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Efficacy of Neulasta® or Neupogen® on H- and GI-ARS mortality and hematopoietic recovery in nonhuman primates after 10 Gy irradiation with 2.5% bone marrow sparing

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Abstract

A nonhuman primate (NHP) model of acute, partial-body, high dose, irradiation with minimal (2.5%) bone marrow sparing (PBI/BM2.5) was used to assess the endogenous gastrointestinal and hematopoietic recovery and the ability of Neulasta® (pegylated granulocyte colony stimulating factor [G-CSF]) or Neupogen® (G-CSF) to enhance recovery from myelosuppression when administered at an increased interval between exposure and initiation of treatment. A secondary objective was to assess the effect of Neulasta® or Neupogen® on the mortality and morbidity due to the hematopoietic acute radiation syndrome (H-ARS) and concomitant acute gastrointestinal (GI)-ARS. NHP were exposed to 10.0 Gy 6 megavolt (MV) linear accelerator (LINAC)-derived photons delivered at 0.80 Gy min⁻¹. All NHP received subject-based medical management. NHP were dosed daily with control article (5% dextrose in water) initiated on day 1 post-exposure, or Neulasta® (300 µg kg⁻¹) administered on days 1 and 8 or days 3 and 10 post-exposure, or Neupogen® (10 µg kg⁻¹) administered daily post-exposure following its initiation on day 1 or day 3 until neutrophil recovery (absolute neutrophil count [ANC] ≥ 1,000 cells µL⁻¹ for 3 consecutive days). Mortality in the irradiated-cohorts suggested that administration of Neulasta® or Neupogen® at either schedule did not affect mortality due to acute GI-ARS or mitigate mortality due to H-ARS (plus GI damage). Following 10.0 Gy PBI/BM2.5, the mean duration of neutropenia (absolute neutrophil count < 500 cells µL⁻¹) was 22.4 days in the control cohort, versus 13.0 and 15.3 days in the Neulasta® day 1–8 and day 3–10 cohorts relative to 16.2 and 17.4 days in the Neupogen® cohorts initiated on day 1 and day 3, respectively. The absolute neutrophil count nadirs were 48 cells µL⁻¹ in the controls, 117 cells µL⁻¹ and 40 cells µL⁻¹ in the Neulasta® days 1, 8 and days 3, 10 cohorts, respectively, and 75 cells µL⁻¹, and 37 cells µL⁻¹ in the Neupogen® day 1 and day 3 cohorts, respectively. Therefore, the earlier administration of Neulasta® or Neupogen® was more effective in this model of marginal 2.5% BM sparing. The approximate 2.5% BM-sparing may approximate the threshold for efficacy of the lineage-specific

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Conflicts of Interest

The authors declare no conflicts of interest

MCM. The PBI/BM2.5 model can be used to assess medical countermeasure efficacy in the context of the concomitant GI- and H-ARS sequelae.

Keywords

gastrointestinal tract; laboratory animals; biological factors; radiation damage

INTRODUCTION

Medical countermeasure (MCM) efficacy should be assessed in the “context of use” as set forth in the FDA guidance document for product development under the animal rule (U. S. Food and Drug Administration). Considering the context of use will help define the evolving clinical treatment scenario relative to the acute radiation syndrome (ARS) and the delayed effects of acute radiation exposure (DEARE). In a mass-casualty radiation event, such as the detonation of an improvised nuclear device, the radiation dose delivered to personnel will be ill-defined, non-uniform, and heterogeneous. These exposure characteristics forecast the potential sparing of tissue in all key organ systems [bone marrow (BM), gastrointestinal (GI), lung, heart and kidney] responsible for the evolution of the ARS and DEARE. Relevant to the study herein, the sparing of a marginal amount of BM may enhance early recovery from the hematopoietic (H) subsyndrome of the ARS (H-ARS) and influence the severity of the GI-ARS (Cole et al. 1967; Gidali and Lajtha 1972; Maloney and Patt 1972; Dewit et al. 1985; Monroy et al. 1988; Mason et al. 1989; Terry and Travis 1989; Wang et al. 1991; Bertho et al. 2005b; Booth et al. 2012; Herodin et al. 2012; MacVittie et al. 2012b). Medical management, based on established clinical triggers, including the use of antibiotics and blood transfusions will be a critical component in stabilizing the clinical treatment scenario noted after near-uniform or non-uniform irradiation (Baranov and Guskova 1990; Guskova et al. 1990; Baranov 1996; Fliedner et al. 2002; Liu et al. 2008; Gourmelon et al. 2010).

Medical management, to be determined in the context of the radiation scenario, will likely be combined with the administration of leukocyte growth factors (LGF) such as Neupogen[®], Neulasta[®], or Leukine[®] (Waselenko et al. 2004; Clark et al. 2005; Timmer-Bonte et al. 2005; Dainiak et al. 2011; U. S. Food and Drug Administration 2015a). The preclinical and clinical, evidence-based database, is consistent and substantial in supporting the use of LGFs and prophylactic antibiotics to mitigate severe myelosuppression and mortality (Butturini et al. 1988; Monroy et al. 1988; Neelis et al. 1997; Nothdurft et al. 1997; Bertho et al. 2005b; Clark et al. 2005; Gafter-Gvili et al. 2005; MacVittie et al. 2005; Timmer-Bonte et al. 2005; Liu et al. 2008; Dainiak et al. 2011; Farese et al. 2012b; Farese et al. 2013; MacVittie and Farese 2013; Farese and MacVittie 2015; Hankey et al. 2015). The majority of preclinical studies assessing MCM efficacy have used bilateral, uniform, total-body irradiation (TBI) at various lethal doses. The TBI exposure geometry however, is unlikely to occur in a nuclear scenario. Therefore, it is important to use a model that is more predictive of a clinical treatment scenario within the context of use for assessing efficacy of MCM and medical management (Monroy et al. 1988; Nothdurft et al. 1997; Bertho et al. 2005a; Bertho et al. 2005b; Herodin et al. 2007; Herodin et al. 2012; MacVittie et al. 2012b; Drouet et al. 2014; MacVittie et al. 2015a). This study used a high-dose, partial-body irradiation (PBI) model

with reduced sparing of approximately 2.5% of bone marrow (BM2.5) relative to the established models using 5% BM sparing or TBI (Farese et al. 2012a; MacVittie et al. 2012b; Farese et al. 2013; Hankey et al. 2015; MacVittie et al. 2015a). The PBI/BM2.5 model permitted the study of MCM against an H-ARS characterized by a greater degree of myelosuppression than observed with 5% marrow sparing, in concert with the concomitant acute GI-ARS and expected multiple organ injuries (MOI) due to the consistent uniform, dose-dependent exposure to key organs, e.g., lung, heart, GI and kidney (MacVittie et al. 2012a; Cohen et al. 2017; Prado et al. 2017).

