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Sargramostim (rhu GM-CSF) Improves Survival of Non-Human Primates with Severe Bone Marrow Suppression after Acute, High-Dose, Whole-Body Irradiation

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Exposure to acute, high-dose, whole-body ionizing radiation results in bone marrow failure (hematopoietic acute radiation syndrome with resultant infection, bleeding, anemia, and increased risk of death). Sargramostim (yeast-derived rhu GM-CSF), a yeast-derived, molecularly cloned, hematopoietic growth factor and pleiotropic cytokine supports proliferation, differentiation, maturation and survival of cells of several myeloid lineages. We evaluated the efficacy of sargramostim in non-human primates (rhesus macaques) exposed to whole-body ionizing radiation at a 50–60% lethal dose. The primary end point was day 60 survival. Non-human primates received daily subcutaneous sargramostim (7 mcg/kg/day) or control. To reflect the anticipated setting of a nuclear or radiologic event, treatment began 48 h postirradiation, and non-human primates received only moderate supportive care (no whole blood transfusions or individualized antibiotics). Sargramostim significantly increased day 60 survival to 78% (95% confidence interval, 61–90%) vs. 42% (26–59%; $P = 0.0018$) in controls. Neutrophil, platelet and lymphocyte recovery rates were accelerated and infection rates decreased. Improved survival when sargramostim was started 48 h postirradiation, without use of intensive supportive care, suggests sargramostim may be effective in treating humans exposed to acute, high-dose whole-body, ionizing radiation in a scenario such as a mass casualty event.

INTRODUCTION

Humans exposed to acute, whole-body ionizing radiation at doses more than 2 Gy develop bone marrow failure [referred to as hematopoietic acute radiation syndrome (H-ARS)] with resultant infection, bleeding, anemia and an increased risk of death (*1–3*). Interventions to reverse these adverse effects are determined by radiation dose (*4*). Persons exposed to doses of 4 to 10 Gy often receive molecularly cloned hematopoietic growth factors, such as granulocyte-macrophage colony stimulating factor (GM-CSF; sargramostim) or granulocyte-colony stimulating factor (G-CSF; filgrastim or pegfilgrastim), which stimulate surviving hematopoietic cells, accelerate bone marrow recovery and probably increase survival (*5*).

Although molecularly cloned hematopoietic growth factors were given in several radiation accidents, randomized trials in humans to assess safety or efficacy cannot be done. Consequently, non-human primate models are typically used to determine if these drugs are safe and effective (*6–9*). The U.S. Food and Drug Administration (FDA) has published guidelines, ''Product Development Under the Animal Rule'' as the basis for evaluating molecularly cloned hematopoietic growth factors in non-human primates after acute whole-body radiation exposure (*10, 11*). The FDA defines death at 60 days after exposure as the primary end point of interest and accelerating neutrophil recovery as a supportive end point (*12*). Based on such studies, the FDA approved filgrastim (G-CSF) (*13*) and pegfilgrastim (pegylated G-CSF) (*14*) for use in victims of radiation accidents. Both drugs were effective when given to non-human primates 24 h after receiving an acute 6–7.5 Gy whole-body dose of radiation (*15–17*). In these studies, non-human primates received intensive supportive care, individualized antibiotics and blood transfusions. Subsequent non-human primate studies of filgrastim without intensive supportive care showed no improvement in survival (*18*). Also, administration of filgrastim at 48 h

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postirradiation accelerated neutrophil recovery but did not improve survival (*19*).

Sargramostim, a yeast-derived recombinant human granulocyte-macrophage colony stimulating factor (rhu GM-CSF), is a pleiotropic cytokine acting on multiple blood cell lineages to support proliferation, differentiation, maturation and survival of granulocytes, macrophages and dendritic cells (*20*). Biological activities associated with GM-CSF are exerted through binding to specific receptors on pluripotent and mature hematopoietic cells. The enhanced functional activity of effector cells triggered by GM-CSF enhances host defenses against pathogens (*21, 22*).

Sargramostim improves survival, accelerates bone marrow recovery and decreases deaths from infections in persons with bone marrow failure under diverse circumstances including after intensive chemotherapy for cancer and after acute high-dose whole-body irradiation in the context of hematopoietic cell transplants (*23–26*). These data suggest sargramostim may accelerate bone marrow recovery after accidental exposure to acute high-dose whole-body irradiation. We conducted a randomized, placebo-controlled, blinded trial in 108 non-human primates exposed to acute high-dose whole-body ionizing radiation to determine the efficacy of sargramostim. Additional background material is provided in the Supplementary Information (https://doi.org/10.1667/RR100131.1.S1).

