2.41**	**(1.07,5.43)**
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	2.31	(1.02,5.23)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	2.94	(1.22,7.13)

Febrile neutropenia (FN)

An animal was considered to have Febrile neutropenia (FN) when the core body temperature was ≥103 degree F and ANC<500/uL. Animals could have some days with FN and some days without FN during the study. Table 10 summarized the number of animals with at least FN for one day during the study.

The counts with imputation are presented in brackets ( ). If an animal died within 60 days, and did not have FN, then this animal was considered to have FN event and is counted as one FN animal.

Majority of the animals in both treatment groups had FN during the 60 day study period. The occurrence of FN is not associated with the treatment

Table 10: Summary of animals with Febrile Neutropenia with and without imputation

	No FN	FN	Total
Control	2 (1)	20 (21)	22
Neupogen	5 (4)	19 (20)	24
Total	7 (5)	39 (41)	46

Supportive care

The number of animals (by treatment) receiving antibiotics, anti-fungal and antibiotics, hydration, blood product, and other medications are summarized by the sponsor. The numbers are similar in Neupogen and Control groups (results not shown).

There is no data with information on supportive care within each subject. The degree of supportive care for each animal may be different, which will affect the survival. This is one limitation of the data.

Transfusion

Animals could have transfusions several times during the study. The observed volume values per transfusion are 18mL, 27mL, and 54mL for the study animals.

Transfusion volume and number of transfusions by treatment were compared, without adjusting for the varying survival days and with adjustment for the survival days.

As shown in Table 11, there is no difference in transfusion between Neupogen and Control groups, without adjusting for the survival days. Notice that the life time of the animals affects the transfusions. After adjusting for survival times, Neupogen group had slightly less transfusion in terms of volume (2mL/day for Neupogen vs. 4mL/day for Control) and number of transfusion (0.05/day for Neupogen vs. 0.9/day for Control) compared with Control group.

Note that the patterns of transfusion for recovered animals and non-recovered animals, and for animals before recovery from an event and after recovery, may be different. This, as a possible confounded factor, is not considered in the above analysis. The difference identified for the Neupogen and Control groups with adjustment may be due to the different patterns of recovery.

Table 11: Summary of transfusion by treatment

Comparison	Volume (mL) mean and 95% CI		Number of transfusions (times), mean and 95% CI	
	Neupogen N=24	Control N=22	Neupogen N=24	Control N=22
Without adjusting survival days	96 (67.2, 125.5)	130 (91.2, 168.9)	2 (1.4, 2.8)	3 (2, 3.5)
Adjust different survival days by using vol/days and #/days	**2 (1.3, 3.1)**	**4 (2.9, 5.3)**	**0.05 (0.03, 0.07)**	**0.09 (0.06, 0.11)**

3.3 Evaluation of Safety

Note that safety evaluation is considered as secondary or exploratory analyses by the sponsor.

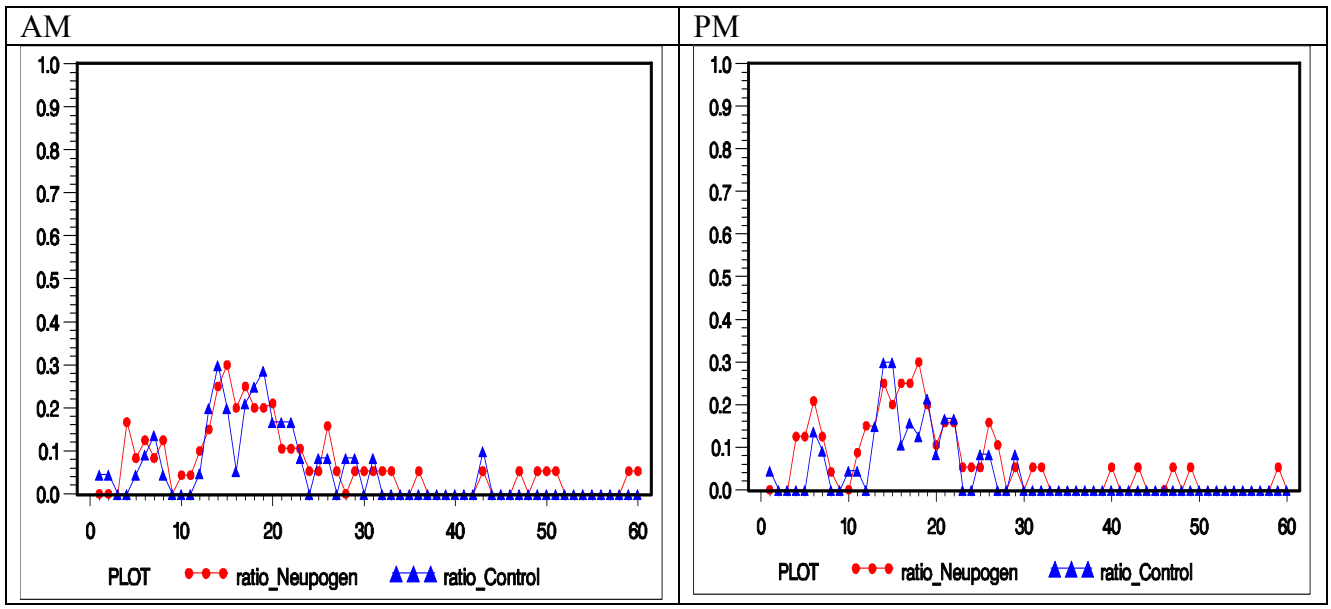
Activity over time

Activity is considered as a safety endpoint in this study. The values of the activity variable is 0 for normal activity, 1 for limited activity, and 2 for recumbent or no activity. The activity values were recorded every day and for AM and PM. Some missing values were imputed by the sponsor using the values close to the missing value. Longitudinal mixed models could be used to evaluate the activity over time by treatment. In this review, we only present the trend plot for the ratio of animals with abnormal activities (values 1 and 2).

The ratio is defined to be the number of animal having abnormal activity on day t over the number of animals still alive on day t-1. The range of the ratio values is from 0 to about 30%.

From Figure 5, there is no difference between Neupogen and Control groups. The patterns are similar to AM and PM activities. There are two peaks of abnormal activities: 0-10 day, 10-25 day. After day 25-30, both groups did not have many abnormal activity cases.

Figure 5: percent of animals with AM/PM abnormal activities (limited or recumbent or no activity) over time (days)



Hemorrhage over time

Hemorrhage over time is considered as a safety endpoint in this study. There are 4 scores for Hemorrhage:

- 0=no blood in cage
- 1=individual blood spots in case (<=10 spots)
- 2=coalescing blood or >10 spots in cage
- 3=estimated to be in excess of 20% of blood volume, life-threatening

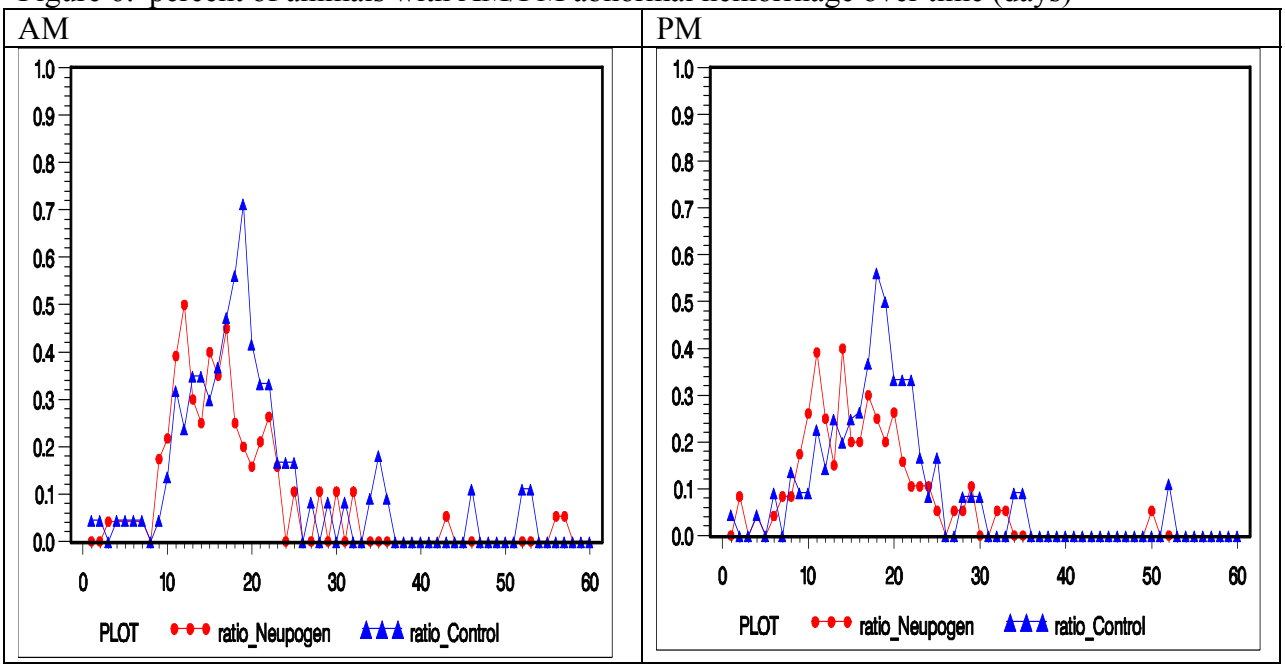
We define 0 as normal, and 1, 2, and 3 as abnormal hemorrhage.

The hemorrhage values were recorded every day and for AM and PM. There are only several missing values and are easily imputed with close values. In this review, we only present the trend plot for the ratio of animals with abnormal hemorrhage.

The ratio is defined to be the number of animal having abnormal hemorrhage on day t over the number of animals still alive on day t-1. The range of the ratio values is from 0 to about 70%.

As shown in Figure 6, from Day 5 to 15, Neupogen group had slightly higher ratio of abnormal Hemorrage. From Day 15 to 25, Control group had slightly higher ratio of abnormal Hemorrhage. After Day 25-30, both treatment groups did not have many abnormal hemorrage cases for the animals still alive. The trends are similar in the two treatment groups and for AM and PM.

Figure 6: percent of animals with AM/PM abnormal hemorrhage over time (days)



Histopathology Evaluation

Histopathology summary (considered as safety evaluation) was obtained for Bone marrow, liver, heart, lung, thymus, spleen, MLN, skin, kidney, small intestine, and large intestine. Results for selected organs are presented in Table 12.

Neupogen group had less animals with abnormal Bone marrow, lung and liver; slightly more animals with abnormal small and large intestine.

The patterns may also be different for animals died during the 60 days study period, and for animals survived >=60 days and had necropsy (postmortem examination) because of the termination of the study (Day 60 through Day 68). This confounding factor is not considered in the analysis and may lead to some bias.

Table 12: Safety evaluation from Histopathology on selected organ (p-value is obtained from fisher exact test)

		Abnormal count and percent (95% CI in %)			
Organ	define abnormal cases	Neupogen		Control	
Bone marrow	1-Mild-moderate depletion, 2-Moderate-severe 3-depletion, Hemorrage	5/24=21%	(7, 42)	11/22=50%	(28, 72)
Lung	Bacterial emboli, Hemorrhagic foci, inflammatory foci, Edema, Fibrosis	7/24=29%	(13, 51)	11/22=50%	(28, 72)
Liver	multiple bacterial emboli, focal hepatocellular necrosis, inflammatory foci, non-specific eosinophilia	2/24=8%	(1, 27)	6/22=27%	(11, 50)
Small intestine	inflammation, dilated lymphatics, lymphangiectasia, hyperplasia, villous fusion, bacteria, hemorrhage, autolysis, necrosis, nematode	21/23=91%	(72, 99)	19/22=86%	(65, 97)
Large intestine	inflammation, dilated lymphatics, Protozoa, hyperplasia, bacteria, hemorrhage	13/24=54%	(33, 74)	7/22=32%	(14, 55)

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

Because of the small sample size (24 Neupogen + 22 Control), we did not evaluate the survival pattern by subgroups. Some important factors (gender, source, "Group") are incorporated in the multivariate modeling process as independent variables for exploration.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

Data issue

The sponsor submitted the data for the animal study AXG 15 according to the request from the Agency. The reviewer could reproduce the primary analysis using the data. Only partial variables for efficacy evaluation (death indicator, time to death, ANC, time of ANC, duration of neutropenia, day of ANC recovery, Febrile neutropenia, number of tranfusions, etc) and baseline information (including Group, id, gender, etc) are included in the submitted electronic data sets. The data sets for supportive care, safety, and partial baseline information (including source, dose,

reason for death, etc) are in pdf files. Data including information on supportive care within each subject (degree of support) is not available.

Primary analysis issue

The primary endpoint specified by the sponsor is overall survival measured at 60-days post randomization. However, the primary endpoint in reality was mortality rate at 60 days evaluated using the chi-square test. With the rejection of the hypothesis that there is no association between the treatment and mortality during 60-day period, using a Chi-squared test for the 2×2 table (treatment as row and mortality as column), we can only claim that the proportion of animals died in 60 days is associated with (not independent) treatment (Neupogen vs. Control). Therefore, approaches for evaluating survival patterns over time, such as logrank test for comparing the two survival curves by treatment, and Cox models with treatment as covariate for evaluating the effect of treatment and estimating the Hazard ratio of Neupogen vs. Control will provide more insight of the change of survival over time. However, this study is an atypical survival study in that animals that were seriously sick as defined by a pre-specified criteria were euthanized, and all animals alive at 60 days were also euthanized immediately there after.

Small sample size and imbalance of baseline information issue

With the small sample size (46 animals), it is difficult to evaluate the balance of the baseline factors (such as gender, source, "Group", etc) and the possible effect of those factors on survival and other important parameters (e.g., ANC, PLT, etc) in the study. The assumption is that the double blind randomization process can avoid baseline imbalance in the treatment (Neupogen) group and the control group. However, with a small sample size, some baseline factors can not be balanced. In addition, the difference in the distribution of the baseline variables by treatment may not be shown to be significant with the small sample size (not powered to show the difference).

Even though the individual baseline variables are not shown to be different, the distribution of the propensity scores (Probability of using Neupogen given some baseline covariates) is shown to be different by kolmogorov-smirnov test (non-parametric method). Therefore, it is necessary to explore the imbalance of the baseline information. A propensity score approach is proposed in this review, to evaluate the survival patterns of the Neupogen vs. Control to adjust for the baseline information.

Also, no difference in safety for Neupogen compared with Control was observed. More data should be collected for evaluating the safety by treatment in future studies.

Missing data issue:

There are no missing values in the major efficacy data (survival and baseline variables). However, there are missing values for some secondary measures (such as activity, hemorrhage,

grading scales, posture, stool, etc), taken over time during the study. The sponsor imputed the missing values if the missing values are not missing at random and ignored the missing values if the missing values are treated as missing as random. Therefore, the analyses on the secondary endpoints such as activity over time, hemorrhage over time, and others may have bias because of the missing values.

5.2 Collective Evidence

After Total Body Irradiation, mortality (natural death or euthanized cases) was significantly decreased in the Neupogen group (5/24=21%) compared to the vehicle control group (13/22=59%). The p-value from Chi-square test for evaluating the association between treatment and mortality is 0.0079. A fisher exact test (more proper for data with small sample size) for this evaluation provided a p-value of 0.0147. The sponsor used an early stopping rule and stopped the study with the 46 animals because the p-value (one-sided p-value 0.0079/2=0.0004) was less than 0.0229 (alpha allocated for interim analysis).

In addition to the primary analysis, supportive analyses were conducted to evaluate the survival over time during the 60-day study period (overall survival). The Logrank test for the two Kaplan Meier (KM) survival curves (Neupogen vs. Control) had a nominal p-value of 0.018, in favor of Neupogen. The Neupogen group had Hazard ratio (HR) 0.31 (95% CI as [0.11, 0.88]) compared with Control group, from Cox model with only treatment as the covariate.
Even though the study is a blinded randomized study, because of small sample size, the baseline information may not be quite balanced in this study. Exploratory analyses with adjustment of baseline information were also conducted. A propensity scoring approach (two-strata method) was used to adjust the baseline information. The propensity scores were obtained from a logistic regression model with treatment as dependent variable, and gender, source, group, and dose variables (chestsum and legsum) as covariates. The 46 animals were grouped into two strata using the propensity scores (cut-off point is 50 percentile). Cox model with treatment as the covariate was conducted for each stratum, and the combined hazard ratio (HR) was obtained as (Neupogen versus Control) 0.31 (0.10, 0.99). Similar results for the HR is obtained using Cox model with treatment and Propensity score as the covariates (propensity approach---linear method).

All the exploratory analyses of survival indicated the advantage of using Neupogen instead of Vehicle (Control), with a small sample of 46 animals, with and without adjusting the baseline information.

In addition to exploratory survival analyses, ANC, platelets (PLT), transfusion, supportive care, and safety were explored.

- For all the 46 animals, Neupogen group had faster recovery from events ANC<500/uL, ANC<1000/uL, and PLT<20000/uL, compared with Control group. The relative rate of recovery for Neupogen vs. Control is 3 (95% CI as (1.3, 6.7)) for ANC<500/uL, 3 (95% CI as (1.4, 6.7)) for ANC<1000/uL, 2.4 (95% CI as (1.1, 5.4)) for PLT<20000/uL.

- The Neupogen group had shorter duration to recover from ANC<500/uL and ANC<1000/uL for the 31 recovered animals. The recovery duration is 14 days (95% CI as (13, 15)) in Neupogen group vs. 19 days (95% CI as (17, 20) in Control group for ANC<500/uL; and 16 days (95% CI as (14, 17) in Neupogen group vs. 21 days (95% CI as (19, 23) in Control group for ANC<1000/uL.

- Neupogen group had similar transfusion in terms of both volume and times compared with Control group.

- Safety endpoints include: activity over time, hemorrhage over time, histopatholoy results on bone marrow, lung, liver, small intestine, and large intestine. There is no significant finding on safety.

There is no data on supportive care within each subject in terms of degree of the care. This is one of the limitations of the study.

5.3 Conclusions and Recommendations

The results from the propensity score analysis and all the supportive analyses conducted by the statistical reviewer showed consistent effect of Neupogen. In conclusion, Neupogen is effective to improve the survival or decrease mortality rate of the animals exposed to total body irradiation (TBI). Neupogen is effective to improve the time to recovery from events ANC<500/uL, ANC<1000/uL, and PLT<20000/uL.

