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A Nonhuman Primate Model of the Hematopoietic Acute Radiation Syndrome Plus Medical Management

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Abstract

The development of medical countermeasures against the hematopoietic sub-syndrome of the acute radiation syndrome requires well characterized and validated animal models. The model must define the radiation dose- and time-dependent relationships for mortality and major signs of morbidity to include other organ damage that may contribute to the morbidity and mortality. Herein, we define these parameters for the nonhuman primate exposed to total-body radiation and administered medical management. A blinded, randomized study (n=48 rhesus macaques) determined the lethal dose response relationship using bilateral, 6 MV linear accelerator photon radiation to doses in the range of 7.20 to 8.90Gy at 0.80Gy minute^{-1}. Following irradiation animals were monitored for complete blood counts, body weight, temperature, diarrhea, and hydration status for 60 days. Animals were administered medical management consisting of intravenous fluids, prophylactic antibiotics, blood transfusions, anti-diarrheals, analgesics and nutrition. The primary endpoint was survival at 60 days post irradiation; secondary endpoints included hematopoietic-related parameters, number of transfusions, incidence of documented infection, febrile neutropenia, severity of diarrhea, mean survival time of decedents and tissue histology. The study defined an LD30/60 of 7.06Gy, LD50/60 of 7.52Gy, and an LD70/60 of 7.99Gy with a relatively steep slope of 1.13 probits per linear dose. This study establishes a rhesus macaque model of the hematopoietic acute radiation syndrome and shows the marked effect of medical management on increased survival and overall mean survival time for decedents. Furthermore, following a nuclear terrorist event, medical management may be the only treatment administered at its optimal schedule.

Keywords

radiation; hematopoietic; ARS; nonhuman primate

INTRODUCTION

The use of antibiotics, fluids, blood products, analgesics and nutrition is the “standard of care” for patients exposed to cytotoxic chemotherapy and cytotoxic “conditioning” for stem

cell transplant (Smith et al. 2006; Timmer-Bonte et al. 2005a). These measures will be similar for personnel exposed to myelosuppressive and potentially lethal doses of radiation (MacVittie et al. 1996a; Ricks et al. 2002a; Nagayama et al. 2002; Waselenko et al. 2004; Baranov et al. 1994; Ortiz et al. 2003; Konchalovsky 2010). There are significant knowledge gaps that marginalize our ability to develop medical countermeasures (MCM) within the criteria established by the Food and Drug Administration (FDA) "Animal Rule" (AR) to mitigate the hematopoietic acute radiation syndrome (H-ARS) (Crawford 2002; FDA and CBER 2009). The FDA published two drafts entitled, "Guidance for Industry – Essential Elements to Address Efficacy Under the Animal Rule" and "Guidance for Industry – Qualification Process for Drug Development Tools (DDT)" (FDA and CBER 2009; FDA 2010). These respective documents provide a description of the component criteria to be addressed for successful use of the FDA AR to gain approval for candidate MCM, in this case to treat potentially lethally irradiated personnel and the potential development of animal models that may be qualified as a DDT within a stated context of use such as a validated model of the H-ARS to be used by sponsors for evaluation of potential MCM to treat lethal consequences of H-ARS. Herein, we describe the dose response relationship (DRR) for a nonhuman primate (NHP) model of the H-ARS plus the administration of supportive care. We focus on the development of a "well characterized" NHP model that defines the DRR and time course of morbidity and mortality as key endpoints for definition of MCM efficacy.

We know that medical management alone can significantly enhance survival of animals exposed to lethal doses of uniform total body irradiation (TBI) (Jackson et al. 1959; MacVittie et al. 1991; Perman et al. 1962; Sorensen et al. 1960; MacVittie et al. 2005; Byron et al. 1964). The relationship between supportive care and lethality over the H-ARS has been shown in canines but has not been established for NHP (Broerse and MacVittie 2001; MacVittie et al. 2005). There have been a number of studies that established the DRR for NHP exposed to TBI with 250 kVp or 2Mev x-rays and mixed neutron and gamma (γ) radiation (Table 1) (Eldred and Trowbridge 1954; Stanley et al. 1966; Wise and Turbyfill 1968; Henschke and Morton 1957; Haigh and Paterson 1956; Schlumberger and Vazquez 1954; Dalrymple et al. 1965). Additionally, there is a single study describing the DRR for Co-60γ radiation-induced lethality in NHP performed in 1967 (Eltringham 1967). These data were provided to Dr. T. MacVittie via personal communication. This data set established the DRR and estimated LD50/30 of 644 rad, with a 95% confidence interval (CI) of [613, 678] and slope of 1.15 probits per linear dose for rhesus macaques exposed to TBI with Co-60 γ-radiation without benefit of supportive care (Table 1). The study using 2Mev TBI compared favorably with that of the Co-60γ radiation and established the DRR for NHP characterized by an LD50/30 estimate of 671, CI [632, 715] and a slope of 0.99 probits per linear dose (Table 1) (Dalrymple et al. 1965). We utilized all of the data (n=418) from the studies using 250kVp x-radiation to assess the DRR and estimated a value for the LD50/30 of 492rad CI [471, 512] and a slope of 0.77 probits per linear dose (Table 1) (Eldred and Trowbridge 1954; Stanley et al. 1966; Henschke and Morton 1957; Haigh and Paterson 1956; Schlumberger and Vazquez 1954).

The effect of medical management on the DRR of NHP exposed to these radiation sources can only be estimated from a few studies. Byron et al compared two lethal doses of 250kVp x-radiation and demonstrated the ability of an antibiotic regimen to significantly increase survival of rhesus macaques exposed to an LD100(Byron et al. 1964). Antibiotics alone decreased the 100% lethality to 72%. Additional data from a study performed by MacVittie et al (unpublished) used TBI with 250kVp x-irradiation established the indirect effect of supportive care, as described herein (Table 1). NHP were exposed to doses of 250kVp TB x-radiation at 5.00Gy, 6.00Gy, 7.00Gy and 9.20Gy and provided supportive care as per an Institutional Animal Care and Use Committee (IACUC)-approved protocol. The resultant DRR was characterized with an LD50/60 of 7.18Gy relative to the composite (n=418),

historical LD50/30 of 492rad noted above for NHP irradiated without the benefit of supportive care (Table 1). Since medical management (alternatively referred to as supportive care) will be the "standard of care" for irradiated personnel, the study herein was performed to determine the DRR for TBI across the H-ARS dose range for NHP administered supportive care. This DRR and associated time course of morbidity and mortality characterized the H-ARS in the NHP. It can be used to choose appropriate doses for developmental and pivotal efficacy studies in the NHP for MCM capable of mitigating lethality throughout H-ARS.

This study was designed to assess the following: a) The DRR and estimated LD50/60 and slopes (probits per linear dose) for rhesus macaques exposed to lethal doses of TBI with linear accelerator (LINAC)-derived 6 MV (average energy, 2 MV) photons and administered medical management, b) The respective "trigger-to-treat" for major aspects of medical management, e.g., administration of antibiotics, whole blood transfusions, rehydration fluids, supplemental nutrition etc. during the time course of morbidity post TBI, c) The effect of medical management on the respective LD50/60 and DRR for TBI alone compared to the historical control data sets, d) The TBI DRR for numerous secondary parameters associated with hematologic and gastrointestinal effects, to include cellular parameters for neutrophils, platelets, incidence of febrile neutropenia, number of transfusions, incidence of diarrhea, loss of body weight, etc. and e) The time course associated with mortality.

MATERIALS AND METHODS

Animals

Male, China-bred, rhesus macaques, n=48, *Macaca mulatta*, [4.7 – 6.2kg body weight (bw)] were exposed to bilateral, uniform TBI. All animals were in good health, sero-negative for simian immunodeficiency virus, simian T cell leukemia virus type 1 and malaria and negative for Herpes B virus and Tuberculosis. Irradiated animals were observed for a 60 day in-life phase post TBI. Another cohort of NHP was maintained for blood donation (males, >5yrs of age, ≥7kg bw).

Housing and Care—Animal holding rooms were maintained at 70°F to 80°F with 30% to 70% relative humidity, using at least 10 air changes per hour of 100% conditioned fresh air with a 12-h light/dark cycle. Animals were maintained in individual stainless steel cages at the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited University of Maryland Testing Facility.

Food and Water—Animals were provided commercial primate biscuits *ad libitum* supplemented with fresh fruit (i.e. apples, bananas, oranges) and primate treats. Following irradiation, all citrus was removed from their diet. The animals had unlimited access to filtered water.

Anesthesia—Ketamine (10 ± 5 mg kg^{-1} of the most recent bw) was administered intramuscularly (IM) prior to procedures to minimize stress and anxiety. If an animal responded poorly to ketamine as a single agent, the ketamine was combined with xylazine (AnaSed®, Fort Dodge, Fort Dodge, IA) (1 ± 0.5 mg kg^{-1}, body weight, IM or SC). If necessary, the anesthetic agents were reversed by administering yohimbine (Yobine®, Lloyd, Inc., Shenandoah, IA,) (0.2 ± 0.1 mg kg^{-1}, IM or IV).

Medical Management

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

Cageside Observations

Veterinarians, who were blinded to the radiation exposure dose, performed cageside observations twice daily, at least 6 h apart. NHP activity, posture, stool consistency, vomit, hemorrhage, respiratory or seizure activity, and alopecia, were graded and recorded.

