Final Technical Report

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Colorado State University

Radiation Leukemogenesis at Low Dose Rates

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This report contains no patentable material or protected data.

**Contents**

**Executive Summary**

The major goals of this program were to study the efficacy of low dose rate radiation exposures for the induction of acute myeloid leukemia (AML) and to characterize the leukemias that are caused by radiation exposures at low dose rate. An irradiator facility was designed and constructed that allows large numbers of mice to be irradiated at low dose rates for protracted periods (up to their life span). To the best of our knowledge this facility is unique in the US and it was subsequently used to study radioprotectors being developed for radiological defense (PLoS One. 7(3), e33044, 2012) and is currently being used to study the role of genetic background in susceptibility to radiation-induced lung cancer.

One result of the irradiation was expected; low dose rate exposures are ineffective in inducing AML. However, another result was completely unexpected; the irradiated mice had a very high incidence of hepatocellular carcinoma (HCC), approximately 50%. It was unexpected because acute exposures are ineffective in increasing HCC incidence above background. This is a potential important finding for setting exposure limits because it supports the concept of an "inverse dose rate effect" for some tumor types. That is, for the development of some tumor types low dose rate exposures carry greater risks than acute exposures.

**Goals and Accomplishments**

The projects originally propose are listed below as goals along with a brief summary of the work done to achieve that goal and the results.

Goal 1: Determine time-dose effects on the induction of AML in CBA/CaJ mice.

Result: This was successfully accomplished and the results were published (publication 3 at the end of the Activities Summary).

Goal 2: Characterize AMLs induced by low dose rate, multiple low dose fractions and acute exposures.

Result: Shortly after the initiation of this program a meeting was held with the external advisory board and Drs. Metting (DOE) and Cucinotta (NASA) at which it was agreed that the low dose rate exposure would be to a total dose of 5 Gy. The Activities Summary section describes the construction of the facility needed to accomplish this, the dosimetry, the experiment itself and the results. The 5 Gy low dose rate exposure yielded only one AML and it was recovered only as a histopathology sample. The characterization of the AMLs resulting from acute exposures was published in publication 4 at the end of the Activities Summary.

Goal 3: Compare radiation damage to hematopoetic stem cells between AML susceptible and AML resistant mouse strains exposed to radiation delivered as acute, low dose rate, and multiple low dose fractions.

Result:  This goal was accomplished only for acute exposures (publications 1 and 2).

Goal 4: Further develop the AML model so that the effects of various radiation exposures on each step of tumorigenesis can be quantified.

Result: The dose response and timing of the Sfpi1 (chromosome 2) deletion and its persistence only in an AML susceptible mouse strain is reported in publications 1 and 2. Continuation of this study after the funding period allowed us to determine the timing of the Sfpi1 R235 mutation.  This work is ongoing

Goal 5: Extend these studies to human hematopoetic stem cells.

Result:  Dr. Belmont was unable to generate a sufficient number of humanized Scid mice to accomplish this goal.  However, we did work out the basic bone marrow cell collection technique and FISH hybridization technique using human:mouse chimeric bone marrows from the mice he did provide.  This work has been reinitiated with a new collaborator.

**Activities Summary**

Radiation Leukemogenesis at Low Dose Rates (DE-FG02-05ER63946) was funded at a supplement to The NASA Specialized Center of Research on Radiation Leukemogenesis (NASA Grant # NAG9 1569). The final report for NAG9 1569 is attached to this report following the Products section.