6 APPENDICES

Table A1. KM curves and nominal pvalues from logrank test for the two curves. Consider random censoring and the censoring time is the observed dead date – the start date of the related event

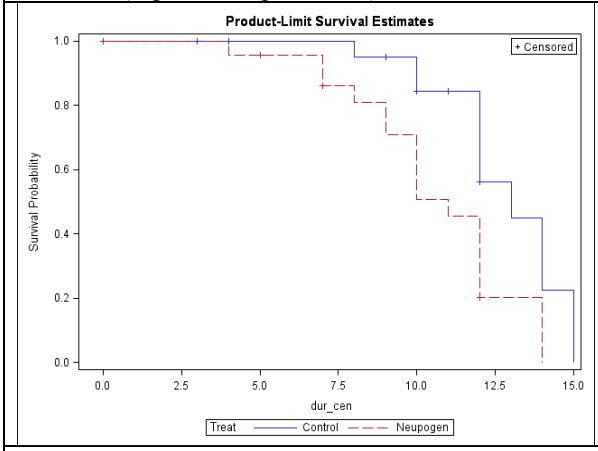
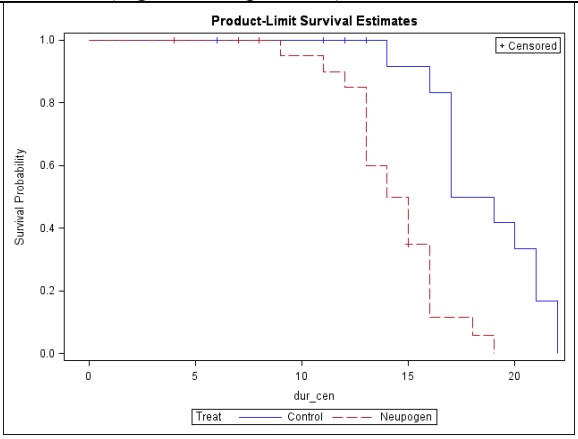
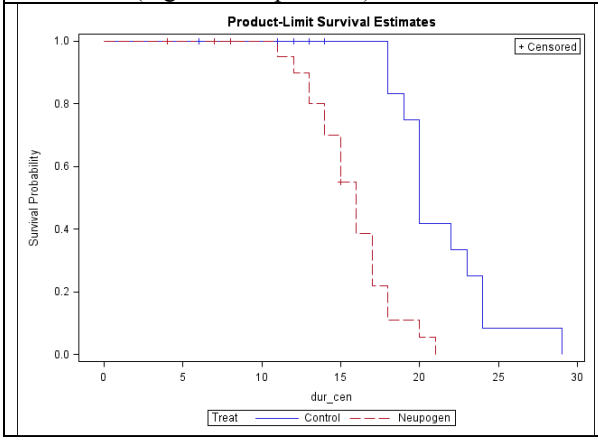
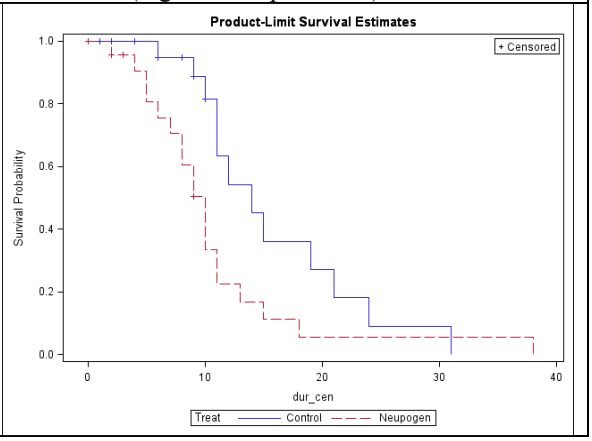
ANC100 (logrank test p =0.0062)	ANC500 (logrank test p<.0001)
	
ANC1000 (logrank test p<.0001)	**PLT 20000 (logrank test p=0.0274)**
	

Table A2: Cox models with and without adjustment of baseline information. Censoring time is not imputed (death date – the start date of the related event)

	HR (Neupogen vs. Control)	95% CI
Event as ANC<100/uL		
Cox model with treatment and covariates	3.09	(1.14,8.39)
Cox model with only treatment	2.41	(1.12,5.20)
Cox model with treatment + PS from logistic model (PS-linear method)	2.53	(1.11,5.78)
PS-2 strata method (Cox model)	1.89	(0.79,4.51)
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	2.84	(1.18,6.80)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	3.45	(1.33,8.96)
Event as ANC < 500/uL		
Cox model with treatment and covariates	11.24	(3.2,39.45)
Cox model with only treatment	5.21	(2.09,12.96)
Cox model with treatment + PS from logistic model (PS-linear method)	5.99	(2.14,16.71)
PS-2 strata method (Cox model)	4.40	(1.68,11.50)
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	10.24	(3.08,34.07)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	11.69	(3.06,44.75)
Event as ANC<1000/uL		
Cox model with treatment and covariates	9.99	(2.85,35.05)
Cox model with only treatment	5.65	(2.28,13.99)
Cox model with treatment + PS from logistic model (PS-linear method)	5.14	(1.90,13.94)
PS-2 strata method (Cox model)	4.51	(1.73,11.80)
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	7.13	(2.39,21.23)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	8.18	(2.44,27.38)
Event as PLT <20000/uL		
Cox model with treatment and covariates	5.12	(1.81,14.48)
Cox model with only treatment	2.19	(1.04,4.58)
Cox model with treatment + PS from logistic model (PS-linear method)	2.30	(1.01,5.27)
PS-2 strata method (Cox model)	2.56	(1.09,6.03)
PS matching, then Cox model = treatment for only matching subjects		
35% std of logit of PS criteria for matching, with 36 matching subjects (KSp=0.13)	2.30	(1.0,5.30)
20% std of logit of PS criteria for matching, with 28 matching subjects (KSp=0.90)	5.50	(1.88,16.07)

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/s/

LAN HUANG
04/01/2013

JYOTI ZALKIKAR
04/01/2013
I concur with the primary reviewer.

RAJESHWARI SRIDHARA
04/01/2013

Summary of Effect of G-CSF and GM-CSF on Hem-ARS in Published Literature

by Yanli Ouyang, PhD
FDA/CDER/OND/Division of Medical Imaging Products
March 22, 2013

Animal Studies

In this section, we summarize the radioprotective effect of granulocyte colony stimulating factor (G-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF) on the hematopoietic acute radiation syndrome (hem-ARS) in published literature in public domains. Databases searched included Embase, Google Scholar, PubMed, and Web of Science. English journal articles regarding effects of G-CSF or GM-CSF on radiation sickness, irradiation sickness, or hem-ARS in animals during recent 30 years were identified. We focus our review on the effect on survival and WBC recovery in studies conducted in NHPs, dogs and mice, because survival and WBC recovery are primary efficacy endpoints and the studies were predominately conducted in these species. We separately summarize G-CSF and GM-CSF data below.

G-CSF

Introduction

Radioprotective effect of G-CSF on hem-ARS was predominately evaluated in NHPs (rhesus), dogs (beagle) and mice (various strains). Studies mainly used recombinant human (rh) G-CSF because rhG-CSF has biologic activity in all species evaluated. Neupogen (filgrastim from Amgen) was the rhG-CSF used in most of the NHPs and dogs studies, with a few studies using pegylated rhG-CSF (Neulasta, pegfilgrastim from Amgen) and other sources of G-CSF. The results we reviewed demonstrated that rhG-CSF consistently enhanced survival in canine and mouse models[1] and/or WBC recovery in all animal species and strains studied regardless of sources of radiation (gamma, x-ray, and mixed neutron and gamma)(1-27).

Collectively, the results of published literature support the survival and WBC recovery benefit of rhG-CSF on hem-ARS. However, the radioprotective effect was dependent on factors such as radiation dose, G-CSF dose, treatment initiation time, and treatment duration. We further discuss below the survival and WBC recovery benefits of rhG-CSF on hem-ARS and the main factors affecting such benefits.

rhG-CSF enhanced survival in canine and mouse models

With the exception of the NHP study under IND 100228, the survival studies were conducted in dogs and mice only. The survival benefit of rhG-CSF treatment was consistently demonstrated in a radiation dose- and rhG-CSF dose-related manner. For

[1] The NIP study based on IND 100228 was excluded in this section because the results were discussed in other sections.

example, in a dog study, dose reduction factor (DRF) was established at 1.73 when compared to controls without supportive care or 1.34 when compared to controls with supportive care (Figure 1) (9). DRF (also referred to as dose modification factor, DMF) was established at 1.06 to 1.2 in mice (Figure 2)(6, 17, 25).

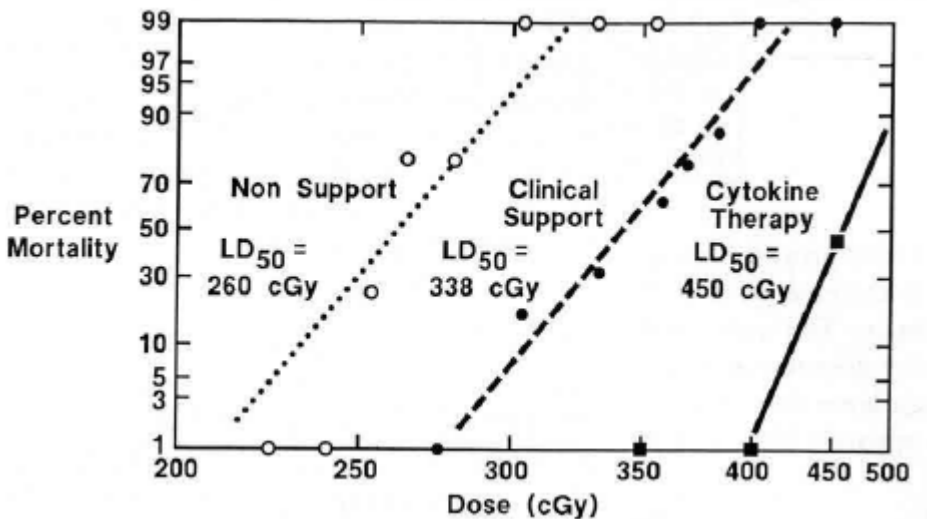

Figure 1. rhG-CSF enhanced survival ($LD_{50/60}$) of ^{60}Co irradiated beagle dogs. -○- represented dogs without supportive care, -●- dogs with supportive care (fluids, antibiotics, and fresh irradiated platelet transfusions) only, -■- dogs with supportive care plus rhG-CSF [10 mcg/kg/day, subcutaneous (SC), daily for 21days starting on Day 1 post TBI]. The figure is adapted from (9).

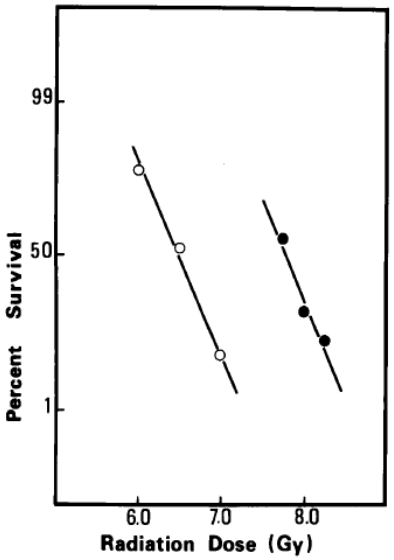

Figure 2. rhG-CSF enhanced survival of X-irradiated ICR-MCH male mice. ○ represented mice in the control group ($LD_{50/30}$, 6.5 Gy) ● mice in the rhG-CSF group [2.25 mcg/mouse (36-40g), ip, BID for 14 days starting on Day 1 post TBI ($LD_{50/30}$, 7.8 Gy)]. DRF was 1.2. The figure is adapted from (6).

We identified only one mouse study in which no survival benefit was demonstrated (26). However, only a single intraperitoneal (ip) rhG-CSF (up to 2.0 mcg/mouse) was administered at 1 or 3 h after 800 cGy ($LD_{95/30}$ dose) ^{60}Co total body irradiation (TBI). In

the same study, single recombinant murine (rm) GM-CSF treatment did not demonstrate survival benefit as well but rhIL-1, rmIFN-gamma, and rhTNF did.

rhG-CSF enhanced WBC recovery

rhG-CSF enhanced WBC recovery in all animal species and strains studied. The effects were measured mainly as decreased duration of neutropenia (a few days), decreased time to absolute neutrophil count (ANC) recovery (a few days), Improved ANC nadir, increased WBC counts, and increased granulocyte/macrophage colony-forming units (GM-CFU) in bone marrow. Table 1 is used as an example to illustrate such effects.

Table 1. Filgrastim or Pegfilgrastim enhanced Neutrophil Recovery in Total-Body X-Irradiated Rhesus Macaques*

Treatment	ANC nadir (μL)	Duration (day) ANC < 100/μL	Duration (day) ANC < 500/μL	Time to recovery (day) 500/μL	Time to recovery (day) 2,000/μL	Antibiotic support (day)
Control, 0.1%AS	19.0 ± 5	9.4 ± 0.7	19.4 ± 1.8	24.4 ± 1.8	28.3 ± 2.6	20.9 ± 0.9
Filgrastim, qd	53.0 ± 17	2.8 ± 0.8[a]	10.5 ± 1.3[a]	16.8 ± 0.9[a]	18.3 ± 0.9[a]	14.3 ± 0.6[a]
Peg-filgrastim, day 1	104 ± 17[a]	1.7 ± 0.7[a]	14.6 ± 1.7[b]	20.0 ± 1.2	30.0 ± 4.2	14.7 ± 1.5[a]
Peg-filgrastim, day 1 and day 7	244 ± 101[a]	1.9 ± 0.7[a]	5.3 ± 1.6[a,c]	10.2 ± 2.6[a,c]	18.1 ± 1.7[a,d]	8.1 ± 2.2[a]

* Monkeys were exposed to 6 Gy X-ray TBI, then subcutaneously administered control protein [0.1% autologous serum (AS), n=10], filgrastim at 10 mcg/kg/d (n=4) until the ANC ≥ 2,000/mcL, pegfilgrastim at 300 mcg/kg (n=9) on Day 1 post TBI, or pegfilgrastim at 300 mcg/kg on Days 1 and 7 post TBI (n=9). Data represented mean values±SEM.
[a] Statistically different from the AS-treated controls ($P < 0.01$).
[b] Statistically different from the AS-treated controls ($P < 0.05$).
[c] Statistically different from pegfilgrastim, day 1 only group ($P < 0.01$).
[d] Statistically different from pegfilgrastim, day 1 only group ($P \leq 0.05$).
The table is adapted from (27).

Factors affecting the radioprotective effect of G-CSF

As aforementioned, factors affecting the radioprotective effect of G-CSF included radiation dose, G-CSF dose, treatment initiation time, treatment duration, and mouse strains and sources. Such factors are further discussed below.

Radiation Dose

The effect of rhG-CSF on survival was related to radiation doses (9, 20; 21). rhG-CSF significantly enhanced the 30 day survival rates of mice receiving TBI at 850 cGy (83.3% vs. 44.0% in controls) or 950 cGy (45.8% vs. 0%) but not the survival at 1050 cGy (6.7% vs. 0%)(
Figure 3) using the same treatment regimen (20). Similarly, rhG-CSF treatment reduced $LD_{100/60}$ to $LD_{0/60}$ in dogs receiving 400 cGy while no survival benefit was noted when dogs receiving 600 cGy (9).

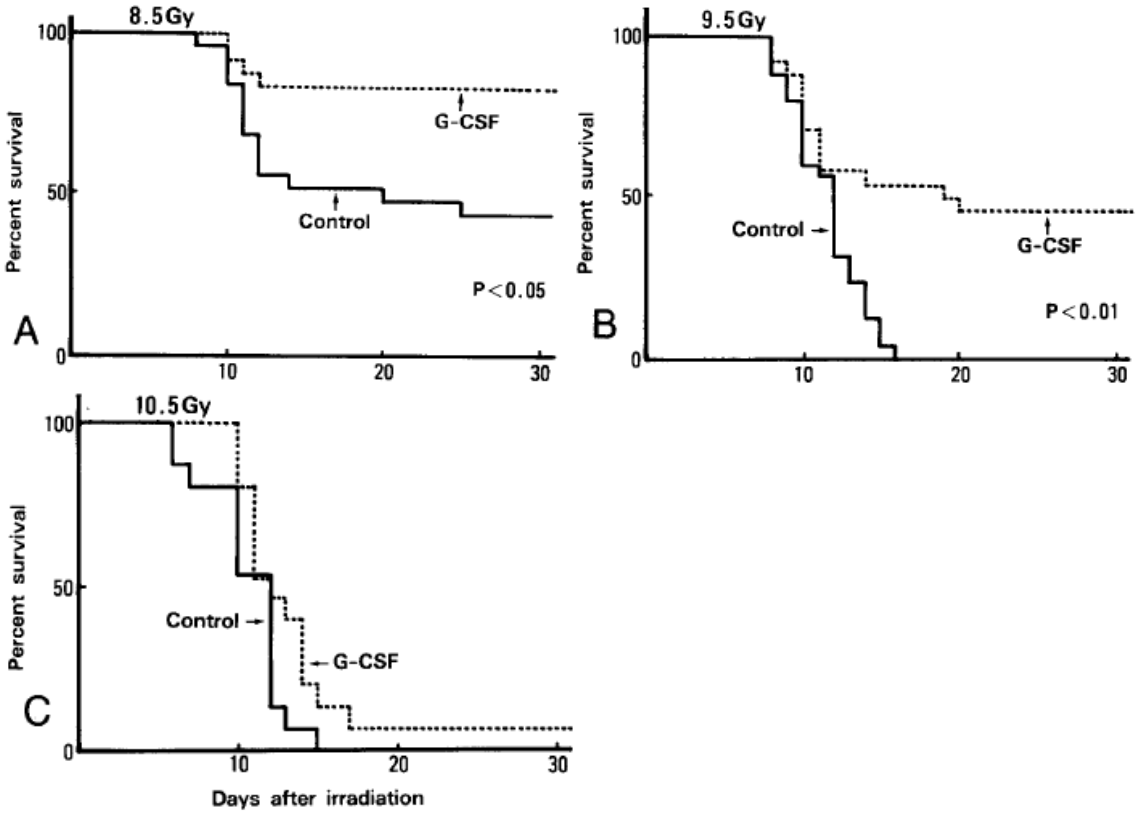

Figure 3. rhG-CSF enhanced 30 day survival in mice in a radiation dose-related manner. Male BDF1 mice were ip administered either saline (control) or rhG-CSF (1.0 mcg/dose/mouse, BID) from Day 0 to Day 6 post TBI at 8.5 Gy (A), 9.5 Gy (B), or 10.5 Gy (C). The 30 day survivals rates were 44.0% (11 of 25), 0% (0 of 25), or 0% (0 of 15), respectively, in control mice receiving TBI at 8.5 Gy (A), 9.5 Gy (B), or 10.5 Gy (C) while the rates were 83.3% (20 of 24), 45.8% (11 of 24), or 6.7% (1 of 15), respectively, in rhG-CSF-treated mice. The figure is adapted from (20).

rhG-CSF Dose

The effect of rhG-CSF on survival was related to rhG-CSF dose (Figure 5A)(21). At 2,000 mcg/kg, rhG-CSF treatment increased survival in BALB/c mice receiving 700 cGy ($LD_{100/30}$ dose) with an estimated 30 day overall survival probability 62% ± 9%. At 200 mcg/kg, the probability was 11% ± 7% only.

rhG-CSF dose used in NHP or dog studies was 10 mcg/kg/day in general while the dose in mice was variable but mainly 2-2.5 mcg/mouse/day.

rhG-CSF Treatment Initiation Time

rhG-CSF was administered at 1h to 24h post TBI in the majority of studies. In general, rhG-CSF treatment was more effective with earlier administration post TBI. However, rhG-CSF still enhanced survival or WBC recovery, even though to a lesser extent, when rhG-CSF was administered at later time points post TBI. For example, a dog study demonstrated that delayed administration of rhG-CSF on Day 9 post $LD_{100/60}$ TBI (400

cGy) was as effective as on Day1. The 60 day survival rates were 100% in the rhG-CSF treatment group that started receiving rhG-CSF on either Day 1 or Day 9 (9). The survival rates were 60% when rhG-CSF was administered on Day 12 and 50% on Day 15.

