SUPPLEMENTARY INFORMATION

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

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Figure S1. Sargramostim accelerates neutrophil recovery when dosed beginning 48 hours after total body irradiation. (A) Following TBI to achieve $LD_{50\text{-}60/60}$, the sargramostim-treated non-human primates (solid line) had significantly accelerated time to neutrophil recovery to ANC ≥500/μL ($P < 0.0001$) and ANC ≥1000/μL ($P = 0.0001$) compared to the control-treated non-human primates (dashed line). (B) Following TBI to achieve $LD_{70\text{-}80/60}$, the sargramostim-treated non-human primates had significantly accelerated time to neutrophil recovery to ANC ≥1000/μL ($P = 0.0206$) compared to the control-treated non-human primates.

Figure S2. Sargramostim accelerates platelet recovery when dosed beginning 48 hours post total body irradiation. (A) Following TBI to achieve $LD_{50\text{-}60/60}$, 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). (B) Following TBI to achieve $LD_{70\text{-}80/60}$, 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.

Figure S3. Sargramostim accelerates lymphocyte recovery when dosed beginning 48 hours after total body irradiation. (A) Absolute lymphocyte count following TBI to achieve $LD_{50\text{-}60/60.}$ (B) Absolute lymphocyte count following TBI to achieve $LD_{70\text{-}80/60.}$

Lymphocyte counts declined drastically immediately after radiation 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 ($LD_{50\text{-}60/60}$ and $LD_{70\text{-}80/60}$, 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).

Figure S4. Sargramostim accelerates reticulocyte recovery when dosed beginning 48 hours post total body irradiation. (A) Absolute reticulocyte count following TBI to achieve $LD_{50\text{-}60/60.}$ (B) Absolute reticulocyte count following TBI to achieve $LD_{70\text{-}80/60.}$ 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 correlated with 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 who received the $LD_{50\text{-}60/60}$ dose. 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 5-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; $LD_{50\text{-}60/60}$ and $LD_{70\text{-}80/60}$, respectively) by day 24.

Supplementary Background Material

Medical Countermeasures. Evaluation and development of colony stimulating factors as medical countermeasures for treatment of bone marrow failure due to acute exposure to high-dose ionizing radiation have largely occurred over the past 20 years to decrease potential morbidity and mortality following a nuclear incident. The US Government has funded the development and acquisition of medical countermeasures for the Strategic National Stockpile (SNS) to treat people inadvertently exposed to potentially lethal levels of radiation (*1*). The SNS Radiation Working Group recommended that individuals inadvertently exposed to more than 2 Gy radiation be treated with colony stimulating factors.

Supplementary Methods

Ethics Statement. All experimental procedures were performed in compliance with Good Laboratory Practice (GLP; 21 CFR Part 58) and in accordance with Institutional Animal Care and Use Committee (IACUC) and the Canadian Council on Animal Care guidelines for use of experimental animals. All protocols included humane euthanasia criteria and were reviewed and approved by the IACUC.

Rationale for Radiation Dose. A total body irradiation (TBI) dose resulting in $LD_{50\text{-}60/60}$ (where 50-60% of the control group is deceased by day 60) was selected for the confirmatory efficacy cohort. The $LD_{50\text{-}60/60}$ radiation dose was consistent with the efficacy studies performed in support of the filgrastim and pegfilgrastim approvals in H-ARS (Neupogen USPI, Neulasta USPI). An exploratory cohort using $LD_{70\text{-}80/60}$ was also included. Based on the survival results in the H-ARS model with similar supportive care levels, the radiation doses needed to achieve the targeted $LD_{50\text{-}60/60}$ and $LD_{70\text{-}80/60}$ were calculated to be 6.55 Gy and 7.13 Gy, respectively.

Rationale for Sargramostim Dosing. The rationale for selecting the sargramostim dose of 7.0 µg/kg/day (84 µg/m^2/day) was that the exposure level and pharmacodynamic effects in non-human primates would generally not exceed that observed in humans receiving the approved sargramostim dose of 250 µg/m^2/day. The dose was based on results from 2 pilot exploratory studies of sargramostim: the first study used healthy non-human primates and the second study used irradiated non-human primates. Both studies showed that exposure would not exceed that observed in humans receiving the approved sargramostim dose of 250 µg/m^2/day (*2, 3*). The

subcutaneous route was selected as it is the intended route of administration of the test item in humans.