Quantitative understanding of cancer risks of ionizing radiation following exposures delivered at low doses and dose rates remains a difficult task despite the many years of study of human populations exposed under a variety of conditions. Data derived from experimental systems have also provided important insights but fundamental questions remain. Early experimental studies in biology related to radiation-induced cancer were largely descriptive in nature. This was mainly related to technical limitations in biological research. As such the ability to directly study low dose effects was limited. However, recent advances in techniques in cell and molecular biology and their application in cancer research are increasing the ability to directly approach important questions and provide a better understanding of cancer risks at low doses and dose rates. To accomplish this, requires adequate quantitative data for cancer induction in well-defined animal models of neoplasia and sufficient understanding of mechanisms involved. This information will enable appropriate biologically-based models to be developed for extrapolation of risks. Currently, there are only a limited number of animal models amenable to this approach. One such model system, which has direct application to human risks, is radiation-induced acute myelogenous leukemia (AML) in mice. There are several reasons for focusing on radiation leukemogenesis and specifically on acute myeloid leukemia. First, in adult populations, AML is one of the principle types of leukemia developing following radiation exposure and its prognosis is poor. Second, there are considerable quantitative dose response and dose rate data for humans following exposure to low LET radiation. Such information is necessary for translating results from animal experiments to humans. Third, there appears to be substantial similarities in dose response and pathogenesis for human and murine AML that can be exploited. Fourth, compared with many models of radiation-induced cancer, the murine AML model is particularly well-defined with respect to quantitative dose response data following exposure to gamma rays, x-rays, neutrons and alpha particles and with respect to potential pathogenic mechanisms. While not extensive at low doses and dose rates, the available dose response and time-dose data are sufficient to facilitate experimental design. Further, while much remains to be learned with respect to the pathogenesis of AML, the mechanisms are sufficiently understood to facilitate the design of cell and molecular studies that can provide insight into radiation effects at low doses and dose rates. The goal of this program is to provide the information required to develop a rational scientific basis for improved estimation of risks for leukemogenesis in humans from exposure to ionizing radiation at low doses and low dose rate.

The primary aim of the low dose rate component of this grant was to provide quantitative data on the induction of AML following low dose rate exposures compared with single acute exposures.

<u>Aim 1.</u> Doses and group sizes for the acute and low dose rate exposures are shown in Table 1.

| Table 1 | | | |
|---|---|---|---|
| **Radiation** | **Delivery** | **Dose (Gy)** | **Group size** |
| 137Cs gamma rays | Acute | 0, 1, 2, 3 | 100 to 400 |
| 137Cs gamma rays | LDR 10 cGy/d | 5 | 236 |

**Construction of the low dose rate facility**

A custom built 8' x 9' x 10' vinyl clean room enclosure (BioBubble) suitable for long term housing of mice in Specific Pathogen Free conditions and meeting the requirements for mouse housing outlined in The Guide for Care and Use of Laboratory Animals (National Research Council) was constructed in a panoramic irradiator room in the Department of Environmental and Radiological Health Sciences at CSU. The unit includes a blower connected to the building air supply vent to redirect air through two pre-filters and a HEPA filter and into the housing enclosure at 40-55 air changes per hour.

Lighting in the irradiator room is equipped with a programmable timer to control the light-dark cycle. The clean room enclosure also contains humidifiers to maintain proper room humidity for rodent housing. Five movable cage racks each holding 10 cages form an arc in the enclosure. Standard ventilated cage rack cages without filter tops are held in the cage racks with their long axes perpendicular to the beam and water bottles distal to the irradiator.

**Dosimetry**

The low dose rate irradiator room houses a Shepherd 600 Ci $^{137}$Cs irradiator. The dose rate delivered to the mice can be varied by the addition or removal of lead attenuators and by repositioning the cages and cage racks. The exposure rate in air was measured at several locations in the room to determine a combination of distance and attenuator that would provided a nominal dose rate of 0.5 cGy/h to mice. Results indicated that the distance from the source with attenuator 5 should be between 3.4m and 4.0 m from the source. This also defined the location of the BioBubble curtain that is used to create the humidity and air exchange rated required by animal care.

A mouse cage was modified to accept a 180 cc ionization chamber. Tests were made to determine how much the cage, water bottle, and food might influence the exposure rate at the location of mice in the cage.

A prototype rack was designed to hold 10 cages (50 mice) in a vertical array. The depth of the rack was 50 cm in order that cages could be arranged in a vertical arc and therefore maintain in a constant cage distance from the source.