In a mouse study, rhG-CSF enhanced survival when single dose rhG-CSF at 1 mg/kg was administered at 2h or 24h post an $LD_{95/30}$ TBI; the survival benefit was diminished when administered at 48h or 72h post TBI (Figure 4)(18).

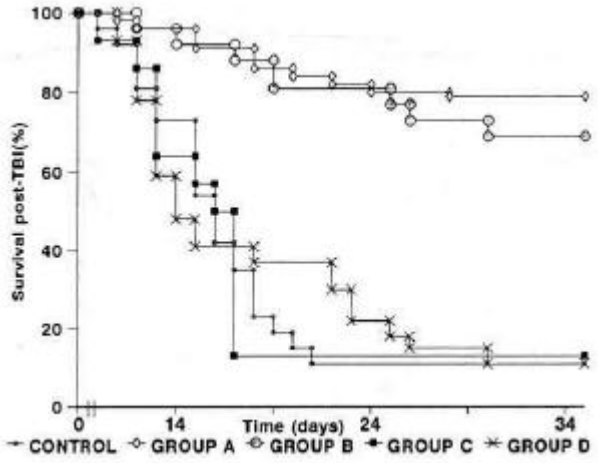

Figure 4. rhG-CSF enhanced survival in mice in a treatment-initiation-time-related manner. Single dose rhG-CSF (from Amgen) was ip injected at 1 mg/kg at 2 (Group A, 46 mice), 24 (Group B, 27 mice), 48 (Group C, 20 mice) or 72 (Group D, 26 mice) hours post $LD_{95/30}$ TBI in female B6D2F1 mice. The figure is adapted from (18).

Duration of rhG-CSF Treatment

The effect of rhG-CSF was related to treatment duration. As illustrated in Table 1, although pegfilgrastim enhanced WBC recovery when administered to monkeys once on Day 1 post 6 Gy TBI, the effect was more profound when administered twice on Days 1 and 7 (27). For example, mean duration of ANC < 500/mcL was 14.6 days with single administration of pegfilgrastim while the duration was reduced to 5.3 days with two administrations.

In most NHP or dog studies, rhG-CSF was subcutaneously administered post TBI for 14 to 24 days (mainly 21 days) and until WBC ≥ 1000 or 2000/mcL.

In mouse studies, shorter duration of treatment with G-CSF or single dose regimen was used. The effect of rhG-CSF was also related to treatment duration. For example, when rhG-CSF was administered at 2 mcg/mouse/day from Days 0-6 post 7.5 Gy TBI, mean WBC counts were 192/mcL on Day 14 (vs. 95/mcL in controls) while rhG-CSF was administered from Days 0-13, the count was 388/mcL (20). The survival rates were 57%, 70%, and 95%, respectively, for G-CSF (2.5 mcg/mouse/day) treatment duration

as 10 (Days 3-12), 11 (Days 1-12), 12 (Days 0-12) days, while the rate was 27% for saline controls with 8.0 Gy TBI (13).

Animal Strains and Sources

Of interest, a study revealed that the effect of rhG-CSF on survival was related to animal strain and source (21). The optimum radioprotective dose of rhG-CSF displayed a pronounced strain and source variation although all mice were matched for age, weight, and sex (Figure 5). Specifically, the optimal radioprotective dose of rhG-CSF was 2,000 mcg/kg for BALB/c mice while the dose was 200 mcg/kg for C3H mice (both strains from the breeding facilities of NIH, Bethesda, MD). rhG-CSF at the optimal radioprotective doses significantly enhanced the survival of BALB/c mice while rhG-CSF only marginally enhanced the survival of C3H mice.

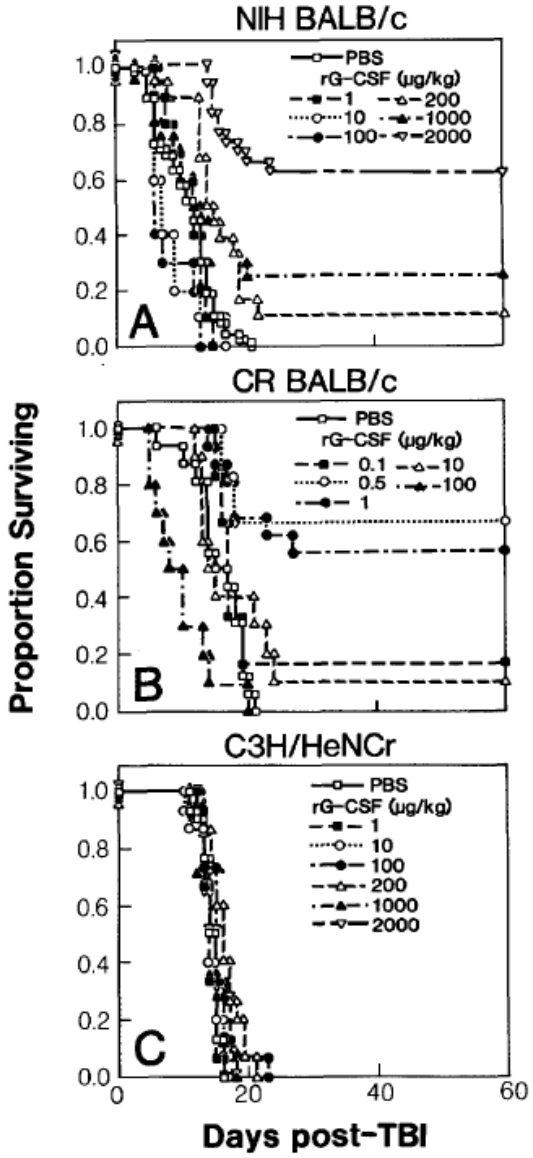

Figure 5. The effect of rhG-CSF (from Amgen) on survival was related to strains and sources in mice receiving $LD_{100/30}$ ^{137}Cs TBI (700 cGy for NIH BALB/c, 650 cGy for CR BALB/c, and 800 cGy for C3H/HcNCr). Survival data were presented as the Kaplan-Meier estimated survival curves. N=86, 16 or 30 in PBS groups for NIH BALB/c, CR BALB/c, or C3H/HcNCr mice, respectively. N=10-29, 6-10 or 15 in rhG-CSF groups for NIH BALB/c, CR BALB/c, or C3H/HcNCr mice, respectively. rhG-CSF doses were labeled in figures. Half of indicated doses were administered subcutaneously and the other half was administered intraperitoneally. Half of the total dose was administered 24 hours before TBI and the other half 30 minutes before TBI. NIH BALB/c: mice from the breeding facilities of NIH, Bethesda, MD; CR BALB/c: mice from Charles River, Inc, Wilmington, DE. The figure is adapted from (21).

As aforementioned, the effect of rhG-CSF on survival was related to animal source (21). The optimal radioprotective dose of rhG-CSF was 2,000 mcg/kg for BALB/c mice from the breeding facilities of NIH (Bethesda, MD, NIH BALB/c) while the dose was 1 mcg/kg for BALB/c mice from Charles River, Inc. (Wilmington, DE, CR BALB/c). The estimated 30 day overall survival probability was comparable for BALB/c mice from both sources at their respective optimal doses [62% ± 9% (18/29) for NIH BALB/c mice receiving 2,000 mcg/kg rhG-CSF vs. 67% ± 19% (9/16) for CR BALB/c mice receiving 1.0 mcg/kg rhG-CSF].

Limitations in Using Published Literature to Support the Efficacy of G-CSF

The limitations in using published literature to support the efficacy of G-CSF are discussed below. Such limitations included inadequate animal PK study for animal-human dose conversion; difficulty in verifying data accuracy, integrity, and adequacy of data presentation, and interpretation; and study design deficiencies.

Inadequate animal PK study for animal-human dose conversion

A PK study was rarely conducted in animal studies reviewed. In a NHP study, a PK study was conducted in 2 animals only, which is not an adequate sample size for calculating PK parameters (27). In addition, there was no detailed information regarding analytical methods and method validations. These deficiencies limited use of scarcely available PK data for animal-human dose conversion.

However, extensive human G-CSF dose regimen information is available. Furthermore, G-CSF treatment is individualized and titrated based on ANC in clinical practice. Therefore, lacking adequate animal PK data should not preclude human dose selection for treatment of hem-ARS.

Difficulty in verifying data accuracy and integrity, and adequacy of data presentation and interpretation

All studies conducted were not in compliance with GLP. Therefore, data accuracy and integrity in the studies can not be verified. Furthermore, because of nature of publications, there was no sufficiently detailed information to verify the adequacy of data presentation and interpretation. However, considering the consistent trends of all publications, collectively, the data are considered credible enough to support the benefit of G-CSF on treatment of hem-ARS.

Study design deficiencies

There are some significant deficiencies regarding study designs. Small sample size (n=2-4) in NHP and dog studies was a common issue. Some studies did not have concurrent controls. Additionally, there was insufficient information provided regarding supportive care and euthanized criteria in majority of publications. These deficiencies limited the usefulness of some publications.

Conclusion

Despite the limitations of published literature, collectively, the results of published literature support the survival and WBC recovery benefit of rhG-CSF on hem-ARS regardless of sources of radiation. However, such radioprotective effects were related to factors such as radiation dose, G-CSF dose, treatment initiation time, and treatment duration.

GM-CSF

Introduction

Radioprotective effect of GM-CSF on hem-ARS was also mainly evaluated in NHPs (rhesus), dogs, and mice. Because of species specificity, rhGM-CSF was used in NHP studies. Both rhGM-CSF and recombinant canine (rc) GM-CSF were used in dog studies. rhGM-CSF has no biologic activity in mouse, therefore, rmGM-CSF was used in mouse studies. Although leukine was used in a few studies, sources of GM-CSF used were more diverse than sources of G-CSF used.

The results demonstrated that GM-CSF enhanced survival in some but not in all studies. GM-CSF enhanced WBC recovery in most studies. Collectively, the results of published literature support the WBC recovery benefit of GM-CSF on hem-ARS. The survival data reviewed appear less consistent for GM-CSF. The survival and WBC recovery benefit of GM-CSF on hem-ARS were further discussed below.

GM-CSF enhanced survival in some animal studies but not in some other studies

The survival studies were conducted in NHPs (rhesus), dogs (beagle) and mice. The survival benefit of GM-CSF treatment was demonstrated in some studies but not in some other studies. For example, in a dog study, dose reduction factor (DRF) was established at 1.73 when compared to controls without supportive care or 1.34 when compared to controls with supportive care (Figure 6)(9). In this dog study, rhGM-CSF (from Immunex, parent company of leukine) was subcutaneously administered at 50 mcg/kg, BID (100 mcg/kg/day) for 21 days beginning on Day 1 after TBI. The rhGM-CSF treatment shifted $LD_{50/60}$ to 450 cGy from 338 cGy.

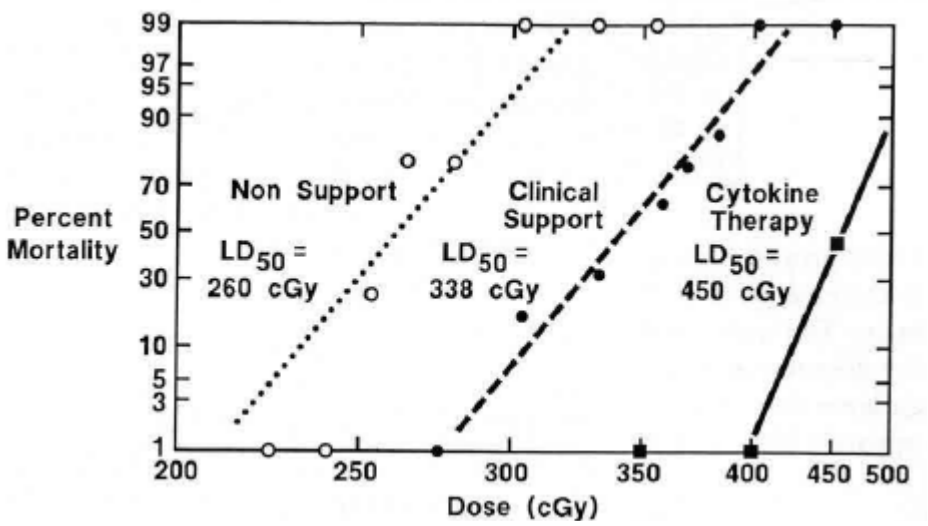

Figure 6. rhGM-CSF enhanced survival ($LD_{50/60}$) of ^{60}Co irradiated beagle dogs. -○- represented dogs without supportive care, -●- dogs with supportive care (fluids, antibiotics and fresh irradiated platelet transfusions), -■- dogs with supportive care and rhGM-CSF [50 mcg/kg, BID (100 mcg/kg/day), SC, daily for 24 days starting on Day 1 post TBI]. The figure is adapted from (9).

rmGM-CSF (from Immunex) treatment also enhanced the survival in a mouse study (24). In this study, mice received 10 Gy TBI followed by allogeneic transplantation. rmGM-CSF was intraperitoneally administered at 200 ng/mouse, BID for 14 days. rmGM-CSF enhanced survival as compared with that of control mice (P=0.05) (Figure 7).

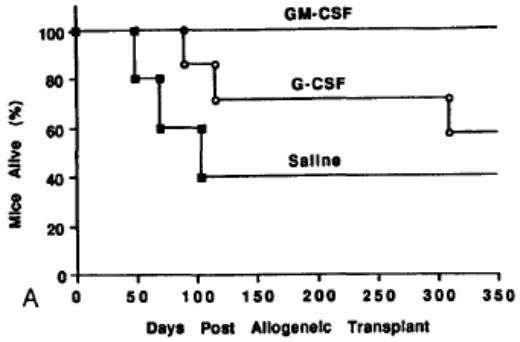

Figure 7. rmGM-CSF enhanced survival of BALB/c mice. The mice received 10 Gy ^{60}Co TBI, followed by 10^7 bone marrow and 10^6 spleen cells from C57BL/6 mice. rmGM-CSF at 200 ng/mouse or rhG-CSF at 100 ng/mouse was intraperitoneally administered twice daily from the day of transplantation to 14 days posttransplantation. The figure is adapted from (24).

However, the survival benefit of GM-CSF was not consistently demonstrated. For example, the survival rate was similar between the rcGM-CSF-treated group (1/10) and non-rcGM-CSF-treated group (1/13) in a dog study (Figure 8)(23). In this study, dogs received 400 cGy ^{60}Co TBI. Within 2 hours of TBI, rcGM-CSF (from Amgen) was subcutaneously administered at a dose of 50 mcg/kg BID for 5 doses and then

continued at 25 mcg/kg BID for 21 days or until death. Nine dogs were dead between Days 11 to 21. The causes of death were reported as pneumonia (n=7) or sepsis (n=2). rcGM-CSF did not enhance the survival or the recovery of neutrophil as evidenced by no difference in survival rate or neutrophil counts between the rcGM-CSF-treated group and non-rcGM-CSF-treated group. The ineffectiveness was not due to rcGM-CSF itself because the same rcGM-CSF (50 mcg/kg/d for 14 days, SC) increased neutrophil counts (3.0 to 9.3 times the baseline counts) in five non-irradiated dogs. In the same study, rcG-CSF enhanced the survival; four of five dogs receiving rcG-CSF (from Amgen) at 10 mcg/kg/d for 21 days after 400 cGy TBI survived (Figure 8).

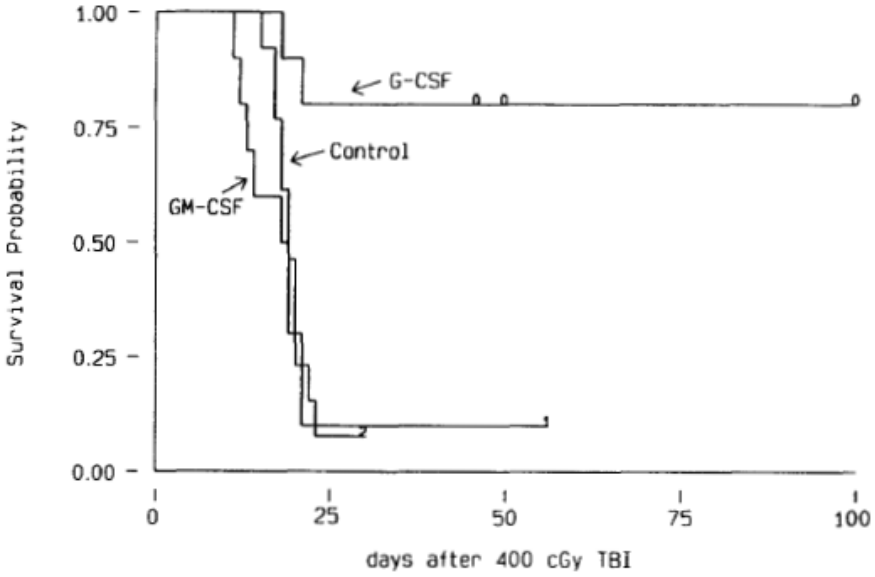

Figure 8. Kaplan-Meier survival curves of dogs in control, rcGM-CSF, or rcG-CSF groups after 400 cGy ^{60}Co TBI. Within 2 hours of TBI, rcGM-CSF was subcutaneously administered at a dose of 50 mcg/kg BID for 5 doses and then continued at 25 mcg/kg BID for 21 days or until death. rcG-CSF was subcutaneously administered at a dose of 10 mcg/kg/d for 21 days or until death. The figure is adapted from (23).