As noted previously, the use of a PBI/BM2.5 model could address several knowledge gaps relative to the use of LGFs in the nuclear radiation exposure scenario. These are assessing the efficacy of LGFs: a) to mitigate myelosuppression consequent to high-dose partial-body exposure with minimal bone marrow sparing, b) with an increasing interval between irradiation and treatment initiation to mitigate myelosuppression and/or mortality of the H-ARS with concurrent GI injury; c) in determining the effect of MCM administration on other organ system sequelae, e.g., the incidence and severity of the acute GI-ARS; and c) assessing the effect of early Neupogen® or Neulasta®, administration during the concurrent evolution of the GI- and H-ARS on the latency, incidence, severity and progression of MOI (co-morbidities and mortality) induced by the threshold exposure for overt manifestation of the DEARE on lung, heart, and kidney injury. This approach permits a more strategic view of organ-specific MCM development and efficacy relative to the acute and delayed effects of high-dose radiation exposure.

Herein, the primary effort is focused on determining the neutrophil and platelet recovery kinetics following a myeloablative dose of 10 Gy with minimal BM sparing and the efficacy of Neulasta® or Neupogen® when administered at an increasing interval between exposure and treatment, to mitigate the myelosuppression of the H-ARS. A secondary objective included early analysis of the effects of Neulasta® or Neupogen®, on mortality associated with the concomitant progression of the acute GI-ARS and H-ARS in concert with prolonged GI damage.

MATERIAL AND METHODS

The animal husbandry, irradiation, medical management, and hematology procedures summarized below for the PBI/BM2.5 model have been previously described in detail (Farese et al. 2012a; MacVittie et al. 2012a; MacVittie et al. 2015a).

Animals

Male Chinese rhesus macaques (*Macaca mulatta*, 4.9 – 8.9 kg, n = 44) were housed and cared for in accordance with the Animal Welfare Act at the University of Maryland's Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility. This study includes comparisons to data sets from published, contemporary studies that served as relevant contemporary controls from models of bone marrow sparing (PBI/BM5: n = 218), TBI (n = 194) and the efficacy of Neupogen® administration (Farese et al. 2012a; MacVittie et al. 2012a; MacVittie et al. 2012b; Farese et al. 2013; MacVittie et al. 2015a).

Irradiation

The NHP were exposed to 10.0 Gy PBI/BM2.5 utilizing 6 MV LINAC-derived photons (2 MV average) at a dose rate of 0.80 Gy min⁻¹ (True Beam™, Varian Medical Systems, 3100 Hansen Way, Palo Alto, CA 94304-1038). Radiation dose calculations were performed by a board-certified medical physicist, based on a sagittal measurement taken at the xiphoid process. Dose was calculated for delivery to midline tissue, including correction factors to account for the inverse-square distance, tissue depth, and sources of photon scatter. Prior to irradiation, NHP were sedated with ketamine, (Ketaset®, 800 5th Street NW, Fort Dodge, IA 50501-7425) (10 ± 5 mg kg⁻¹ of the most recent body weight) intramuscularly (IM) and secured in a supine restraint device, and then administered xylazine (AnaSed®, Lloyd Laboratories, 604 West Thomas Avenue, Shenandoah, IA 51601) ($0.5 - 1.0 \pm 0.1$ mg kg⁻¹, body weight, IM or SC) to ensure proper radiation field placement would be maintained. The NHP were positioned with one half (50%) of their tibiae, plus ankles, and feet outside of the beam field. Half of the prescribed dose was delivered with an anteroposterior (AP) beam, and half with a posteroanterior (PA) beam. All irradiations were performed between the hours of 8:00 AM and 1:00 PM. In vivo dosimetry was performed using a microStar® system with nanoDots™ (Landauer, Inc., 2 Science Rd, Glenwood, IL 60425).

Animal cage-side observations

The research staff performed cage-side observations at least 6 hours apart, twice daily. Activity, posture, stool consistency, emesis, hemorrhage, alopecia, respiratory status, and seizure activity were observed, scored, and recorded. An additional general health assessment was also performed daily.

Medical Management

All NHP were administered medical management in accordance with an Institutional Animal Care and Use Committee (IACUC)-approved protocol of defined clinical triggers to start and stop treatments. This included hydration fluids, antibiotics, analgesics, anti-diarrheals, anti-pyretics, anti-emetics, anti-ulceratives, nutritional support, and blood transfusions. Specific details as to initiation, dosages, and duration of treatments were described previously (MacVittie et al. 2015a). Herein, Invanz® (ertapenem sodium) (Merck & Co. Inc., 1 Merck Dr., Whitehouse Station, NJ 08889) was administered in place of Primaxin® when microbial resistance was demonstrated to enrofloxacin, gentamicin, and ceftriaxone.

Mortality/Survival

Acute mortality/survival for GI-ARS was defined through 15 days and H-ARS was defined through 60 days post-irradiation, respectively. Animals were euthanized in accordance with IACUC-approved clinical criteria.

Neulasta® or Neupogen® Administration

Neulasta® or Neupogen® (Amgen, Inc., 1 Amgen Center Dr, Thousand Oaks, CA 91320) were dosed (10.2 ± 0.3 µg kg⁻¹) using the previous day's body weight and injected subcutaneously using four (4) treatment cohorts: Neulasta® was injected once on d1 (24h)

and d8 or d3 (72h) and d10; Neupogen[®] was injected once a day starting at d1 (24h) or d3 (72h) post-irradiation. Dosing of Neupogen[®] was stopped when an absolute neutrophil count (ANC) $< 1,000$ cells μL^{-1} was observed for 3 consecutive days. Control animals were dosed with an identical volume of 5% dextrose (D5W) (Baxter Healthcare Corporation, 1 Baxter Pkwy, Deerfield, IL 60015) in water starting at 24 hours post-irradiation.

Hematology and bacteriology

Peripheral blood was obtained by venipuncture for complete blood counts (CBC) (AcT diff2[™], Beckman Coulter, Inc., 11800 SW 147th Avenue, Miami, FL 33196). A WBC differential, which included verification of all electronically generated WBC $< 1,000$ cells μL^{-1} was performed by trained personnel using light microscopy and a blood-film stained (Hema- Tek II[™], Bayer Corp., Diagnostic Division, 511 Benedict Ave., Tarrytown, NY 10591-5097) with Wright-Giemsa Stain Pack (Fisher Scientific, 2000 Park Lane Dr., Pittsburgh, PA, 15275) on all CBC samples. The ANC was calculated (Microsoft Excel 2007) based on the WBC count obtained by CBC and the neutrophil count obtained by manual differential.