The rack was filled with 10 cages.  Positions were labeled from 1 to 10 beginning at the lowest cage location.  Exposure rates were measured at all locations with the modified cage and 180 cc ionization chamber.  It was determined that the exposure rates in the lower locations were higher the upper location when the distance from the source was constant.  This was attributed to scatter from the floor and indicated that cages would not necessarily be located in an arc centered at the source.

Forty eight LiF (TLD-100) dosimeters were obtained and sorted according to weight. These were exposed to a uniform fluence of photons at a distance of 200 cm from the ^{137}Cs source and a 90cm above the floor corresponding to the center of the source.
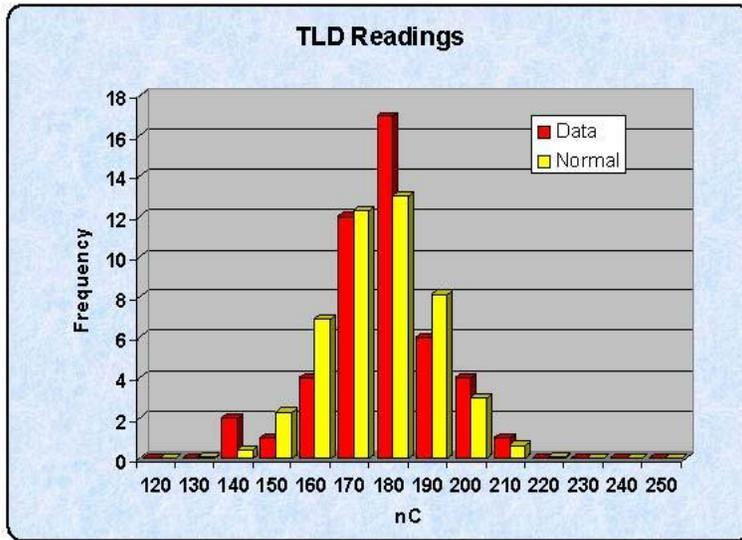


 Figure 1

For comparison purposes, a Normal distribution is also plotted in the figure.  The coefficient of variation ($\sigma/\mu$) for these data is 7.5%.  The exposures were repeated two more times for different total exposures.  In each case the data were normally distributed with a CV of 9.6% and 8.5%.  There was no indication that the CV was dependent on the total exposure.

 The data for each exposure was rearranged to determine the rank of each individual TLD.  This was done to determine if the same dosimeter was consistently reading high or low.  The ranks for each TLD following exposure 1 and exposure 3 are shown as a scatter plot in Fig. 2.
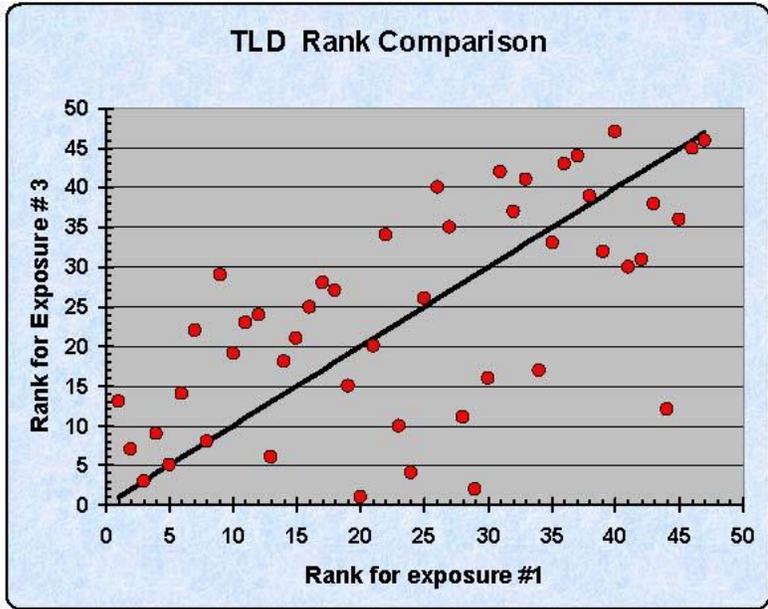
Figure 2

The black line represents the case where a given TLD would have the same rank from exposure to exposure There is no indication that a given TLD consistently reads low or high.  This implies that scatter in TLD readings is random and that the uncertainty in the TLD heating and read-out system is approximately 10%

 The batch of 48 TLDs was then calibrated using the 60cc ionization chamber as a reference.  The result used for this report is 0.021 cGy/nC.