Similarly, in a male NHP study, the survival rate was similar between the rhGM-CSF-treated and non-rhGM-CSF-treated groups (28). Four of five animals survived beyond 30 days in both rhGM-CSF-treated and non-rhGM-CSF-treated groups. The dogs received tibiae shielded irradiation (mean midtissue dose 425 cGy, ^{60}Co) and supportive care (antibiotics and platelet transfusions). All 7 animals receiving TBI (800 cGy, without tibiae shield) died during Days 8 to 17. rhGM-CSF (6.25 x 10^6 U/mg, from Genetics Institute) was intravenously administered as a single dose of 50,000 U on the day of pump implantation (on either Day 3 or 4 post irradiation) then subcutaneously infused at 72,000 U/kg/day daily for 7 days through an implanted pump. However, rhGM-CSF treatment decreased time to ANC recovery (ANC >1 x 10^6/mL) from 22 days to 18 days, and enhanced the ANC level (Figure 9). Furthermore, rhGM-CSF enhanced granulocyte-macrophage progenitor cell activity (as GM-CFU) in bone marrow 10 folds than that in the non-rhGM-CSF treatment group on Days 10 and 20 (Figure 10).

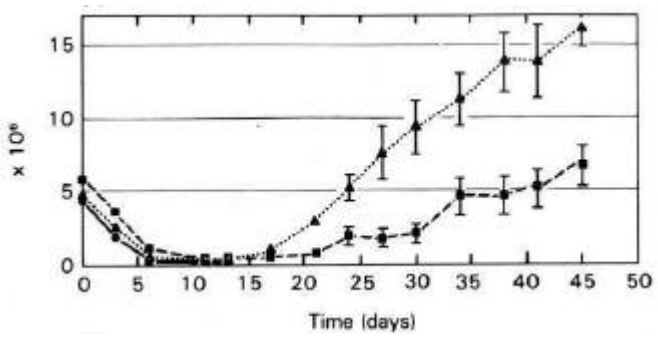

Figure 9. rhGM-CSF enhanced the recovery of peripheral blood granulocytes (x 10^6 /mL) in monkeys receiving shielded irradiation and rhGM-CSF treatment. Values were mean ± SEM. ●: animals with TBI, ■: animals with tibiae shielded irradiation, ▲: animals with tibiae shielded irradiation and rhGM-CSF treatment. rhGM-CSF was intravenously administered as a single dose of 50,000 U either on Day 3 or 4 post irradiation then subcutaneously infused at 72,000 U/kg/day (6.25 x 10^6 U/mg) daily for 7 days through an implanted pump. The figure is adapted from (28).

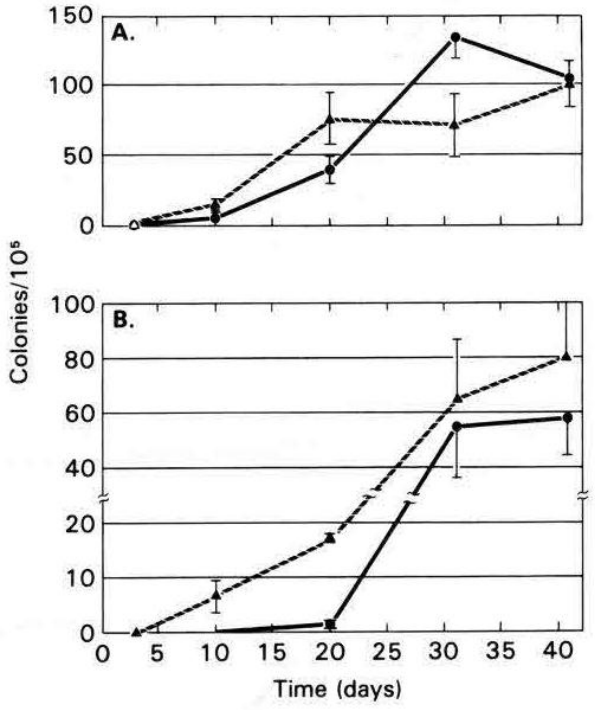

Figure 10. rhGM-CSF enhanced the recovery of granulocyte-macrophage progenitor cell activity in bone marrow of tibia (A) and iliac crest (B). ● : animals with tibiae shielded irradiation, ▲: animals with tibiae shielded irradiation and rhGM-CSF treatment. The treatment regimen was the same as described in Figure 9 legend. The figure is adapted from (28).

In addition, single dose of rmGM-CSF at up to 10 mcg/mouse administered either 20h pre-TBI or 1 or 3 hr post-TBI did not demonstrate survival benefit in mice (26, 29).

Reasons for the inconsistency of survival benefit of GM-CSF cannot be determined. The variations in sources of GM-CSF and study designs may contribute to this inconsistency.

rhGM-CSF enhanced WBC recovery

GM-CSF enhanced WBC recovery in majority of studies conducted in NHPs (rhesus)(11; 28, 30), dogs (beagle)(9, 31, 32), and mice (19, 21, 24, 33). The effects were also measured mainly as decreased duration of neutropenia (a few days), decreased time to ANC recovery (a few days), improved ANC nadir, increased WBC counts, and increased GM-CFU in bone marrow. Figure 11 is used as an example to illustrate such effects in addition to Figure 9 and Figure 10.

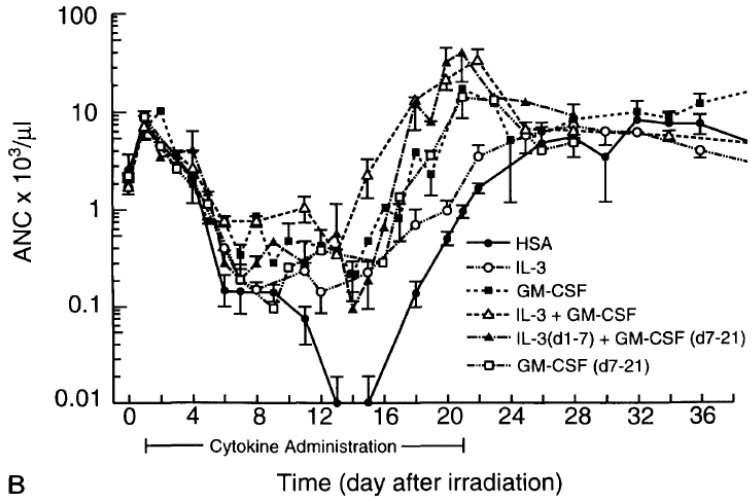

Figure 11. rhGM-CSF reduced the duration of neutropenia (defined as ANC < 1,000/mcL) and the depth of the nadir. The values were ANC ± SEM. Male Rhesus monkeys were exposured to 450 cGy TBI (mixed fission neutron:gamma radiation) then subcutaneously administered 25 mcg/kg/day (12.5 mcg/kg, BID) human serum albumin (HAS, control), IL-3, rhGM-CSF, or IL-3 plus rhGM-CSF daily from Day 1 to Day 21 post TBI. Supportive care including antibiotics and platelet transfusions were used to reduce $LD_{70/30}$ to $LD_{0/30}$. The figure is adapted from (30).

Conclusion

The results of published literature support WBC recovery benefit of GM-CSF on hem-ARS. The results were less consistent regarding the survival benefit.

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The Use of Leukocyte Growth Factors (LGFs) for Treatment of Radiation Injury from Radiological/ Nuclear Accidents, 1986-2012

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March 31, 2013

I. Summary
II. General
-Table 1. Sites of Radiation Incidents where Leukocyte Growth Factors were used
-Table 2. Significant Radiation Incidents since 1986 where LGFs were not used
III. Radiation Incidents since 1986 where LGFs were used
IV. Consensus Guidelines and Recommendations for treatment of ARS
V. Conclusion
Appendix: Abbreviations and some websites dealing with radiation injury
References

I. Summary

The purpose of this report is to review and evaluate the use of Leukocyte Growth Factors (LGFs, i.e. G-CSF, GM-CSF, and pegylated G-CSF) for the treatment of radiation-induced myelosuppression associated with radiological/ nuclear incidents. LGFs have been utilized for the treatment of radiation-induced myelosuppression since 1986. Radiation accident reports show that LGFs have been used in a wide variety of accident situations. There have been a larger number of serious accidents involving sealed radioactive sources such as Cobalt-60 or Cesium-137 than accidents involving the nuclear power industry, accidents in the radiation therapy of patients, or accidents in other radiation industries. Most of the accidents with sealed sources took several days to diagnose the cause of injury or sickness was due to radiation. LGFs, if given to these victims, were usually started several days after an incident. While the number of victims for each sealed source accident has been relatively few, this contrasts with the large number of victims affected by each nuclear power industry accident.

The consensus guidelines described in Section IV of this report recommend starting LGFs as soon as possible. That has been a challenge. It has also been a challenge to determine for each accident who might benefit from LGFs. The estimated dose of radiation to individual victims has varied and in some cases may not have been accurate. Also dose to each individual may have varied to different parts of their bodies. The 1999 Tokaimura, Japan criticality accident is an example of a nuclear accident where diagnosis and start of treatment were made early. Despite optimum medical care the worker who received 16-25 Gy died on day 83 of multiple organ failure, and the worker who received 6-9 Gy died on day 211 of multiple organ failure.

Treatment of Radiation Injury is complex. Despite limitations the general conclusion by several authors is that LGFs improve time to white blood cell recovery and for some victims may improve survival. Some authors believe that G-CSF and possibly GM-CSF treatment reduces platelet counts. The IAEA recommends that platelet counts be monitored when G-CSF is being administered. (IAEA, Gilan, 2002).

II. General

Although the first LGFs were not approved by the US FDA until 1991, the first known use of LGFs was for the Chernobyl, Ukraine nuclear power plant disaster in 1986. A year later in 1987 LGFs were used in Goiania, Brazil for an abandoned radiation source accident. In this paper I briefly describe chronologically all the radiation incidents since 1986 for which I have been able to identify the use of LGFs for treatment of radiation-induced myelosuppression. Where I have found a description of possible benefits and risks of LGFs, I report these also. In Section IV of this report I provide some consensus guidelines and recommendations for the treatment of the hematopoietic syndrome of the acute radiation syndrome (ARS). My sources of information have been from a search of the literature, a review of the International Atomic Energy Agency (IAEA) website and other websites (AFRRI, REAC/TS, REMM, and REMPAN) and discussion with colleagues.

Table 1. Sites of Radiation Incidents where Leukocyte Growth Factors were used

YEAR	LOCATION & TYPE	VICTIMS Receiving LGF	Radiation Dose Range	Deaths/ Results
1986	Chernobyl, Ukraine. Nuclear Power Plant	GM-CSF started weeks after exposure per RP Gale, 2011 and 2013	Unpublished	Unpublished
1987	Goiania, Brazil. Cesium-137	8 GM-CSF started 24-48 days after exposure	2.5-6.0 Gy	4/8 died, ages 6-37.
1989	San Salvador, El Salvador. Cobalt-60	3 GM-CSF	3.0-8.1 Gy	1/3 died after 197 days
1990	Soreq, Israel. Cobalt-60	1 GM-CSF, IL-3, & BMT*	> 10 Gy	1/1 died day 36
1991	Nesvizh, Belarus. Cobalt-60	1 GM-CSF & IL-3	10 Gy	1/1 died day 113 due to pulmonary
1996	Gilan, Iran. Iridium-192	1 G-CSF	4.5 Gy whole body, 30 Gy skin epidermis	0/1, no death.

YEAR	LOCATION & TYPE	VICTIMS Receiving LGF	Radiation Dose Range	Deaths/ Results
1998-1999	Istanbul, Turkey. Co-60	7 G-CSF	0.9-3.1 Gy	0/7, no death
1999	Henan Province China. Co-60	3 GM-CSF, 1 also EPO	2.4-6.1 Gy	0/3, no death
1999	Tokaimura, Japan. Criticality accident	3 G-CSF 1 also GM-CSF 2 with SCT* 1 TPO, EPO	2-25 Gy	2/3 deaths at day 83 and 211.
1999	Yanango, Peru. Iridium-192	1 G-CSF	1.5 Gy total body, up to 9966 Gy to skin	0/1, no death.
2000	Samut Prakan, Thailand. Co-60	9 both G-CSF & GM-CSF	2 Gy to > 6 Gy	3/9 died
2000	Meet Halfa, Egypt. Iridium-192	5 G-CSF	3.5-4 Gy	0/5 no death
2005	Nueva Aldea, Chile. Iridium-192	1 G-CSF for only 3 days.	1.5 Gy whole body, 1600 Gy Buttocks	0/1, no death.
2006	Fleurus, Belgium Cobalt-60	1 peg-G-CSF, SCF and peg-EPO	4.2-4.8 Gy mean dose	0/1, no death
2006	Dakar, Senegal Iridium-192	1 peg-G-CSF, SCF and peg-EPO	3.4 Gy mean dose, range 1.3-75 Gy	0/1 no death
TOTAL	13 accidents where LGFs were used	17 GM-CSF + Chernobyl 17 G-CSF 10 Both 2GMCSF+ IL3 2 peg-G-CSF with SCF 48 Total + Chernobyl	0.9 to > 10 Gy	11 Deaths out of 48 plus Chernobyl (unpublished)

* Abbreviations: See Appendix

Table 2. Significant Radiation Incidents since 1986 where LGFs were not used: (Victims did not have significant myelosuppression, or for some reason treatment was not given)

YEAR	LOCATION	Reference
1992	Hanoi, Vietnam	IAEA Report
1993	Tomsk, Russia	IAEA Report
1994	Tammiku, Estonia	IAEA Report

1996	San Jose, Costa Rica	IAEA Report
1997	Lilo, Georgia	IAEA Report
1997	Sarov, Russia	IAEA Report
2000	Panama	IAEA Report
2002	Cochabamba, Bolivia	IAEA Report
2004	Shandong Jining, China	Johnston's Archive 2012
2006	London, U.K. (Alexander Litvinenko)	News media, Polonium-210 poisoning
2010	Turmero, Venezuela	IAEA Nuclear Safety Review 2010
2011	Fukushima, Japan	IAEA Information Sheets

III. Radiation Incidents since 1986 where LGFs were used

1986: Chernobyl, Ukraine, USSR, Treatment with GM-CSF

The Chernobyl nuclear power plant accident on April 26, 1986 led to the greatest release of radioactive material in history. After the meltdown and explosion of one of the nuclear power plants at Chernobyl, a fire continued for several days spewing radioactive material into the atmosphere. Winds carried the material over a large area of Europe. Severe radiation effects were felt almost immediately. Of 600 workers present on the site the morning of the accident, 134 received high doses (0.8-16 Gy) and suffered from radiation sickness. Of these 134 victims, 28 died within the first three months, and another 19 died between 1987 and 2004 of various causes not necessarily associated with radiation exposure. In addition, according to the UNSCEAR 2008 Report, the majority of the 530,000 registered recovery operation workers received doses of between 0.02 Gy and 0.5 Gy between 1986 and 1990. That cohort is still at potential risk of late consequences such as cancer and other diseases. (UNSCEAR, 2008)

Dr. Robert Gale, an American hematologist-oncologist and bone marrow transplant physician, was allowed to perform bone marrow transplants in Moscow on 13 of the most seriously exposed victims. It does not appear that bone marrow transplants were beneficial because each of the victims eventually recovered his own bone marrow cells. He and his Soviet colleagues infused human fetal liver cells in a few other victims. (Gale, 2011)

Several days after the accident, there were some victims who had persistence of low white blood cell counts. White blood cells (granulocytes) can not be effectively transfused. GM-CSF at that time was a new drug which had shown some benefit in animals receiving myelosuppressive doses of chemotherapy or radiation. Dr. Gale and Dr. Andrei Vorobiev of the USSR administered LGFs (GM-CSF) for the first time in history to some of these victims of radiation-induced myelosuppression. They first tested GM-CSF on themselves before administering it to any Chernobyl victim. Dr. Vorobiev developed severe sternal pain which lasted for a few hours from the drug, but Dr Gale tolerated GM-CSF without difficulty. They then offered it to some of the victims. Per Dr. Gale "GM-CSF proved useful in treating the (Chernobyl) radiation victims….. This

approach is now a standard intervention for radiation accident victims." (Gale, 2013, Note: No additional information about the Chernobyl victims who received GM-CSF is provided in this reference.)

Dr. Gale has described a caveat to the use of hematopoietic growth factors (e.g. LGFs). He has stated "This approach can only succeed if sufficient numbers of immature bone marrow cells survive radiation damage. This survival might be possible after low radiation doses, but not after very high doses. When few or no target cells survive, the medical approach shifts to bone marrow replacement…." (Gale, 2011, pp 13-14).

1987: Goiania, Brazil, Treatment with rhuGM-CSF (Leukine and non-Leukine)

The next published use of LGF for radiation induced myelosuppression was for the Goiania Brazil radiation accident. This accident involved whole-body exposure, internal contamination and local radiation injuries. On September 13, 1987 two individuals in Goiania found an abandoned source of cesium-137 which had been used for radiation teletherapy. At the time of discovery the source contained 50.9 TBq (1375 Curie). They took the source home, ruptured the shielding, and sold part of it to a scrap yard owner. The scrap yard owner admired the sources blue glow in the dark, brought it home and shared pieces with friends and relatives. Some of these victims rubbed the material on their skin and unknowingly swallowed some as they ate with their hands.