Clinical Observations

The NHP was anesthetized and clinical parameters such as body weight, body temperature, complete blood count (CBC) (Beckman Coulter Ac·T diff™, Beckman Coulter, Inc., Miami, FL) including a manual white blood cell (WBC) differential performed on a Wright-Giemsa-stained blood film), dehydration status, presence of mouth ulcers, and observation of blood in the stool are assessed.

Analgesics

Buprenorphine HCl (Hospira, Lake Forest, IL) (IM at 0.01mg kg^{-1} up to 0.02mg kg^{-1}, BID) was administered whenever mouth ulcers or bloody stools were observed and from study day 5 to 35. Mouth ulcers were cleansed with hydrogen peroxide or Nolvasan solution and rinsed with saline. Bupivacaine gel, a mixture of 0.1ml of 25% Bupivacaine HCl (Marcaine®, Hospira, Lake Forest, IL) with a dab of surgical lubricant (Surgilube®, Fougera®, Melville, NY), was applied to the area with a cotton-tipped applicator.

Anti-Ulcerative

Sucralfate (Carafate®, Axcan Scandipharm Inc., Birmingham, AL & Nostrum Laboratories, Inc., Edison, NJ) was administered (1g day^{-1} BID) from study day 5 to 35 or if bloody stool was observed.

Anti-diarrheals

Following the observation of diarrhea, Loperamide Hydrochloride (Imodium, McNeil Consumer Healthcare, Fort Washington, PA), (0.1-0.2mg kg^{-1} PO BID) was administered. If diarrhea persisted for three (3) successive days during Imodium treatment or if watery stool without any signs of formed stool were observed, diphenoxylate hydrochloride (Lomotil, Pfizer Inc, New York, NY, 0.1mg kg^{-1} PO BID for 3 days) was administered. If diarrhea persisted after three (3) days, Imodium treatment was re-administered.

Antibiotics

Antibiotics were initiated when the absolute neutrophil count (ANC) was $<500\ \mu L^{-1}$ and continued until the animal maintained an ANC $>500\ \mu L^{-1}$ for 48 hours. The primary antibiotic was enrofloxacin (Baytril®, Bayer HealthCare LLC, Shawnee Mission, KS). Additionally, gentamicin sulfate (GentaMax®, Phenoix Scientific, Inc., St. Joseph, MO, 5mg kg^{-1} QD IM or IV) was administered in combination with Baytril when the body temperature ≥103°F and was continued for 24 h. Rocephin (Roche Laboratories Inc., Nutley, NJ) or Primaxin (Merck & Co Inc., Whitehouse Station, NJ) was administered when microbial resistance was demonstrated to enrofloxacin or gentamicin.

Antipyretic

Carprofen (Rimadyl®, Pizer Inc., New York, NY, 2.2mg kg^{-1} BID or 4.4mg kg^{-1} QD, IM, IV, or PO) was administered when a body temperature of ≥104°F was observed. It was continued for 48 h after the first day the temperature was <104°F.

Nutritional Support

On all days post-irradiation animals received fresh fruit, soft food, and bottles containing diluted fruit juice or oral rehydration (Prang™, Bio-Serv®, Frenchtown, NJ). Animals that were observed to have weight loss ≥10% of their baseline body weight received Bio-Serv® certified Rhesus Liquidiets at 15ml kg^{-1} by oral gastric gavage (OG). Volume was reduced to 7ml kg^{-1} if the animal was also receiving OG reverse osmosis (RO) water for hydration.

Blood Product Support

Whole blood, anti-coagulated with 10% citrate, dextrose phosphate with adenine (CPD-A) (Sigma-Aldrich, St. Louis, MO) was obtained from healthy, male NHPs, bw ≥ 7.0 kg. Blood was filtered through a 70 micron cell strainer (BD Falcon™, BD Biosciences, Chicago, IL) and irradiated to 2500 cGy (Gammacell® Elite 1000, Best Theratonics, Ottawa, ON, Canada) prior to use. Transfusions of whole blood were administered at 7-14ml kg^{-1}, IV, using a 18 micron blood filter (Hemo Nate® Filter, Utah Medical Products, Inc., Midvale, UT) following a decrease of ≥5% in hematocrit (HCT) resulting in a HCT ≤25% over a 24 h time period, HCT is <20%, or there was obvious signs of uncontrolled hemorrhage.

Fluid Support

Fluid support was provided based on a grading system delineated as mild, moderate, or severe dehydration. Mild: presence of tacky mucus membranes or a skin tent time (STT) or capillary refill time (CRT) ≥2 but <3s. Mild animals received a bolus of lactated Ringer's solution (LRS) (10-15mL kg^{-1}) by slow IV push and reverse osmosis (RO) water (10-15mL kg^{-1}) by oral gastric feeding tube (OG). Moderate: NHPs displaying any of the mild criteria plus dry mucous, >3% increase in HCT from the day before (not transfusion related), sunken eyes, or STT or CRT ≥3sec. Moderate animals received a bolus of LRS (20-30mL kg^{-1}) over 15-20 min by slow push and RO water (7-10 mL kg^{-1} by OG). Severe: NHPs displaying any of the mild and or moderate criteria plus pale mucous membranes, >5% increase in HCT from the day before (not transfusion related), a rapid and weak pulse, cold extremities, lethargy, or rapid breathing. Severely dehydrated animals received fluids as described for moderate dehydration with the addition of a slow IV infusion (15±5 mL kg^{-1} h^{-1}) administered over a period of 2-4 h. Animals are placed in a restraint device at this time and allowed to awaken. Midazolam HCl (Bedford Laboratories™, Bedford, OH) (0.2mg kg^{-1}) may be administered to calm the NHPs while in the restraint.

Euthanasia

Veterinarians, who were blinded to the radiation dose, adhered to a specific set of criteria as outlined below when determining if euthanasia was appropriate prior to the end of the study (d60). Any NHP that was recumbent or exhibited decreased or absent responsiveness to touch, hemorrhage from the gastrointestinal (GI) tract in excess of 20% of the estimated blood volume in any 24 h period, or signs of unrelieved pain were humanely euthanized. Any NHP which experienced any combination of the following observations such as respiratory distress, decreased food and water intake, reluctance to move for >24 h, and severe dehydration were also humanely euthanized. Animals were euthanized using Drug Enforcement Agency (DEA) Class III euthanasia solution (Euthasol®, [Virbac AH Inc., Ft. Worth, TX] 0.27ml kg^{-1} IV). Expiration was confirmed by a lack of heart beat, absent femoral artery pulse, and lack of chest respiration.

NHP irradiation

On the day prior to the irradiation, food was removed from the animals approximately 18 h prior to exposure to minimize the occurrence of radiation-induced emesis. On the day of irradiation, NHP were administered an antiemetic, Zofran (Glaxo SmithKline, Research Triangle Park, NC) or Ondansetron, (Hospira, Inc., Lake Forest, IL) at 1-2mg kg^{-1}, PO, IV or IM, 45-90min before TBI. The NHP were secured under ketamine-induced anesthesia in a Plexiglas supine restraint device and transported from the NHP housing area to a LINAC facility.

The restrained-NHP were exposed to TBI with 6 MV LINAC-derived photons. Bilateral TBI was delivered in the anterior-posterior and posterior-anterior direction at mid-dose, at a dose rate of 0.80Gy m^{-1}, to the midline tissue dose (MLTD). The NHP, n=48, were irradiated at doses of 7.20Gy, 7.55Gy, 7.85Gy, 8.05Gy, 8.40Gy, and 8.90Gy. Animals were observed via in-room cameras throughout the entire procedure. Following TBI, NHPs were anesthetized, transported back to the NHP housing area, administered a second dose of Zofran or ondansetron (1-2mg kg^{-1}, IM) within 35-45 m post TBI and returned to their home cage. Dosimetry was performed prior to and during each irradiation. Depth dose measurements were made at the center of a cylindrical phantom that approximates the mean diameter of the experimental rhesus macaque. Three phantoms of increasing size are available. The phantom is made of 0.32 cm Lucite and filled with water. Dose measurements were performed with paired 0.5-cc ion chambers, specifically an A-150 plastic tissue-equivalent chamber with methane-based, tissue-equivalent gas in a magnesium chamber with argon gas. Actual animal irradiations were monitored with ionization chambers.

Experimental endpoints

Primary endpoint: The primary clinically relevant parameter was 60 day all-cause mortality. *Secondary endpoints:* Secondary endpoints included neutrophil- and platelet (PLT)-related parameters to include: cell nadirs, the day of and duration of neutropenia (ANC<500 μL^{-1}, ANC<100 μL^{-1}) and thrombocytopenia (PLT<20,000 μL^{-1}), time to recovery to an ANC > 1,000 μL^{-1} and PLT > 20,000 μL^{-1}. Other parameters included mean survival time (MST) of decedents, the first day and incidence of febrile neutropenia (FN) defined as body temperature ≥ 103°F and an ANC<500 μL^{-1}, number of days with fever (body temperature ≥ 103°F), incidence of documented infection (both in-life blood cultures, and blood and major organs at necropsy), incidence and severity of diarrhea, hydration status, and body weight loss.

Statistical Methods

Data Description—Data was collected for 60 days on 48 male, rhesus macaques exposed to TBI in 7 dose groups of 2-8 NHP each. Figure 1: 60 day survival was collected for each group (Co60, 2 Mev x-rays, Linac 6 MV) and a dose normal probit fit made to each group. Total NHP in each group were 90, 84, and 48 respectively with 8 to 15 subjects per radiation dose. All probit fits were significant indicating a strong dose mortality effect ($P<0.01$). Analysis was performed using R statistical software (version 2.13.1).