A polyethylene mouse phantom was designed to simulate the midline dose to mice in a cage.  It was a cylinder 1 inch in diameter and 2.25 inches long, weighing 21g.  Each phantom could accommodate two TLDs.  The phantoms are shown in Fig. 3.



Figure 3

Measurements were taken to determine the response of the TLD in the phantom. Fig 4 shows the results for bare TLDs and inside a mouse phantom with the axis oriented perpendicular and parallel to the direction pointing toward the source.
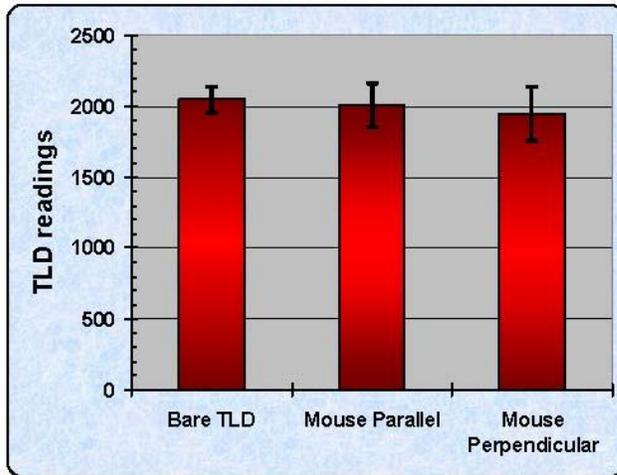


Figure 4

The data show that scatter within the phantom compensates any attenuation through the mouse.

Phantoms were placed in the front, center, and rear of a cage to determine variations in dose rate. Fig. 5 shows the results of these measurements.
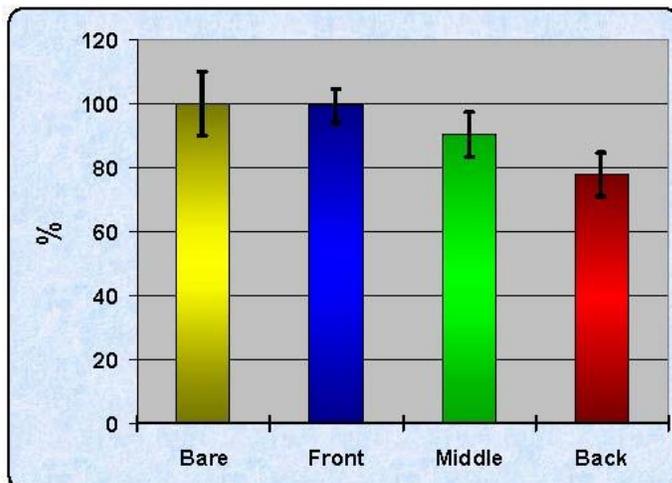


Figure 5

These data show that there is approximately a 20% variation from the front to the rear of the cage. The reduction in dose rate is caused by a combination of distance from the source and shielding by mice in the front of the cage

The rack was tested for stability and durability. Some modifications were proposed. A total of 5 racks were then fabricated and positioned into the room. Each rack was filled with 10 cages containing a full water bottle and food bin. Measurements were taken using the mouse phantoms to identify the position of each rack and cage to yield an average dose rate of 0.5 cGy/h.

It became clear that cages and racks were coupled to each other in terms of scattering. In effect, moving one rack changed the dose rate in adjacent racks. Converging on a suitable arrangement became an iterative process.