Sixteen days passed between the rupture of the source container, and discovery of the accident and notification of authorities. Cesium-137 was found to have been spread to parts of the city. From September 30 until December 21, 1987 authorities offered screening to concerned personnel. 112,800 people in this city of one million went for screening. Of this total 249 persons had some degree of contamination. Further study of contaminated individuals revealed that 152 had internal contamination. 49 individuals required medical treatment. 20 victims were hospitalized, and of these, 8 had severe bone marrow impairment. Of the victims found to have internal contamination, 46 were treated with Radiogardase (Prussian Blue, or ferric ferrocyanide). (Gusev 2001, pp 355-360)

RhuGM-CSF was used to treat the eight individuals with severe bone marrow impairment. Initiation of rhuGM-CSF therapy occurred from 24 to 48 days following radiation exposure, and the estimated radiation exposure doses ranged from 2.5 to 6.0 Gy. All eight individuals who received rhuGM-CSF had neutrophil counts $\leq 0.5 \times 10E9/L$ prior to starting rhuGM-CSF, which was dosed at 500 $\mu g/m^2$ IV daily until the ANC exceeded 2 x 10E9/L. Subsequently it was continued at half the dose for an additional three days. Overall, 50% (4/8) of exposed individuals survived. rhuGM-CSF was initiated in the four surviving patients within five days of developing neutropenia and prior to onset of infectious complications. By contrast, individuals who did not survive were colonized with gram-negative bacteria prior to receiving rhuGM-CSF.

The Discussion Section in the Butturini paper stated that **three observations suggested the rHuGM-CSF aided granulocyte recovery:** 1. There was a rapid rise in granulocytes within 12 hours of injection in several individuals, 2. there was a decline in granulocytes after dose attenuation or discontinuation, and 3. there were different patterns of recovery in treated and untreated persons. (Butturini, 1988).

1989: San Salvador, El Salvador, Treatment with non-Leukine GM-CSF

Three individuals developed severe neutropenia following an industrial radiation accidental exposure to 3.0 - 8.1 Gy from a Cobalt-60 radiation source. At 24, 26, and 32 days following exposure, each patient received bacterially-derived rhuGM-CSF (Schering-Plough) at a daily dose of 240 μg/m² IV over 2 hours until the ANC reached 1,500/mm³. All three patients responded to rhuGM-CSF such that they had an ANC > 1,500/ mm³ within 9 to 20 days after starting rhuGM-CSF and none experienced severe infections.

The IAEA report states that the legs and feet of two of the three men were so seriously injured that amputation was required. The worker who had been most exposed (Patient A) died 6.5 months (197 days) after the accident. "His death was attributed to residual lung damage due to irradiation, exacerbated by a pneumothorax from catheter placement. His family refused an autopsy so the exact cause of death is unknown. Based on cytogenetic analyses, the dose estimate for patient A by REAC/TS was 7.97 Gy. The dose estimates for Patient B and C were 3.77 and 2.92 Gy respectively. (IAEA, 1990)

Per Thiery, "The spontaneous recovery of haemoglobin and platelets was greater that that of neutrophils, which bears to the fact that GM-CSF stimulates granulocyte precursors." Mild side effects included tremor and weakness for the patient who died on day 197 after receiving 8.1 Gy. (Thiery 1995)

1990: Soreq, Israel, Treatment with GM-CSF

One victim received an estimated whole body dose of > 10 Gy from a Cobalt-60 source. GM-CSF was started about 9 hours after exposure and continued for 18 days at a dose of 250 μ/m²/day. A bone marrow transplant was performed on day 4. The victim was also treated with IL-3 days 5-18. Growth factors were discontinued on Day 18 due to normalization of the white blood cell counts. The victim died on day 36. Some of the post mortem findings were compatible with acute Graft versus host disease (GVHD). However the role of GVHD in the death of the patient could not be fully addressed. Data were thought to indicate that a combination of GM-CSF and IL-3 may lead to early and effective engraftment and maturation of donor marrow cells. (Thiery 1995, IAEA 1993)

1991: Nesvizh, Belarus, Treatment with GM-CSF

One victim received an estimated whole body of about 10 Gy from a Cobalt-60 source. GM-CSF was given days 2-6 and 16-41 at a dose of 250 μ/m²/day. IL-3 was given days 6-41. Neutrophil recovery started on day 21, reticulocytes appeared 10-12 days later. No platelet recovery occurred. Granulocytes reached a level of 5 x 10E9/liter on day 40. The patient died on day 113 from pneumonia and acute respiratory failure. Per Thiery, "Haematopoietic recovery was incomplete but results suggest a real improvement for the growth factor therapy." (Thiery 1995)

1996: Gilan, Iran, Treatment with G-CSF

On July 24, 1996 a worker picked up an industrial radiography pigtail (short metal cable resembling a pigtail) and placed it in his chest pocket for 1.5 hours. He did not

know that this pigtail was a highly radioactive source of Iridium-192. About 1.5 hours after picking up the source the victim stated having dizziness, nausea, lethargy and a burning sensation in his chest. The worker believed that the source was a possible cause of his symptoms, and he put it back where he had found it. The radiographer who had accidentally lost this source found it and the victim and notified authorities. Authorities requested blood samples on 600 employees. Everyone's blood test returned normal except the exposed worker.

On July 27 the exposed worker continued to have burning in his chest and also had a continued drop in his lymphocyte counts, so he was hospitalized. He had progressive drops in his white blood cell and platelet counts. Cytogenetic dosimetry indicated **a whole body dose of about 4.5 Gy**. He was treated with prophylactic antibiotics, and transfused with 7 units of platelets on Day 20. On Day 22 he was started on G-CSF (Leucomax) **400 mcg twice daily,** subcutaneously.

On Day 24 he was transferred to Paris for a possible bone marrow transplantation. In Paris platelet transfusions and antibiotics were continued. G-CSF was continued at a rate of **300 mcg daily** for 10 more days until the white blood cell count showed marked improvement. A skin graft for the chest lesion was performed on Day 63. He returned to Iran on Day 95.

The IAEA report concludes:

"In effect, intervention with cytokines probably made little contribution to the eventual recovery as treatment was initiated at a stage where bone marrow recovery was likely to be already under way. However, the use of G-CSF probably accelerated the process, thereby reducing to some degree the risk of intercurrent infection. Administration of G-CSF, as reported previously, appeared to inhibit the recovery of platelet numbers; this is suggested by the almost immediate rise in platelet count after the therapy was discontinued…. "

The IAEA report recommends:

"In the case of non-homogeneous whole body irradiation (i.e. the situation in most accidents), bone marrow stimulating cytokine treatment should be initiated at the earliest opportunity. G-CSF may be the drug of first choice, but if this drug is used, particular attention should be given to the monitoring of platelet counts." (IAEA, Gilan, 2002)

1998-1999: Istanbul, Turkey, Treatment with G-CSF

In December 1998 and January 1999 two packages used to transport cobalt-6**0** teletherapy sources were sold as scrap metal. The persons who purchased the packages broke open the shielded containers and later suffered from the acute radiation syndrome (ARS). Eventually a total of 10 adults showed signs and symptoms of acute radiation exposure.

Individuals started dismantling the shielded containers on December 13, 1998. Six of 10 persons involved in dismantling the source had vomiting that night. The accident victims consulted several doctors over the next 4 weeks. The diagnosis of radiation injury was not made until January 8, 1999. A public health announcement led to 404 persons seeking evaluation over the period January 9-15, 1999. Ten patients were diagnosed with ARS. On or about January 12, 1999 the seven most severely affected patients were

started on G-CSF and continued on G-CSF for 5-12 days. Five patients had life-threatening thrombocytopenia and were transfused with "massive" platelet and whole blood transfusions. All patients survived, and by February 24, 1999, after 45 days in the hospital, the five most severely affected patients were discharged.

Per the IAEA report, "…although patient 5 recovered rapidly after a massive platelet transfusion of 24 units, all other patients showed marked recovery in platelet counts only after completion of G-CSF treatment. Such a delay in recovery of platelets had previously been reported and is sometimes attributed to a negative impact (on platelets) of G-CSF…..However, such a recovery chronology could also be consistent with spontaneous recovery of the platelet lineage…(IAEA, 2000, p. 39). Estimated radiation dose based on dicentric analysis for the 7 worst victims was 0.9-3.1 Gy. The five worst victims were estimated to have received 2.2-3.1 Gy. (IAEA, 2000)

1999: Henan Province China, Treatment with non-Leukine GMCSF

Three individuals in Henan Province, China were accidentally exposed to high doses of Cobalt-60. All three received rhuGM-CSF starting when the total white cell count was below 1000. All had increase in their white cell counts and survived. Details are as follows:

In April 1999 an old cobalt-60 source was accidentally sold as scrap metal. A scrap metal dealer, Patient C, bought the radiation source and took it home where he left it in a bedroom. His wife, Patient A, and 8-year old son, Patient B, within a few hours developed nausea and vomiting. Patient C also developed vomiting. Medical providers first treated the patients for "food intoxication". However, by day 3, the source was discovered and the patients were transferred to an appropriate hospital for treatment of radiation injury. Dose estimates were 6.1 Gy for Patient A- the wife, 3.4 Gy for Patient B- the 8 year old son, and 2.4 Gy for Patient C- the scrap metal dealer. Patient A's GM-CSF dose was 400 mcg/m² per day from day 9-33. Patient B's dose was 200mcg/m² from day 18-33, and Patient C received 400 mcg/m² from day 26-35. EPO was given to patient A 120 U/kg/day from day 10-36. Gamma globulin, whole blood and fresh platelets were provided to each patient. Patient A was given testosterone for 7 days to delay her menstruation which may have caused significant blood loss. She was noted to have a moderate degree of hepatosplenomegaly and pain on day 60 but liver function tests were normal. Laminated air-flow rooms were used with each patient to prevent exogenous infection. Patient A had received asymmetrical irradiation for about 20 hours. The author, Liu, states that GM-CSF "is helpful for the recovery of the bone marrow." The article does not give any follow-up information, but apparently all 3 patients survived. The author states in the abstract "In our view, GM-CSF should be given as early as possible with enough dosage for promoting early hematological reconstruction." (Liu, 2008, J Radiat Res).

1999: Tokaimura, Japan, Treatment with G-CSF, GM-CSF, Stem Cell Transplants, EPO, and TPO

On September 30, 1999 a criticality accident occurred at a chemical processing facility in Tokaimura, Japan. Enriched uranium was being poured into a tank in an amount about

seven times the recommended amount. Criticality occurred and 3 workers received prompt high doses of neutron and gamma radiation. Several other workers and members of the public received lower doses of radiation. 161 residents within 150 meters of the accident were evacuated and about 310,000 people in the Ibaraki Prefecture were advised to stay indoors for about 18 hours as a precaution. 43 people had their radiation dose assessed based on chromosomal analyses. Whole body radiation counters were also used.

The three workers who received the highest dose of radiation were workers A, B, and C. Worker A developed loss of consciousness for about 30 seconds and vomiting and diarrhea during the first hour after the event. He was promptly hospitalized. On days 7 and 8 he received peripheral blood stem cell transplantation (PBSCT) from a family member with identical HLA. The report states that hematopoietic factors such as G-CSF, erythropoietin (EPO), thrombopoietin (TPO) and blood components were administered as needed. He went on to develop severe radiation skin damage, GI bleeding, and respiratory failure due to pulmonary edema. On day 58 he had a cardiopulmonary arrest. He died of multiple organ failure on day 83. Total body dose estimate was 16-25 Gy.

Worker B also developed vomiting within 1 hour of the accident. He was transferred to a Tokyo hospital where he had umbilical cord blood transplantation. The report states "Cytokines were also applied, such as G-CSF, GM-CSF…, TPO, and EPO." His own bone marrow recovered about 2 months after the incident. On day 153 he developed MRSA pneumonia which led to acute respiratory distress syndrome. He also developed a CMV infection. GI bleeding started on day 145 and continued until his death on day 211 due to multi organ failure. Total body dose estimate was 6-9 Gy.

Worker C was the supervisor who was not as close to the accident as workers A and B. He developed nausea but no vomiting. He was hospitalized under reverse isolation. He was on G-CSF until Day 28. His neutrophils had reached a nadir on day 20. Platelets had decreased slower than the other 2 victims, but still necessitated platelet transfusions on days 17, 20, and 23. He left the hospital on Day 82. Total body dose estimate was 2-3 Gy. (IAEA, 2008, pp 33, 77-80)

1999: Yanango, Peru, Treatment with G-CSF, GM-CSF,

On February 20, 1999 a serious radiological accident occurred when a welder picked up an Iridium-192 industrial radiography pigtail source and placed it in his pocket. The welder did not know that the source was radioactive. He put the source in his pocket for several hours. At night he went home and unknowingly exposed his wife and children to radiation. He had nausea but no vomiting. Within a few hours he developed pain and redness in his right thigh. He saw a physician who thought the patient had a bug bite. A few hours later the industrial radiographer discovered that his radiography source was missing. He went to the welder's house and was given the pigtail source.

On February 21, 1999 the patient was admitted to a hospital in Lima, Peru. Over the next few days a large blister developed over the right thigh and buttock. On day 34 the patient was found to have a drop in his neutrophils down to 1440 (normal 2500-7000), and lowering of his total leucocytes to 1500 (normal 4000-11000). On day 35 G-CSF (Leucomax) was started at 300 mcg per day. G-CSF was continued until Day 42 when it was stopped due to a significant rise in white blood cells.

On day 98 (May 28, 1999) the patient was transferred to France for wound grafting.

On August 16, 1999, radical surgery was necessary for purulent necrosis. The right hip was disarticulated, and the right leg was amputated with a hemipelvectomy. On October 17, 1999 the patient returned to Peru from France, approximately 8 months after the incident.

At the time of the accident the source was 9.6 x 10E11 Bq (26 Ci). The patient's total dose, assuming homogeneous whole body radiation, was estimated to be 1.5 Gy. Doses to the skin were calculated to have been up to 9966 Gy. The dose to the rim of the lesion was estimated to be 25 Gy.

Per Dr. Igor Gusev's text: "Most cases of local radiation injury do not have significant bone marrow depression. G-CSF was given at day 34 postexposure, but whether this had a beneficial effect is unclear. The bone marrow did improve, but this was at a time when spontaneous recovery would have been expected." (Gusev 2001, and IAEA 2000)

2000: Samut Prakan, Thailand Treatment with G-CSF and GM-CSF

On January 24, 2000 several individuals took a cobalt-60 teletherapy head from an unsecured warehouse. They took the source to one person's home, and attempted to disassemble it. On February 1, 2000 two individuals took the partially disassembled source to a junkyard in Samut Prakarn Province. A worker at the junkyard disassembled the source. By the middle of February several involved individuals felt ill and sought medical attention. Medical providers fortunately suspected radiation as the cause of the illnesses, and notified authorities who found the source capsule intact on February 20, 2000. At the time of recovery the source was estimated to have an activity of 15.7 TBq (425 Curie) of cobalt-60.

Ten victims, ranging in age from 18-75, presented with symptoms of vomiting. Some of them also had epilation and burns. Total body doses were estimated to range from 1 Gy to > 6 Gy. Four individuals were found to have received > 6 Gy. Nine victims who received 2 Gy or more were treated with both G-CSF (lenogastrim) and GM-CSF. For each of the victims receiving LGFs, treatment started with G-CSF and later GM-CSF was added. G-CSF was started at 250 or 500 mcg/day (5-10mcg/kg/day) and in some cases increased to 1000 mcg/day (20mcg/kg/day) if white blood cell (WBC) counts remained low. GM-CSF started at 300 mcg/day and was increased to 500 or 600 mcg/day depending on the response of the white blood cells. Both G-CSF and GM-CSF were stopped if the WBC counts were adequate.

Despite heroic efforts, four victims died. Victim P5 died of septic shock 47 days after exposure. He also had burns due to radiation. Victim P6 died 38 days after exposure also from septic shock. Victim P8 died 53 days after exposure due to ARDS. He had positive blood cultures. (Ricks 2001, pp 283-301, and IAEA, Samut Prakarn, 2002)

2000: Meet Halfa, Egypt, Treatment with G-CSF

On May 5, 2000 a resident of Meet Halfa village found an industrial gamma radiography source which had been lost sometime before. Unaware that the item was radioactive, he took it home and shared it with his wife, sister, 2 sons and 2 daughters. The family believed the source to be a precious metal and handled it occasionally over the following weeks. On June 5 the 9-year old younger son died and was found to have

marked bone marrow failure and extensive inflammatory skin lesions. On June 10, a fact-finding mission from the Ministry of Health found that 4 other members of the family had similar signs and symptoms. An exact diagnosis was not known, but the family was admitted to a hospital for observation. On June 16 the father died with bone marrow failure and extensive skin lesions. On June 25 authorities discovered high levels of radiation in the family home. By June 28 a source was found and identified as Iridium-192.

The IAEA was notified and offered to assist, but, because the situation was under control, the IAEA offer was declined. Estimated protracted whole body radiation exposure doses to the family members was as follows:

Father	7.5 to 8 Gy	Died before diagnosis
Younger son	5 to 6 Gy	Died before diagnosis
Sister	3.5 to 4 Gy	
Wife	3.5 to 4 Gy	
Elder daughter	3.5 to 4 Gy	
Elder son	3.5 to 4 Gy, localized	
Younger daughter	3.5 to 4 Gy, localized	

Per Anas El-Naggar, all 5 surviving family members were treated similarly after diagnosis. Medical management included patient isolation in a laminar air-flow tent, attention to hygiene, and nutrition, and G-CSF (Neupogen) 10 mcg/kg per day. There were no additional deaths. (Ricks, Egypt, 2002)

2005: Nueva Aldea, Chile, Treatment with G-CSF

On December 14, 2005 a radioactive source containing Iridium-192 fell out of gamma radiograpy equipment being used at a construction site. It was later found and handled by 3 workers, A, B, and C. At the time of the accident the activity of the source was 3.33 TBq (90 Ci). Within one day the source was recovered and authorities notified. Based on extensive history and dosimetry calculations, worker A was determined to have received a total body dose of 1.3-1.5 Gy, and a dose of up to 1600 Gy to the surface of his buttocks adjacent to a pocket where he had placed the source. Workers B and C were estimated to have received < 0.5 Gy whole body dose (IAEA, p. 30-31). Biodosimetry was also performed from blood samples on 34 individuals who had worked near the exposed source. One of these workers was calculated to have received 0.17 Gy. The other 33 workers all received <0.1 Gy.

Worker A was admitted to a hospital on December 15, 2005. G-CSF was started on December 18, 2005 and stopped on December 20, 2005. Per the IAEA report "….this administration (of G-CSF) was not fully justified, taking into account the radiological data: the level of whole body dose and the inhomogeneous character of the exposure. This radiological information was not fully known at the time of GCSF administration …..After collecting radiological data, …the IAEA Assistance Mission experts and the staff of the hospital analyzed the haematological and radiological information, and decided to stop the administration of GCSF on 20 December 2005."