Peripheral blood samples for bacteriology cultures were collected on days when febrile neutropenia (FN), defined as an ANC < 500 cells μL^{-1} coincident with a rectal body temperature > 103.0 °F (39.4 °C), was observed. Blood was cultured in aerobic and anaerobic bottles and analyzed with a BACTEC 9050 Microbial Detection System (Becton Dickinson, 1 Becton Drive Franklin Lakes, NJ 07417-1880).

Statistics

Probit fits were made to mortality data, and confidence intervals were calculated for lethal dose (LD) and slope values according to the methods of Finney using the R statistical software (version 2.13.1) (Finney 1947). A comparison of slopes and LD50s was made using Wald statistics. Means values and associated standard errors for continuous and count data were calculated and plotted using Microsoft Excel 2010.

RESULTS

The Dose Response Relationships (DRRs) for GI-ARS and H-ARS using PBI/BM5 or TBI plus Medical Management: A data-based rationale for using 10 Gy and the PBI/BM2.5 protocol.

GI-ARS

The established DRR for the acute GI-ARS using the PBI/BM model with 5% BM sparing (n=126) resulted in an LD50/15 at 12.01 Gy [95% confidence interval: 11.71, 12.52], which was significantly different ($p = 0.02$) than the estimated value of 11.33 Gy for TBI-induced GI-ARS (n = 61)(Farese et al. 2012a; MacVittie et al. 2012b). The corresponding slope for the PBI/BM5 DRR was 1.01 [0.60, 1.42], compared to 0.92 [0.70, 1.34] for the TBI DRR (Table 1, Fig. 1). The slopes were not significantly different ($p = 0.46$) from each other. The sparing of approximately 5% BM significantly increased the LD50/15 to 12.01 Gy relative to 11.33 Gy for the TBI-induced GI-ARS and supported the observation that functional

hematopoiesis mitigated mortality of the acute GI-ARS (Dewit et al. 1985; Mason et al. 1989; Terry and Travis 1989; Farese et al. 2012a; MacVittie et al. 2012b).

H-ARS

The database used to determine the H-ARS DRR for PBI/BM5 and TBI exposure protocols included additional NHP from recent efficacy studies (Farese et al. 2013; Farese et al. 2014; Hankey et al. 2015). Sparing approximately 5% BM in the PBI protocol increased the LD50/60 for TBI, from 7.54 Gy [7.24, 7.75] (composite n=133) to 10.88 Gy [10.48, 11.3] (n=92), an increase of 3.34 Gy noted for the TBI protocol (Table 1, Fig. 1) (Farese et al. 2012a; MacVittie et al. 2012a; Farese et al. 2013; Hankey et al. 2015; MacVittie et al. 2015a).

Mortality due to GI- and H-ARS

PBI/BM2.5 vs PBI/BM5 or TBI at 10 Gy. GI-ARS: The DRR for the PBI/BM5 protocol estimated that 10 Gy would induce less than 10% mortality due to the acute GI-ARS through 15d post exposure (MacVittie et al. 2012a). Recent use of the PBI/BM5 protocol resulted in 6.7% (1/15) mortality for the acute GI-ARS with a mean survival time of 13d post exposure (MacVittie et al. 2015a). Reduction of the BM spared from approximately 5% to 2.5% in the PBI/BM2.5 protocol resulted in 8.3% (1/12) mortality through 15d post exposure at 10 Gy with a mean survival time of 14d post exposure (Table 2). Mortality due to the acute GI-ARS at 10 Gy was equivalent in the PBI/BM5 and PBI/BM2.5 exposure protocols.

H-ARS

The DRR for the PBI/BM5 protocol estimated that 10 Gy would induce approximately 25% mortality through 60d post exposure (MacVittie et al. 2012a; MacVittie et al. 2015a). Reduction of the spared-BM from approximately 5% to 2.5% in the PBI/BM2.5 protocol resulted in increased ($p=0.052$) mortality to 58.3% (7/12) through 60d post exposure at 10 Gy relative to actual 26.7% (4/15) observed with 5% BM sparing (Table 2) (MacVittie et al. 2015a). These animals survived the acute GI-ARS but retained unresolved GI damage that may contribute to the mortality through 60d post exposure (MacVittie et al. 2015a). Note that 55% (6/11) succumbed during the period from d16 to d60. TBI at 10 Gy is uniformly lethal due to the combined GI- and H-ARS.

The Kaplan-Meier plot illustrates the differential survival probability over the 60d time course post 10 Gy for PBI/BM2.5 and PBI/BM5 exposure protocols (Fig. 2). The increased mortality or change in cumulative survival probability throughout the early time course, d10–30, of the concomitant GI and hematopoietic syndromes underscored the relative lack of efficacy in the approximate 2.5% BM vs the 5% BM to regenerate hematopoiesis.

Hematologic response

PBI/BM2.5 vs PBI/BM5 or TBI at 10.0 Gy. The hematologic response for control cohorts exposed to 10 Gy varied considerably relative to the three exposure protocols. The differences can be best illustrated by the relative time course of neutropenia and thrombocytopenia (Fig. 3a, b). Severe, grade 3 neutropenia ($ANC < 500/\mu L$) and thrombocytopenia (platelet count $< 20,000/\mu L$) occurred at d4.5 and d9–10 respectively,

regardless of the exposure protocol. The respective nadirs and recovery time to ANC $> 1,000/\mu\text{L}$ or platelet count $> 20,000/\mu\text{L}$ were significantly different and underscored the relative value of each model to assess MCM efficacy in the context of coincident H-ARS and GI damage. Decreasing the amount of active BM spared from 5% to 2.5% resulted in a more prolonged recovery time of critical hematologic parameters.

GI-ARS mortality and survival time

Neulasta[®] or Neupogen[®] administration at an increased interval post-exposure. GI-ARS mortality at day 15 in the 10.0 Gy control cohort was 8.3% ($n = 1/12$) relative to the Neulasta[®] d1-d8 or d3-d10 cohorts of 0% (0/8) and 12.5% (1/8) or Neupogen[®] cohorts of 25% (2/8) and 12.5% (1/8), when starting administration on day 1 or day 3, respectively (Table 3, Fig. 4).