***Sample Size Power Calculations*.** For the $LD_{50\text{-}60/60}$ (6.55 Gy) part of the trial, 36 non-human primates per group provided 90% power at a 1-sided alpha level of 5% to demonstrate a significant difference in mortality rate at day 60: 25% in the sargramostim group and 60% in the control group. For the exploratory $LD_{70\text{-}80/60}$ (7.13 Gy) part of the trial, 18 non-human primates per group provided approximately 75% power at a 1-sided alpha level of 10% to demonstrate a significant difference in mortality rate at day 60: 25% in the sargramostim group and 60% in the control group. The mortality rate at day 60 in the control group was selected based on available historical data (*4*) and results from previous sargramostim studies.

***Overall Survival (in days)*.** Overall survival (OS) was defined as the time from TBI to death, and non-human primates still alive at day 60 were censored. OS was analyzed with the Kaplan-Meier method. The median OS and probabilities of surviving at different time points (at 15, 30, and 45 days) were provided with corresponding 95% CIs. For exploratory purposes, OS in the sargramostim group was compared to the control group using the log-rank test. The Kaplan-Meier OS curves were constructed. The estimates of the hazard ratio and corresponding 95% CI were determined using the Cox proportional hazard model. A 2-sided 0.05 alpha level was used.

***Absolute Neutrophil Count-related Parameters*.** These included absolute neutrophil count (ANC) nadir (lowest post-TBI value), duration of severe neutropenia, time to ANC recovery, and incidence/duration of febrile neutropenia. ANC nadir, day of onset, and duration of severe neutropenia were summarized with descriptive statistics. The

analysis of time to ANC recovery was similar to that described for OS. Number (%) of non-human primates with febrile neutropenia was provided, and day of onset and duration was similar to that described for ANC-related parameters.

***Platelet-related Parameters*.** These included platelet nadir (lowest post-TBI value), duration of severe thrombocytopenia, and time to thrombocytopenia recovery. The analysis of platelet-related parameters was similar to that described for ANC-related parameters.

Additional Hematology-related Parameters. These included nadir (lowest post-TBI value), duration of cytopenia, and time to recovery for each of the following blood cells: leukocytes, lymphocytes, erythrocytes, and reticulocytes. The analysis of these additional hematology-related parameters was similar to that described for ANC-related parameters.

***Post-TBI Infection*.** The number and percent of non-human primates with post-TBI infection (ie, positive blood cultures or tissue, or evidence of sepsis at necropsy) were summarized with descriptive statistics.

Experimental Environment. The room environment was maintained at 21 ± 3°C with relative humidity of 50 ± 20%, a 12 hours light/12 hours dark cycle, and 10–15 air changes per hour. Temperature and relative humidity were monitored continuously.

Body Weight and Temperature. Body weights were recorded prior to non-human primate assignment, the day prior to irradiation, and every 3 days thereafter. Body temperature was taken the day prior to irradiation and every 3 days after irradiation until end of study, or when clinically justified and authorized by veterinarian.

Clinical Observations. Cage-side clinical signs were recorded on all non-human primates at least twice daily throughout study. Detailed clinical examinations were performed prior to assignment, the day prior to irradiation, and every 6 days thereafter.

Histology. Histopathological evaluation was performed by a blinded board-certified veterinary pathologist followed by peer review (unblinded) from a second board-certified veterinary pathologist. Tissues from heart, lymph nodes, bone marrow, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, kidneys, liver, lungs, spleen, thymus, injection site, and abnormal findings were collected and fixed in formalin, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin-phloxine.

Exploratory Endpoints. These endpoints were summarized with descriptive statistics by time points and included body weight, temperature (including number of days of fever ≥103°F), and other laboratory parameters. Cage-side observations (clinical signs) were summarized by findings and severity grade, when available. Number (%) of non-human primates developing anti-sargramostim neutralizing antibodies was provided. Gross necropsy and histopathological findings for select organs were also provided.

Study Schedule. The study initiation date was October 29, 2015, and the experimental start date and randomization occurred on October 30, 2015. Irradiation (day 0) was performed on November 24-26 and December 1-3, 2015. Dosing of control or sargramostim was initiated (day 2) on November 26-28 and December 3-5, 2015. The last necropsy was performed on February 01, 2016. The experimental completion date was June 13, 2017, with finalization of the Pathology Report.