Descriptive statistics—Frequency and percent are presented for count data; mean, standard deviation, standard error, median, minimum and maximum are presented for continuous data. All secondary parameters are collated as appropriate. Respective means and/or medians relative to number of survivors in each radiation dose cohort were reported. The descriptive analysis was performed using SAS version 9. Table and graphs were constructed using Microsoft Office Excel 2010.

RESULTS

Historical data base for H-ARS dose response relationship (DRR)

Two complete studies have been performed to determine the H-ARS DRR in rhesus macaques that used a radiation quality comparable to the LINAC-derived photons used herein without the addition of supportive care (Table 1) (Eltringham 1967; Dalrymple et al. 1965). Dalrymple et al used 2Mev x-radiation (HVL of 7.5mm Pb) whereas Eltringham used Co-60 γ-radiation (Dalrymple et al. 1965; Eltringham 1967). Although Eltringham's study was published in abstract form only, the data from the complete DRR was provided as personal communication to TJ MacVittie.

The respective LD50/30 values for the 2Mev x- and Co-60 γ-radiation studies were 671rad [632, 715] and 644rad [613, 678] determined for exposure at the MLTD (Fig 1). The respective slopes were 0.99 probits per linear normal dose [0.61, 1.37] and 1.15 probits per linear normal dose [0.74, 1.56] MLTD (Table 1). Dalrymple et al used seven dose cohorts (range 360-802rad, n=84) exposed to TBI at 10.7rad per m^{-1}, whereas Eltringham irradiated seven dose cohorts (range 400-790 rad, n=90) at 54.6rad per m^{-1}. The respective overall mean survival time (MST) of decedents was 14.7 days (range 11-20d) and 14.4 days (range 6-27d) for the 2Mev x-radiation and Co-60 γ-radiation studies. No deaths were observed in the 30-60 day in-life phase of the studies.

The ratio of the respective LD10/60 and LD90/60 for each study provides another measure of the steepness of the slope. In these studies the 2Mev dose response provided respective estimates of 542rad and 800rad for the LD10/60 and LD90/60 and a resultant ratio of 1.48 (Table 1). The LD10/60 and LD90/60 for the Co-60 γ-radiation study were 533rad and 756rad; the resultant ratio is 1.42 (Table 1). The dose differential between 10% and 90% mortality for the 2Mev x- and Co-60 γ-radiation studies was 258rad and 223rad, respectively (Table 1).

The Current Study: Radiation dose and lethality for H-ARS dose response relationship (DRR)—Thirty-two (32) of 48 total animals (66.6%) succumbed to the H-ARS and associated sequelae over the dose range from 7.20Gy to 8.90Gy. Radiation dose was a significant predictor of mortality ($P = 0.01$) with increased mortality rates at the higher doses (Fig 1, Table 2). In the current study, the animals were exposed to 6 MV LINAC photons, n=8 per six doses of TBI, and received medical management as described in Methods. The historical data sets showing the dose response, calculated LD50/30 values and 95% CI of rhesus macaques exposed to TBI from Co-60 γ-radiation or 2 MeV x-radiation are presented as a comparison for the current study data set (Eltringham 1967; Dalrymple et al. 1965).

Lethal doses within the H-ARS. The estimated LD30/60, LD50/60, and LD70/60 [95% CI], were respectively, 7.06Gy [5.01, 7.50], 7.53Gy [6.50, 7.88], and 7.99Gy [7.60, 8.65] and a slope of 1.13 probits per linear dose [0.33, 1.93] as MLTD (Table 3). The radiation DRR will be used to design efficacy trials for MCM against the lethal H-ARS in adherence to the criteria of the FDA AR (Crawford 2002; FDA and CBER 2009). The ratio of the lethal dose for few (10%) to that for many (90%), provides an estimate of the slope for lethality across the H-ARS. Estimation of the LD10/60 (6.39Gy) [2.74, 7.06] relative to the LD90/60 (8.66Gy) [8.23, 10.73] determines the respective ratio between the lethal doses for “few” to that for “many” animals (Table 1). The LD90:LD10 is 1.36 [1.05, 1.67]. The dose differential between 10% and 90% mortality is approximately 2.27Gy, but the difference between 30% survival and 70% survival is only 0.93Gy. This is a very steep slope and underscores the necessity of accurate dosimetry and irradiation protocols.

LD50/60 plus medical management: The estimated value for the LD50/60 using TBI with 6 MV LINAC photons plus medical management is 7.53Gy [6.50, 7.88] (Table 3). A retrospective comparison of the DRR's noted in the aforementioned studies indicated that supportive care alone will enhance the LD50/60 value and survival across the lethal H-ARS range (Fig. 1 and Table 2). An indirect comparison of the data presented herein and the historical data base for the 2Mev x- and Co-60 γ-radiation suggested an average dose modification factor for the LD50/60 of approximately 1.13 for administration of supportive care as defined by our protocol. The data demonstrated that supportive care became less effective at the highest TBI dose of 8.90Gy. It remains to be determined if the addition of an effective MCM will increase survival at the high-lethal doses of TBI.

Mean Survival Time (MST) of Decedents within the H-ARS: The MST of decedents for each radiation dose ranges from 16.2d to 22.2d (Table 2). The overall MST of decedents across all doses was 19.4d. The comparison of MST for decedents that received supportive (current study) relative to those that did not (*historical data noted earlier*) indicated an approximate increase of 5.0d (19.4d *vs.* 14.5d). This value becomes significant when considered in the context of administering a potential MCM to lethally irradiated animals that are receiving effective supportive care. In this case, the candidate MCM would have the benefit of 5 additional days to invoke its mechanism of action on marrow regeneration, stabilization of the niche and/or production of mature cells such as neutrophils or platelets.

Duration of radiation-induced cytopenia

Neutrophil-related parameters—Neutrophils provide the first line of defense against opportunistic infection. Lethal doses of TBI administered in this study reduced the circulating ANC to < 500 μL^{-1} within approximately 5 days after TBI, irrespective of the radiation dose (Table 4). Antibiotics were administered prophylactically, e.g., when the ANC < 500 μL^{-1} because it is anticipated that the ANC will continue to decrease to values < 100 μl^{-1}. This is severe grade 4 neutropenia and the animal is at greatest risk for infection and sepsis. Furthermore, these values determine the validity of administering antibiotic prophylactically. The ANC in all lethally irradiated animals herein, decreased from a mean ANC < 500 μL^{-1} to a mean ANC < 100 μL^{-1} within the next 1.4 to 2.7 days and continued to decrease in all dose cohorts with the exception of one animal (7.85Gy exposure), to absolute neutropenia. The average nadir for the 7.85Gy cohort was 5 μL^{-1} (Table 4). The mean duration of grade 4 neutropenia (ANC < 100 μL^{-1}) over all dose cohorts, for survivors or non-survivors when the ANC recovered to ≥ 100 μL^{-1} prior to death ranged from 9.8 to 12.7 days (Table 4); the range of the individual animals with an ANC < 100 μL^{-1} was from day 5 to day 23 (Table 5). The range over all dose cohorts for the mean duration of ANC < 500 μL^{-1} was 14.3 to 24.0 d (Table 4); the range of the individual animals was from d3 to d46 (Table 5). Only the surviving NHP attained recovery to an ANC ≥ 500 μL^{-1}. The mean decrease, nadir, and neutrophil recovery for animals exposed to doses of TBI that approximate the LD30, LD50, and LD70/60 through their survival are comparable (Fig 2).

Febrile Neutropenia (FN) and Antibiotic Requirements. Antibiotics are administered when the ANC <500 μL^{-1} with the expectation that the ANC will be < 100 μL^{-1} within 2-3 days and remain at that level or lower for 7-10 day duration (Ricks et al. 2002b; Hughes et al. 2002) (Table 6). FN is defined as the ANC <500 μL^{-1} and the core body temperature ≥103.0°F. This protocol follows the recommendations in Infectious Disease Society of America guidelines for treatment of humans experiencing severe neutropenia (Hughes et al. 2002). The first day the animals experience FN and the duration of FN is variable and ranges from 7-12, 5-11, 4-11, 5-16, 7-14 and 5-11days for the TBI dose-cohorts of 7.20, 7.55, 7.85, 8.05, 8.40, 8.90Gy, respectively. The range of means for first day of FN across all cohorts was 7.1-10.9days.

On the average, the earliest a cohort experienced FN was 7.1d while it is noted that within cohorts there were animals that experienced FN as early as d4 and d5 post TBI (Table 6). The percentage of animals which experienced a core body temperature ≥103.0°F at any point during the study was 91.2% (44/48).

Platelet-related parameters—Lethal doses of TBI induced a severe decrease in PLT levels for all radiation dose cohorts. The decrease, nadir, and recovery of PLT (through their survival) following exposure to TBI that approximate the LD30, LD50, and LD70/60 is similar (Fig 3).

The first day the PLT count decreased to < 20,000 μL^{-1} or < 10,000 μL^{-1} was not different within all dose cohorts and ranged from 8.6 to 9.7d (Table 7). The PLT counts continued to decrease to near absolute levels of ≤ 1 μL^{-1} and the duration of thrombocytopenia (PLT < 20,000 μL^{-1}) over all dose cohorts, ranged from 8.7 to 20.5d. The animals required a range of 18.0 to 30.0d to recover to PLT counts ≥ 20,000 μL^{-1} and during this period, required a range of 0.5 to 6.5 blood transfusions (Table 7). Because a PLT count in an animal may be elevated as a result of a whole blood transfusion the following rule is followed when determining endogenous PLT recovery post-irradiation: If the animal's PLT count is < 20,000 μL^{-1} prior to transfusion, yet the PLT count increases to ≥ 20,000 μL^{-1} for three consecutive days following the transfusion, endogenous recovery will be acknowledged on the third day post-transfusion.