Summary:

The final source configuration consisted of two Pb plates labeled # 1 and #5

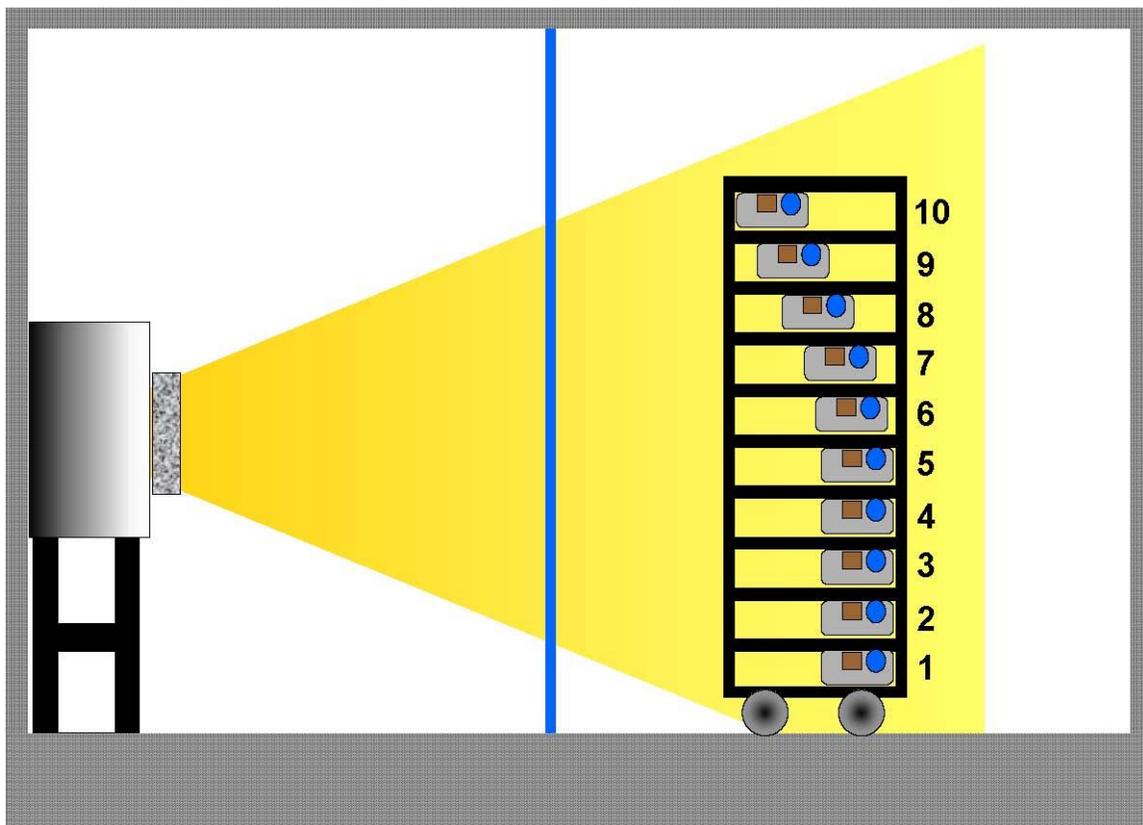 Fig. 6 shows an elevation view of the rack and cage positions.