H-ARS Mortality

PBI/BM2.5 at 10.0 Gy plus Neulasta[®] or Neupogen[®]. The 10.0 Gy control cohort ($n=12$) had 58.3% mortality (7/12) within 60 days post exposure. The mean survival time (MST) for decedents was 26 ± 5 days. The cohorts administered Neulasta[®] in the d1-d8 or d3-d10 or Neupogen[®] initiated on d1 or d3 all had 50% (4/8) mortality at day 60 and respective decedent MST of 29d, 18d, 26d and 25d (Table 3, Fig. 4). The study was not powered to detect a significant difference in mortality however, we note that the treatment with Neulasta[®] or Neupogen[®] while not significantly increasing survival did not exacerbate mortality when administered in concert with the concomitant and prolonged, acute GI-ARS subsyndrome.

Hematologic parameters and neutrophil kinetics

PBI/BM2.5 at 10.0 Gy plus Neulasta[®] or Neupogen[®]. The administration of Neulasta[®] or Neupogen[®] initiated on their respective schedules in the PBI/BM2.5 protocol resulted in mitigation of neutropenic parameters relative to the control cohort (Table 4, Fig. 5a, b).

ANC nadir and mean duration of neutropenia. The mean nadir and the mean day the nadir was reached was $48 \text{ cells } \mu\text{L}^{-1}$ at day 8.8 for the control cohort, and $75 \text{ cells } \mu\text{L}^{-1}$ at day 6.7 and $37 \text{ cells } \mu\text{L}^{-1}$ at day 8.5 for the cohorts where Neupogen[®] was initiated on day 1 or day 3, respectively (Table 4, Fig. 5a). The mean number of days the ANC $< 500 \mu\text{L}^{-1}$ in the control cohort, was 18.0 days. Neupogen[®] administration initiated at either day 1 or day 3 reduced the mean number of days the ANC $< 500 \mu\text{L}^{-1}$ to 12.7 days and 14.6 days, respectively. Neulasta[®] administered in the d1-d8 or d3-d10 cohorts reduced the mean number of days the ANC $< 500 \mu\text{L}^{-1}$ to 10.9 days and 13.8 days, respectively. The mean nadirs and the mean days the nadirs were reached were $117 \text{ cells } \mu\text{L}^{-1}$ at day 7.6 and $40 \text{ cells } \mu\text{L}^{-1}$ at day 7.9 for the Neulasta[®] cohorts administered at d1-d8 or d3-d10, respectively (Table 4, Fig. 5b).

The mean time to recovery to ANC $> 1,000 \mu\text{L}^{-1}$. The mean time to recovery to an ANC $> 1,000 \mu\text{L}^{-1}$ was 27.0 days for the control cohort. Neupogen[®] administration initiated at day 1 or day 3 reduced the respective mean recovery times to an ANC $> 1,000 \mu\text{L}^{-1}$ to 20.5 days and 22.2 days. Neulasta[®] administered in the d1-d8 or d3-d10 cohorts reduced the duration

of mean time to recovery to an ANC $1,000 \mu\text{L}^{-1}$ to 17.1 days and 20.3 days respectively (Table 4, Fig. 5a, b).

Incidence of FN. The incidence (the number of animals who met the criteria on at least one study day) of FN in the 10.0 Gy control cohort was 83.3% (10/12). The incidence of FN in the day 1 and day 3 Neupogen[®] cohorts were 75% (6/8) and 50% (4/8), respectively. The mean first day of FN was day 12.1 in the control cohort and day 9.7 and day 11.5 in the day 1 and day 3 Neupogen[®] cohorts, respectively (Table 5). The incidence of FN in the day 1–8 and day 3–10 Neulasta[®] cohorts was 50% (4/8) and 62.5% (5/8) respectively. The mean first day of FN was day 7.3 and day 9.8 in the day 1–8 and day 3–10 Neulasta[®] cohorts, respectively (Table 5).

Platelet recovery kinetics

The sparing of approximately 2.5% of active BM significantly reduced the severely thrombocytopenic effect of TBI at 10.0 Gy (Fig 3b). Administration of Neupogen[®] or Neulasta[®] in either of the respective cohorts beginning on day 1 or day 3 or d1-d8 and d3-d10 in the 10.0 Gy PBI/BM2.5 protocol did not affect platelet recovery relative to that noted in the control cohort (Figs. 6a, b; Table 6). The respective nadirs, duration of thrombocytopenia, recovery time to a platelet count $>20 \times 10^3 \mu\text{L}^{-1}$ were equivalent across all cohorts.

DISCUSSION

MCM efficacy must be assessed in the context of the clinical management of patients that have experienced the acute radiation-induced ARS and latent or overt DEARE in a nuclear radiation accident or terrorist scenario (Baranov and Guskova 1990; U. S. Food and Drug Administration 2015a; U. S. Food and Drug Administration 2015b). The exposure scenario will likely be heterogeneous, non-uniform and ill-defined. These conditions forecast the sparing of variable amounts of critical organ tissue that will define the severity of the ARS and DEARE. The sparing of BM, GI, lung, heart and/or kidney tissue, can have dramatic effects on the incidence, latency, severity, progression and resolution of organ-specific and organ-interactive sequelae (Ainsworth et al. 1965; Cole et al. 1967; Wise and Turbyfill 1968; Rauchwerger 1972; Monroy et al. 1988; Mason et al. 1989; Terry and Travis 1989; Geraci et al. 1990; Guskova et al. 1990; Geraci et al. 1992; Liao et al. 1995; Bertho et al. 2005a; Novakova-Jiresova et al. 2005; van Luijk et al. 2007; Drouet et al. 2008; Granton et al. 2014; Boittin et al. 2015). Non-uniform and/or heterogeneous exposure with or without medical management will change the organ-specific dose response relationships (DRR) for morbidity and mortality throughout the ARS and DEARE (Ainsworth et al. 1965; Wise and Turbyfill 1968; Evans et al. 1987; Baranov and Guskova 1990; Guskova et al. 1990; Herodin et al. 2007; MacVittie et al. 2012a).

The research platform using the PBI/BM5 protocol shifted the mortality DRR for both the acute GI- and H-ARS in mouse and NHP (Booth et al. 2012; MacVittie et al. 2012a). The respective DRRs in the NHP shifted the LD50/60 for TBI-induced H-ARS from 7.54 Gy to 10.88 Gy and the LD50/15 for GI-ARS from 11.33 Gy to 12.01 Gy (Farese et al. 2012a; MacVittie et al. 2012a; MacVittie et al. 2012b). A model using PBI with BM sparing permits

analysis of the concurrent, time- and dose-dependent development of organ-specific sequelae in the ARS and DEARE. Since the LD50/60 for the H-ARS is shifted to the dose range for the GI-ARS and delayed, multi-organ injury (MOI), the H-ARS evolves in concert with the dose-dependent GI-ARS and results in a combined subsyndrome of hematopoietic myelo- and immune-suppression and early and prolonged GI damage (MacVittie et al. 2012a; MacVittie et al. 2014; MacVittie et al. 2015a; Fish et al. 2016). Furthermore, the GI- and H-ARS evolve during the latent periods for delayed appearance of MOI to lung, heart and kidney (MacVittie et al. 2012a; de Faria et al. 2015; Cohen et al. 2017).