Transfusion Requirements. The necessity and requirements for whole blood transfusions is more complicated since the decision to transfuse is usually multifactorial. A decision to transfuse generally requires knowledge of the most recent and current HCT and PLT counts, as well as the presence of active bleeding by an NHP. The peripheral blood PLT count and number of transfusions per TBI dose cohort are shown in Table 7. A key study parameter is the time to the administration of the first transfusion following irradiation, which ranges between day 9 and d11 for the 6 irradiation cohorts. The mean value for the time interval between the day of TBI and the first transfusion for all animals which required whole blood transfusions (n=47 of 48) is 12.1d. This timeframe lends further credence to the treatment value of supportive care and authenticates that there is a reasonable time interval between the event and the need for this to occur.

Incidence and Severity of Diarrhea

The incidence and consistency of stool from each animal was observed at least twice daily by an individual blinded to the radiation exposure dose. If stool was present it was scored 0 for formed stool, 1 for soft stool, 2 for loose and/or watery stool, and 3 for bloody diarrhea, where a 0 indicates a normal condition and 3 indicates the most severe condition. Although there is not a definitive relationship between radiation exposure dose, all animals experienced some degree of diarrhea during the study and at a minimum 75% of each cohort was graded ≥2 (Table 8).

Incidence and Severity of Loss of Body Weight

Animals were weighed daily from d0 through d25 and thereafter when anesthetized for scheduled sample collection of medical management. Weight loss ≥ 15% from d0 occurred in 45.8% of all NHP. Although the number of NHP exposed at 7.55Gy experienced the greatest percentage of weight loss of all dose exposure groups (62.5% versus a range of 25.0% to 50.0%), no animal in this cohort progressed to a ≥ 25% weight loss from d0 (Table 9). In general, 50% of all NHP exposed at ≥7.85Gy experienced weight loss ≥ 15% from d0 and 46.7% of these animals went on to experience weight loss ≥ 25% from their d0 body

weight. Additionally, the mean number of days that NHP exposed at ≥7.85Gy experienced a body weight loss ≥ 15% from d0 was 13days vs 3.4 days for the 7.55Gy cohort (Table 9).

Microbiology. Peripheral Blood Culture in association with febrile neutropenia (FN)

Blood cultures were obtained when FN (ANC <500 μL^{-1} and body temperature ≥103°F) was observed or any day the body temperature was ≥105°F. Additional blood cultures were collected if FN persisted for 5 consecutive days after a previous blood culture collection. The treatment regimen was altered if an organism was isolated from a blood culture that demonstrated resistance to the current treatment antibiotic.

There was a marked variability in microbial culture results from peripheral blood obtained at the time of FN (data not shown). Nearly half (approximately 43%, 59 of 137) of the peripheral blood-derived culture results were negative for the presence of bacteria. Seventy-nine percent (79%, 38/48) of the animals had at least one (1) positive blood culture during the study. The number of animals with positive blood cultures over radiation dose ranged between five (5) and seven (7). Seventy eight percent (78%, 61 of 78) of the cultures were positive for either gram negative or gram positive bacteria, whereas fifty-seven percent (57%, 78 of 127) were gram positive.

The number of animals that had an organism(s) isolated from their blood cultures that were resistant to at least one of the following antibiotics, baytril, gentamicin, rocephin or claforan is enumerated in Table 10 by radiation dose. There were 30 animals from the 48 study animals (62.5%) that had at least one organism that was resistant to at least 1 of the above mentioned antibiotics.

Microbiology of Organs and Blood Obtained at Necropsy

Microbiology was assessed on all animals that either expired or were euthanized, as per protocol, during the in-life phase or at the end of the study. In addition to peripheral blood, the liver, spleen, lung and kidney were assayed for presence of gram positive and gram negative bacteria. A total of 9 animals, chosen from each radiation dose cohort (7.2Gy n=3, 7.55Gy n=3, 7.85Gy n=1, 8.05Gy n=1, 8.40Gy n=1) except 8.90Gy were not terminated at the end of the study (d60) in order to examine long term radiation effects. All four organs harvested from NHP (n=8) that expired prior to euthanasia were positive for either gram negative and/or gram positive organisms (7.20Gy n=1, 7.85Gy n=3, 8.40Gy n=1, 8.9Gy n=3).

Twenty-one (21) NHP that were humanely euthanized between d12 and d30 (7.2Gy n=1, 7.55Gy n=4, 7.85Gy n=2, 8.05Gy n=5, 8.40Gy n=5 and 8.90Gy n=4) had a necropsy performed immediately after euthanasia. Nineteen of these NHP (90.5%) had either gram negative and/or gram positive organisms in all four organs. One NHP only had yeast in liver and spleen and another NHP had both gram positive and gram negative bacteria in liver, spleen, and lung, but not kidney. The incidence of gram negative or positive organisms present in all animals euthanized before d31, was 38.1% gram positive bacteria only, 28.6% gram negative bacteria only, 28.6% both gram positive and gram negative bacteria. The results from blood cultures obtained at necropsy generally duplicated what was observed in the organ cultures. However, three NHP had both gram negative and positive organisms in their blood ebut only one type of gram delineated organisms was detected in their tissue. One NHP had gram positive blood culture but both gram positive and negative organism in its tissue. Another NHP that had a gram negative blood culture but only yeast was detected in the liver and spleen. Yeast and both gram negative and positive organisms were detected in the blood culture, liver and kidney in another NHP.

Three NHP were humanely euthanized on d31, d45, and d52 and the necropsy was performed immediately thereafter with no evidence of bacteria in any of their organs. However, two of the three NHP had either a gram negative or gram positive organism in their blood culture at necropsy. Seven (7) NHP survived the in-life phase of the study, were humanely terminated and underwent a necropsy between d65 and d113 post TBI. Five (5) of these NHP had no bacteria present, one NHP had gram positive bacteria in a kidney (d76), and a second NHP (d93) had gram positive organism(s) present in liver, spleen, lung and blood but not kidney at necropsy.

Gross Necropsy and Histology

A total of thirty-one animals had a necropsy performed immediately following humane euthanasia. Gross observations at necropsy and histological examination of tissue were performed. Necropsies were also performed on eight NHP that succumbed prior to euthanasia. All of these animals had findings of bacteria, hemorrhage, and necrosis in multiple organs and histological examination was confounded by autolysis. *Gross Observations.* Radiation-induced pathology was observed in every NHP euthanized during the in-life phase of the study. All NHPs euthanized during the in-life phase generally had numerous petechiae over the face, arms, legs, and trunk as well as a lean body mass and marked loss of body weight. Other sporadic findings included ecchymosis, dehydration, enlarged spleens, and oral ulcerations. *Histological Assessment.* All major sequelae including cytopenia, bacteremia/sepsis, and hemorrhage were radiation dose- and time-(post-TBI) dependent.

Bone Marrow—Bone marrow isolated from femoral bone and sternum showed evidence of significant radiation effects on the hematopoietic system. The bone marrow (BM) sections from the femur and sternum were markedly depleted of myeloid, erythroid, and megakaryocytic cells in all NHPs euthanized during the in-life phase of the study. The severity of myeloid and erythroid hypocellularity increased with radiation dose. Animals surviving the duration of the study had active myeloid and erythroid cells with moderate to normal cellularity and evidence of mitotic activity.

Lymphoid tissue—Severe depletion of lymphocytes was noted in lymph nodes, spleen, and thymus. The extent of lymphopenia in the lymphoid tissue appears to be dose dependent and to some extent organ dependent. Diffuse lymphocyte depletion was noted in lymph nodes and thymus, while moderate lymphocyte depletion was observed in the spleen. However the thymus was non-observable in several NHP.

Gastrointestinal (GI) tissue—Areas of hemorrhage, ecchymosis and petechiae were evident with increasing doses of radiation in both the small and large intestine. Hemorrhagic areas were observed on both serosal and mucosal surfaces although this was variable among animals. The consistency and severity of the damage increased relative to the dose of irradiation.

Other organs (liver, heart, lung, spleen, kidney)—The majority (81.0%) of the twenty-one (21) NHP that were humanely euthanized between d12 and d30 (see Microbiology of Organs and Blood Obtained at Necropsy) had evidence of bacterial emboli or hemorrhage in at least one of these organs. No significant findings were observed in 33.3% of liver, 61.9% of heart, 23.8% of lung, and 61.9% of kidney, whereas depleted lymphocytes was the only finding in 33.3% of spleen. Necropsies performed on NHP > 31d post-TBI (n=8) and exposed to 7.20Gy to 8.05Gy TBI generally revealed no significant findings with regard to “other organs”. NHP exposed to higher radiation doses were found to have limited inflammation in the heart and kidney combined with areas of fibrosis in spleen

and kidney (n=1, 8.40Gy, d93 TBI) and only limited inflammation in the lung and kidney (n=1, 8.90Gy, d45 TBI).

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

CONCLUSIONS

1. The dose of uniform, TBI with average 6 MV LINAC-derived photons was a significant predictor of lethality. The doses of TBI used herein allowed us to estimate the respective LD30, LD50, and LD70/60 required for the design of supportive efficacy and pivotal efficacy trials for MCM that mitigate the lethality associated with the H-ARS.
2. The respective LD30, LD50, and LD70/60 were estimated to be 7.06, 7.53 and 7.99Gy.
3. Compared to literature values determined in studies designed to assess the lethality DRR of rhesus macaques without the benefit of supportive care, supportive care as administered herein increased the LD50/60 and survival from high-lethal doses within the H-ARS and increased the MST of decedents relative to historical controls.