MATERIALS AND METHODS

Test/Control Items

Sargramostim (yeast-derived rhu GM-CSF, Leukine®; Partner Therapeutics Inc., Lexington, MA) was stored in refrigeration at 5.5°C to 6.6°C. The control was sterile water for injection, USP (SWFI; Baxter International Inc., Deerfield, IL) purchased commercially and stored at room temperature. Sargramostim (250 mcg/vial) was reconstituted fresh on each dosing occasion in SWFI to obtain a nominal concentration of 250 mcg/ml. The solution was gently swirled at room temperature until complete dissolution of test item, then placed on wet ice pending use.

Animals

Non-human primates were obtained from commercial breeders in the province of Kunming, China, an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited Chinese breeding farm (age 3 years 1 month to 5 years 4 months; weight 2.9 to 6.2 kg at time of dosing). Non-human primates [a total of 108 rhesus macaques (*Macaca mulatta*)] were randomized in this study. Non-human primates were fed standard certified commercial chow (Harlan Teklad Certified Hi-Fiber Primate Diet no. 7195C; Madison, WI) twice daily. Treats or fruits/vegetables were provided for animal enrichment. Municipal tap water exposed to ultraviolet light and purified by reverse osmosis was provided *ad libitum*. Procedures involving the care and use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and were conducted in accordance with the principles outlined in the current Guidelines published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals, an NRC publication. The CiToxLAB North America facility is accredited by the Canadian Council on Animal Care and AAALAC.

Irradiations

Non-human primates were irradiated using a 60-cobalt gamma source (Theratron® 1000; Best® Theratronics Ltd., Ottawa, Canada). The radiation dose was calibrated using an acrylic phantom placed in the same experimental setup used for non-human primate irradiation. Body measurements were performed to deliver the prescribed dose to the midline at the level of the xiphoid process. Measurements were performed using an ion chamber with a solid water phantom build-up. Dosimetry was determined in a Farmer ionization chamber connected to an electrometer included in each irradiation session. nanoDot™ dosimeters were used for confirmation. Dosimetry results (i.e., average of frontal and dorsal) obtained using the Farmer electrode chamber and NanoDot chips were within –1.4% to +1.3% and –10.2% to +5.6%, respectively, of the targeted radiation dose.

Non-human primates were acclimated for a minimum of 6 weeks prior to total-body irradiation (TBI) and then received a single uniform dose of 6.55 or 7.13 Gy delivered at 0.5 Gy min^{-1}. Irradiation time for each non-human primate was calculated individually based on body dimensions in accordance with facility standard operating procedures. Irradiation was divided in two parts, with each non-human primate receiving the first half of the dose antero-posteriorly and the second half postero-anteriorly to produce homogenous dose distribution.

Supportive Care

Non-human primates were monitored continuously for untoward clinical signs. They received moderate supportive care by administration of the following prophylactic drugs: ondansetron for emesis suppression on day 0 before and after TBI, enrofloxacin on days 5–27 and sucralfate on days 5–30. Symptomatic moderate supportive care based on individual animal need consisted of: analgesics for pain management (buprenorphine and/or bupivacaine); parenteral fluids for dehydration (Ringer's lactate with or without 5% dextrose and/or Pedialyte®/Gastrolyte®); nutritional support for weight loss, anorexia and/or mouth lesions (e g., crushed cookies, fruit, juice); and cutaneous care (hydrotherapy, iodine and/or sterile water flush). Moderate supportive care was provided as indicated by cage-side and clinical observations according to the approved protocol.

Experimental Design

The study was designed with reference to ICH M3(R2): Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals; FDA Guidance for Industry October 2015: Product Development Under the Animal Rule Guidance for Industry; and ICH S6(R1): Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

Animal Assignment

Male and female non-human primates were separately assigned to dose groups using a randomized stratification process based on body weights during the acclimation period. A total of 108 non-human primates (54 male/54 female) were randomized to the sargramostim/control and radiation dose groups: 72 (36 male/36 female) received 6.55 Gy (targeted $LD_{50–60/60}$; lethal radiation dose for 50–60% within 60 days postirradiation); and 36 (18 male/18 female) received 7.13 Gy (targeted $LD_{70–80/60}$; lethal dose for 70–80% within 60 days postirradiation).

