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<th>Task Last Updated: FY 04/29/2008</th>
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<td>PI Name: Ullrich, Robert Ph.D.</td>
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<td>PI Address 2: Comprehensive Cancer Center, MS 1048</td>
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<td>PI Web Page:</td>
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<td>City: Galveston</td>
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Page 1 of 4
**Task Description:**

The goal of this program is to provide information required to develop a rational scientific basis for estimation of risks for leukemogenesis in humans from exposure to radiation during space flight. This will be accomplished using several coordinated research activities. A key activity will be to develop quantitative data on the relative leukemogenic effectiveness of specific HZE particles and of high-energy protons compared with gamma rays in a mouse model of radiation-induced acute myeloid leukemia (AML). Extrapolation of risks from this mouse model to risks in humans requires additional information, and a major component of research activities in this program are designed to provide such information. A major research effort of this program is focused on studies of cellular and molecular mechanisms and processes involved in radiation-induced AML. These studies will not only improve the basic understanding of the pathogenesis of AML but will also provide information on cellular and molecular biomarkers that may be useful in predicting individuals at increased risk. These activities will focus on the use of molecular and molecular cytogenetic approaches, the development of new model systems, and comparative analyses of murine and human AML to facilitate the translation of results obtained in mice to human AMLs. Problems of extrapolation are not limited to biological factors. There are clear dosimetric differences that also must be considered when extrapolating data obtained from mouse studies to human risks. To address these issues this project also has an important physics and dosimetry component that includes modeling of dose to target cells in murine and human bone marrow.

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The need for better estimates of cancer risk associated with the space environment is clear. The problems associated with obtaining reliable estimates are also clear. Quantitative data on carcinogenic effects of radiations associated with the space environment must rely on animal models, and, at present, extrapolation of such animal data to risks in humans is problematic. This program directly addresses these issues (which are described in the Critical Path Roadmap as questions 28a through 28g and 28i) in a number of ways.

In project 1, this program will provide quantitative data on the relative effectiveness of protons, specific HZE particles, and gamma rays on the induction of AML in a well-defined murine model that has been shown to have substantial similarities to human AML both with respect to dose response characteristics for induction after radiation exposure and to its pathogenesis. Information on effects on latency will also be obtained. An advantage of the murine AML model is that there is already information on potential initiating lesions. Quantification of these early events, their transmissibility and on progression of initiated cells is invaluable in the extrapolation of results from murine to human AML.

Project 2 focuses on RBE estimates for induction of initial lesions involving chromosome 2 and examine the impact of different radiation qualities on the persistence, clonal expansion and progression of these initiated cells.

Projects 3 and 4 of this program will provide the fundamental knowledge needed to facilitate the application of the data derived from projects 1 and 2 for human risk estimates.

Project 3 is designed to identify, quantify, and characterize cytogenetic and molecular targets and early biomarkers in murine AML. This project will also determine the impact of factors such as radiation quality, time after exposure, tissue and cell lineage specific effects, and individual susceptibility on these fingerprints and biomarkers.

Project 4 serves as the primary link between murine and human AML. The aim of project 4 is two-fold. First, to develop comparative molecular and molecular cytogenetic information on potential common targets involved in the pathogenesis of murine and human AML. Second, to develop model systems to facilitate translation of results from mice to human AML. The program outlined is a dynamic scientific program focused on achieving long-term goals. As such the specific projects outlined in this proposal are meant as a starting point for a comprehensive analysis of radiation-induced AML. Based on progress and results from the studies described in this proposal, new directions will arise, projects may be completed, and new projects initiated. Changes are coordinated through consultations with our advisory groups and with appropriate NASA officials who may be involved in scientific and programmatic oversight. This year all of the investigators in the program met at CSU along with the members of the internal and external advisory committee for a comprehensive program review and to develop strategic plans for the next year.

In addition to these research projects, this program contains two cores. Core A coordinates the distribution of cells, tissues and other biological materials needed for all the projects and coordinate irradiation of animals, tissues and cells. Core C provides support to the research projects and cores within this program, to coordinate interactions among investigators and institutions, and to provide scientific and operational management and fiscal oversight.

The projects are briefly described below. Details of progress for each project during this reporting period follow this description.