The estimated LD50/60 herein is 7.53Gy, whereas the historical values were estimated to be 644rad and 671rad. The respective slopes for the DRR were 1.13, 1.15 and 0.99 probits per linear dose and not significantly different from each other (P=0.83). The overall estimated MST for all decedents herein was 19.4d compared to the historical value of 14.5d from the studies conducted without supportive care administration to the NHP.

4 The data suggested that spontaneous recovery from TBI of 8.90Gy is not possible with moderate supportive care alone, as described herein. It remains to be determined if additional level of care and/or the use of MCM will increase survival at the high-lethal dose range.

5 All animals that receive lethal doses of TBI in range of 7.20Gy to 8.90Gy and either survived or succumbed but whose ANC returned to levels ≥100 μL^{-1} following their ANC nadir experienced grade 4 neutropenia (ANC < 100 μL^{-1}) for a minimum 8d duration. The remainder of the animals succumbed while experiencing grade 4 neutropenia.

DISCUSSION

Dose response relationship, radiation quality, and the LD50

The successful development of a MCM against the potentially lethal H-ARS sequelae will require well-characterized animal models. Valid animal models are imperative to define the dose- and time-dependent incidence, as well as severity and latency of the major sequelae of the H-ARS. However, there are no contemporary studies that determine the lethal DRR for the H-ARS in the nonhuman primate. The literature contains several studies, performed decades ago, that define the DRR for the lethal H-ARS in NHP using 250kVp x-radiation (Eldred and Trowbridge 1954; Stanley et al. 1966; Henschke and Morton 1957; Haigh and Paterson 1956; Schlumberger and Vazquez 1954). Two additional studies, using 250kVp x-radiation contributed limited data sets at one or three dose levels that can be utilized to

confirm aspects of the dose range for the H-ARS and initiation of the GI-ARS (Allen et al. 1964; Allen et al. 1966). A single study was performed in 1965 that used 2Mev x-radiation that resulted in a different DRR consistent with the marked increase in energy of the x-radiation (Dalrymple et al. 1965).

We used the total animal cohort from the five 250kVp x-radiation studies, n=418, to establish a composite DRR. The estimated LD50/30 was 492rad. Comparison of the LD50/30 from the composite, low energy, 250kVp cohort, to the LD50/30 of 671rad determined from the Dalrymple study using 2 Mev x-radiation suggested an approximate RBE of 1.37. The respective DRRs for 250kVp and 2Mev energy x-radiation were also characterized by slopes of 0.77 and 0.99 probits per linear dose that are not significantly different from each other. Additionally, all of these studies were performed without benefit of supportive care.

There are no published studies performed using either Co-60 γ-radiation or comparative energy photons from a 6MV LINAC device that define the DRR for the H-ARS. Furthermore, there has never been a study conducted in rhesus macaques that determined the DRR for H-ARS in the presence of supportive care. Herein, we defined the DRR, slope, and time course of morbidity and mortality for rhesus macaques exposed to TBI with 6MV LINAC-derived photons plus administration of supportive care.

The DRR for 6MV LINAC-derived photon TBI plus supportive care defined a steep slope of 1.13 probits per linear dose and a respective LD50/60 of 7.53Gy [6.50, 7.88]. A comparison of the dose differential between the LD10/60 and LD90/60, another measure of the slope, provides a differential of only 2.27Gy that separates 10% from 90% mortality. These values can be compared to the respective historical data of Dalrymple et al and Eltringham that exposed rhesus macaques to 2 Mev x-ray and Co60 γ-radiation without benefit of supportive care (Eltringham 1967; Haigh and Paterson 1956). The respective LD50/60 values were 671rad and 644rad. The respective slopes for Dalrymple et al and Eltringham were 0.99 and 1.15 probits per linear dose and the dose differential between LD10 and LD90 were 258rad and 223rad. It is of interest that the respective slopes and LD50/60 values that define the DRR between the current study and the historical data sets of similar radiation quality for Co-60 γ-radiation and 2Mev x-radiation are not significantly different from each other (P=0.83) in spite of the study cohort herein receiving benefit of supportive care. The steep slopes most likely reflect the homogeneity of the experimental NHP in age, health status, and care, as well as the well-controlled radiation protocols with sources of similar high energy.

Supportive care in the medical management of lethally irradiated personnel

The historical data sets noted within provide valuable insight into the relatively consistent DRR of NHP and relative biologic effect (RBE) of 250kVp x-radiation vs. 2Mev x-radiation and Co-60 γ-radiation performed over time and different research sites. The studies also provided a data base that permits insight into the relative value of supportive care administered to the macaques exposed to lethal TBI.

An RBE for supportive care in the rhesus macaque model can only be appreciated in comparison between our contemporary studies and those performed decades ago without benefit of supportive care. There have been no contemporary studies of the DRR for rhesus macaques exposed to TBI without supportive care. The two historical studies noted above have respective LD50/60 values of 6.71Gy and 6.44Gy relative to 7.53Gy estimated from our study presented herein. The respective RBE for supportive care would be approximately 1.12 and 1.17. There is a consistent data base demonstrating that supportive care alone can significantly enhance survival of animals exposed to lethal doses of radiation (Jackson et al.

1959; MacVittie et al. 1991; Perman et al. 1962; Sorensen et al. 1960; MacVittie et al. 2005; Conard et al. 1956; Taketa 1962). Studies performed over 50 years ago showed the efficacy of good supportive care centered on systemic antibiotics and fresh platelet transfusions (Jackson et al. 1959; Sorensen et al. 1960; Perman et al. 1962; Bagdasarov et al. 1959). An additional data set in canines expanded the aforementioned studies over the complete DRR suggesting an RBE of 1.3 for the increase in LD50/30 relative to the lack of supportive care (Jackson et al. 1959; Broerse and MacVittie 2001). It should be noted that the addition of a MCM, G-CSF to the treatment regimen increased the survival of canines across the DRR and resulted in an increased LD50; thus supporting an additive effect of MCM to that noted for supportive care alone (MacVittie et al. 1990; MacVittie et al. 2005). Additionally, the canine study showed similar slopes for the DRR with or without supportive care concordant with that herein, related to the historical data sets.

The use of antibiotics, fluids, blood transfusions, analgesics, and nutrition is the standard of care for treating severe myelosuppression in the clinic and in the treatment of radiation accident victims (Waselenko et al. 2004; Ricks and Fry 1990; Villinger et al. 2001; Baranov et al. 1994; Timmer-Bonte et al. 2005a; Liu et al. 2008; Browne et al. 1990a; Gourmelon et al. 2010; Hirama et al. 2003). This will not change in the large casualty situation for personnel exposed to high-dose radiation (Waselenko et al. 2004; Meineke and Fliedner 2005; Gourmelon et al. 2010). Severe neutropenia, an ANC < 500 μL^{-1} is predictive of impending infection. The most severe infections and all bacteremias occur when the patient has an ANC < 100 μL^{-1} and are most prevalent in patients with prolonged periods of bone marrow aplasia (Bodey et al. 1966; Hughes et al. 1990; Ricks et al. 2002b; Browne et al. 1990b; Gafter-Gvili et al. 2005; Timmer-Bonte et al. 2005b). Lethal radiation exposure induces significant marrow myelosuppression with subsequent profound and prolonged neutropenia. These conditions predispose the victim to infection and thereby necessitate the prophylactic administration of antibiotics in afebrile but severely neutropenic, irradiated personnel. It is recognized that afebrile patients who are neutropenic and are expected to become profoundly neutropenic (ANC <100 μL^{-1}) should have prompt empirical antibiotic therapy (Bodey et al. 1966; Hughes et al. 1990; Waselenko et al. 2004; Ricks et al. 2002b; Gafter-Gvili et al. 2005; Hughes et al. 2002; National Comprehensive Cancer Network (NCCN) 2005; Timmer-Bonte et al. 2005a; Hughes et al. 1997; Timmer-Bonte et al. 2005b). The prophylactic use of antibiotics in this model is based on the fact that all lethally irradiated NHP reached an ANC <500 μL^{-1} within 5 days post TBI. The ANC continued to decrease within the subsequent two days and reached an ANC <100 μL^{-1} by seven days post TBI. The duration of ANC <100 μL^{-1} was 12.0 days. These parameters support the use of prophylactic antibiotics as recommended by the IDSA guidelines and others (Ricks et al. 2002b; Gafter-Gvili et al. 2005; Hughes et al. 2002; National Comprehensive Cancer Network (NCCN) 2005).

Blood transfusions and fluids were administered to NHP as per signs and cellular parameters indicative of serious morbidity during the time course post TBI. The value of “replacement therapy” to include blood transfusions or platelets and fluids to control radiation-induced morbidity and mortality was underscored decades ago (Furth et al. 1953; Jackson et al. 1959; Sorensen et al. 1960). Sorensen et al reported on the effectiveness of several antibiotics in conjunction with fresh, whole blood transfusions and parenteral fluids to control dehydration and hemorrhage in reducing lethality from 90% to 20% in lethally irradiated canines (Sorensen et al. 1960). We have not determined the relative effectiveness of the individual components of supportive care but the effectiveness of these regimens supports the concept that infection and hemorrhage are the two primary factors in the lethal consequences of acute radiation exposure in the H-ARS. There is no doubt that dehydration can exacerbate these consequences along the time course of morbidity and that fluids are required on a daily basis. It is important to note that ARS is a continuum as the radiation

dose increases. Herein, the incidence of diarrhea, the incidence and severity of body weight loss, and the histological data from animals that expired prior to euthanasia or were euthanized prior to the end of study provides evidence that the range of the H-ARS does infringe upon the G-ARS.