Figure 6

The blue line represents the Bio Bubble barrier. The cage locations reflect the enhanced dose rate caused by scatter from the floor. Fig. 7 shows a plan view of the final arrangement.
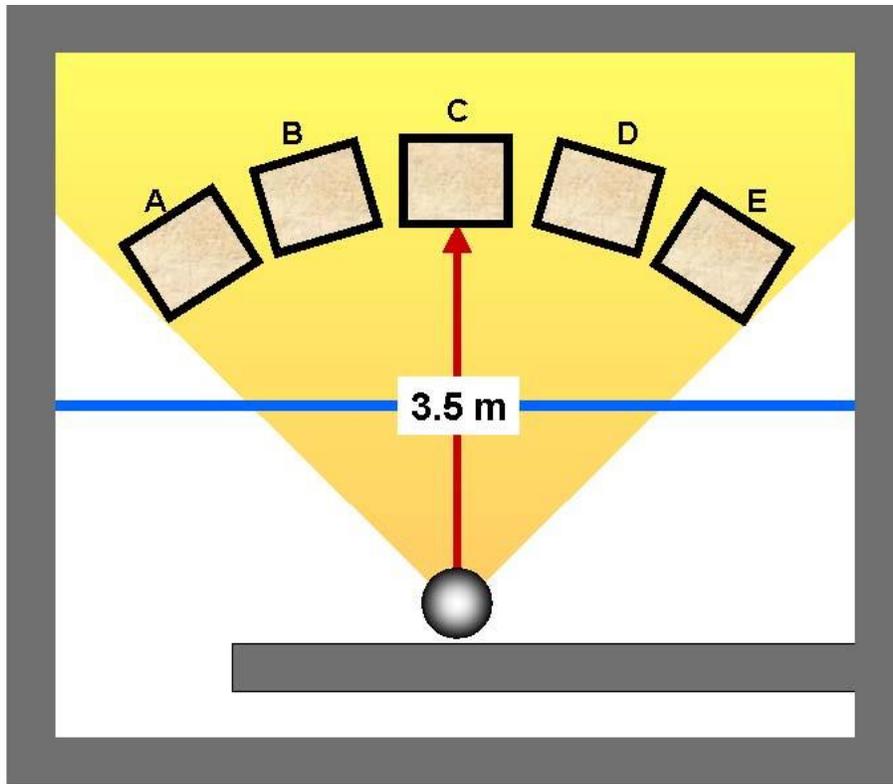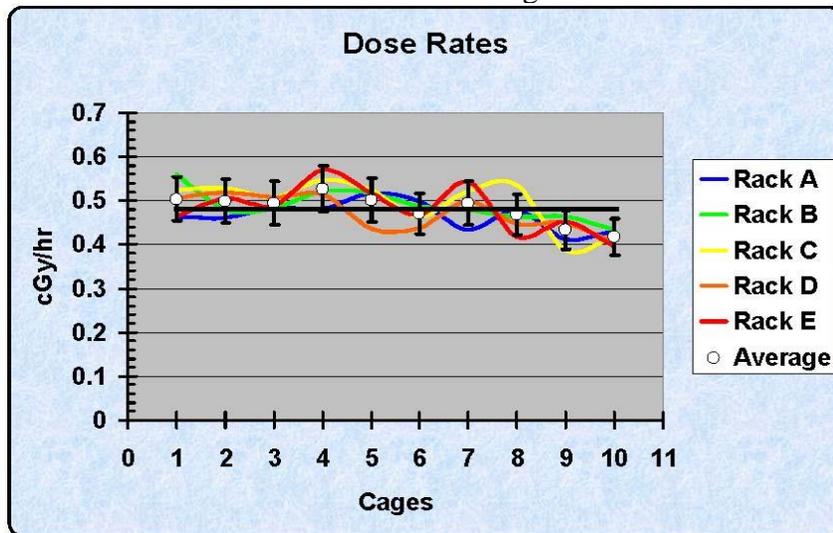
Figure 7

The front of the racks are located 350 cm from the source except for rack E, which is located at 340 cm.

Fig. 8 shows the dose rate in the center of each cage measured with mouse phantoms and



TLDs.

Figure 8

The circled represent the dose rate for each cage location (1 to 10), averaged over all racks (A-E). The variations reflect the uncertainty in the TLD measurements (~10%) as well as variations due to positioning in the cage.

The mean of all the measurements is

$$\dot{D} = 0.48 \pm 0.044 \text{ cGy/h}$$

This is shown as the horizontal line in Fig. 7.

**Tumor incidence and life span of low dose rate irradiated mice**

A total of 236 male CBA/CaJ mice, 8 to 12 weeks of age, were irradiated in two groups in the Low Dose Rate Irradiation Facility. The 5 Gy dose was delivered at 10cGy/day over 50 days with the $^{137}$Cs source exposed for 20.8 hours/day. The source was shielded 3.2 hours/day to allow health monitoring, cage change outs and routine maintenance. Irradiation of the first group of mice was completed January 25, 2007 and the second group was completed on March 27, 2007.