Supplementary Results

Histology. Macroscopic and microscopic findings particularly in the gastrointestinal tract and lymphoid organs, consistent with exposure to lethal TBI, were observed in both groups. Increased hematopoietic activity was observed in multiple tissues at study termination in association with sargramostim treatment in non-human primates receiving 6.55 Gy ($LD_{50\text{-}60/60}$) or 7.13 Gy ($LD_{70\text{-}80/60}$) of irradiation.

Clinical Signs. Expected radiation-induced clinical signs were observed (ie, decreased appetite, decreased activity, weakness, hunched posture, dehydration, diarrhea, skin wound, dyspnea, tremors, petechiae, and buccal ulceration) over the course of the study for all groups. Between days 15 and 20 (period with higher mortality rate) in those exposed to the $LD_{50\text{-}60/60}$ dose, the sargramostim group had fewer non-human primates presenting most of these clinical signs. At the higher irradiation dose ($LD_{70\text{-}80/60}$), the difference between sargramostim and control groups was not as evident. After day 25, the occurrence of adverse radiation-induced clinical signs decreased dramatically in all groups.

No drug-induced anti-sargramostim antibodies were detected in this study.

Supplementary Tables and Figures

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

Radiation Dose Sex	Control	Sargramostim
$LD_{50-60/60}$		
Male, % (n/N)	50 (9/18)	83 (15/18)
Female, % (n/N)	33 (6/18)	72 (13/18)
$LD_{70-80/60}$		
Male, % (n/N)	33 (3/9)	67 (6/9)
Female, % (n/N)	0 (0/9)	56 (5/9)

$LD_{50-60/60,}$ lethal radiation dose for 50-60% within 60 days after radiation;
$LD_{70-80/60}$, lethal radiation dose for 70-80% within 60 days after radiation.

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

Radiation Dose Day	Control	Sargramostim
$LD_{50-60/60}$		
Day 15	0.89 (95% CI, 0.73-0.96)	0.92 (95% CI, 0.76, 0.97)
Day 30	0.42 (95% CI, 0.26-0.57)	0.83 (95% CI, 0.67, 0.92)
Day 45	0.42 (95% CI, 0.26-0.57)	0.81 (95% CI, 0.63, 0.90)
$LD_{70-80/60}$		
Day 15	0.56 (95% CI, 0.31-0.75)	0.83 (95% CI, 0.57, 0.94)
Day 30	0.17 (95% CI, 0.04-0.37)	0.67 (95% CI, 0.40, 0.83)
Day 45	0.17 (95% CI, 0.04-0.37)	0.61 (95% CI, 0.35, 0.79)

$LD_{50-60/60}$, lethal radiation dose for 50-60% within 60 days after radiation; $LD_{70-80/60}$, lethal radiation dose for 70 80% within 60 days after radiation.

Figure S1. Sargramostim accelerates neutrophil recovery when dosed beginning 48 hours after total body irradiation.
(A) Following TBI to achieve $LD_{50\text{-}60/60}$, the sargramostim-treated non-human primates (solid line) had significantly accelerated time to neutrophil recovery to ANC ≥500/μL ($P < 0.0001$) and ANC ≥1000/μL ($P = 0.0001$) compared to the control-treated non-human primates (dashed line).
(B) Following TBI to achieve $LD_{70\text{-}80/60}$, the sargramostim-treated non-human primates had significantly accelerated time to neutrophil recovery to ANC ≥1000/μL ($P = 0.0206$) compared to the control-treated non-human primates.
ANC, absolute neutrophil count; $LD_{50\text{-}60/60}$, lethal radiation dose for 50-60% within 60 days after radiation; $LD_{70\text{-}80/60}$, lethal radiation dose for 70-80% within 60 days after radiation; SEM, standard error of mean; TBI, total body irradiation.