Blinding

All study personnel were blinded to treatment assignment, with exception of the staff members responsible for randomization, preparation of test items and dosing. Unblinded personnel were not involved in clinical evaluation or euthanasia decisions. The pathologist was blinded at time of necropsy and initial macro- and microscopic evaluation.

Treatment and Route of Administration

Sargramostim (7 mcg/kg/day) or the control were administered subcutaneously (s.c.). The first injection was performed 48 ± 1 h postirradiation, and then daily until absolute neutrophil count (ANC) returned to 1,000/μl or higher for three consecutive days, or when ANC reached 10,000/μl or higher. Dose was explored in pilot studies using non-human primates as the model in agreement with FDA. The 7 mcg/kg dose level was chosen based on two GLP-compliant pharmacokinetic studies that were specifically designed to identify a dose of sargramostim that resulted in a systemic exposure in non-human primates (with and without irradiation) that was less than or similar to that achieved with the currently approved 250 mcg/m^2 human s.c. dose. There was evidence of this dose level having a protective effect against H-ARS in a pilot exploratory study; CiToxLAB study 2014-2313 (sponsor's reference TSK0143). This dose was then used for all studies of GM-CSF in non-human primate models for medical countermeasure evaluation.

Survival

Non-human primates were evaluated for death at least twice daily. At 60 days postirradiation, surviving non-human primates were humanely sacrificed and necropsies completed. Non-human primates in pain or distress that could not be relieved before day 60 were humanely euthanized based on clinical judgment of the clinical veterinarian in consultation, if possible, with the study director and the sponsor who were blinded to experimental treatments. Non-human primates were euthanized if one of the following criteria was observed: respiratory distress; complete anorexia for 3 days with deteriorating conditions; loss of weight more than 20% of baseline; severe dehydration with hypothermia or hyperthermia; recumbent in the cage with decreased or absent responsiveness to touch; severe pain that could not be relieved with analgesia.

Hematology Studies

Blood samples were obtained on days 1–30, 35, 40, 45, 50, 55 and 60 postirradiation. A complete blood count was performed using a clinical hematology analyzer (Advia® 120; Siemens Medical Solutions USA, Inc., Malvern, PA). Blood samples were obtained on days 16, 21 and 30 and at unscheduled euthanasia (if prior to day 30) for anti-drug antibody evaluation [enzyme-linked immunosorbent assay (ELISA)] with affinity-purified, rabbit anti-Leukine polyclonal antibody (Sanofi Genzyme, Bridgewater, NJ).

Clinical Observations

Cage-side clinical signs were recorded on all non-human primates at least twice daily. Detailed clinical examinations were performed prior to assignment, the day prior to irradiation, and every six days thereafter.

Necropsy and Microbiology

A necropsy was performed on all non-human primates, and macroscopic findings were noted. Microbial analyses were performed on blood and tissues from liver, lungs, spleen, kidney, heart and brain (IDEXX BioResearch, Columbia, MO).

Statistical Design

The primary objective of this work was to measure survival rate at day 60 postirradiation at $LD_{50–60/60}$, defined as the proportion of non-human primates alive on that day. Survival rates at day 60 were compared between sargramostim and control using a one-sided Fisher exact test at the 2.5% significance level. Survival rates were summarized with descriptive statistics with 95% confidence intervals (CI). Secondary objectives were to measure overall survival, neutrophil- and platelet-related parameters (nadir, duration of cytopenia and time to recovery), and infection rates. Primary and secondary end points were also analyzed in an exploratory cohort irradiated at $LD_{70–80/60}$. Analyses were performed on the intent-to-treat population of all randomized non-human primates that received irradiation. Data from $LD_{50–60/60}$ efficacy cohort and $LD_{70–80/60}$ exploratory cohort were analyzed separately using the same methods. Analyses were performed using SAS version 9.2 (Cary, NC) running on Windows version 7 (Microsoft® Corp., Redmond, WA).

Sample Size Power Calculations

Using 36 non-human primates per group, the $LD_{50–60/60}$ (6.55 Gy) part of the trial provided 90% power at a one-sided alpha level of 5% to demonstrate a mortality rate at day 60 of 25% in the sargramostim arm and 60% in the control arm. For the exploratory $LD_{70–80/60}$ (7.13 Gy) part of the trial, using 18 non-human primates per group, provided approximately 75% power at a one-sided alpha level of 10% to demonstrate a mortality rate at day 60 of 25% in the sargramostim arm and 60% in the control arm. The mortality rate at day 60 in the control arm was selected based on available historical data (*15*) as well as previous data with this model at the test facility. Given the historical data and study objective, this number of animals used for this pivotal efficacy study was consistent with guidelines when an animal model is used to support regulatory submissions as a surrogate to an efficacy clinical trial under the FDA Animal Rule.

