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Project Title:	Space Radiation Exposure and Risk Mediated by Clonal Hematopoiesis		
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Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **Cancer**:Risk of Radiation Carcinogenesis		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	22903-3390	**Congressional District:**	5
Comments:			
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	Per the Principal Investigator (PI): Eunbee Park has left the project. We have 2 bachelor of science graduates and an MD/PhD student working in part on this project.		
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Task Description:	During spaceflight, astronauts are exposed to many stresses that alter multiple physiological systems. The recent NASA Twins Study provided a highly detailed analysis of how prolonged, low orbit space travel may contribute to genotoxic stress, elevated DNA damage responses and genomic instability in leukocytes. The observed genomic instability during and after flight suggests that the ionizing radiation exposure caused DNA damage to hematopoietic stem cells that replenish blood cells throughout life. Thus, it is conceivable that these alterations will contribute to the development of hematologic malignancies and other chronic diseases through changes in immune cell function. Furthermore, these effects may be particularly magnified when traveling beyond Earth's geomagnetic field where there is increased exposure to high atomic number and high energy radiation. Recent epidemiological studies have documented the prevalence of somatic mutations within the cells of the hematopoietic system in healthy individuals. These acquired DNA mutations accumulate with age and, in some instances, can provide a competitive advantage to the mutant cell thus allowing for its clonal expansion. This phenomenon is known as clonal hematopoiesis of indeterminate potential (CHIP). While the mutational landscape of CHIP has only partially been deciphered, some of these clonal expansions can be attributed to somatic mutations in driver genes that are recurrently mutated in blood malignancies. These driver genes include epigenetic regulators (TET2, DNMT3A, ASXL1), spliceosome components (SF3B1, SRSF2), signaling proteins (JAK2), and DNA damage response molecules (TP53, PPM1D). Studies show that CHIP is associated with an increased risk of all-cause mortality. While there is a marked increase in the frequency of hematological cancer in individuals with CHIP, which is to be expected, the major cause of the increased mortality in these populations appears to be an increase in cardiovascular diseases including coronary heart disease, stroke, and early-onset myocardial infarction. Studies in the applicants' laboratories have provided evidence for a causal link between CHIP, derived from mutations in TET2, DNMT3A, JAK2, TP53 and PPM1D genes, and cardiovascular, metabolic, and renal pathologies. In some instances, it was shown that the pathological effects of a CHIP driver mutation (TET2, TP53 and PPM1D) could be mitigated with specific anti-inflammatory drugs. Of particular relevance to the proposed studies, there is an accelerated form of clonal hematopoiesis that is observed in individuals that have undergone myelosuppressive treatment and is referred to as "therapy-related clonal hematopoiesis." Under these conditions, it has been shown that there are hematopoietic clonal expansions with a very high frequency of mutations in PPM1D andTP53, both of which are classic DNA damage response genes. In individuals undergoing cytotoxic therapy, the hematopoietic system is likely under extreme stress, and it is thought that mutations in genes such as TP53 and PPM1D confer the mutated hematopoietic stem cell with a survival advantage against genotoxic stress induced by chemotherapy. Recent work from the applicants' laboratories have shown that this form of CHIP can synergize with the genotoxic agent's direct effect on the cardiovascular system to promote a more robust cardiomyopathic phenotype. While the impact of space travel on CHIP is completely unknown, it is reasonable to speculate that space radiation in combination with other space travel-related stresses will lead to radiation-specific and gene-specific accelerations of clonal hematopoiesis. Further, these forms of CHIP may increase the risk of leukemogenic and cardiovascular pathologies in a radiation- and gene-specific manner.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	While the impact of space travel on CHIP is completely unknown, it is reasonable to speculate that space radiation in combination with other space travel-related stresses will lead to radiation-specific and gene-specific accelerations of clonal hematopoiesis. Further, these forms of CHIP may increase the risk of leukemogenic and cardiovascular pathologies in a radiation- and gene-specific manner.