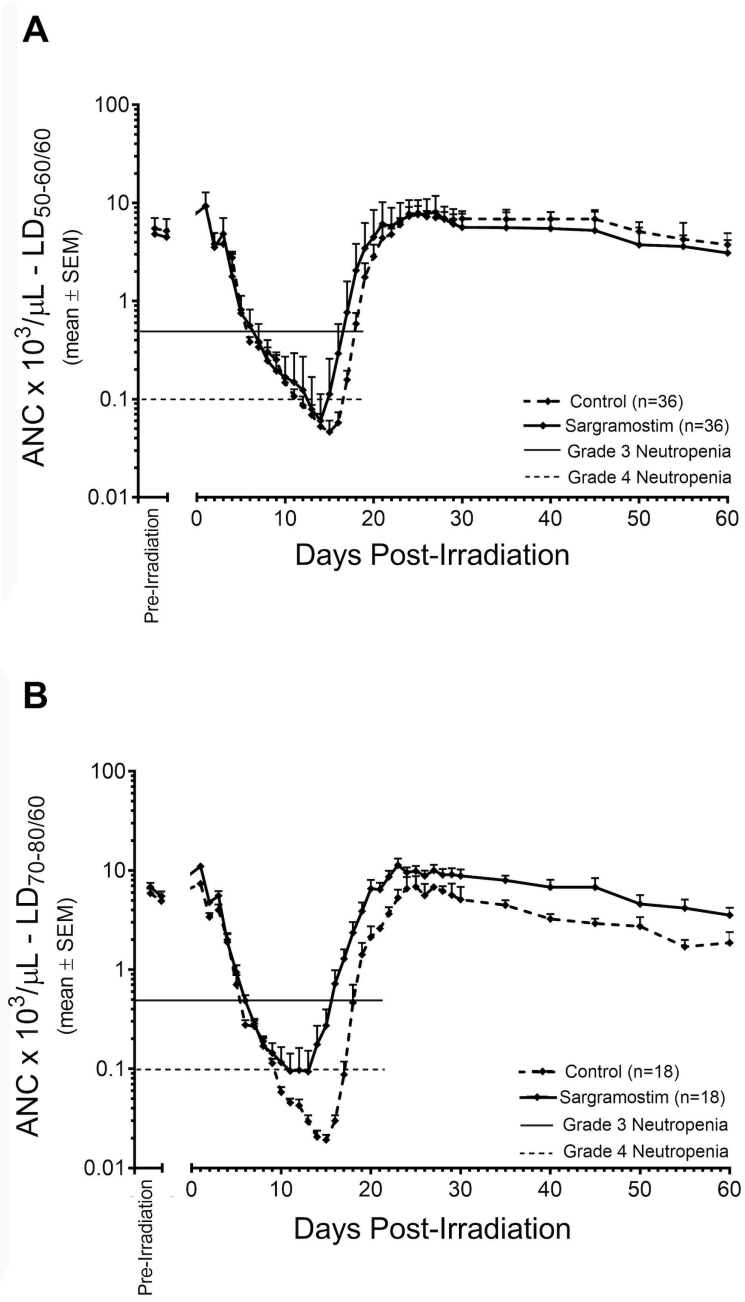
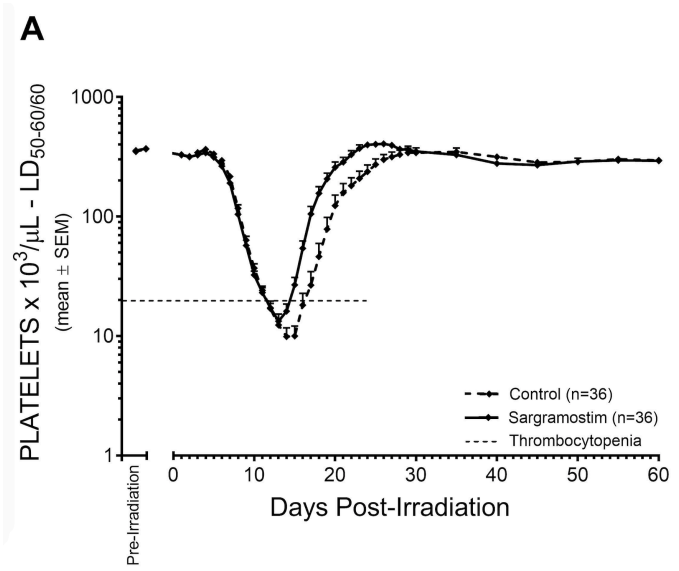