Worker A developed a progressive necrotic wound on his buttocks, and was transferred to a burn hospital in Paris on December 28, 2005. There he consented to

experimental treatment with surgical excision followed by two administrations of mesenchymal stem cells (MSCs) for his local injury. Within 3 months he had almost complete healing of the wound on his buttock. On May 4, 2006 Worker A returned to Chile. (IAEA, Nueva Aldea, 2009)

2006 Fleurus, Belgium, Treatment with pegylated G-CSF and SCF

On March 11, 2006 an alarm went off in a facility for the sterilization of medical devices. A cobalt-60 source producing approximately 5000 Gy per hour was out of its security position. The operator's whole body was exposed to this source for about 22 seconds. The victim began vomiting a few hours after the incident, but did not think it was related to radiation exposure. Eighteen days after the incident, the victim consulted a physician because of persistent nausea, diarrhea, headache and hair loss. A diagnosis of accidental radiation exposure was subsequently made. The patient was admitted to Percy Hospital in France where he was noted to have the hematoloogical syndrome with a 26% drop in hemoglobin, a platelet nadir of 2,000 per mm^3, and a leukocyte nadir of 400 mm^3. Eight days after hospitalization the victim developed septicemia. Whole body radiation dose was estimated at 4.2-4.8 Gy with a range to different parts of the body of 1.5-6.4 Gy.

Treatment with peg-G-CSF was initiated on day 28 after exposure. On days 32 and 33 post-exposure the victim received peg-EPO and recombinant human SCF (Stemgen). Cytokines had an immediate effect. The patient had complete resolution of the hematopoietic syndrome on day 43. (Gourmelon 2010)

2006 Dakar, Senegal, Treatment with pegylated G-CSF

In June-August, 2006, an industrial radiation device with Iridium-192 was used, but its source did not properly retract into its shielded storage container. It was later discovered that the source was not secure. It was estimated that 63 people had received radiation from the source. The most severely irradiated patient was admitted to the Percy Hospital in France on August 25, 2006. He was found to have a leukocyte nadir of 700 per mm^3 and a platelet nadir of 8,000 per mm^3. Radiation dose reconstruction estimated that the mean dose was 3.4 Gy, but dose was very heterogeneous ranging from 1.3 Gy to the liver, up to 75 Gy to the skin of the left arm. He was diagnosed with the hematopoietic syndrome with a severe cutaneous radiation syndrome.

Treatment was initiated with peg-G-CSF, rh-SCF (Stemgen), and peg-EPO. The patient rapidly recovered a normal blood count. The cutaneous syndrome required additional management. (Gourmelon, 2010)

IV. Consensus Guidelines and Recommendations for treatment of ARS

There are consensus documents in the United States, Europe and Global (international) which recommend the use of LGFs for radiation-induced myelosuppression.

Global:

The World Health Organization (WHO) convened a panel of experts in 2009 to develop a harmonized approach to the medical management of acute radiation exposure. One of their considerations was the management of the hematopoietic syndrome (HS). Dr. Nicholas Dainiak was the first author of their publication. Their recommendation, based on their analysis and review, was a strong recommendation for the administration of granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor. They made a weak recommendation for the use of erythropoiesis-stimulating agents or hematopoietic stem cell transplantation. In their conclusion they stated that "Assessment of therapeutic interventions for HS in humans exposed to nontherapeutic radiation is difficult because of the limits of the evidence." (Dainiak, 2011)

United States:

American Society of Clinical Oncology (ASCO) 2006 Clinical Guidelines for use of white blood cell growth factors include the following recommendation: "12. Special Comments on Growth Factors As a Treatment for Radiation Injury, 2005 recommendation, Current recommendations for the management of patients exposed to lethal doses of total body radiotherapy, but not doses high enough to lead to certain death due to injury to other organs, includes the prompt administration of CSF or pegylated G-CSF. …" (Smith, JCO, 2006)

In 2004 Dr. Jamie Waselenko authored recommendations similar to the ASCO recommendations in her publication "Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group." (Waselenko 2004).

The Radiation Injury Treatment Network (RITN) has similar guidelines. (Confer DL, 2012)

Europe:

The article "Consensus conference on European preparedness for haematological an other medical management of mass radiation accidents" states "There are presently several cytokines available for the treatment of bone marrow failure caused by irradiation: granulocyte growth factor (G-CSF) (including pegylated forms), erythropoietin, IL-11, keratinocyte growth factor (KGF) and stem cell factor. In addition, several thrombopoietin (TPO) agonists and a peptibody active on megakaryocytopoiesis are in clinical trials. There was consensus that G-CSF and KGF should be used.… TPO and IL-11 should not be combined. Treatment should be continued for 14-21 days." (Gorin 2006)

V. Conclusion

Among radiation incidents from 1986- present I have identified more than 48 victims who were treated with LGFs. The results of treatment are hard to determine. The number of victims who received LGFs was small for each accident. Treatment generally started late after the accident. In many cases the victims did not know they had been exposed to high doses of radiation until hours or days later. In general, authors stated that

the use of LGFs appeared to be beneficial, and would have been more effective if given earlier.

The series of accidents are heterogeneous. Results for the efficacy of LGFs with radiation-induced myelosuppresssion are suggestive but not conclusive. Is there any risk of survival being worse with LGFs? Some authors questioned if G-CSF delayed platelet recovery. Thiery stated in his 1995 publication… "G-CSF is well tolerated, has less side effects than GM-CSF or IL-3, and allows the rise of fully functional granulocytes. The broader action of GM-CSF and IL-3 and especially their possible role in thrombopoiesis stimulation, added to their proven action on granulopoiesis in vivo, could be beneficial for the patients. …..It has been suggested after the Brazil accident that, when internal exposure is involved, the use of growth factors would stimulate haematopoiesis-induced progenitors or stem cells to progress in the cell cycle, while the cells are irradiated. The combination of haematopoietic growth factors inducing mitosis and simultaneous prolonged radiation exposure might result in the depletion of the stem cell pool. This was not confirmed by observations. This hypothesis could, however, be important in situations where internal contamination persists during treatment." (Thiery, 1995, pp 112-113)

Appendix: Abbreviations and some websites dealing with radiation injury (Note: all websites here and in references were accessed during March 2013):

-AFRRI	Armed Forces Radiobiology Research Institute http://www.afrri.usuhs.mil
- BMT	Bone marrow transplant
-CDC	Centers for Disease Control and Prevention, Radiation Emergencies http://www.bt.cdc.gov/radiation/
-Co-60	cobalt 60
-Cs-137	cesium-137
-EPO	erythropoietin
-G-CSF	granulocyte colony stimulating factor (a type of LGF)
-GM-CSF	granulocyte-macrophage colony stimulating factor (a type of LGF)
-GVHD	Graft versus host disease
-Gy or cGy	Gray or centiGray (units of radiation dose)
-IAEA	International Atomic Energy Agency, Accident Response http://www-pub.iaea.org/books/IAEABooks/Publications_on_Accident_Response
-Ir-192	Iridium-192
-LGF	leukocyte growth factors
-PBSCT	peripheral blood stem cell transplantation
-peg-G-CSF	pegylated (long acting) G-CSF
-REAC/TS	Radiation Emergency Assistance Training Site, http://orise.orau.gov/reacts/
-REMM	Radiation Emergency Medical Management www.remm.nlm.gov
-RITN	Radiation Injury Treatment Network http://ritn.net/

-SCT	Stem cell transplant
-TPO	thrombopoietin
-UNSCEAR	United Nations Scientific Committee on the Effects of Atomic Radiation, Accessed at: http://www.unscear.org/

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RADIATION ONCOLOGY: EXECUTIVE SUMMARY

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March 2013

This document reviews the literature on the use of leukine growth factors (Granulocyte Colony Stimulating Factor [GCSF] and Granulocyte Macrophage Colony Stimulating Factor [GMCSF]) in the radiation oncology clinical setting (where possible, Radiation Therapy [RT] only--no chemotherapy). For the methods used in the literature search and the criteria used to determine which articles would be further described in this document, please see page 4.

The majority of reports identified are confounded by inclusion of either concurrent chemotherapy or a significant previous chemotherapy history. Due to the small numbers of articles and subjects, it is not possible to draw any definitive conclusions regarding the utility of the products as supportive of its use as a medical countermeasure. However, it appears that one may state that GCSF administration may increase the white blood cell count, at least temporarily, in patients who are treated with fractionated radiation therapy; unplanned treatment breaks due to neutropenia may thus be avoided. However, the ultimate clinical implications are unknown.

Strengths

The four articles identified in our literature search which included at least some subjects undergoing radiation therapy without chemotherapy or other confounding factor are described in Table 1.

Limitations

Please note the inherent differences in clinical scenarios (ARS vs. therapeutic radiation) being compared to demonstrate utility of the growth factors may limit interpretation and applicability. For example, in cancer therapy, the total dose is fractionated into daily small doses over several weeks and limited to a specific region of the body while an accidental exposure is typically one large dose fraction (e.g. 2 – 10 Gy) to potentially the entire body (i.e., an unlimited body region). From a radiobiological point of view, these are vastly different situations as radiation toxicity is time (fractionation), dose (total), and volume (partial vs. whole organ/body) dependent.

There is little information available on the use of these growth factors in the therapeutic RT-only setting, and the studies available vary with respect to:

- radiation dose (total)
- treatment intent – adjuvant vs. definitive vs. palliative
- treatment port – region/volume of the patient that is treated with radiation

- fractionation
- disease treated
- growth factor utilized (GCSF vs. GMCSF)
- endpoints
    - GCSF studies usually are evaluating white blood cell (WBC) count or treatment interruption
    - GMCSF studies are usually evaluating mucositis
- Timing of growth factor intervention
    - prophylactic measure – before signs/symptoms occur
    - therapeutic measure – after signs/symptoms occur
    - during RT vs. during a treatment break from RT
- Route of administration of growth factor - orally vs. subcutaneously
- Wide range of growth factor doses administered

As mentioned above, the articles described below are not exhaustive, but considered representative by the reviewer. References in the tables are numbered sequentially across tables. The LGF in some publication titles are abbreviated.

LIST OF TABLES

Table 1. RT + GCSF Studies Potentially Supportive of Use in Acute Radiation Syndrome (ARS) 7

Table 2. RT + GCSF Studies Potentially Supportive of GCSF Efficacy in Reducing Mucositis Severity 13

Table 3. RT + GCSF Studies that may NOT Support Use in ARS 16

Table 4. RT + GMCSF Studies Potentially Supportive of GMCSF Efficacy in Reducing Mucositis Severity 21

Table 5. RT + GMCSF Studies NOT Supportive of GMCSF Efficacy in Reducing Mucositis Severity 23

ABBREVIATIONS

AE	adverse event
ANC	absolute neutrophil count
ARS	acute radiation syndrome
chemo	chemotherapy
c.i.	continuous infusion
CSI	craniospinal irradiation
CSRT	craniospinal radiotherapy
CTCAE	Common Terminology Criteria for Adverse Events
DHBI	double half-body irradiation
EORTC	European Organization for Research and Treatment of Cancer
f/u	follow up
Fx	fraction
FU	fluorouracil
GCSF	Granulocyte Colony Stimulating Factor
GI	gastrointestinal
GMCSF	Granulocyte Macrophage Colony Stimulating Factor
Gy	Gray (unit of radiation dose)
Gy/Fx	Gray per fraction
H&N	head and neck
I-131	Iodine-131 (therapeutic isotope)
IMRT	Intensity-Modulated Radiation Therapy
LGF	leukine growth factor
μg/kg/d	micrograms per kilogram per day
μg	micrograms
mg/kg	milligrams per kilogram
$mg/m^2/d$	milligram per meter squared per day
op	operation
OS	overall survival
PO	oral
post	after
QOL	quality of life
RT	Radiation Therapy
RTOG	Radiation Therapy Oncology Group
SQ	subcutaneous
SWOG	Southwest Oncology Group
tx	treatment
UTI	urinary tract infection
WBC	white blood cell
WHO	World Health Organization

METHODS

A literature search was requested via the FDA library website:

> Use (safety and effectiveness) of leukine growth factors (neupogen, leukine, neulasta or generic names) in patients treated with radiation therapy (no chemotherapy) studies, case reports if studies not available.

The search was limited to 1980 - 2013 based on the following US approval dates of the products:

- Neupogen – 1991
- Leukine – 1991
- Neulasta – 2002

Please note that the search results included articles that evaluated Leucomax and Mielogen, neither of which is approved in the US. However, when applicable, articles utilizing those drugs are included in the discussion below.

Pubmed and Embase search:

The librarian retrieved 330 + 38 records from Pubmed and Embase using the search terms below:

> 77 records – i.e. those indexed to neoplasms/radiotherapy
> 253 records – the remainder of the set (i.e. those not indexed to neoplasms/radiotherapy as a major point of the article)
> 38 records – newly added records to Pubmed not indexed with MeSH headings yet

Below is a typed list of search terms:

> ((Recombinant Proteins/therapeutic use [mesh] OR DNA, Recombinant/therapeutic use [mesh])
> AND (granocyte OR lenograstim OR neupogen OR filgrastim OR leukine OR sargramostim OR molgramostim OR pegfilgrastim OR neulasta))
> OR
> Granulocyte colony-stimulating factor/therapeutic use [mesh] OR granulocyte-macrophage colony-stimulating factor/therapeutic use [mesh] OR colony-stimulating factors/therapeutic use [MeSH:noExp]
> AND
> Radiation-protective agents/therapeutic use [mesh:noexp] OR radiotherapy [mesh] OR radiotherapy [subheading]
> = 330 records

For the non-indexed material in Pubmed, the following words were searched.

> Neupogen OR filgrastim OR leukine OR sargramostim OR molgramostim OR pegfilgrastim OR neulasta OR lenograstim OR granocyte OR granulocyte colony stimulating factor OR granulocyte colony stimulating factors OR granulocyte macrophage colony stimulating factor OR granulocyte macrophage colony stimulating factors OR colony stimulating factor OR colony stimulating factors
> AND
> Radiotherapy OR radiation therapy
> = 1,772 records
>
> The records were then limited to "NOT medline [sb]" to get 38 records.

All the articles found in the search were reviewed for applicability/relevance to the topic. Articles not included in this review did not include radiation therapy only patients (were primarily chemotherapy articles), did not include use of a growth factor, and/or had insufficient details to evaluate findings.

Articles in librarian search: 330
Articles excluded based on criteria mentioned above: 302
Articles included in the review below: 330-302= 28

Table 1. RT + GCSF Studies Potentially Supportive of Use in Acute Radiation Syndrome (ARS)

Reference	Design	Dose	Results	Critique	Supportive of Use?
1 Granulocyte colony-stimulating factor treatment of leucopenia during fractionated radiotherapy. *Eur J Cancer* (1993) 29A(14): 1927-1931 LGF: Neupogen (filgrastim)	N=11 total 5 curative 6 palliative 5 concurrent chemo 2 extensive chemo history **4 RT only – no prior chemo history** 1 Hodgkin's 1 uterine sarcoma 1 metastatic lung cancer 1 metastatic thyroid with prior I-131 therapy and prior RT	GCSF: 5 mcg/kg body weight SQ **when leukocyte count was <2000** and continued through RT RT: **4 patients RT only;** variable doses, % bone marrow treated, and anatomic regions Chemo: 7/11 subjects received prior and/or concurrent chemo	↑ leukocyte count in 10/11 patients Post discontinuation of GCSF, leukocyte counts dropped to subnormal levels in 2-3 days (unknown how long this persisted)	○ Small numbers ○ Confounded by prior and concurrent chemo ○ GCSF given concurrently with RT after leukocyte rebound in 4 patients ○ RT interrupted in 4 patients (2 RT only) but not in 7 others ○ RT variable doses, % bone marrow treated anatomic region, and therapeutic intent	Possibly
2 Value of granulocyte colony stimulating factor in	N=12 total N=8 craniospinal RT/	GCSF: 4-5 ug/kg SQ **initiated when ANC was < 1500** (*only when needed, not consecutively, once initiated as in the study*	Increased neutrophil count in all patients No unscheduled	○ Small numbers ○ Confounded by prior chemo in 4 lymphoma patients	Probably

Reference	Design	Dose	Results	Critique	Supportive of Use?
radiotherapy induced neutropenia: Clinical and laboratory studies. *Eur J Cancer* (1995) **31A**:302, LGF: Neupogen (filgrastim)	No chemo 5 of the 8 pts were **pediatric** (ages 1-6 years) N=4 extended field RT/ previous chemo for lymphoma	*above*) o CSRT pts received 2-6 injections during RT o lymphoma pts received 3-6 injections during RT	treatment breaks in RT only pts due to neutropenia	o RT interrupted in 3 patients due to low platelets (2) and urinary tract infection (1)	
3 Effect of G-CSF as an adjunct to large-field radiotherapy: a phase I study. *IJROBP* (1996) **35**(1):137-42. LGF trade name: not specified	Open label, phase 1, noncontrolled, nonrandomized **Prophylactic** GCSF – given before WBC drop N=30 Hodgkin's (12); Non-Hodgkin's lymphoma (14); Ovarian (2); Anaplastic germinoma (2) Patients with bone metastases or bone marrow involvement excluded Endpoint: neutropenia according to WHO criteria	RT: 30-50% marrow; variable doses Chemo: yes; variable prior to RT GCSF: 300 ug SQ (not weight based) Fri/Sat/Sun begins post 5th fraction **6 patients RT only [2 GCSF+ arm; 4 GCSF- arm]** all others had prior chemo	Longer treatment duration for subjects without GCSF (14/15 patients in GCSF (-) group required a treatment break ANC > for patients with GCSF Lower platelet nadirs in GCSF group Adverse Events: musculoskeletal pain in 6/15 patients	o Confounded by prior chemo, RT, and underlying disease o GCSF dose not weight based; LGF trade name not specified o Unusual dosing regimen o GCSF initiated at beginning of RT in prophylactic manner	possibly

Reference	Design	Dose	Results	Critique	Supportive of Use?
4 Effect of GCSF in Hodgkin's Disease Patients Treated with Radiation Therapy *IJROBP* (1994) **28**(2):445-50. LGF trade name: Filgrastim	N= 7 patients 2Female/5 male aged 27-72 years with Hodgkin's Disease, no prior therapy, plan for RT only (no chemo) Historical control used (N=100) 5 nodular sclerosing; 1 lymphocyte predominant; 1 mixed cellularity WBC, ANC and platelets checked 3x/week	GCSF: 3 ug/kg/d SQ beginning Day 1 of the second course of RT – then dose decreased to 2 ug/kg/day for ANC ≥ 10,000/mm3, and 1 ug/kg/day for ANC ≥ 15,000/mm3 and discontinued for ANC ≥ 20,000/mm3 and resumed at 1 ug/kg/day once the ANC was < 20,000. RT: Subtotal lymphoid mantle: ~45 Gy 1-2 week break then sub-diaphragmatic, para-aortic, and common iliac: ~35-43 Gy	WBC and ANC nadir higher in GCSF group during second RT course. No difference during first RT course. Platelets no difference between groups AEs: musculo-skeletal pain in 3/7 patients 1/7 patients developed radiation pneumonitis (known RT toxicity) No RT treatment breaks	○ Small numbers ○ GCSF given in a prophylactic manner ○ Historical controls but at least from same institution ○ No follow up available regarding WBC, ANC, survival or local control	Possibly

Additional information and/or comment on the references in Table 1 are provided below.