The human response to potentially lethal radiation exposure

The most extensive, anecdotal experience on potentially lethal, near uniform radiation exposure of humans and the use of variable aspects of supportive care in managing acute radiation disease (ARD) is derived from a selected population cohort that were exposed to a nuclear weapon while in two school buildings, Chinzei and Shirayama in Nagasaki, Japan, the radiation accident at Chernobyl, and selected reports of accidental exposure in Russia prior to Chernobyl (MacVittie et al. 1996b; Anno et al. 2003; Anno et al. 1989; Ricks and Fry 1990; Baranov et al. 1994; Browne et al. 1990a). The victims in each of these radiation exposure scenarios and other scenarios with fewer victims, all received supportive care (Liu et al. 2008; Hirama et al. 2003). The major variables lie in the extent of care, the time interval from exposure to administration of care, the age and nutritional status of the exposed population, presence of combined injury such as trauma and/or burns, radiation exposure geometry, and radiation dose. The data base, considering the selected weapon-exposed cohorts, Chernobyl victims and controlled animal data suggests that the most likely LD50/60 of 4.00Gy for the human population can be raised to 6.00 MLTD with the benefit of supportive care (Anno et al. 2003). Anno suggested that “A mass casualty medical care DMF for LD50 of 1.5 appears to be reasonable...” (Anno et al. 2003).

Supportive care may be the best MCM available in the context of the nuclear scenario. It has demonstrated efficacy in enhancing survival and mean survival time, but it may also be the only MCM administered at its optimal therapeutic schedule. The data herein for the NHP indicated that the first day to antibiotics was day 5, while the average day to the first transfusion was day 12. The time course for a trigger-to-treat for the key signs of morbidity in the human victim would permit a reasonable period post exposure for triage to occur with consequent access to victims for optimal administration of supportive care.

CONCLUSIONS

We have established the DRR for NHP exposed to TBI plus supportive care. The slope is steep and not significantly different from that shown for historical data sets for DRR with TBI of similar radiation quality. Although the administration of supportive care does not apparently influence the slope of the DRR, we have demonstrated that the DRR plus supportive care results in increased survival and MST relative to historical controls. These data show that the dose differential between 10% and 90% mortality is approximately 2.27Gy which underscores the need for accurate dosimetry and a well-defined radiation exposure protocol. These data demonstrate that the time course of the H-ARS is characterized by dose- and time-dependent severe lineage specific cytopenia, followed by consequent febrile neutropenia, infection, and hemorrhage of varying degree.

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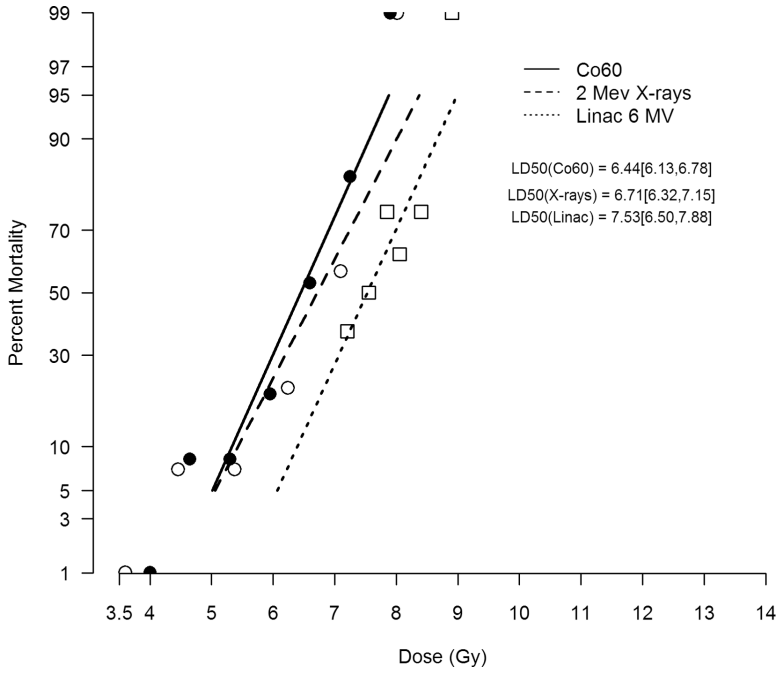

Figure 1. Dose response relationship for total-body irradiated (tbi) rhesus macaques

The 60 day mortality dose response relationship (DRR) for the hematopoietic sub-syndrome of the ARS in rhesus macaques, presented as probit percent mortality vs TBI dose (Gy) (on a linear scale). The graph contains two historical data sets showing the DRR and calculated LD50/30 values [95% CI] of rhesus macaques exposed to TBI from Co-60 γ-radiation or 2 MeV x-radiation (Eltringham 1967, Dalrymple 1969). These cohorts were not administered supportive care. The current study used TBI with 6MV LINAC-derived photons at a dose rate of 0.80 Gy/min with administration of supportive. The in-life phase was 60 days post TBI.

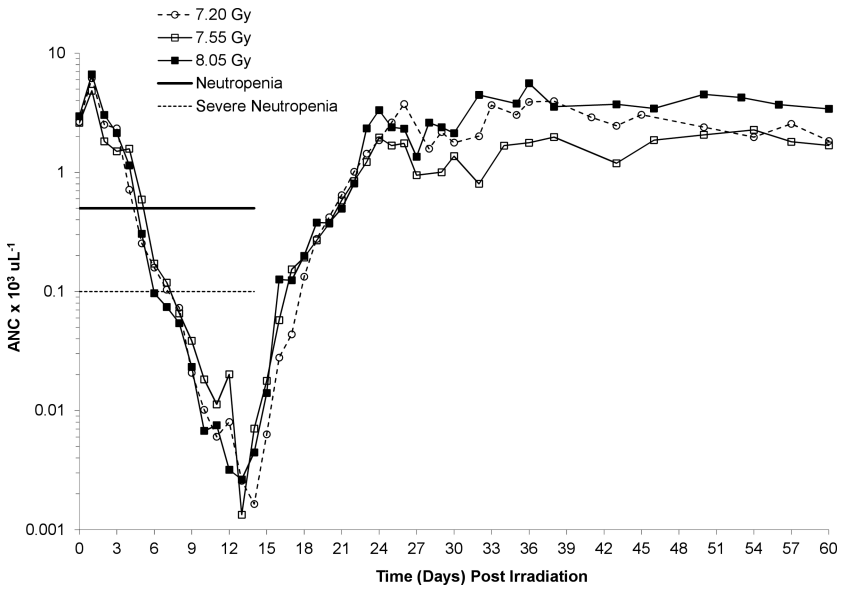

Figure 2. Mean absolute neutrophil counts in rhesus macaques following total-body irradiation

Animals were exposed to total body irradiation (TBI) with 6MV LINAC-derived photons at a dose rate of 0.80 Gy/min and administered supportive care. Shown are the decrease in the absolute neutrophil count (ANC) in the peripheral blood of rhesus macaques (n=8/radiation dose) as a function of time post TBI and dose (Gy). The respective dose of TBI and survivors/total NHP are: 7.20Gy 5/8, 7.55Gy 4/8, 8.05Gy 3/8. The radiation doses shown approximate the LD30, LD50, and LD70/60 estimated from the resultant data set.

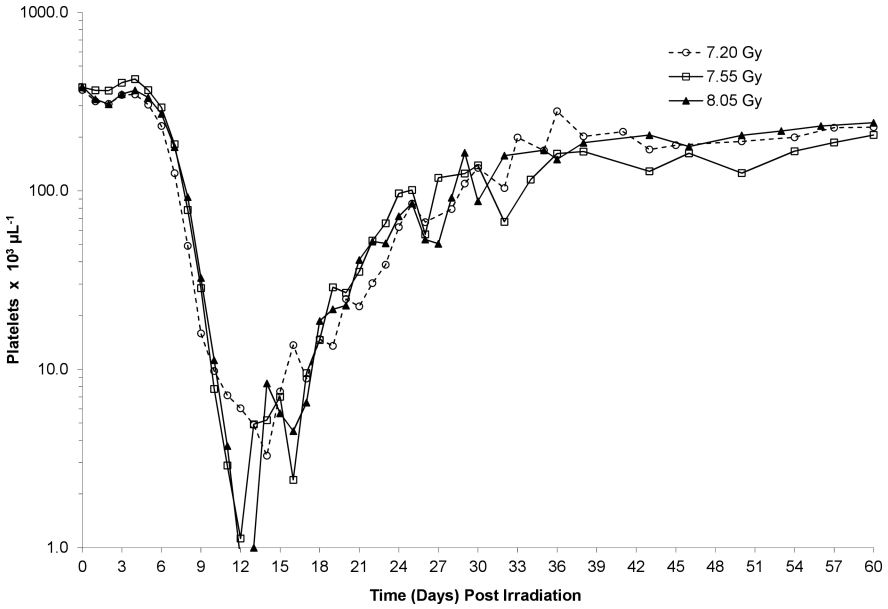

Figure 3. Mean platelet counts in rhesus macaques following total-body irradiation

Animals were exposed to total body irradiation (TBI) from LINAC-derived photons at a dose rate of 0.80 Gy/min with administration of supportive care as defined in the material and Methods. Shown are the changes in the platelet count (PLT) × 10^3 μL^{-1} in the peripheral blood of rhesus macaques (n=8/radiation dose) as a function of time post TBI and dose (Gy). The respective dose of TBI and survivors/total NHPs are: 7.20Gy 5/8, 7.55Gy 4/8, 8.05Gy 3/8. TBI, of 6 MV LINAC-derived photons was administered at 0.80Gy min^{-1}. The radiation doses shown approximate the LD30, LD50, and LD70/60 estimated from the resultant data set.