The mice were monitored until they became moribund or reached 800 days of age. Three mice died during or within a few days after irradiation and an additional 4 were lost due to accident. These animals were excluded from the study analyses. Necropsies were performed on all of the mice, and tissues suitable for histopathology were collected from all but one. A total of 228 mice were evaluated for tumors.

Survival to 800 days of age was compared between the low dose rate irradiated mice, unirradiated mice, and mice that received acute exposures of 1, 2, or 3 Gy. Kaplan-Meier survival analyses are shown in Fig. 9 and 10. There was no significant survival difference between low dose rate irradiated mice and the unirradiated controls whereas an acute exposure of 3 Gy yielded significant life shortening (Pairwise Multiple Comparison Procedures, Holm-Sidak method, P = 0.017).

The project was designed to study the efficacy of low dose rate radiation for inducing acute myeloid leukemia (AML). Only a single mouse developed AML for an incidence of <1%. Unexpectedly, 113 mice developed hepatocellular carcinoma (HCC) and an additional 8 mice had liver tumors that were likely HCC but could not be confidently distinguished from hepatocellular adenomas. Thus, the HCC incidence from the low dose rate exposure was 50 to 53%.

The background level of HCC is about 12%, and in other work this NASA/DOE jointly funded program we found that acute exposure to gamma ray doses up to 3 Gy barely increases it (publication 4 below). It is possible that HCC exhibits a so called "inverse dose rate effect," but
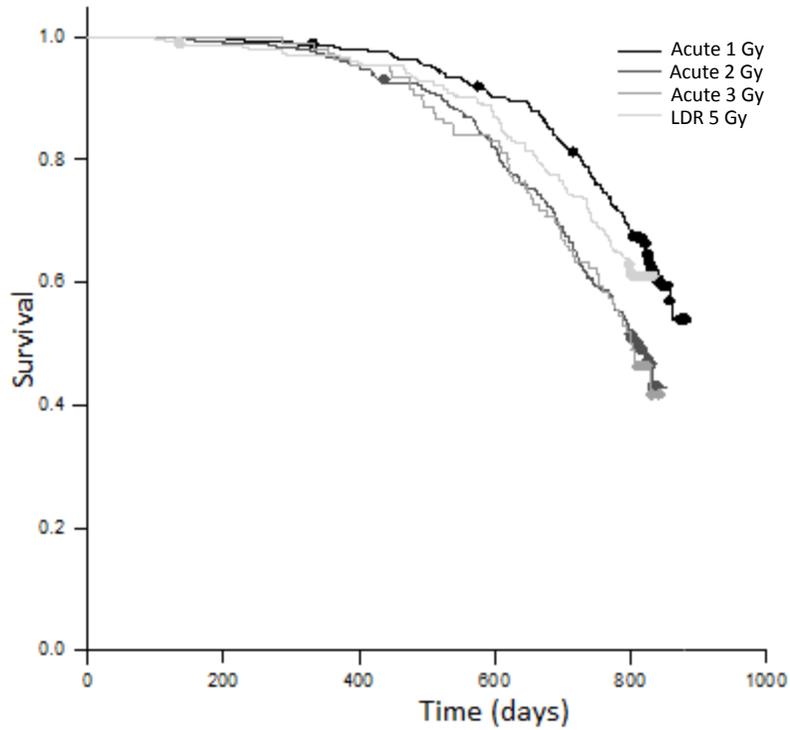
12