The reduced amount of bone marrow spared to approximately 2.5% provided a model of H-ARS with increased myelosuppression and mortality in concert with an equivalent degree of dose-dependent early mortality due to the coincident GI-ARS and immunosuppression (MacVittie et al. 2012a; MacVittie et al. 2014; MacVittie et al. 2015a).

Mortality and myelosuppression due to concomitant H- and GI-ARS: PBI/BM2.5 relative to PBI/BM5 and TBI. Homogeneous, uniform TBI at 10 Gy induced a 100% lethal H-ARS in the NHP (Henschke and Morton 1957; Dalrymple et al. 1965; Farese et al. 2012a; MacVittie et al. 2015b; Thrall et al. 2015). The PBI/BM5 exposure protocol using 10 Gy resulted in 27% mortality as a consequence of combined GI- and H-ARS within the 60d time course for the control-treated cohort (MacVittie et al. 2012a; MacVittie et al. 2015a). Reduction of the spared BM from approximately 5% to 2.5% resulted in increased mortality to 58% over the 60d time course relative to 27% noted for the PBI/BM5 protocol but similar to the approximate 50% recorded after homogeneous TBI at 7.50 Gy of LINAC-derived photons (Farese et al. 2012a; Farese et al. 2013; Farese et al. 2014; Hankey et al. 2015). Sparing less BM, to approximately 2.5%, resulted in a lower nadir, an increased mean duration of neutropenia ($ANC < 500 \mu L$), increased time to ANC recovery ($ANC > 1,000 \mu L$), reduced the time to FN and increased the incidence of FN relative to the PBI/BM5 protocol control cohorts.

It is important to note that the approximate 2.5% of tibial BM is not completely spared and received approximately 0.50 Gy as noted for the PBI/BM5 protocols (Farese et al. 2012a; Farese et al. 2013; Farese et al. 2014; Hankey et al. 2015). This is a significant exposure relative to the estimated D_{37} or D_0 of 0.60 Gy for hematopoietic stem and progenitor cells in the NHP and served to diminish the regenerative capacity of the spared BM (van Bekkum 1991). The recovery pattern is similar to that noted for sparing 5% BM (PBI/BM5). However, the lower nadir resulted in longer recovery kinetics that were equivalent to those parameters noted after TBI at the 7.54 Gy LD50/60 (Farese et al. 2012a; MacVittie et al. 2012b; Farese et al. 2013; Hankey et al. 2015; Thrall et al. 2015).

Administration of Neupogen® or Neulasta® when initiated at either of the d1 protocols lessened the nadir and enhanced the recovery kinetics relative to the control cohort. Whereas, delaying the initiation of treatment with either MCM until d3, slowed the recovery kinetics. Although, all treatment protocols with either Neupogen® or Neulasta® resulted in increased recovery of ANC relative to the control cohort. The earlier administration of either MCM was more effective in initiating granulopoiesis and recovery of neutrophils than the delayed administration when the spared BM was reduced to approximately 2.5% relative to

that observed with approximate 5% sparing (MacVittie et al. 2015a). This is in contrast to the ability of Neupogen® to stimulate equivalent recovery of granulopoiesis when administered up to 5 days post PBI/BM5 at 10 Gy or 3 days post PBI/BM5 at 11 Gy (MacVittie et al. 2015a).

The ability of surviving BM hematopoietic progenitor cells (HPC), either localized to a marrow site or dispersed throughout the total marrow space, to accelerate hematopoietic recovery after highly myelosuppressive irradiation has been consistent and is model independent, be it partial-body with marrow shielding/sparing, or unilateral, non-uniform total-body exposure in several animal species (Maillie et al. 1966; Cole et al. 1967; Wise and Turbyfill 1968; Gidali and Lajtha 1972; Maloney and Patt 1972; Monroy et al. 1988; Wang et al. 1991; Geraci et al. 1992; Bertho et al. 2005a; Booth et al. 2012; Herodin et al. 2012; MacVittie et al. 2012a; Drouet et al. 2014; Boittin et al. 2015). The critical condition is the differential dose distribution throughout the tissue and the basic assumptions that hematopoietic recovery from the H-ARS is dependent on the survival of a critical fraction of HPC that are equally distributed throughout the active marrow tissue, independent of location and equivalent in regenerative ability (Bond and Robinson 1967; Taketa et al. 1970; Bond et al. 1991; Campbell et al. 2015). Shochat and Rom-Kedar emphasized a similar concept called “acute marrow capacity” in an attempt to model G-CSF efficacy after intensive chemotherapy (Shochat and Rom-Kedar 2008). The post-chemotherapy, acute marrow capacity is the critical variable relative to neutrophil kinetics, degree of neutropenia and maximal granulopoietic response that can be stimulated by G-CSF. It is apparent that the reduction of spared-BM from approximately 5% to approximately 2.5% has approached the threshold of viable marrow capacity that can effectively mitigate morbidity and mortality associated with the high-dose myeloablation of greater than 95% of remaining BM and induction of a concurrent acute GI-ARS in NHP shown herein. The surviving fraction of surviving HPC, representing the marrow capacity, must be able to balance the endogenous and exogenous, G-CSF, pressure to differentiate and regenerate. G-CSF will stimulate HPC differentiation, proliferation, enhanced mitotic activity in the neutrophil lineage, reduced transit time through the marrow and amplification of the post-mitotic pool entering the blood (Chatta et al. 1994; Basu et al. 2002; Mackey et al. 2003; Engel et al. 2004; Zhuge et al. 2012).