Figure S2. Sargramostim accelerates platelet recovery when dosed beginning 48 hours post total body irradiation.
(A) Following TBI to achieve $LD_{50\text{-}60/60}$, the time to platelet recovery was accelerated in the sargramostim-treated non-human primates (solid line) and demonstrated a significant 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).
(B) Following TBI to achieve $LD_{70\text{-}80/60}$, 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.
ANC, absolute neutrophil count; $LD_{50\text{-}60/60}$, lethal radiation dose for 50-60% within 60 days after radiation; $LD_{70\text{-}80/60}$, lethal radiation dose for 70-80% within 60 days after radiation; SEM, standard error of mean; TBI, total body irradiation.



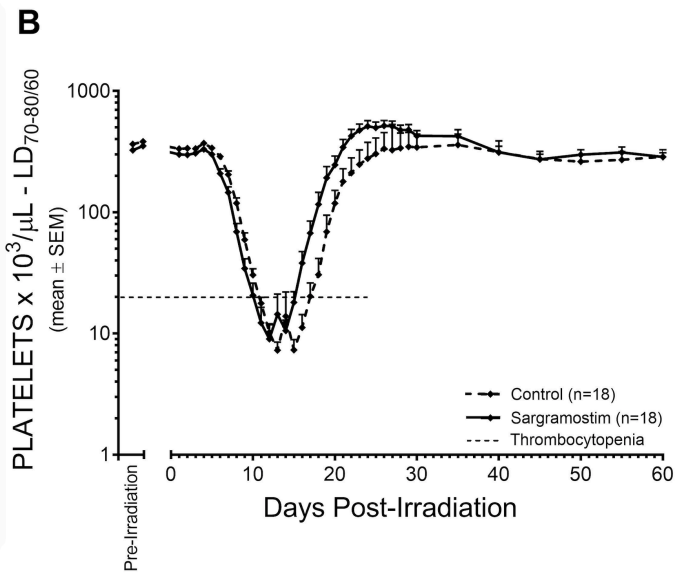


Figure S3. Sargramostim accelerates lymphocyte recovery when dosed beginning 48 hours after total body irradiation.
(A) Absolute lymphocyte count following TBI to achieve $LD_{50\text{-}60/60}$.
(B) Absolute lymphocyte count following TBI to achieve $LD_{70\text{-}80/60}$.
Lymphocyte counts declined drastically immediately after radiation 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 ($LD_{50\text{-}60/60}$ and $LD_{70\text{-}80/60}$, 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).
$LD_{50\text{-}60/60}$, lethal radiation dose for 50-60% within 60 days after radiation; $LD_{70\text{-}80/60}$, lethal radiation dose for 70-80% within 60 days after radiation; LYM, lymphocyte; SEM, standard error of mean; TBI, total body irradiation.