Additional methods are provided in the Supplementary Information (https://doi.org/10.1667/RR100131.1.S1).

RESULTS

Survival

Day 60 survival with $LD_{50–60/60}$ was 78% (95% CI, 61–90%) for non-human primates receiving sargramostim versus 42% (95% CI, 26–59%; $P = 0.0018$) for controls (Fig. 1A) with a survival hazard ratio (HR) = 0.31 (95% CI, 0.14–0.70). For the non-human primates exposed to $LD_{70–80/60}$, 11 of the 18 receiving sargramostim lived to day 60 versus only 3 of 18 controls ($P = 0.0076$) with a survival HR = 0.29 (95% CI, 0.12–0.73; Fig. 1B). The benefit of sargramostim was seen in male and female non-human primates at both dose levels but survival rates were higher in males (Supplementary Table S1; https://doi.org/10.1667/RR100131.1.S1). Survival probabilities at days 15, 30, and 45 are shown in Supplementary Table S2. Most non-human primates dying before day 60 (43 of 51; 84%) had two or more positive tissue cultures with the same bacterial strain, suggesting infection as the likely cause of death. These deaths were less frequent in non-human primates receiving sargramostim, 5 of 8 vs. 19 of 21 non-human primates receiving controls ($P = 0.1119$) with $LD_{50–60/60}$ and 4 of 7 vs. 15 of 15 non-human primates ($P = 0.0227$) with $LD_{70–80/60}$. The two most frequently isolated bacteria were *Staphylococcus aureus* and *Escherichia coli*.

Hematologic Parameters

A marked increase in neutrophils followed by a rapid decrease was apparent in all groups at both radiation doses. For $LD_{50–60/60}$, a neutrophil count lower than 500/μL was reached at a mean of 6.3 days (range, 4–10 days and 4–16