**Project 1. Induction of AML in CBA/Caj mice (project leader, Dr. Robert L. Ullrich).**

This project is designed to quantitatively compare the leukemogenic effects of irradiation with gamma rays, select HZE particles, and protons using the CBA/Caj murine model of AML. These experiments are designed to provide sufficient quantitative data to permit the determination of their relative effectiveness by comparing slope constants obtained over specified dose ranges rather than fully defining the dose response relationships for each of these radiations. Over this project a large number of animals have died or were sacrificed at 800 days of age and analysis of life shortening and AML frequencies are underway. We also finished dosimetry for the low dose rate (LDR) irradiation groups and are beginning irradiations. This is the time at which we should expect to begin to see AML and indeed, we are beginning to find mice with AML in these groups. Likewise for the acute gamma rays group, we are beginning to see the development of AML. Besides AML we have also observed a high frequency of liver tumors and some hemangiomomas and hemangiosarcomas. The relationship of radiation dose and quality on the frequency as well as the malignancy and metastatic potential for these tumors is also being examined.

Project 2: Quantification and characterization of specific cytogenetic changes associated with initiation, progression, and development of AML (Project leader, J.S. Bedford).

The aim of Project 2 is to measure the relative biological effectiveness (RBE) of select HZE radiations relative to gamma-radiation over specified dose ranges with respect to the production of chromosomal abnormalities that have
been shown in previous studies (for x- or gamma-ray exposures) to be associated with radiation induced AML in CBA/CaJ mice. For x- or gamma-radiations, virtually all (at least 95%) of the cells of leukemias that do develop in the irradiated CBA mice contain a deletion of the PU.1 gene located on chromosome 2. During the past 2.5 years we have been measuring the loss of a small (200kb) segment of mouse chromosome 2 where this gene resides, in bone marrow cells sampled periodically after irradiation in the two mouse strains for the different doses and different radiations. There is some question as to whether the dose response is linear, though it cannot be ruled out. The slope of the dose response relationship in the lower dose region was perhaps somewhat less than that seen after 1 day, but the dose response appeared to plateau for higher doses. After 1 year, we have scored only a few mice from the CBA group, but it is immediately apparent that the percent of bone marrow cells with PU.1 loss was greatly increased relative to mice sampled at both the 1 day, and especially 1 month after irradiation.

We cannot, with any certainty, explain the differences in the dose responses for cells sampled from mice at different times after irradiation. One possibility is that cells experiencing nuclear traversals by the HZE iron ions are much more likely to be eliminated from the population after several divisions, as would be the case for the samples taken 1 month after irradiation. The samples taken after 1 year, on the other hand, may result from a small growth advantage of cells that did survive. We are continuing our investigations into these possibilities.

Project 3: Molecular and cytogenetic fingerprints and biomarkers. (project leaders, Drs. Joel Bedford, Susan Bailey, Wei-Wen Cai, and Michael Story).

The goals of Project 3 deal with investigations aimed at gaining a better understanding of factors at the cell and molecular level that may be involved in radiation induced murine AML in general, and for HZE radiation exposures in particular. The overall long-term focus of the studies in project 3 is on developing comparative information on radiation leukemogenesis in mice and humans. The studies outlined are subdivided into five sub-projects.

Project 3A. Work this past year has involved laying the groundwork to investigate the possible role of the structural organization of chromatin in cytogenetic damage associated with AML. We have isolated and tested the hybridization of several BACs and other markers along mouse chromosome 2 that will be used to measure the proximity of the two breakpoint cluster regions surrounding the PU.1 gene. Using Metamorph software with a deconvolution package to simulate confocal microscopy, we can begin to measure interphase breakpoint cluster distances as previously outlined.

Project 3B. This project involves utilization of microarray-based comparative genomic hybridization of normal vs. tumor DNA to identify regions of genomic deletions and amplifications common to the radiation-induced AML. The aim is to use mouse genome tiling BAC to characterize the genomic abnormalities in the mouse leukemia or other tumor samples collected in this project. While most of the mice subjected to heavy ion radiation are still in the process of developing leukemia or other type of tumors we are currently analyzing samples from Simon Boufleur’s lab in the UK. On the new tilling arrays about 60 % of the clones have an independent overlapping clone. The redundant clones are expected significantly increase the confidence level of abnormalities detected by single clones. Most significant progress has been made with regard to the automation of scanning and image quantitation. This improvement has significantly increased our array analysis throughput. Other aspects of our technology such as blocking and hybridization of arrays have also been improved over the last year benefiting from the support of this project. We expect in the coming year we will be focusing on routine analysis of the leukemia samples. The first set of histologically confirmed AML cases have recently been received and we should have some preliminary results from this sub-project soon.