Table 1

Dose response relationship for rhesus macaques exposed to total body irradiation. The dose response relationship (DRR) for rhesus macaques exposed to total-body irradiation from several different sources derived from a comparable historical data set for Co-60 γ-radiation, 2Mev x-radiation, and 250kVp x-radiation (1 (Eltringham 1967), 2 (Dalrymple et al. 1965), 4 (Eldred and Trowbridge 1954; Stanley et al. 1966; Henschke and Morton 1957; Haigh and Paterson 1956; Schlumberger and Vazquez 1954), 5 unpublished T. MacVittie et al) relative tc the current data set (3) described herein. Shown are the data sets with respective radiation source, total number (n) of animals in the experiment, respective LD10, LD50, and LD90 [95% CI] , a nd slopes for each DRR [95% CI].

Data Set	Radiation Source	n	LD10 (Gy) [95% CI]	LD50 (Gy) [95% CI]	LD90 (Gy) [95% CI]	Slope [95% CI]
1	Co60	90	5.33 [4.64, 5.71]	6.44 [6.13, 6.78]	7.56 [7.15, 8.32]	1.15 [0.74,1.56]
2	2 Mev X-rays	84	5.42 [4.56, 5.88]	6.71 [6.32, 7.15]	8.66 [8.23, 10.73]	0.99 [0.61, 1.37]
3	LINAC 6MV Photon	48	6.39 [2.74, 7.06]	7.53 [6.50, 7.88]	8.66 [8.23, 10.73]	1.13 [0.33, 1.93]
4	250 kVp X-ray	418	3.27 [2.81, 3.61]	4.92 [4.71, 5.12]	6.58 [6.31, 6.93]	0.77 [0.70, 0.84]
5	250 kVp X-ray	72	6.05 [5.38, 6.43]	7.18 [6.71, 8.97]	8.32 [7.46, 12.09]	1.13 [0.76, 1.50]

Table 2

Percent lethality and mean survival time of decedents following total-body irradiation in rhesus macaques

Rhesus macaques were exposed to total-body irradiation (TBI) using 6MV LINAC-derived photons at a dose rate of 0.80Gy min^{-1} in a blinded and randomized study. The TBI was delivered as 50% in the anterior (AP), then 50% in the posterior (PA) directions for total dose cohorts (n=8 each) of 7.20, 7.55, 7.85, 8.05, 8.40 and 8.90Gy. Animals were observed for 60d post TBI for cageside observations and euthanized under protocol criteria for all-cause mortality by veterinarians that were "blinded" to the radiation dose for each animal. The percent lethality, number of decedents versus the total number, mean survival time (days) of decedents ± standard error (sem), median survival time (days) of decedents are reported for each radiation cohort. All animals received IACUC-approved supportive care as per defined signs of morbidity.

Radiation Exposure (Gy)	**7.20**	**7.55**	**7.85**	**8.05**	**8.40**	**8.90**
% Lethality	38%	50%	75%	63%	75%	100%
Decedents/total	3/8	4/8	6/8	5/8	6/8	8/8
Mean survival time of decedents (days)	20.0±5.5	18.3±2.1	22.2±6.0	16.2±3.0	17.5±1.6	21.1±3.6
Median survival time of decedents (days)	15.0	18.5	16.5	14.0	17.5	18.0

Table 3

The estimated LD30, LD50 and LD70/60 for all rhesus macaques administered supportive care post total-body irradiation

The dose response relationship for the hematopoietic sub-syndrome of the ARS is characterized by the lethal dose for 30%, 50% and 70% at sixty days post irradiation. The 95% confidence interval was calculated based on the survival of animals following TBI to 7.20, 7.55, 7.85, 8.05, 8.40 and 8.90Gy (n=8 each) with 6MV LINAC-derived photons at dose rate of 0.08Gy min^{-1}. All NHP receive IACUC-approved supportive care as per defined signs of morbidity.

LD30/60 = 7.06Gy [5.01, 7.50]
LD50/60 = 7.53Gy [6.50, 7.88]
LD70/60 = 7.99Gy [7.60, 8.65]

Table 4

Neutrophil-related parameters for rhesus macaques following total-body irradiation. Animals were exposed to total body irradiation (TBI) from LINAC-derived photons at a dose rate of 0.80 Gy/min. The mean, standard error (sem), median and range (where applicable) for neutrophil-related parameters are reported for each radiation cohort. The day of the occurrence of an absolute neutrophil count (ANC) < 500 μL^{-1} or <100 μL^{-1} for each irradiation dose is shown. The duration of neutropenia is defined as an ANC <500 μL^{-1} or <100 μL^{-1}. The durations (d) do not include data from decedent animals unless recovery occurred to that level, e.g., ANC ≥ 500 μL^{-1} prior to death. The duration of neutropenia was estimated as the number of days that a subject has an observed or an imputed ANC <500 μL^{-1}. Any single observed ANC that was ≥ 500 μL^{-1} and was immediately preceded and followed by ANC < 500 μL^{-1} was counted as a day of severe neutropenia. The time to recovery was estimated as the number of days from study day 1 until the first 2 consecutive observed or imputed ANC after the nadir was ≥ 1,000 μL^{-1}. The time to recovery of ANC >1000 μL^{-1} ranged from 21.7d to 42.0d post TBI. There are no significant differences in these values relative to radiation dose. There were only two survivors in the 8.40Gy cohort, responsible for the 42.0d value for this parameter. The average recovery time to an ANC >1000 μL^{-1} for all survivors is approximately 26.2d. The ANC nadir was the first lowest observed or imputed ANC that occurred at least 2 days after irradiation. TBI dose and survivors/total NHPs are: 7.20Gy, 5/8; 7.55Gy, 4/8; 7.85Gy, 2/8; 8.05Gy, 3/8; 8.40Gy, 2/8; 8.90Gy, 0/8.

TBI Dose (Gy)		First day (d) and range ANC < 500 μL^{-1} or 100 μL^{-1} (n=8)		Duration (days and range) ANC < 500 μL^{-1} or 100 μL^{-1}		Recovery to ANC ≥ 1000 μL^{-1}	ANC Nadir (μL^{-1}) (n=8)
		< 500 μL^{-1}	< 100 μL^{-1}	< 500 μL^{-1}	< 100 μL^{-1}	≥ 1000 μL^{-1}	
7.20	Mean[a]	4.6±0.3	7.3±0.3	16.0±0.5[b]	11.5±1.3[c]	23.4±0.8[b]	0
	Median	NA	NA	16.0[b]	10.5[c]	24.0[b]	0
	Range	d4-6	d6-9	d15-18	d9-18	d21-24	NA
7.55	Mean[a]	5.5±0.6	7.1±0.4	24.0±7.3[d]	9.8±1.0[d]	26.7±3.7[e]	0
	Median	NA	NA	17.0[d]	9.5[d]	23.0[e]	0
	Range	d3-6	d5-8	d16-46	d8-12	d22-23[f]	NA
7.85	Mean[a]	4.6±0.3	6.5±0.4	14.3±1.5[e]	10.3±0.9[e]	21.7±1.2[e]	0.5±0.5
	Median	NA	NA	14.0[e]	10.0[e]	21.0[e]	0
	Range	d4-6	d6-8	d12-17	d9-12	d20-24	0-4
8.05	Mean[a]	5.0±0.0	6.5±0.3	15.0±1.0[e]	10.3±0.9[e]	24.7±2.7[e]	0
	Median	NA	NA	16.0[e]	10.0[e]	22.0[e]	0
	Range	NA	d6-8	d13-16	d9-12	d22-23	NA
8.40	Mean[a]	5.0±0.3	6.4±0.4	17.0±2.0[g]	12.7±2.3[e]	42.0±18[g]	0
	Median	NA	NA	NA	NA	NA	NA
	Range	d4-6	d5-8	d15 or d19	d8-15	d24 or d27	NA
8.90	Mean[a]	4.5±0.2	6.3±0.3	DNO	DNO	DNO	0
	Median	NA	NA	NA	NA	NA	0

TBI Dose (Gy)		First day (d) and range ANC < 500 μL^{-1} or 100 μL^{-1} (n=8)		Duration (days and range) ANC < 500 μL^{-1} or 100 μL^{-1}		Recovery to ANC ≥ 1000 μL^{-1}	ANC Nadir (μL^{-1}) (n=8)
		< 500 μL^{-1}	< 100 μL^{-1}	< 500 μL^{-1}	< 100 μL^{-1}	≥ 1000 μL^{-1}	
	Range	d4-5	d5-8	DNO	DNO	DNO	NA

DNO= Did not observe; NA= all numbers were equal to the mean value or in the case of the median where it is not applicable due to n=2.

[a] Mean data shown with the standard error of the mean (±SEM)

[b] n=5

[c] n=6

[d] n=4

[e] n=3

[f] one animal survived but the ANC after the nadir did not attain ≥ 1,000 μL^{-1} at study day 60

[g] n=2

Table 5

Incidence and severity of neutropenia in total-body irradiated rhesus macaques

Animals were exposed to total-body irradiation (TBI) from 6MV LINAC-derived photons at a dose rate of 0.80 Gy/min. Shown are the occurrence of grade 3 or 4 neutropenia post TBI: The first and final day that grade 3 or 4 neutropenia occurred in an animal within each radiation cohort. The first and final day when an ANC <500 μL^{-1} or <100 μL^{-1} occurred in an animal in each radiation group. Additionally, the range of each of these occurrences for each irradiation group is presented. TBI dose and survivors/total NHPs are: 7.20Gy, 5/8; 7.55Gy, 4/8; 7.85Gy, 2/8; 8.05Gy, 3/8; 8.40Gy, 2/8; 8.90Gy, 0/8.