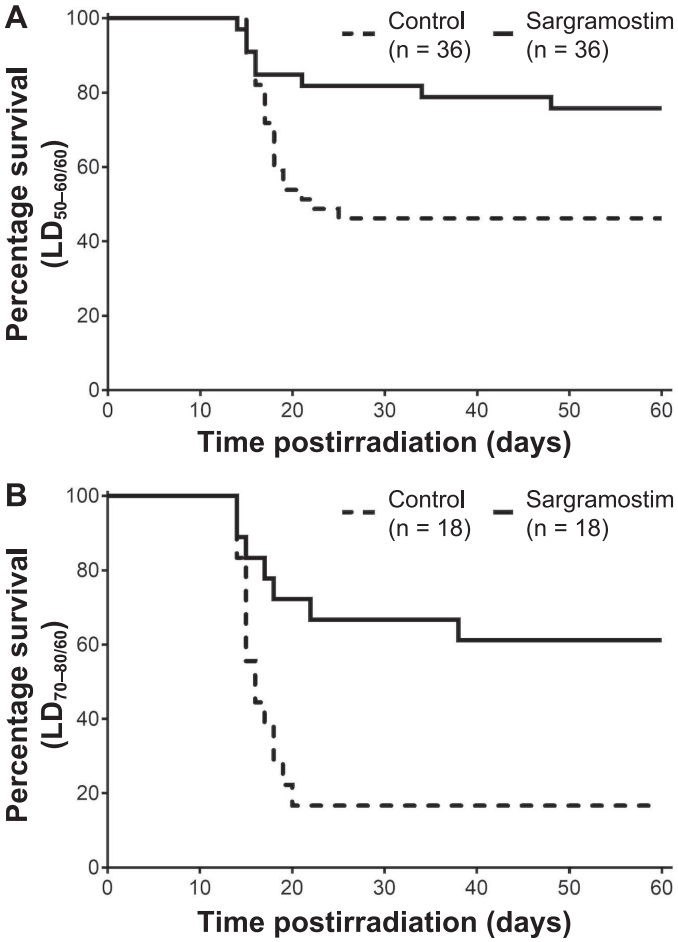

FIG. 1. Sargramostim increases survival when dosed beginning 48 h after TBI. Panel A: After TBI to achieve $LD_{50–60/60}$, 15 of 36 (41.7%) control-treated (dashed line), 28 of 36 (77.8%) sargramostim-treated (solid line) non-human primates survived to day 60 ($P = 0.0018$, Fisher's exact one-sided). The median survival time for the control group is 20 days. The median survival time for the sargramostim group is not estimable. Comparison of the two survival curves indicated a significant difference in favor of the sargramostim-treated group ($P = 0.0023$). Cox proportional hazards model has a hazard ratio (sargramostim versus control) of 0.31 (95% CI, 0.14–0.70). Panel B: After TBI to achieve $LD_{70–80/60}$, 3 of 18 control-treated and 11 of 18 sargramostim-treated non-human primates survived to day 60 ($P = 0.0076$, Fisher's exact one-sided). The median survival time for the control group is 16 days. The median survival time for the sargramostim group is not estimable. Comparison of the two survival curves indicated a significant difference in favor of the sargramostim group ($P = 0.0036$). The Cox proportional hazards model has a hazard ratio (sargramostim versus control) of 0.29 (95% CI, 0.12–0.73). $LD_{50–60/60}$, lethal radiation dose for 50–60% within 60 days postirradiation; $LD_{70–80/60}$, lethal radiation dose for 70–80% within 60 days postirradiation. TBI = total-body irradiation.

days, respectively) postirradiation in both the sargramostim and control cohorts (Table 1). At $LD_{70–80/60}$, a neutrophil count lower than 500/μL was reached at mean of 6.6 days (range, 4–17 days) for the sargramostim cohort and 5.4 days (range, 4–6 days) for controls. The nadir was less deep and recovery was earlier in sargramostim cohorts at both radiation doses (Tables 1 and 2).

Platelet levels started to decrease on day 6 in all cohorts. At both radiation doses, the nadir value in the sargramostim-treated non-human primates was higher than that of controls. Interval to platelets 20,000/μL or higher was briefer in sargramostim-treated non-human primates compared to controls (Tables 1 and 2). Supplementary Figs. S1 and S2 (https://doi.org/10.1667/RR100131.1.S1) display more rapid neutrophil and platelet recoveries in non-human primates receiving sargramostim.

Lymphocyte counts declined rapidly after irradiation followed by a sustained but less severe decrease, reaching lowest mean levels at day 13 for both sargramostim-treated cohorts and on days 16 and 17 for controls ($LD_{50–60/60}$ and $LD_{70–80/60}$, respectively). Nadir lymphocyte counts were higher in sargramostim-treated non-human primates compared to controls and with an earlier recovery (Supplementary Fig. S3; https://doi.org/10.1667/RR100131.1.S1).

Infections

$LD_{50–60/60}$: compared to controls there were fewer documented bacterial infections in the sargramostim cohort, 32% (95% CI, 27–38%) vs. 63% (95% CI, 58–69%; $P < 0.0001$) (Table 3). $LD_{70–80/60}$: there were fewer documented bacterial infections in non-human primates receiving sargramostim compared to controls, 37% (95% CI, 30–46%) versus 84% (95% CI, 77–89%; $P < 0.0001$). (Table 3).

Additional results are provided in the Supplementary Information (https://doi.org/10.1667/RR100131.1.S1).

DISCUSSION

Bone marrow failure causes substantial morbidity and death after exposure to acute high-dose, whole-body irradiation (*27*). Studies of non-human primates that have received TBI indicate that intensive supportive care with individualized antibiotic therapy and blood transfusions improves survival (*6, 27*). However, it is anticipated in a mass causality situation that such resources may not be available because of extensive infrastructure disruptions and lack of appropriately trained medical personnel (*27–29*). Consequently, our study used experimental conditions designed to mimic this scenario.

Our results show a substantial increase in survival when non-human primates are given sargramostim beginning 48 h postirradiation at doses expected to be lethal in 50–60% and 70–80% of non-human primates by day 60. Rates of neutrophil, platelet, and lymphocyte recovery were increased and infection rates decreased. While the effects of sargramostim on myeloid progenitor cells are well known, results affecting lymphoid cells were unexpected. Benefits were achieved with only modest antibiotic support and without blood transfusions or individualized antibiotics.