Project 3C involves a study to investigate the possibility that HZE radiation induces chromosomal instabilities in the clonal progeny of surviving bone marrow cells after mice are exposed to HZE radiations. This sub-project also would investigate the long term stability of complex aberrations in such spleen colonies, since exposure to other high LET radiations suggest that even for low dose exposures, more than 90% of all aberrations are complex. We will also use another aliquot of the spleen samples for assessment of microsatellite instability. Project 3-D. Project 3-D has received 4 control samples and 10 samples from leukemic mice, 3 of which were lost because of degraded RNA. This left us with 4 normal and 7 leukemic samples. We used these samples to examine expression on our array platform (Illumina mouse whole genome). Within we examined technical replicates. All the replicates were most similar. Normal spleen samples were all found under one branch. However, leukemic samples showed the greatest diversity as a group. We will also examine signal transduction pathways when we have more samples.

Project 4. Molecular and cytogenetic targets in murine and human AML. (Project leaders, Drs. Ulrich, Bedford, Nagarajan, and Belmont).

The goals of project 4 are to identify common cytogenetic and molecular targets in mice and humans that are involved in the development of radiation-induced AML. Studies in this project are closely integrated with studies in projects 2 and 3 which focused on focus on cell and molecular factors of importance for murine leukemogenesis. Project 4 also was designed to provide a link between murine and human AML that will be necessary to extrapolate from mouse studies to estimate human risks. Project 4 contains two sub-projects.

Project 4A. This project aims to compare the mouse vs. human changes involved in leukemogenesis. Tumors have now appeared in appreciable numbers of animals from HZE irradiated and some of the gamma irradiated mice, so we are now beginning to have material for analysis of PU.1 loss along with the pathology and histology necessary for diagnosis of AML as opposed to other tumors. All the animals (CBA mice) irradiated with gamma-rays that have been diagnosed with AML have a very high percentage of PU.1 loss in cells harvested from the enlarged spleens. In very striking contrast to the results for gamma-irradiations, cells harvested from the enlarged spleens of animals irradiated with 1GeV/n ion rays that were later diagnosed with AML did not have an appreciable proportion with PU.1 loss. The interesting finding here, of course is that the AMLs resulting from the HZE radiations may arise through a different pathway. The results so far suggest an even greater need to carry out the more detailed cytogenetic analysis of chromosomal changes, possible induction of instabilities (chromosomal and microsatellite) as judged by the spleen colony assays, and the changes that may be seen in the expression arrays as well as the amplification and deletion analysis from the array CGH analyses for AMLs induced by gamma vs HZE irradiations in these mice.

Project 4B. Deletions of chromosomes 5q, 7q, 11q,17p and 20q are recurrent anomalies in human acute myelogenous leukemia (AML), secondary to radiation and or alkylating agent therapy. As part of the NSCOR, our focus in the past twelve months has been on two goals:

(I) To characterize the expression of candidate leukemia suppressor genes within the minimally deleted interval in 7q. Quantitative PCR suggests decreased expression for HIC/MDFIC, TES and CAV in AML cell lines. Interestingly these
genes span the FRA7G fragile site. Among these, the transcription factor HIC/MDFIC is of particular interest because of its potential role in enhancing GMCSF sensitivity in myeloid progenitors. We will be interested in evaluating HIC, TES and CAV expression in the murine heavy ion induced AML models developed in other parts of the consortium.

(II) To characterize genetic pathway in AML patients secondary to radiotherapy for primary malignancy. This subset is distinguished from secondary AML studied by others in that these patients did not receive alkylating agents. We have examined 145 patients for recurrent, non-random anomalies. A number of interesting conclusions can be drawn from this analysis: (i) Deletions of chromosome 5 and 7 co-segregate in these patients (ii) A significant fraction of these cases (>30%) harbored no gross cytogenetic anomalies and (iii) Recurrent anomalies of chromosome 11q, found in a small subset raise a potential leukemia suppressor role for genes encoded in this interval.

Bibliography Type: Description: (Last Updated: 09/11/2017)

### Abstracts for Journals and Proceedings

Peng Y, Warner C, Weil M, Ullrich R, Bedford J. "Leukemogenesis and progressive changes in bone marrow cells with loss of PU.1 on chromosome 2 in CBA/Caj and C57BL/6 mice after irradiation with 1 Gev/n Fe ions or gamma-rays." COSPAR Colloquium on Space Radiation Biology, Xi’an China, July 22, 2006. COSPAR Colloquium on Space Radiation Biology, Xi’an China, July 22, 2006., Jul-2006