TBI Dose (Gy)		ANC (d) < 100 μL^{-1}		ANC (d) < 500 μL^{-1}	
		First day (n=8)	Final day	First day (n=8)	Final day
7.20	Day	6	23[a]	4	22[b]
	Range	d6-9	d15-23	d4-6	d18-22
7.55	Day	5	18[b]	3	46[c]
	Range	d5-8	d15-18	d3-6	d20-46
7.85	Day	5	17[d]	4	20[d]
	Range	d5-8	d14-17	d4-6	d15-20
8.05	Day	6	18[d]	5	20[d]
	Range	d6-8	d15-18	NA	d17-20
8.40	Day	6	21[d]	4	23[e]
	Range	d6-8	d15-21	d4-6	d20,23
8.90	Day	5	DNO	4	DNO
	Range	d5-8	DNO	d4-5	DNO

NA=all numbers equal to the mean; DNO=did not observe

[a] n=6

[b] n=5

[c] n=4

[d] n=3

[e] n=2

Table 6

Febrile neutropenia and antibiotic requirements for rhesus macaques following total-body irradiation. Animals were exposed to total-body irradiation (TBI) from 6MV LINAC-derived photons at a dose rate of 0.80 Gy/min. Shown are: Incidence, duration of febrile neutropenia (FN), days on antibiotic regimen, and days core body temperature was > 103.0 F. Febrile neutropenia (FN) is defined as the ANC <500 μL^{-1} and the core body temperature (CBT) ≥103.0°F. The mean of the first day of the occurrence of FN for each irradiation dose is shown. The duration of FN and the numbers of days an animal required antibiotic administration was determined for the survivors only. However, all animals in the study did experience FN. The mean numbers of days an animal's CBT ≥103.0°F is shown for all animals regardless of survival. The standard error (sem) is reported for each parameter. TBI dose and survivors/total NHPs are: 7.20Gy, 5/8; 7.55Gy, 4/8; 7.85Gy, 2/8; 8.05Gy, 3/8; 8.40Gy, 2/8; 8.90Gy, 0/8. The duration of FN or antibiotic requirements for the 8.90Gy cohort was not observed (DNO) due to 100% lethality.

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

DNO=did not observe.

[a] n=5

[b] n=4

[c] n=2

[d] n=3

Table 7

Incidence and duration of thrombocytopenia and transfusion requirements in total-body irradiated rhesus macaques

Animals were exposed to total body irradiation (TBI) from 6MV LINAC-derived photons at a dose rate of 0.80 Gy/min. Shown are: The mean, standard error (sem) and range of the first day thrombocytopenia, defined as a platelet (PLT) count < 20,000 μL^{-1}, is observed in an animal in each radiation cohort, duration of thrombocytopenia, platelet nadir, day recovery from thrombocytopenia occurs, number of whole blood transfusions administered (54mL), and first day a transfusion is observed in an animal in each radiation cohort are displayed. All but one animal (1/48) received a transfusion. Additionally, the range of each of these occurrences is shown for each radiation dose cohort. TBI dose and survivors/total NHP are 7.20Gy, 5/8; 7.55Gy, 4/8; 7.85Gy, 2/8; 8.05Gy, 3/8; 8.4Gy, 2/8; 8.90Gy, 0/8.

TBI Dose (Gy)		First day platelet count (d) < 20,000 μL^{-1} (n=8)	Duration (d)[a] < 20,000 μL^{-1}	Nadir[b] (PLT μL^{-1}) (n=8)	Recovery to platelet count ≥ 20,000 μL^{-1}	# Transfusions[b] (54mL) (n=8)	First day (d) transfusion occurred within radiation cohort (n=8)
7.20	Mean±sem	8	12.6±1.8[c]	0.5±0.4	24.3±1.4[c]	2.3±0.6	9
	Range	d8-10	d8-12	0-3	d18-27	0.5-5.5	d9-15
7.55	Mean±sem	8	19.8±9.5[d]	0.4±0.2	29.5±10.2[d]	2.0±0.7	11
	Range	d8-11	d8-48	0-1	d18-60	0.5-6.5	d11-15
7.85	Mean±sem	7	13.7±3.2[e]	0±0	22.7±3.2[e]	1.8±0.3	11
	Range	d7-9	d11-20	NA	d19-29	0.5-3	d11-13
8.05	Mean±sem	9	8.7±0.3[e]	0.4±0.2	19.0±0.8[e]	1.5±0.5	10
	Range	d9-11	d8-9	0-1	d18-20	0-4	d10-14
8.40	Mean±sem	8	20.5±1.5[f]	0.1±0.1	30.0±2.0[f]	2.3±0.6	11
	Range	d8-10	19,22	0-1	28,32	0.5-5.5	d11-13
8.90	Mean±sem	9	DNO	0.1±0.1	DNO	3.2±0.9	11
	Range	d9-10	DNO	0-1	DNO	1-9	d11-15

DNO=did not observe due to 100% lethality in the 8.90Gy cohort.

[a] Durations (d) do not include data from decedent animals unless recovery occurred to that level, e.g., PLT ≥ 20,000 μL^{-1} prior to death

[b] The platelet nadir and number of transfusions includes both survivors and non-survivors

[c] n=5

[d] n=4

[e] n=3

[f] n=2

Table 8

The incidence and severity of diarrhea in rhesus macaques exposed to total-body irradiation. Animals (n=48) were exposed to total body irradiation (TBI) from 6MV LINAC-derived photons at a dose rate of 0.80Gy/min. The TBI ranged across the hematopoietic syndrome (7.20Gy, 7.55Gy, 7.85Gy, 8.05Gy, 8.40Gy or 8.90Gy, n=8 per dose). Animals were observed daily for the occurrence of diarrhea. Grade 1 indicted soft stool, grade 2 indicated loose and/or watery stool, grade 3 indicated bloody diarrhea. The highest grade of diarrhea was determined for each animal in the study. All animals experienced some degree of diarrhea; Grade 1 = 6, Grade 2 = 25, Grade 3 = 12.

	Grade 1			Grade 2			Grade 3		
Radiation Dose (Gy)	**#/Grp**	**%**	**Range (days) or day**	**#/Grp**	**%**	**Range (days) or day**	**#/Grp**	**%**	**Range (days) or day**
7.20	1	12.5%	9	5	62.5%	6-54	2	25.0%	6,19
7.55	2	25.0%	13,38	4	50.0%	4-60	2	25.0%	17,23
7.85	0	0	NA	6	75.0%	4-18	2	25.0%	18,23
8.05	2	25.0%	5,36	4	50.0%	4-15	2	25.0%	8,28
8.40	1	12.5%	60	6	75.0%	7-24	1	12.5%	23
8.90	0	0	NA	5	62.5%	8-45	3	37.5%	14-24

Table 9

The incidence and severity of body weight loss in rhesus macaques exposed to total-body irradiation. Animals (n=48) were exposed to total body irradiation (TBI) from 6MV LINAC-derived photons at a dose rate of 0.80Gy/min. across the dose range for the hematopoietic syndrome (7.20Gy, 7.55Gy, 7.85Gy, 8.05Gy, 8.40Gy or 8.90Gy, n=8 per dose). Animals were weighed daily or when anesthetized for supportive care. Shown are the numbers of animals per cohort, the percentage animals that experience the degree of weight loss for each cohort, and the number of days of the occurrence of bw loss for each animal. If an animal was not weighed on a particular day the days between measurements were imputed.

Radiation Dose (Gy)	≥ 15% body weight loss			≥ 25% body weight loss		
n	%	# of days	n	%	# of days	
7.20	2	25.0%	3, 12	1	12.5%	3
7.55	5	62.5%	1, 2,3,5,6	0	0	NA
7.85	3	37.5%	3, 6, 32	2	25.0%	1, 19
8.05	4	50.0%	1,6,8,16	1	12.5%	5
8.40	4	50.0%	1,1,39,45	2	25.0%	4, 19
8.90	4	50.0%	1,3,7, 25	2	25.0%	1, 4

Table 10

The incidence of antibiotic resistance observed in blood cultures from total-body irradiated rhesus macaques. Animals (n=48) were exposed to total body irradiation from 6MV LINAC-derived photons at a dose rate of 0.80Gy/min across the dose range for the hematopoietic syndrome (7.20Gy, 7.55Gy, 7.85Gy, 8.05Gy, 8.40Gy or 8.90Gy, n=8 per dose). Blood cultures were obtained per protocol guidelines. Shown are the number and percent of animals in each radiation cohort from which an organism was obtained by blood culture which was resistant to an antibiotic. Some organisms were resistant to multiple antibiotics.

Radiation Dose (Gy)	Baytril (Enrofloxacin)	Gentamicin	Rocephin (Ceftriaxone)	Claforan (Cefotaxime)	Primaxin (Imipenem)	Total # Animals	Percent
7.20	3	1	0	0	0	3	37.5%
7.55	6	4	2	0	0	6	75.0%
7.85	2	2	1	0	0	3	37.5%
8.05	4	2	3	1	0	4	50.0%
8.40	6	3	2	0	0	7	87.5%
8.90	7	2	4	1	0	7	87.5%