While no direct comparison of data exists to form a definitive conclusion, sargramostim may offer advantages

TABLE 1
Neutrophil and Platelet Parameters for Non-Human Primates Administered Control or Sargramostim Beginning 48 h after TBI: $LD_{50–60/60}$

Blood cell	Parameter		Control	Sargramostim
Neutrophils	Day of onset[a]			
	ANC < 500/μl	Mean ± SE	6.3 ± 0.31	6.3 ± 0.26
		Range	4–16	4–10
		n	36	36
	ANC < 100/μl	Mean ± SE	11.2 ± 0.31	11.2 ± 0.41
		Range	8–17	7–16
		n	36	34
	Duration, days[b]			
	ANC < 500/μl	Mean ± SE	11.5 ± 1.0	9.9 ± 0.76
		Range	2–17	2–16
		n	18	33
	ANC < 100/μl	Mean ± SE	5.9 ± 0.61	4.7 ± 0.42
		Range	2–11	2–12
		n	20	32
	Day of recovery[c] to			
	ANC ≥ 500/μl	Median (95% CI)	19 (18–20)	17 (16–18)
		P value		$P < 0.0001$
	ANC ≥ 1,000/μl	Median (95% CI)	20 (19–20)	18 (17–18)
		P value		$P = 0.0001$
	ANC nadir[d] (/μl)	Mean ± SE	20.3 ± 2.8	34.4 ± 6.0
Platelets	Duration, days[e]			
	PLT < 20,000/μl	Mean ± SE	6.0 ± 0.62	4.8 ± 0.46
		Range	2–12	2–11
		n	17	28
	Day of recovery[e] to			
	PLT ≥ 20,000/μl	Median (95% CI)	18 (18 to NE)	16 (NE to NE)
		P value		$P = 0.0008$
	Platelet nadir[d] (/μl)	Mean ± SE	6,944.4 ± 1,326.7	11,805.6 ± 1,821.7

[a] Day of onset includes data from non-human primates that developed neutropenia.
[b] Durations do not include data from decedent non-human primates unless recovery occurred prior to death, nor from non-human primates that did not develop neutropenia.
[c] Day of recovery does not include data from decedent non-human primates unless recovery occurred prior to death, nor from non-human primates that did not develop neutropenia.
[d] Nadir includes both survivor and non-survivor values.
[e] Durations and day of recovery do not include data from decedent non-human primates unless recovery occurred prior to death, nor from non-human primates that did not develop thrombocytopenia.
Abbreviations: ANC = absolute neutrophil count; $LD_{50–60/60}$ = lethal radiation dose for 50–60% within 60 days after irradiation; NE = not estimable; PLT = platelet count; SE = standard error.

in the anticipated treatment setting. Sargramostim, filgrastim and pegfilgrastim were studied in non-human primate models at TBI doses expected to be lethal in 50% of non-human primates by day 60. Although decreased deaths compared to controls were observed for sargramostim and filgrastim, only sargramostim significantly improved survival at day 60 when compared to controls, when administered 48 h postirradiation with moderate supportive care (*19*). Published studies of filgrastim and pegfilgrastim indicate improved survival when given with intensive supportive care and beginning 24 h after TBI (*15, 17*). There was no improved survival when filgrastim was initiated at intervals greater than 24 h postirradiation or with only moderate supportive care (*18, 19*). As reported elsewhere, the need for intensive supportive care with G-CSF may limit its efficacy in a mass casualty situation with limited resources (*29*). A potential disadvantage of sargramostim (and filgrastim) is the need for daily dosing compared to pegfilgrastim, which is given weekly.

Sargramostim accelerates recovery and improves function of more myeloid lineages than filgrastim and pegfilgrastim (*26, 30, 31*). Our study and others report increased day 60 survival with sargramostim but not with filgrastim despite similar acceleration of neutrophil recovery (*18, 19*). Multiple hematologic parameters including platelet, neutrophil and lymphocyte counts independently correlate with survival outcomes in H-ARS models (*7, 32*). Stickney *et al.* reported that the relationship between neutrophil recovery and survival in H-ARS remains uncertain (*33*). Better survival in non-human primates receiving sargramostim might reflect the drug's pleiotropic effects on cells beyond neutrophils, namely dendritic cells and macrophages, which have essential roles linking the innate and adaptive immune responses (*20, 25*). The results of an exploratory analysis in the current study support this hypothesis, highlighting the close interactions between neutropenia, thrombocytopenia and lymphopenia. We evaluated the percentage of days alive with lymphopenia or thrombocytopenia versus

TABLE 2
Neutrophil and Platelet Parameters for Non-Human Primates Administered Control or Sargramostim Beginning 48 h after TBI: $LD_{70–80/60}$