It is difficult to draw definitive conclusions based on the differences in the clinical scenarios as well as the paucity of data in subjects treated only with RT (no chemo). It appears that the utility of GCSF is when the WBC (or ANC) is low to allow subjects to complete their treatment in a timely fashion. The long term consequence of GCSF use in the radiation setting with respect to bone marrow exhaustion remains unknown, but it may be a concern as hematopoietic cells are sensitive to radiation such that one fraction may be sufficient to kill the newly stimulated cell as it passes through the radiation beam. One observation appears to be that GCSF does not impede the delivery of RT. Note that these studies did not address issues, at all or in sufficient detail, to

draw conclusions regarding febrile neutropenia, local control, and survival to address applicability to acute radiation syndrome clinical scenario.

Reference 1

- 4 patients with RT only (#1, 2, 7, and 11)
- Leucocyte counts increased (in all 4 patients) and RT treatment breaks were avoided in 2 patients due to neutropenia.
- The figure below documents the treatment of patient #1. Note that the leucocyte count appears to struggle with the opposing effects of GCSF to increase the leucocyte count and RT that decreases the count.

Leucocyte counts returned to subnormal levels within 2-3 days after the discontinuation of GCSF in these patients (duration unknown).

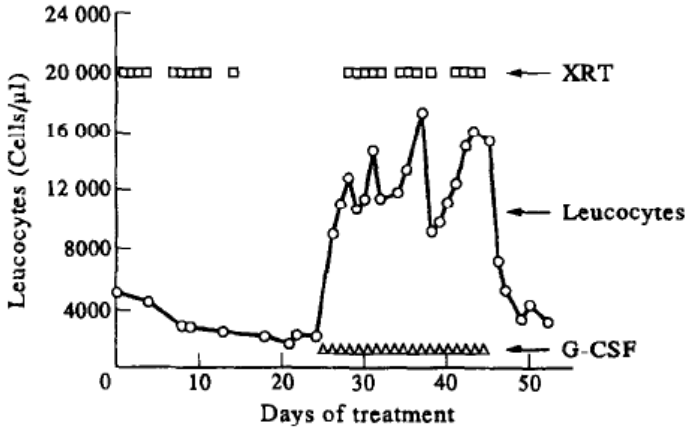


Fig. 1. Leucocyte counts of patients treated with fractionated radiotherapy and G-CSF. The change of circulating leucocyte counts over treatment time from the beginning of radiotherapy is shown for one representative patient (patient no. 1). Leucocyte counts are indicated in open circles. Each dose of G-CSF (5 µg/kg/day) is shown as a triangle. Fractions of radiotherapy are given in open squares. The application of G-CSF induced a rapid increase of leucocyte counts, allowing the continuation of radiotherapy. Similar curves have been obtained for the other patients, apart from patient no. 5 who reacted adversely.

Fig.1 reproduced from Eur J Cancer 1993 Vol 29A, No 14, pp 1927-1931

Regarding Reference 1, it appears that the growth factor is able to increase the leucocyte count but that once cells are circulating they are killed by the subsequent fraction of RT. This may ultimately lead to a reduction in the ability of the marrow to recover (i.e. utilize reserve) as evidenced by the subnormal cell count after discontinuation of growth factor.

Reference 2

- 5 of 8 patients were pediatric (ages 1-6 years)
- GCSF utilized when needed (not continuously)
- Absolute neutrophil count [ANC] peaked the day after each GCSF injection and then steadily declined. Further GCSF injections were administered if the ANC reached treatment threshold (<1500)

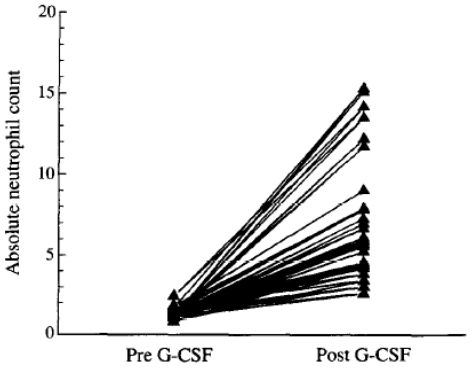


Figure 1. Craniospinal patients—absolute neutrophil counts immediately before and one day after each G-CSF dose given during craniospinal irradiation.

Figure 1 reproduced from Eur J Cancer 31A:302, 1995

Regarding Reference 2, the GCSF was only used when needed as opposed to the study above in which GCSF is given daily once initiated. The GCSF resulted in a temporary increase in the WBC. Conceivably, this administration schedule may be more practical than the one utilized in the study previously discussed above as it is potentially a) more economical, b) conserves bone marrow reserves, c) less cumbersome for staff and patients, and d) achieves the same result (increased leucocyte count). It is unknown if the patients WBC returned to normal or subnormal levels after completion of GCSF and RT.

Reference 3

An increase in the ANC was observed in all patients. Upon discontinuation of GCSF, the ANC dropped daily. Patients who received GCSF were able to complete their treatment without interruption (N=14). One subject had treatment interrupted for low platelets. GCSF was given on the weekend to avoid unexpected side effects due to the simultaneous interaction of fractionated radiation and stimulation of hematopoietic stem cells (cells that were stimulated and circulating could be damaged/killed by a subsequent fraction of radiation). See "Fig. 1" above under Reference 1.

Reference 4

Regarding Reference 4, GCSF was given in a prophylactic manner beginning on Day 1 of the second course of RT and then the GCSF dose was modified based upon the ANC. An increase in ANC was seen over historical controls during the second course of RT (see figure below). There was no difference in ANC between the groups during the first course of RT. As noted in other articles, no differences were seen in platelet levels. None of the 7 patients required an unplanned treatment break. The authors did not comment on a comparison of abdominal side effects between groups.

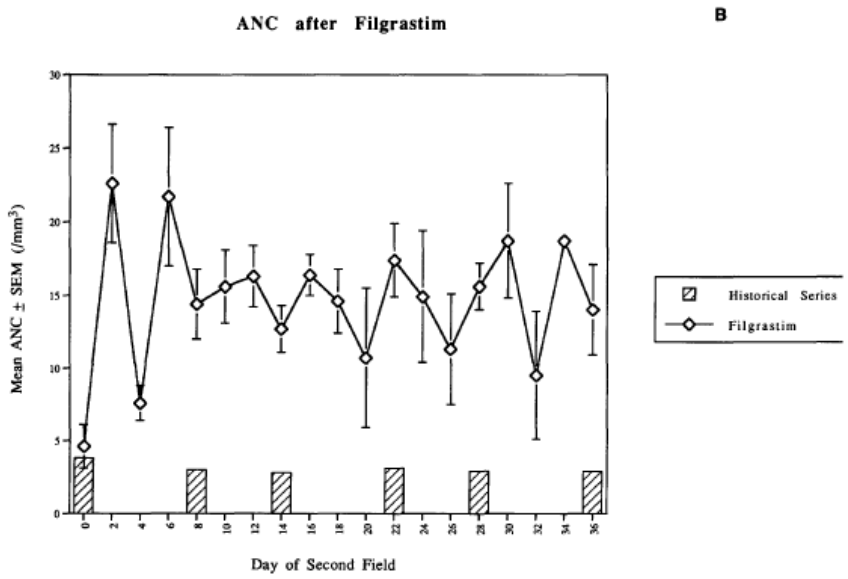


Figure (above) reproduced from IJROBP Vol. 28(2), 1994

Table 2. RT + GCSF Studies Potentially Supportive of GCSF Efficacy in Reducing Mucositis Severity

Note: It is unclear that mucositis as an endpoint is useful information to consider when evaluating the potential of GCSF to improve survival after an acute radiation accident. However, the information is included here for completeness and reader reference.

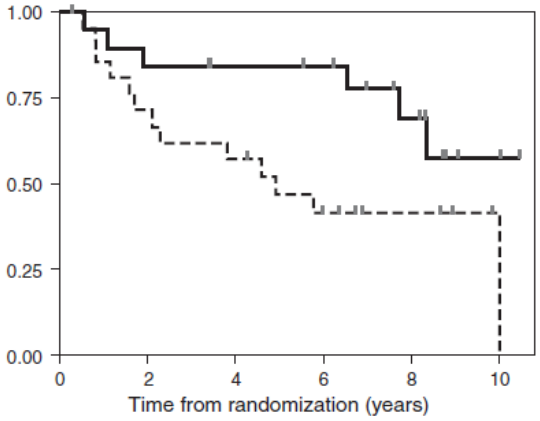
Reference	Design	Dose	Results	Critique	Supportive of Use?
5 Double-Blind, Placebo-Controlled, Randomized Trial of GCSF During Postoperative RT for Squamous Head and Neck Cancer *Cancer J* (2006) **12**(3):182-188 LGF trade name: not specified	Single institution, randomized, double-blind, placebo-controlled phase 3 trial **post operative** adjuvant RT stage II-IV Head & Neck cancer Stratified by disease site N=41 (19 GCSF) primary endpoint: percutaneous endoscopic gastrostomy (PEG) placement as defined by >10% weight loss Secondary endpoint: Severity of mucositis – prespecified criteria	GCSF: 3 ug/kg/d SQ beginning 3 days prior to RT through end of RT GSCF dose modified/held pending WBC RT: 63 Gy primary site and involved neck, subclinical risk 54Gy; 1.8 Gy/fraction	**GCSF arm showed trends toward** lower rates of PEG placement (0% vs. 14%, P = 0.2) and decreased **severity of mucositis** (P= 0.13), and had shorter mean RT duration (48.4 ± 4.32 days vs. 51.6 ± 1.84 days, P = 0.005). Unplanned analysis: OS with median duration of f/u ~7 yrs. Deaths: GCSF: (6); placebo (13) > # T4 patients in placebo arm; > # N2 and N3 disease in GCSF arm all subjects completed	○ Mucositis was secondary endpoint ○ Difficult accrual ○ GCSF arm worse actors ○ Small #s ○ LGF not specified ○ WBC not an endpoint and no long term follow up re: WBC ○ GCSF given prophylactically Note that this study was post operative adjuvant RT as opposed to definitive therapy Local control and survival were not planned efficacy endpoints	Unclear due to unusual endpoints, slow accrual, no long term follow up with respect to WBC

Reference	Design	Dose	Results	Critique	Supportive of Use?
			RT Adverse Events: ↑WBC, bone pain in GCSF arm		
6 Filgrastim and its potential use in the reduction of radiation induced oropharyngeal mucositis: An interim look at a randomized, double blind, placebo controlled trial *Cytokines, cellular & molecular therapy* (1999) **5**:175-80 LGF trade name: Neupogen	N=14 (54 planned) Head & Neck cancer (does not specify definitive or adjuvant RT) Randomized Double blind Placebo controlled Independent blinded observer scored mucositis using WHO and Hickey scales Treatment for mucositis was allowed Endpoint: severity of mucositis NO CHEMO	RT: at least 50 Gy planned GCSF: 3 ug/kg/d beginning day 1 and throughout RT GCSF dose titrated to ANC between 10-30 (max dose GCSF 12 ug/kg/d)	Mucositis less severe with GCSF	○ Interim analysis ○ Survival and local control not evaluated ○ Study not completed Note: this study titrated ANC similar to the Reference 4 Note the different end points of each study	Possibly – for mucositis endpoint

TABLE 4 Patterns of Recurrence

Pattern of Recurrence	G-CSF (n = 19)	Placebo (n = 22)
Local-Regional (L-R) only	1	1
Distant only	2	4
Both (L-R & Distant)	0	2
2nd primary malignancy	2	3
Unknown cause of death	1	3
No evidence of disease*	13	9

*Continuously disease-free through last follow-up.



Reprinted from article 5 (2006 Cancer Journal 12:182-188)

Reference 5

This is the one study that evaluated survival, albeit, in a post-hoc (unplanned) analysis. The GCSF arm, which had patients with more advanced disease, had improved survival. While the statisticians determined the difference to be statistically significant even when controlling for multiple variables including Tumor and Nodal status, the clinical significance is unknown as the study enrolled post operative subjects (no gross disease) and had difficulty with accrual. Note the 4 subjects (1 GCSF; 3 placebo) the cause of death was unknown. A pubmed search was performed to find a follow up article yielded no results.

Table 3. RT + GCSF Studies that may NOT Support Use in ARS

Reference	Design	Dose	Results	Critique	Supportive of Use?
7 GCSF During Large Field RT Reduces Bone Marrow Recovery Capacity *Eur J Med Res* (2006) **11**:322-328 LGF trade name: Neupogen (filgrastim) Labeled dose of filgrastim for reference: 5 -10 mcg/kg/day depending upon indication	N=10, Prospective, randomized, RT only vs. RT + **concurrent** GCSF; Endpoint: CD34+ cells measured by flow cytometry on day 4 and colony forming unit (CFU) day 14 post agar inoculation; Follow Up: 1, 3, & 18 months Stopping rules: 1. ↓ of CD 34+ cells > 50% of baseline level 2. platelets < 30,000/ul RT: ≥8 thoracic vertebrae, the complete vertebral column, abdomen or pelvis (~25% of bone marrow volume)	GCSF: 12.5 ug/kg body weight SQ on days 1-4 CD34+ and progenitor cells determined on day 4 RT: variable doses and anatomic regions; not standard fractionation based on US practices Chemo: 4/5 subjects in each arm had received prior but not concurrent chemo	***Long term follow up revealed a persistent decrease in CD34+ cells in GCSF group at all follow up time points.*** Study stopped early as it met (negative) stopping rules. GCSF group had ↓ in monocytes but ↑ in neutrophils. No difference between arms for eosinophils, basophils, or lymphocytes.	○ Small numbers ○ Variable RT doses and regions ○ GCSF given Day 1-4 concurrent with RT (before needed) ○ Confounded by prior chemo ○ GCSF dose > labeled recommendation of 5-10 mcg/kg/day	No May indicate concurrent GCSF + RT utilizes bone marrow progenitor cell reserve

Reference	Design	Dose	Results	Critique	Supportive of Use?
8 Granulocyte Colony Stimulating Factor for Prevention of Cranio-spinal Radiation Treatment Interruption among Central Nervous System Tumor Patients *Asian Pac J Cancer Prevention* (2010) **11**(6): 1499 LGF trade name: not specified	Prospective, randomized GCSF vs. no GCSF N=40 Brain tumors (31/40 medulloblastoma) 16/40 had prior chemo RT discontinued when WBC<2000; platelets <100,000 Endpoint: total # of RT interruption days	GCSF: 1 dose/week during RT (amount not specified) RT: Posterior fossa 54Gy; spine 36 Gy	Average WBC > GCSF (~4000) than control (~3000) Average platelets not significantly different RT interruption < GCSF (7 days) than control (11 days) among pts who received chemo (8 patient in each group), the GCSF group only one patient had RT break, while 6 of 8 patients in the control group had RT break due to WBC count	○ GCSF dose and trade name not specified ○ ANC may be better endpoint than WBC ○ Platelet cut off of 100,000 is high ○ Perhaps utility is in patients with prior chemo ○ Concurrent administration of GCSF and RT ○ GCSF dose justification unknown ○ 40% of control arm received GCSF	Unclear – insufficient information in article and significant number of control arm received GCSF

Reference	Design	Dose	Results	Critique	Supportive of Use?
9 Intensified hyper-fractionated accelerated radiotherapy limits the additional benefit of simultaneous chemotherapy--results of a multicentric randomized German trial in advanced head-and-neck cancer. *IJROBP* (2001) **50**(5): 1161-71 LGF trade name: not specified	5 centers (1995-1999) N=263 Stage 3 or 4 oro- or hypo- pharynx, unresectable Randomized, prospective, phase 3 Primary endpoint: 1 yr survival with local control Arm A (N=113): hyperfractionated, accelerated, combined modality tx (concurrent 5-FU/carboplatin) Arm B (N=127): hyperfractionated, accelerated RT (no chemo) GCSF: each arm had 2nd randomization to + or – GCSF prophylactically for assessment of mucositis efficacy GCSF was allowed for all patients if WBC $\leq$2000 57% pts had	RT: 70 Gy am: 1.8 Gy pm: 1.5 Gy concomitant boost beginning week 4 not IMRT Chemo: weeks 1 + 5 5FU 600 mg/m^2/d c.i. Carboplatin 70 mg/m^2/d GCSF: 263 ug SQ days 15-19 (except in 1 center due to cost)	Minimum f/u 15 months 23/240 did not start therapy Arm A: 4/116 did not receive RCT as planned Arm B: 1/124: did not receive RT as planned GCSF was stopped after interim analysis showing a trend toward reduced local control Multivariate Cox analysis showed GCSF use as a poor prognostic indicator	○ Study was not powered to detect GCSF effect – only survival ○ Patients with low WBC were allowed GCSF as treatment (number unknown) ○ Confounded by chemo in Arm A ○ LGF not specified	No - Worse outcome with GCSF with respect to local control when given concurrent and prophylactically with RT