Several NHP models have effectively demonstrated the efficacy of marrow-sparing relative to high doses of myelosuppressive irradiation, high dose rate, specific marrow site and total-body marrow space (Monroy et al. 1988; Drouet et al. 2004; Bertho et al. 2005a; Herodin et al. 2007; Drouet et al. 2008; Drouet et al. 2014; Herodin et al. 2014). Monroy et al used both high-dose (8 Gy) and high-dose rate ($5.0\text{--}7.35\text{ Gy min}^{-1}$) bilateral exposure in a model with variable shielding of femora and tibiae to demonstrate effective marrow regeneration and increased stimulation with GM-CSF (Monroy et al. 1988). The 100% mortality after TBI at 8.0 Gy was reduced to 20% given the heterogeneous, non-uniform exposure. Bertho et al. delivered 8.5 Gy or 10.0 Gy, uniform exposure to the torso with a reduced dose of 4.5 Gy to the right arm (Bertho et al. 2005a). This study demonstrated the efficacy of lenograstim (G-CSF) administered within 6h post exposure in the animals that received 8 Gy relative to only partial hematopoietic recovery in the cohort that received 10 Gy, a threshold dose for the acute GI-ARS. This is similar to the PBI/BM2.5 protocol shown herein and the PBI/BM5

and TBI models where the 10 Gy exposure induced a coincident GI-ARS and H-ARS (MacVittie et al. 2012a; MacVittie et al. 2012b; MacVittie et al. 2015a).

The acute GI-ARS mortality was equivalent to that noted for the PBI/BM5 protocol and less than the TBI-induced mortality confirming the fact that a marginal amount of functional hematopoiesis can mitigate the GI-ARS (Dewit et al. 1985; Mason et al. 1989; Terry and Travis 1989; Vriesendorp et al. 1992; MacVittie et al. 2012a; MacVittie et al. 2012b; MacVittie et al. 2015a). However, the administration of Neupogen® or Neulasta®, which stimulated recovery of hematopoiesis, had no additional effect on the incidence or severity of the acute GI-ARS. This suggested that the active, functional BM must be present prior to the acute time course of the GI-ARS and regenerating during the development of the acute GI-ARS. We do not know if the recovered hematopoietic system will mitigate the prolonged GI damage that may persist through at least 180d post exposure (Booth et al. 2012; MacVittie et al. 2012a). Recent studies have focused on the cell subpopulation and its secretory potential that may initiate mitigation of the acute GI-ARS via regulation of both intestinal stem cell regeneration and restorative nature of the intestinal niche (Saha et al. 2011). Saha and colleagues recently demonstrated the efficacy of BM-derived adherent stromal cell transplantation to mitigate the acute GI-ARS when administered 24 hrs post 100% lethal TBI (Saha et al. 2011). It was proposed that anti-inflammatory growth factors are produced that induce regeneration of both intestinal stem cells and the intestinal niche.

The PBI/BM2.5 model was utilized to further assess: a) the potential threshold for hematologic recovery kinetics consequent to a further reduction in BM spared and thus viable marrow capacity on the severity and time course of the H-ARS with concurrent GI injury; b) the ability of Neulasta® or Neupogen® to mitigate the greater degree of myelosuppression noted minimal BM sparing and an extended delay between exposure and the initiation of treatment; and c) the effect of Neulasta® or Neupogen® on the severity of the acute GI-ARS at a threshold level of 10 Gy. These questions provided realistic data on the efficacy of LGFs as MCM to be employed in the context of a clinical scenario imposed by a nuclear radiation event. Spared BM will provide a ready source of multi-lineage and lineage-specific progenitor cells as targets for either spontaneous, endogenous G-CSF or LGF-induced hematopoietic recovery. The timely recovery of hematopoiesis and utility of LGFs and other MCM administered at an increased time interval post exposure will depend of the amount of BM spared and endogenous regeneration.

The relative quality of hematopoietic and intestinal stem cells post exposure in the lethal dose range as well as those surviving the lower doses in the partial-body spared BM or unilateral models has not been fully examined. Recent studies in murine models have shown a significant loss of the potential for multilineage differentiation for HSC that survive the potentially lethal TBI in the H-ARS range (Chua et al. 2012). The competitive re-transplantation of the phenotypically defined HSC at 19 months post exposure, relative to age-matched controls, resulted in a markedly skewed differentiation pattern in the engrafted host. The ability to assess intestinal stem cell quality in long-term survivors cannot rely on a competitive repopulation assay. Booth et al., assessed crypt microcolony recovery in surviving mice 200d post lethal 13 Gy exposure using a PBI/BM5 protocol (Booth et al. 2015). The surviving mice were re-exposed to TBI and crypt clonogenic recovery was

assessed relative to previously un-irradiated age-matched controls. It was noted that recovery of crypts in the small intestinal regions had not returned to pre-irradiation levels within the 200d post exposure. Interestingly, it was noted that there was improved crypt survival and regeneration in the pre-irradiated cohorts relative to the age-matched controls.

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Figure 1. Mortality dose response relationships (DRR) for TBI and PBI/BM5 H-ARS and GI-ARS exposure protocols

Rhesus macaques were exposed to 7.2 Gy to 12.5 Gy utilizing 6 MV LINAC-derived photons in the TBI or PBI/BM5 exposure protocols. Animals were euthanized according to IACUC-approved clinical criteria. Probit analysis demonstrated the DRR for mortality consequent to the range of TBI or PBI/BM5 exposures. Probit DRRs are shown for cumulative contemporary data set: TBI H-ARS, TBI GI-ARS, PBI/BM5 H-ARS and PBI/BM5 GI-ARS.

Figure 2. Kaplan-Meier survival curves for control cohorts exposed to 10Gy PBI/BM5 and PBI/BM2.5 protocols

Kaplan-Meier curves demonstrate the time- and exposure protocol-dependent outcome after 10 Gy using PBI/BM5 (n=15) or PBI/BM2.5 (n=12) protocols. Nonhuman primates were exposed utilizing 6 MV LINAC-derived photons. Animals were observed through the 60 day study duration. Comparison of the survival curves using the one-sided log-rank (Mantel-Cox) test showed the relative survivals at 60d were significantly different ($p=.052$).

Figure 3. a, b. ANC and platelet kinetics post 10.0Gy PBI/BM2.5 vs PBI/BM5 or TBI control cohorts

Rhesus macaques were exposed to 10.0 Gy PBI/BM2.5 and PBI/BM5 or TBI at 10.0Gy utilizing 6 MV LINAC-derived photons. A comparison between exposure protocols for ANC or platelets at 10Gy is shown in 3a and 3b respectively.

Figure 4. Kaplan-Meier survival curves for non-human primates (NHP) exposed to 10Gy partial-body irradiation with 2.5% bone-marrow sparing (PBI/BM2.5)

Kaplan-Meier curves demonstrate the time- and treatment-dependent outcome after 10 Gy using PBI/BM2.5 (n=12) protocol. NHP were exposed utilizing 6 MV LINAC-derived photons. The control cohort received 5% dextrose in water. The other four cohorts were administered Neulasta® once on d1 (24h) and d8 or d3 (72h) and d10 or Neupogen® once a day starting at d1 (24h) or d3 (72h) post-irradiation. All NHP received medical management and were observed through the 180 day study duration.