Blood cell	Parameter		Control	Sargramostim
Neutrophils	Day of onset[a]			
	ANC < 500/μl	Mean ± SE	5.4 ± 0.15	6.6 ± 0.68
		Range	4–6	4–17
		n	18	18
	ANC < 100/μl	Mean ± SE	9.6 ± 0.20	9.2 ± 0.36
		Range	8–11	7–12
		n	18	17
	Duration, days[b]			
	ANC < 500/μl	Mean ± SE	7.4 ± 3.12	11.6 ± 0.55
		Range	2–16	7–15
		n	5	14
	ANC < 100/μl	Mean ± SE	8.8 ± 0.37	5.9 ± 0.79
		Range	8–10	2–10
		n	5	13
	Day of recovery[c] to			
	ANC ≥ 500/μl	Median (95% CI)	19 (19–20)	17 (16–18)
		P value		$P = 0.2076$
	ANC ≥ 1,000/μl	Median (95% CI)	19 (18–20)	17 (17–18)
		P value		$P = 0.0206$
	ANC nadir[d] (/μl)	Mean ± SE	15.0 ± 1.67	23.3 ± 6.57
Platelets	Duration, days[e]			
	PLT < 20,000/μl	Mean ± SE	6.8 ± 1.11	6.6 ± 0.59
		Range	5–10	2–10
		n	4	15
	Day of recovery[e] to			
	PLT ≥ 20,000/μl	Median (95% CI)	20 (17 to NE)	16 (15–17)
		P value		$P = 0.0002$
	Platelet nadir[d] (/μl)	Mean ± SE	5,000.0 ± 1057.2	4,722.2 ± 955.9

[a] Day of onset includes data from non-human primates that developed neutropenia.
[b] Durations do not include data from decedent non-human primates unless recovery occurred prior to death, nor from non-human primates that did not develop neutropenia.
[c] Day of recovery does not include data from decedent non-human primates unless recovery occurred prior to death, nor from non-human primates that did not develop neutropenia.
[d] Nadir includes both survivor and non-survivor values.
[e] Durations and day of recovery do not include data from decedent non-human primates unless recovery occurred prior to death, nor from non-human primates that did not develop thrombocytopenia.
Abbreviations: ANC = absolute neutrophil count; $LD_{70–80/60}$ = lethal radiation dose for 70–80% within 60 days after irradiation; NE = not estimable; PLT = platelet count; SE = standard error.

neutropenia during the first 30 days postirradiation, the time window with the highest mortality. Both neutrophil and lymphocyte recovery were associated with improved survival. However, the data suggest that the risk of death increases with prolonged lymphopenia even though duration of neutropenia is briefer. A similar observation was made in relationship to platelet recovery versus neutropenia and lymphopenia. Results of our study agree with the observations of Stickney *et al.* and suggest neutrophil recovery contributes to better survival but the severity and duration of thrombocytopenia and lymphopenia are also important. In a clinical study of GM-CSF in allogenic hematopoietic cell transplant recipients who received GM-CSF, G-CSF, or both, 100-day cumulative mortality and infection-related mortality were significantly less and platelet recovery briefer in the GM-CSF cohorts. However, neutrophil recovery was slightly longer in these cohorts (*34*). Studies of the effects of acute radiation on hematologic and immune biomarkers may increase understanding of the relationship between infection risk and death and suggest effective treatment (*18, 35, 36*).

In conclusion, data from a non-human primate model of a radiation accident suggest sargramostim improves survival in a setting mimicking a large-scale nuclear and radiological event.

SUPPLEMENTARY INFORMATION

Supplementary Background Material.

Supplementary Methods.

Supplementary Results.

Table S1. Survival rate at day 60 by sex of non-human primates administered control or sargramostim beginning 48 h after total-body irradiation.

Table S2. Survival probability at days 15, 30 and 45 for non-human primates administered control or sargramostim beginning 48 h after total-body irradiation.

TABLE 3
Neutropenia, Fever, Febrile Neutropenia and Bacteriology for Non-Human Primates Administered Control or Sargramostim Beginning 48 h after TBI

Radiation dose	Parameter		Control	Sargramostim
$LD_{50–60/60}$	Incidence of neutropenia (ANC < 500/μl)	% (n/N)	100 (36/36)	100 (36/36)
	Incidence of fever ≥103°F (without neutropenia)	% (n/N)	8 (3/36)	11 (4/36)
	Incidence of febrile neutropenia (ANC < 500/μl concurrent with ≥103°F core body temperature)	% (n/N)	6 (2/36)	11 (4/36)
	Day of febrile neutropenia onset	Mean ± SE	18	12.4 ± 0.89
		Range		9–15
	Bacteriology samples taken	% (n/N)	53 (312/589)	47 (277/589)
	Samples positive for bacteria	% (n/N)	63 (197/312)	32% (89/277)
		95% CI	58–69%	27–38%
		P value		$P < 0.0001$
$LD_{70–80/60}$	Incidence of neutropenia (ANC < 500/μl)	% (n/N)	100 (18/18)	100 (18/18)
	Incidence of fever ≥103°F (without neutropenia)	% (n/N)	0 (0/18)	0 (0/18)
	Incidence of febrile neutropenia (ANC < 500/μl concurrent with ≥103°F core body temperature)	% (n/N)	6 (1/18)	11 (2/18)
	Day of febrile neutropenia onset	Mean	12	12
	Bacteriology samples taken	% (n/n)	53 (164/311)	47 (147/311)
	Samples positive for bacteria	% (n/n)	84 (137/164)	37 (55/147)
		95% CI	77–89%	30–46%
		P value		$P < 0.0001$