Figure 9. Comparison of survival to 800 days for a 5 Gy
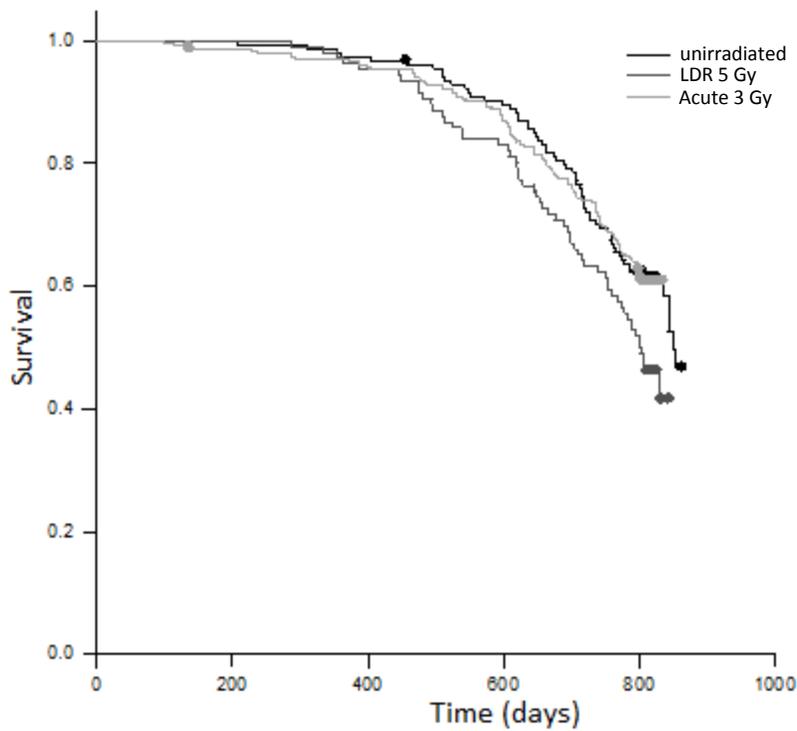low dose rate exposure and acute exposures to 1, 2, or 3 Gy.



Figure 10. Comparison of survival to 800 days for unirradiated mice
and mice receiving 3 Gy acute or 5 Gy low dose rate exposures.

without a group

without a group of mice exposed to 3 Gy at low dose rate or 5 Gy acutely this isn't certain.

Assuming that an inverse dose rate effect is responsible for the high incidence of HCC in low dose rate irradiated mice, what is the underlying mechanism? Hepatitis was common in irradiated mice without liver tumors suggesting chronic inflammation might play a role in HCC in this model. It is also intriguing that whereas acute exposure to low LET radiation has a minimal effect on HCC incidence, acute exposure to high LET radiation is quite effective. We found increased HCC incidence in mice irradiated with 0.1 to 1 Gy of 1 GeV $^{56}$Fe ions with a peak incidence of 35% at 0.4 Gy. It is possible that the same mechanism is responsible for both the low dose rate and high LET enhancement.

**Products Developed**

**Publications**

1. Peng Y, Brown N, Finnon R, Warner CL, Liu X, Genik PC, et al. Radiation leukemogenesis in mice: loss of PU.1 on chromosome 2 in CBA and C57BL/6 mice after irradiation with 1 GeV/nucleon 56Fe ions, X rays or gamma rays. Part I. Experimental observations. Radiat Res 2009; 171(4):474-483.

2. Peng Y, Borak TB, Bouffler SD, Ullrich RL, Weil MM, Bedford JS. Radiation leukemogenesis in mice: loss of PU.1 on chromosome 2 in CBA and C57BL/6 mice after irradiation with 1 GeV/nucleon 56Fe ions, X rays or gamma Rays. Part II. Theoretical considerations based on microdosimetry and the initial induction of chromosome aberrations. Radiat Res 2009; 171(4):484-493.

3. Weil MM, Bedford JS, Bielefeldt-Ohmann H, Ray FA, Genik PC, Ehrhart EJ, et al. Incidence of acute myeloid leukemia and hepatocellular carcinoma in mice irradiated with 1 GeV/nucleon (56)Fe ions. Radiat Res 2009; 172(2):213-219.

4. Steffen LS, Bacher JW, Peng Y, Le PN, Ding LH, Genik PC, et al. Molecular characterisation of murine acute myeloid leukaemia induced by 56Fe ion and 137Cs gamma ray irradiation. Mutagenesis 2013; 28(1):71-79.

**Equipment**

A low dose rate irradiator facility capable of irradiating up to 250 mice at low dose rate for protracted was designed and constructed. It is describe in the Activities Summary.