Figure 5. a, b. ANC kinetics post 10.0Gy PBI/BM2.5 for control, Neupogen®- or Neulasta®-treated cohorts

Rhesus macaques were exposed to 10.0 Gy PBI/BM2.5 utilizing 6 MV LINAC-derived photons. Animals were administered control article (5% dextrose in water) or Neupogen® or Neulasta® beginning at the indicated times post 10 Gy. Five cohorts were observed relative to ANC responses: control (n=12), Neupogen® initiated treatment day 1 (24h) (n=8) or day 3 (72h) (n=8) and continued daily through effect (ANC $> 1,000 \mu\text{L}^{-1}$ for three consecutive days) and Neulasta® initiated treatment on day 1 (24h) and then again on day 8 (n=8) or Neulasta® initiated on day 3 (72h) and then again on day 10 (n=8). The ANC (mean values) were calculated using values obtained from an automated CBC and manual WBC differential.

Figure 6. a, b. Platelet kinetics post 10.0Gy PBI/BM2.5 for control, Neupogen®- or Neulasta®-treated cohorts

Rhesus macaques were exposed to 10.0 Gy PBI/BM2.5 utilizing 6 MV LINAC-derived photons. Animals were administered control article (5% dextrose in water) or Neupogen® or Neulasta® beginning at the indicated times post 10 Gy using the PBI/BM2.5 protocol. Five cohorts were observed: control (n=12), Neupogen® initiated treatment on day 1 (24h) (n=8) or day 3 (72h) (n=8) and continued daily through effect (ANC < 1,000 µL⁻¹ for three consecutive days) and Neulasta® initiated treatment on day 1 (24h) and then again on day 8 (n=8) or Neulasta® initiated on day 3 (72h) and then again on day 10 (n=8). Platelet counts were obtained from an automated CBC.

Table 1

Mortality due to H- and GI-ARS TBI and PBI/BM5 exposure protocols

Mortality estimated from respective dose response relationships for H- and GI-ARS consequent to TBI or PBI/BM5 exposure protocols. H-ARS TBI DRR is a cumulative data base. H-ARS PBI/BM5. GI-ARS TBI. GI-ARS PBI/BM5. Values in table are derived from linear (no dose transform) normal probit fits. Values in [] are 95% confidence intervals, and “n” is the number of subjects in each cumulative analysis.

Models	LD10(Gy)	LD50(Gy)	LD90(Gy)	Slope	n
H-ARS TBI	6.41[4.57,6.89]	7.54[7.24,7.75]	8.66[8.24,10.27]	1.14[0.45,1.82]	133
H-ARS PBI/BM5	9.21[7.87,9.79]	10.88[10.48,11.3]	12.56[11.97,13.91]	0.76[0.44,1.09]	92
GI-ARS TBI	9.93[8.47,10.54]	11.33[10.81,11.75]	12.73[12.21,13.9]	0.92[0.70,1.34]	61
GI-ARS PBI/BM5	10.74[10.12,11.06]	12.01[11.71,12.52]	13.28[12.71,14.57]	1.01[0.60,1.42]	126

Table 2
Mortality and mean survival time (MST) through GI- and H-ARS at 10 Gy PBI/BM2.5 versus PBI/BM5

Rhesus macaques were exposed to 10 Gy 6MV LINAC-derived photons in the PBI/BM2.5 or PBI/BM5 exposure protocols. Animals were euthanized according to a defined set of clinical criteria per IACUC-approved protocol. Mortality displayed as decedent/total per cohort (%). The MST days (d) for each cohort is displayed +/- SEM.

Group	Acute GI (d1-15)	MST (d)	H+GI-ARS (d16-60)	MST (d)	Acute GI + H+GI-ARS (d1-60)	MST (d)
PBI/BM2.5	1/12 (8.3%)	14	6/11 (54.5%)	29 ± 5	7/12 (58.3%)	26 ± 5
PBI/BM5.0	1/15 (6.6%)	13	3/14 (21.4%)	31 ± 6	4/15 (26.7%)	26 ± 6

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Table 3
Mortality and mean survival time (MST) of decedents through GI- and H-ARS subsyndromes following PBI/BM2.5 at 10 Gy with Neupogen or Neulasta administration

Rhesus macaques were exposed to 6MV LINAC-derived photons using the PBI/BM2.5 exposure protocol. Animals were administered control article (5% dextrose in water) or Neupogen® or Neulasta® beginning at day 1 (24h) or day 3 (72h) post exposure. Animals were euthanized according to a defined set of IACUC-approved criteria. Mortality (decedent/total NHP in cohort) rates were collated at 15d, 16–60d and 1–60 inclusive post-irradiation. The MST (d) of decedents in each interval is displayed +/- SEM.

Group	Acute GI (d1–15)	MST (d)	H+GI-ARS (d16–60)	MST (d)	Acute GI + H+GI-ARS (d1–60)	MST (d)
Control (n=12)	1/12 (8.3%)	14	6/11 (54.5%)	29 ± 5	7/12 (58.3%)	26 ± 5
Neupogen 24h (n=8)	2/8 (25.0%)	12 ± 1	2/6 (33.3%)	40 ± 17	4/8 (50.0%)	26 ± 10
Neupogen 72h (n=8)	1/8 (12.5%)	15	3/7 (42.8%)	28 ± 8	4/8 (50.0%)	25 ± 7
Neulasta 24h (n=8)	0/8 (0%)	NA	4/8 (50.0%)	29 ± 4	4/8 (50.0%)	29 ± 4
Neulasta 72h (n=8)	1/8 (12.5%)	14	3/7 (42.8%)	19 ± 1	4/8 (50.0%)	18 ± 1

Latency, duration, and severity of neutropenia post 10 Gy in the PBI/BM2.5 protocol for control, Neupogen®- or Neulasta®-treated cohorts

Rhesus macaques were exposed to 10.0 Gy utilizing 6 MV LINAC-derived photons in the PBI/BM2.5 protocol. Animals were administered control article (5% dextrose in water) or Neupogen® or Neulasta® beginning at the indicated times post-irradiation. Five cohorts were observed: control (n=12), Neupogen® initiated treatment on day 1 (24h) (n=8) or day 3 (72h) (n=8) and continued daily through effect (ANC 1,000 μL^{-1} for three consecutive days) and Neulasta® initiated treatment on day 1 (24h) (n=8) or Neulasta® initiated on day 3 (72h) and then again on day 10 and then again on day 10 (n=8). The ANC was calculated daily using values obtained from an automated CBC and manual WBC differential. The duration of neutropenia (ANC <500 μL^{-1}) was assessed as the number of days that the observed or transposed ANC was below 500 μL^{-1} . Any single observed ANC that was 500 μL^{-1} and was immediately preceded and followed by an ANC <500 μL^{-1} was counted as a day of neutropenia. Recovery was defined as the observation of an ANC 1,000 cells μL^{-1} for 3 consecutive days of post-nadir. NHP that did not recover were excluded from the duration calculations. The values displayed are means $\pm$ sem.