Reference	Design	Dose	Results	Critique	Supportive of Use?
	gastrostomy tube placed Routine mouth washes were recommended and regular oral swabs were performed. RTOG/EORTC AE criteria				
10 The effect of GCSF on oral mucositis in head and neck cancer patients treated with hyper fractionated radiotherapy *Oral Oncology* (1999) **35**(2):203-8 LGF trade name: not specified	N=26 (13 each arm) consecutive patients without prior RT or chemo At least 50% of oropharynx in treatment volume Head & Neck cancer stage 3&4 RT (no. 1-13) vs. RT + GCSF (no. 14-26) Endpoints: Daily mucositis, median mucositis score, day of highest mucositis, requirement of parenteral nutrition, weight loss, treatment break, number of days of RT interruption were analyzed during RT treatment. WHO toxicity	RT: hyper-fractionated 74.4 Gy/62 fxs or 73.6 Gy/46 fxs GCSF: began first day of RT and given daily throughout treatment Initial dose: 3 ug/kg/day then adjusted to maintain ANC between 20,000 and 25,000 ul	No benefit other than decreased treatment breaks RT only: grade 4 mucositis and tx break 69% RT+GCSF: grade 4 mucositis and tx break 23% 2 patients reported mild bone pain	○ Not randomized ○ No mention of local control or survival ○ Mucositis was endpoint ○ Neutropenia, fever, infection not evaluated	No

Reference	Design	Dose	Results	Critique	Supportive of Use?
	scale				
11 Hyperfractionated radiation therapy and 5-fluorouracil, cisplatin, and mitomycin-C (±GCSF) in the treatment of patients with locally advanced H&N carcinoma. *Cancer* (1997) **80**:266–276 LGF trade name: not specified	N=70 Stage 3 17 Stage 4 53 FU=41 mo (12-80 mo) GCSF added after 34 pts enrolled RTOG acute toxicity scale Grade 1: erythema or dry desquamation; Grade 2: patchy exudative mucositis or patchy moist desquamation; Grade 3: confluent moist fibrinous mucositis or moist desquamation with severe pain requiring analgesic; and Grade 4: ulceration, necrosis, hemorrhage, or requiring hospitalization.	5FU 1000 mg/m^2/24hr x 72 hrs Mito-C 8 mg/m^2 Cisplatin 50 mg/m2 RT 1.2 Gy BID to 74.4 Gy total GCSF 5 ug/kg/day Monday thru Friday beginning wks 2-4, 6, and 7	Grade 3/4 mucositis =65% in both arms of study Grade 3/4 leukopenia GCSF (-) 45% GCSF (+) 36% G-CSF administered during the second phase of delivery the protocol did not reduce the severity or duration of mucositis and did not reduce the incidence of grade 3/4 leukopenia or leukopenic fever.	○ No benefit with GCSF ○ GCSF not started until half way thru trial ○ Concurrent chemo ○ No diff in local control ○ GCSF brand not specified One case of Myelodysplastic syndrome 15 mo post treatment (treatment arm unknown)	No due to concurrent chemo

Table 4. RT + GMCSF Studies Potentially Supportive of GMCSF Efficacy in Reducing Mucositis Severity

Reference	Design	Dose	Results	Critique	Supportive of Use?
12 **GMCSF** improves double hemibody irradiation (DHBI) tolerance in patients with stage III multiple myeloma: a pilot study *Br J Hematology* (1995) **89**: 191-195 LGF trade name: not specified though Schering Plough stated as manufacturer	N=10 with stage IIIA multiple myeloma 2/10 DHBI first line treatment SWOG response criteria prespecified WHO criteria for GMCSF AEs Historic controls without GMCSF	GMCSF: 5 ug/kg/d SQ day 0-15 post RT RT: hemibody 7 weeks apart Dose: 8 Gy	9/10 completed DHBI Compared to historical controls, mean neutrophil count was higher 2nd hemi irradiation interval shorter with GMCSF Greater % of subjects completed DHBI with GMCSF Stomatitis less severe (grade 1) with GMCSF compared to controls Follow up: 11 months (2-33) No infections and decreased platelet transfusions in GMCSF	○ Prior chemo in 8 of 10 patients ○ Small #s ○ Used historical controls	probably

Reference	Design	Dose	Results	Critique	Supportive of Use?
			group		
13 Comparison of **GMCSF** and sucralfate mouthwashes in the prevention of radiation-induced mucositis: a double-blind prospective randomised phase III study. *Int J Radiat Oncol Biol Phys* (2002) **54**(2):479–485. LGF trade name: not specified	Prospective, double-blind, randomized, single center, phase 3 study N=40 Adjuvant RT GMCSF vs. sucralfate Beginning p 1st week of RT (10 Gy) until end of RT Swish and swallow RTOG scale for evaluation NO PRIOR CHEMO	RT: 50-60 Gy GMCSF: 37.5 ug **orally** QID Sucralfate: 1.0 gram po QID	GMCSF group: o Mucositis less severe o Less pain o No treatment breaks o < pain medication o No hospitalization o No PEG placement No WBC differences between arms	o Local control and overall survival not assessed o No follow up	Yes - orally

Table 5. RT + GMCSF Studies NOT Supportive of GMCSF Efficacy in Reducing Mucositis Severity

Reference	Design	Dose	Results	Critique	Supportive of Use?
14 Oral Administration of **GMCSF** in the Management of Radiotherapy-induced Esophagitis *Clinical Cancer Research (*1999) **5**:3970–3976 LGF trade name: Mielogen	Phase 2 N=36 stage IIIB non small cell lung cancer **15 no chemo** 21 pre-RT chemo WHO toxicity scale for esophagitis Endpoint: Dysphagia changes after 5 days of oral GMCSF	RT: 60 Gy GMCSF: 800 ug **orally** in 4 divided doses x 5 days beginning after documentation of grade 3 dysphagia	Regression of dysphagia to grade 0/1 was observed in 19of 36 (52%) patients, whereas grade 2 dysphagia persisted in 12 of 36 (33%) patients. Progression of dysphagia to severe grade 4 was seen in 5 of 36 (14%) patients. 22% required repeat treatment for dysphagia	Very difficult administration schedule Not randomized Results not separated for RT only	Unclear
15 Local application of **GMCSF** for the treatment of oral mucositis *European Journal of Cancer* (2001) **37**: 2003–2009 LGF trade name: Leucomax	prospective, randomized, open parallel-grouped, single centre study N=35 evaluable Stratified by RT only or RT/chemo (#s unknown) Stage 3 & 4 Head & Neck cancer GMCSF vs. hydrocortisone	RT: 60 Gy split course Chemo: 5 FU/mito C GMCSF: 400 ug **orally** q day at start of mucositis grade 1 Hydrocortison e wash: 250 ml	No difference between arms	o Chemo for unknown % of pts o Study ended early for lack of efficacy o Concomitant meds allowed (nystatin, analgesics, antifungals; Aluminum Formate; Arnica; Chamomile; Sage) o Corrections for	no

Reference	Design	Dose	Results	Critique	Supportive of Use?
	mouthwash Mucositis: WHO criteria Endpoints: degree of oral mucositis, the perception of pain, the incidence of secondary infections and the change in hematological parameters	q day po at start of mucositis grade 1 Swish & swallow		multiple comparisons were not performed o Split course RT is uncommonly utilized	
16 Efficacy and safety of granulocyte-macrophage colony-stimulating factor **(GM-CSF)** on the frequency and severity of radiation mucositis in patients with head and neck cancer. *Int J Radiat Oncol Biol Phys* (1997) **37**:1005–1010 LGF trade name: Leucomax	RT only (no chemo) N=10 8 buccal mucosa 2 posterior tongue All patients were in-patients	GMCSF 1 ug/kg/d SQ beginning after 20 Gy RT 2 Gy to 66 Gy	No patient developed Grade 3 mucositis	o Unusual patient population (buccal) o Small #s o Inpatients o No patient developed G3 mucosits – uncommon event	unclear

Reference	Design	Dose	Results	Critique	Supportive of Use?
17 Evaluation of efficacy and safety of **GM-CSF** in the prophylaxis of mucositis in patients with head and neck cancer treated with RT. *J* *Cancer (*1995) **41A**:431 LGF trade name: not specified	N=10 >T2N1M0 RT +/- GMCSF	RT 200 cGy/day GMCSF 1 ug/kg/d SQ beginning week 3 of RT thru end of RT	Pain 41% control 6% GMCSF Oral mucositis not evaluated	o Abstract only o Small #s o Mucositis not evaluated	unclear
18 Therapeutic efficacy by **GMCSF** on mucositis in patients with oral and oropharyngeal tumors treated with curative RT *Med Onc* (2005) **22**(3): 247-56 LGF trade name: Leucomax Funded by grant from Schering-Plough	Multicenter, randomized, phase 3 trial N=92 (48+44) - 51 total patients in primary endpoint analysis RT+GMCSF vs. RT only GMCSF begins after mucositis score of 1.5 Mucositis prespecified Primary endpoint: mucositis score 2 weeks post GMCSF	GMCSF: 4 ug/kg/d SQ at time of mucositis score $\geq$1.5 until end of RT RT: 60 Gy; $\geq$64 Gy; and Hyperfractionated, accelerated 64.6 Gy	Same unplanned tx break decrease in mucositis score > GMCSF group GMCSF group had less weight loss	o >\# women in control group o Definitive and adjuvant RT groups but balanced between arms o Did not evaluate local control or survival o Short f/u o Unusual endpoint o Unusual grading system	unclear

Reference	Design	Dose	Results	Critique	Supportive of Use?
19 **GMCSF** and sucralfate in prevention of radiation-induced mucositis: a prospective randomised study. *Int J Radiat Oncol Biol Phys* (2000) **46**:525–534 LGF trade name: Leucomax	Open, prospective, randomized study GMCSF+sucralfate Sucralfate only (control) N=40 Prior chemo not allowed	**GMCSF** 150 (<70 kg) -300 (>70 kg) ug SQ beginning p 10 Gy thru RT course Sucralfate 1 gram po 6x/day RT median 66 Gy Daily vs BID RT (1.6 Gy) 23 pre-op RT 10 post-op RT 7 RT only	No difference in frequency or severity of mucositis Local skin reaction > in GMCSF group	o No benefit from GMCSF o Worse skin reaction in GMCSF group o RT varied o Unknown if sucralfate was a good choice for a control arm	no
20 Efficacy and safety of **GMCSF** on frequency and severity of mucositis in patients with H&N ca *IJROBP* (1995) **37(**5): 1005-10 LGF trade name: Leucomax	RT: definitive Head & Neck cancer stages 3 and 4 N=10 Mucositis grading scale prespecified Pilot study	RT: ~66 Gy cobalt (range 60-70) GMCSF: 1 ug/kg/d SQ beginning p 20 Gy thru end of RT	No grade 3 mucositis	o Nonrandomized o Small #s o Old RT technique	unclear

Reference	Design	Dose	Results	Critique	Supportive of Use?
21 **GMCSF** mouthwashes heal oral ulcers during H&N RT *IJROBP* (1998) **41**(4): 747-754 LGF trade name: Leucomax	N=12 definitive RT for Head & Neck cancer GMCSF began with ulceration Mucositis criteria specified – WHO Retrospective control group No chemo	RT: 72 Gy GMCSF: 300 ug oral mouthwash daily x3 upon ulceration – if Complete Response or No Response mouthwash stopped – if Partial Response continued for 3 days	8/12 GMCSF had disappearance of ulcerations 3/12 progressed 1/12 Partial response	○ Unusual GMCSF dosing regimen ○ Retrospective control group	unclear
22 A Pilot Study of the Effect of **GMCSF** on Oral Mucositis in Head & Neck cancer patients during RT: A Preliminary Report *IJROBP* (1998) **42(**3): 551-56 LGF trade name: Mielogen	N=17 Head & Neck cancer Mucositis scale prespecified No placebo arm	RT: 50-70 Gy GMCSF: 400 ug po q day from time of pain (usually after end of week 2) until end of RT	descriptive	Multiple primary tumor sites; 3 patients had chemo; GMCSF oral administration; Pilot study – no placebo arm; Short f/u; Small #s No systemic evaluation	unclear

Reference	Design	Dose	Results	Critique	Supportive of Use?
23 Treatment of irradiation-induced mucositis with **GMCSF** in patients with head and neck cancer *Anticancer Research* (1999) **19**(1B):799-803. LGF trade name: Leucomax	N=16 Locally advanced Head & Neck cancer Retrospective 16 historical controls Post op RT + GMCSF vs. post op RT only Mucositis assessed by prespecified scales NO CHEMO	RT: 60 Gy post operative GMCSF: 5 ug/kg/day SQ for 5 days starting after 20 Gy and symptoms of mucositis	Pain improved with GMCSF AEs: bone pain and elevation of alkaline phosphatase	o Retrospective, historical control group o Small #s	unclear
24 Oral pseudo-membranous candidiasis, herpes simplex virus-1 infection, and oral mucositis in head and neck cancer patients receiving radiotherapy and **GMCSF** mouthwash. *J Oral Path & Med* (2001) **30** (8):471-80 LGF trade name: Mielogen	N=61 18/61 concurrent chemo 10/61 post op RT Patients receiving H&N RT (variable diseases – oral squamous cell, nasopharyngeal, non-Hodgkin's lymphoma, salivary adeno-carcinomas, laryngeal carcinoma, and osteosarcoma. No control group	GMCSF mouthwash: 400 ug orally once per day at time of mucositis until end of RT	46/61 received GMCSF for ulcers	o Oral mouthwash o Variable malignancies o Confounded by chemo o Variation in RT port and dose o Article difficult to read due to references back to previously published articles o Descriptive statistics only	Unclear

Reference	Design	Dose	Results	Critique	Supportive of Use?
25 Therapeutic effect of oral recombinant human **GMCSF** in radiotherapy-induced esophagitis *Hepatogastroent* (2003) **50**(53):1297-1300 LGF trade name: Leucomax	N=97 -> 48 of whom developed esophagitis symptoms, had endoscopy. 25 of the 48 were treated with GMCSF for grade 3 esophagitis then had repeat endoscopy within 3 days of GMCSF Chest or Head and Neck cancer treated with definitive RT Chemo: concurrent (N=29) or sequential (N=32) Length of esophagus in RT port: 9-18 cm GMCSF orally administered for endoscopy proven grade 3 esophagitis byKuwahata scoring system Esophagitis symptoms used RTOG scale	GMCSF: 400 ug in water po in three divided doses for 5-10 days post endoscopy proven grade 3 esophagitis RT=50-66 Gy	26 had grade 3 esophagitis 25/25 rec'd GMCSF 23/26 RT continued 21/23 Esophagitis improved; 2 no response 21 RT w/o break 2 patients (NSCLC treated with concurrent chemo and radiation) developed esophageal stricture ~2 months after RT and GMCSF	Endoscopy proven grade 3 toxicity (good); Oral administration; Different primary tumors; Confounded by chemo use	unclear
26	N=33	GMCSF: 100 ug/day SQ post grade 1	1 death progressive	o Not randomized or placebo	unclear

Reference	Design	Dose	Results	Critique	Supportive of Use?
The optimal use of **GMCSF** in radiation induced mucositis in head and neck squamous cell carcinoma *J Cancer Res Ther* (2005) 1(3): 136-41 LGF trade name: Leucomax	27 definitive RT 6 post op RT Chemo: None GMCSF: prespecified starting criteria Toxicity grading pre-specified (CTCAE V2)	mucositis/dys-phagia/pain x 6 days or until symptoms improved RT: 66 Gy definitive; 57 Gy adjuvant	disease 3 local recurrence 1 unrelated death Descriptive statistics hard to interpret No grade 4 pain, dysphagia or mucositis No RT interruptions	controlled o Small #s o Did not evaluate WBC as an endpoint	
27 Randomized phase 2 study of **GMCSF** to reduce mucositis caused by accelerated RT of laryngeal cancer *Br J Radiol* (2006) **79**: 608 LGF trade name: not specified	prospective, randomized, observer blind phase 2 T1 N0 or T2 N0 glottic carcinoma N=29 f/u=3 weeks RTOG scoring system for mucositis	GMCSF: 150 ug SQ daily x 2 weeks beginning on day 15 of RT (final wk of RT + 1st wk of follow up) Placebo injection not used RT: 50 Gy/16 fx/21 days (3.125 Gy/fx)	3 recurrences in control; 1 in GMCSF; 1 MI in GMCSF arm 3 second malignancies in control; 1 in GMCSF arm	o more T2 patients in the GMCSF arm (larger RT field) o difficult accrual o 2 pts discontinued GMCSF o Small #s o LGF not specified	Unclear – probably not
28 The Impact of Concurrent	Prospective, double blind, randomized, placebo controlled,	RT: 60-70 Gy Chemo: concurrent or	There was no difference in reasons for RT discontinuatio n between the	o GM-CSF: trade name not specified o Large # of	No

Reference	Design	Dose	Results	Critique	Supportive of Use?
GMCSF on RT Induced Mucositis in H&N Cancer Pts: A Double Blind, Placebo controlled prospective phase 3 study by RTOG 9901 *IJROBP* (2007) **67**(3): 643-650 LGF trade name: not specified	multicenter, phase 3 study 50% of oral cavity, oropharynx, or both must be in RT port Chemo cisplatin allowed (induction or concurrent) T1 and 2 glottic tumors excluded N=115 Follow up 48 wks post day 1 of RT NCI-CTC defined mucositis Washington Quality of life Head & Neck Endpoint: severity and duration of mucositis	prior cisplatin allowed **GMCSF**: 250 ug/m2 SQ ○ 1 week pre RT until 2 weeks post RT ○ M, W, and F 2 hrs post RT ○ Held chemo days	study arms or in the distribution of acute mucositis scores between the arms. Ninety percent of GM-CSF and 93% of placebo patients completed RT as planned. Days of RT interruption due to toxicity or other reasons did not seem to vary between the arms. No difference in toxicity 43% of GMCSF arm completed RT compared to 78% of placebo arm due to toxicity The average acute mucositis score of the 9 sites (for the GM-CSF arm was 0.73 (0 to 2.9). The average acute mucositis score for the placebo arm	patients in treatment arm discontinued use ○ Role of cisplatin in negative study?	

Reference	Design	Dose	Results	Critique	Supportive of Use?
			was 0.86 with a range of 0 to 3.2. No Difference. Grade 3/4 mucositis was 45% and 47% of the GMCSF and placebo patients respectively. No difference between the arms. QOL: no difference		

There are multiple studies evaluating the effect of GMCSF administered orally or subcutaneously on mucositis, as noted in Table 5 that are not clearly supportive of its use in the radiation clinic or translate to use in the acute radiation setting. It is difficult to draw any definitive conclusions as there appear to be a greater number of negative or inconclusive articles; however, the data appear to suggest a decrease in grade 3 and 4 mucositis, probably subcutaneous administration > oral. It is unclear if this information may represent a benefit to the GI system in an acute radiation syndrome scenario where a) the radiation dose is delivered in one fraction rather than receiving continued injury with additional fractions and b) a larger and different (esophagus has squamous cells) portion of the GI tract is irradiated.