Abbreviations: ANC = absolute neutrophil count; $LD_{50–60/60}$ = lethal radiation dose for 50–60% within 60 days after irradiation; $LD_{70–80/60}$ = lethal radiation dose for 70–80% within 60 days after irradiation; SE = standard error.

Fig. S1. Sargramostim accelerates neutrophil recovery when dosed beginning 48 h after TBI. Panel A: After TBI to achieve $LD_{50–60/60}$, the sargramostim-treated non-human primates (solid line) had significantly accelerated time to neutrophil recovery to ANC ≥500/μl ($P < 0.0001$) and ANC ≥1,000/μl ($P = 0.0001$) compared to the control-treated non-human primates (dashed line). Panel B: After total-body irradiation to achieve $LD_{70–80/60}$, the sargramostim-treated non-human primates had significantly accelerated time to neutrophil recovery to ANC ≥1,000/μl ($P = 0.0206$) compared to the control-treated non-human primates.

Fig. S2. Sargramostim accelerates platelet recovery when dosed beginning 48 h after total-body irradiation. Panel A: After total-body irradiation to achieve $LD_{50–60/60}$, the time to platelet recovery was accelerated in the sargramostim-treated non-human primates (solid line) and demonstrated a significant decrease in the time to thrombocytopenia recovery (platelet count ≥20,000/μl; $P = 0.0008$) compared to the control-treated non-human primates (dashed line). Panel B: After total-body irradiation to achieve $LD_{70–80/60}$, the time to platelet recovery was accelerated in the sargramostim-treated non-human primates and demonstrated a significant decrease in the time to thrombocytopenia recovery (platelet count ≥20,000/μl; $P = 0.0002$) compared to the control-treated non-human primates.

Fig. S3. Sargramostim accelerates lymphocyte recovery when dosed beginning 48 h after TBI. Panel A: Absolute lymphocyte count after total-body irradiation to achieve $LD_{50–60/60}$. Panel B: Absolute lymphocyte count after TBI to achieve $LD_{70–80/60}$. Lymphocyte counts declined drastically immediately after irradiation followed by a sustained but less severe decrease, reaching lowest mean levels at day 13 for both sargramostim groups and on days 16 and 17 for control groups ($LD_{50–60/60}$ and $LD_{70–80/60}$, respectively). Lymphocyte recovery was initiated earlier in sargramostim-treated non-human primates (solid line) and the nadir was also higher compared to control-treated non-human primates (dashed line).

Fig. S4. Sargramostim accelerates reticulocyte recovery when dosed beginning 48 h after TBI. Panel A: Absolute reticulocyte count after total-body irradiation to achieve $LD_{50–60/60}$. Panel B: Absolute reticulocyte count after total-body irradiation to achieve $LD_{70–80/60}$. Baseline mean reticulocyte counts were between approximately 65,000 to 85,000/μl for all non-human primates. Reticulocyte levels began to decline the day after irradiation reaching similar nadir on day 8 in all non-human primates. This was followed by a compensatory increase up to approximately day 13, which was concurrent to bone marrow recovery and is a characteristic of the regenerative response to radiation-induced anemia. The magnitude of the increase was greater in sargramostim-treated non-human primates and was also more pronounced in those that received $LD_{50–60/60}$. A second moderate decline in reticulocyte count was observed up to approximately day 16, which may have resulted from iron sequestration typically observed with acute inflammation. Afterward, a marked increase (approximately five-fold from baseline levels) was noted, reaching mean reticulocyte counts above 450,000/μl in both sargramostim-treated non-human primates (solid line) and between 181,000 and 236,000/μl in control-treated non-human primates (dashed line; $LD_{50–60/60}$ and $LD_{70–80/60}$, respectively) by day 24.

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