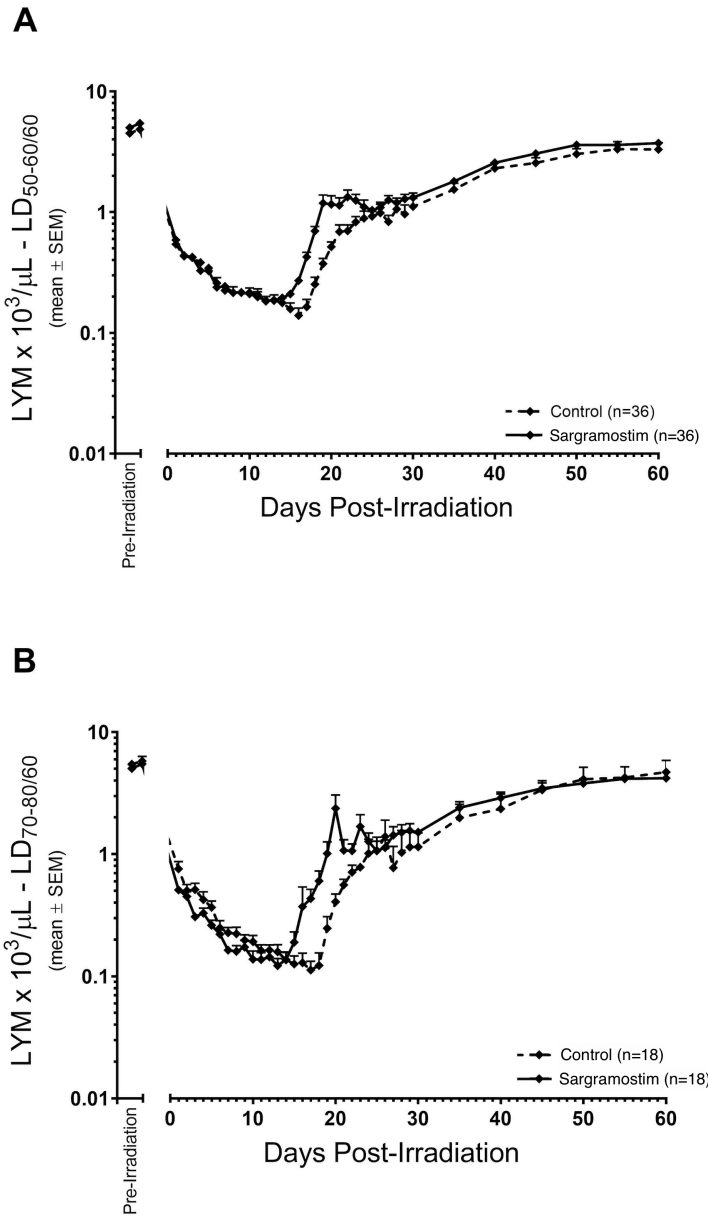
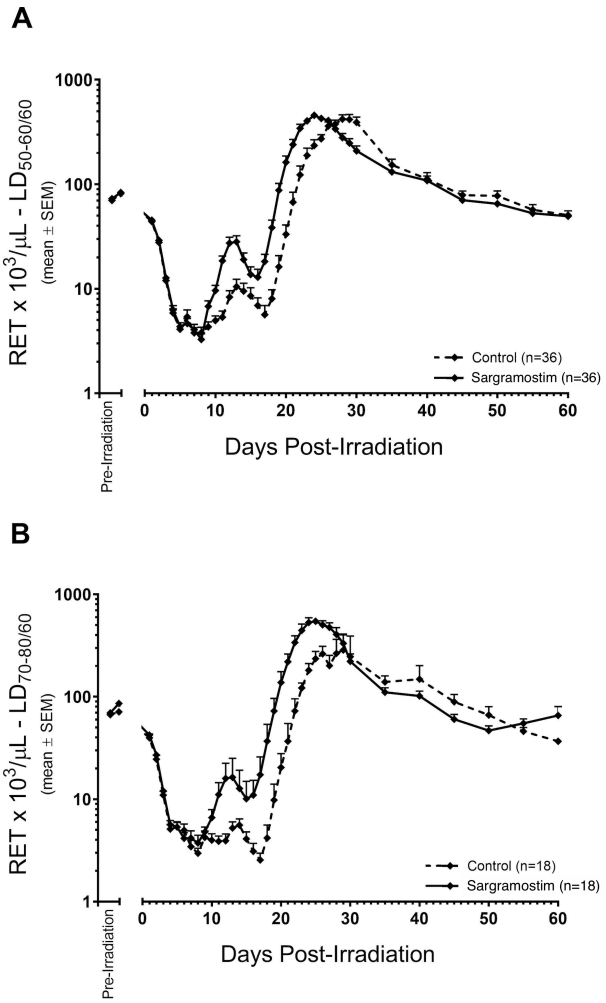


Figure S4. Sargramostim accelerates reticulocyte recovery when dosed beginning 48 hours post total body irradiation.
(A) Absolute reticulocyte count following TBI to achieve $LD_{50\text{-}60/60}$.
(B) Absolute reticulocyte count following TBI to achieve $LD_{70\text{-}80/60}$.
Baseline mean reticulocyte 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 correlated with 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 who received the $LD_{50\text{-}60/60}$ dose. 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 5-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; $LD_{50\text{-}60/60}$ and $LD_{70\text{-}80/60}$, respectively) by day 24.
$LD_{50\text{-}60/60}$, lethal radiation dose for 50-60% within 60 days after radiation; $LD_{70\text{-}80/60}$, lethal radiation dose for 70-80% within 60 days after radiation; RET, reticulocyte; SEM, standard error of mean; TBI, total body irradiation.



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