Treatment (n)	1 st Day ANC<500 μL^{-1}	1 st Day NC 500 μL^{-1}	Number of (days) ANC<500 μL^{-1}	1 st Day of Recovery to ANC 1000 μL^{-1}	Duration of Neutropenia (days)	Nadir (ANC μL^{-1})	Day of Nadir
10 Gy Control (12)	4.5 $\pm$ 0.2	22.1 $\pm$ 1.1	18.0 $\pm$ 1.3	27.0 $\pm$ 2.0	22.4 $\pm$ 2.0	48 $\pm$ 10	8.8 $\pm$ 0.6
10 Gy Neupogen 24h (8)	4.1 $\pm$ 0.2	16.2 $\pm$ 1.2	12.7 $\pm$ 1.4	20.5 $\pm$ 2.4	16.2 $\pm$ 2.3	75 $\pm$ 30	6.7 $\pm$ 0.6
10 Gy Neupogen 72h (8)	4.6 $\pm$ 0.3	19.2 $\pm$ 1.7	14.6 $\pm$ 1.9	22.2 $\pm$ 1.6	17.4 $\pm$ 1.7	37 $\pm$ 9	8.5 $\pm$ 1.0
10 Gy Neulasta 24h (8)	4.1 $\pm$ 0.1	15.3 $\pm$ 1.6	10.9 $\pm$ 1.9	17.1 $\pm$ 1.5	13.0 $\pm$ 1.7	117 $\pm$ 40	7.6 $\pm$ 1.0
10 Gy Neulasta 72h (8)	5.0 $\pm$ 0.2	18.0 $\pm$ 2.2	13.8 $\pm$ 2.7	20.3 $\pm$ 2.3	15.3 $\pm$ 1.9	40 $\pm$ 16	7.9 $\pm$ 0.7

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Incidence, latency, and duration of FN with bacteriology post-PBI/BM2.5 protocol for control, Neupogen®- or Neulasta®-treated cohorts

Rhesus macaques were exposed to 10.0 Gy utilizing 6 MV LINAC-derived photons in the PBI/BM2.5 protocol. Animals were administered control article (5% dextrose in water) or Neupogen® or Neulasta® beginning at the indicated times post-irradiation. Five cohorts were observed: control (n=12), Neupogen® initiated treatment on day 1 (24h) (n=8) and continued daily through effect (ANC 1,000 μL^{-1} for three consecutive days) and Neulasta® initiated treatment on day 1 (24h) (n=8) and then again on day 8 (n=8) or Neulasta® initiated on day 3 (72h) and then again on day 10 (n=8). FN was defined as ANC < 500 cells μL^{-1} with rectal body temperature 103.0 °F (39.4 °C), and blood cultures were collected on days when FN was observed. The time values displayed are means $\pm$ sem.

Table 5

Treatment (n)	Febrile Neutropenia (FN)		Incidence of Infection (% of Cultured NHP)	
	Incidence	1 st Day	# of Days	Gram+ Gram-
10 Gy Control (12)	83.3%	12.1 $\pm$ 2.1	2.7 $\pm$ 1.0	50% 0%
10 Gy Neupogen 24h (8)	75.0%	9.7 $\pm$ 1.0	2.0 $\pm$ 0.5	17% 17%
10 Gy Neupogen 72h (8)	50.0%	11.5 $\pm$ 1.3	2.8 $\pm$ 0.6	75% 0%
10 Gy Neulasta 24h (8)	50.0%	7.3 $\pm$ 1.2	3.8 $\pm$ 1.7	50% 0%
10 Gy Neulasta 72h (8)	62.5%	9.8 $\pm$ 1.9	6.4 $\pm$ 2.2	80% 0%

Table 6
Latency, duration, and severity of thrombocytopenia post 10 Gy in the PBI/BM2.5
protocol for control, Neupogen®- or Neulasta®-treated cohorts

Rhesus macaques were exposed to 10.0 Gy utilizing 6 MV LINAC-derived photons in the PBI/BM2.5 protocol. Animals were administered control article (5% dextrose in water) or Neupogen® or Neulasta® beginning at the indicated times post-irradiation. Five cohorts were observed: control (n=12), Neupogen® initiated treatment on day 1 (24h) (n=8) or day 3 (72h) (n=8) and continued daily through effect (ANC 1,000 μL^{-1} for three consecutive days) and Neulasta® initiated treatment on day 1 (24h) and then again on day 8 (n=8) or Neulasta® initiated on day 3 (72h) and then again on day 10 (n=8). Platelet counts were obtained from an automated CBC. Thrombocytopenia was defined as a platelet count $<20,000 \mu\text{L}^{-1}$. Recovery was defined as the observation of a platelet count $\geq 20,000 \mu\text{L}^{-1}$ for 3 consecutive days of post-nadir. The “first day to recovery parameter” accounts for the effect of whole blood transfusions. The values displayed are means $\pm$ sem.

Treatment (n)	1 st Day of Thrombocytopenia	1 st Day of Platelet Recovery*	Nadir (Platelet count μL^{-1})	Day of Nadir
10 Gy Control (12)	8.7 $\pm$ 0.1	27.0 $\pm$ 1.7 (n=8)	2,000 $\pm$ 246	12.1 $\pm$ 0.9
10 Gy Neupogen 24h (8)	8.8 $\pm$ 0.3	29.6 $\pm$ 7.2 (n=5)	3,875 $\pm$ 666	10.5 $\pm$ 0.7
10 Gy Neupogen 72h (8)	9.3 $\pm$ 0.3	28.6 $\pm$ 3.2 (n=5)	2,000 $\pm$ 378	11.8 $\pm$ 0.7
10 Gy Neulasta 24h (8)	8.4 $\pm$ 0.3	23.4 $\pm$ 1.2 (n=7)	2,125 $\pm$ 350	10.1 $\pm$ 0.3
10 Gy Neulasta 72h (8)	8.9 $\pm$ 0.4	22.2 $\pm$ 3.2 (n=6)	2,375 $\pm$ 865	10.3 $\pm$ 0.6

* Only the animals whose platelet count recovered to $20,000 \mu\text{L}^{-1}$ are included.