	During spaceflight, astronauts are exposed to many stresses that alter multiple physiological systems. The recent NASA Twin Study provided a highly detailed analysis of how prolonged, low-orbit space travel may contribute to genotoxic stress, elevated deoxyribonucleic acid (DNA) damage responses, and genomic instability in leukocytes. The observed genomic instability during and after flight suggests that ionizing radiation exposure caused DNA damage to hematopoietic stem cells that replenish blood cells throughout life. Thus, it is conceivable that these alterations will contribute to the development of hematologic malignancies and other chronic diseases through changes in immune cell function. Furthermore, these effects may be particularly magnified when traveling beyond Earth's geomagnetic field, where there is increased exposure to high atomic number and high-energy radiation. During the current year, we were able to participate in the Spring and Summer 2023 NASA Space Radiation Laboratory (NSRL) campaigns. In the months leading up to this campaign, we prepared the mice that were used as bone marrow donors. Recipient mice were purchased from Jackson Laboratory. Fourty-eight of these recipient mice received TP53 wild-type bone marrow cells, via the murine adoptive transfer bone marrow transplant (BMT) approach. The re-analysis of this condition was necessary because we discovered that the commercial DNA sequencing service had a mix-up of samples, leading to the "contamination" of wild-type TP53 with mutant TP53 cells used in the BMT. However, these "contaminated" mice will be useful for the final analysis as we were able to determine the ratio of wild-type to mutant cells used for the BMT, and thus this cohort represents a lower gene dosage (that will be informative when compared with the PPMID cohort that is described below). Approximately 2 months after bone marrow transplantation, the mice were transported to Brookhaven National Laboratory (BNL) and exposed to one of four types of radiation: no radiation, 100cGy gamma, 100cGy simGCRsim, or 100cGy SPEsim. One member of the Walsh lab traveled to BNL to complete these irradiation sessions. After the irradiation sessions, the mice were transported back to the University of Virginia, and we are completing serial blood sampling and echocardiography. Currently, this wild-type TP53 cohort is 6 months post radiation exposure. Thus far, in addition to baseline sampling, this cohort of mice has had flow cytometry and whole blood analysis performed at 1 month post-irradiation, and 4 months post-irradiation. Echocardiography was performed before irradiation and approximately every 6 months thereafter. Body weights are measured monthly. In addition to this cohort, the first TP53 mutant cohort and the PPM1D (mutant and wild-type) cohort have completed the study time course. We also continued to monitor and perform serial sampling for the TET2 (mutant) cohort which is currently 17 months post radiation exposure. The donor chimerism trends that were noted in the 2022 progress report held true until the study ended. In the male TP53 mutant cohort, the effect of radiation on mutant cell expansion in white blood cells (WBCs) was strongest in the following order: gamma and simGCRsim, followed by SPEsim. In the female mutant cohorts, the effect of radiation was strongest with gamma, and SPEsim and simGCRsim were slightly less. Overall donor chimerism appeared to be greater in the female groups compared to the male groups. Donor chimerism also reached a plateau.

Task Progress:	In the PPM1D cohort, the mutant cells did not expand without radiation exposure. The overall percent chimerism was much lower than TP53 and TET2, but the donor cell expansion was still similar to TP53 as a percentage increase. The effect of radiation on mutant cell expansion in WBCs followed similar trends as the TP53 cohort. In both the male and female groups, gamma and simGCRsim showed the greatest expansion of the mutant cells. Similar to what was observed with the TP53 cohort, there appeared to be a sex-specific effect where overall donor chimerism appeared to be greater in the female groups compared to the male groups. Donor chimerism also reached a plateau in the PPM1D cohort. In the TET2 cohort, the effect of radiation on mutant cell expansion in WBCs still does not appear to be radiation specific. The mutant cell expansion occurs at the same rate regardless of the radiation type. Similar to TP53 and PPM1D there does appear to be a sex-specific effect where there is slightly greater expansion in the female groups. Unlike TP53 and PPM1D donor chimerism does not appear to be reaching a plateau. Some interesting survival trends also emerged in the TP53, PPM1D, and TET2 cohorts. Survival was substantially lower in the simGCRsim and gamma TP53 mutant male groups. This speed and level of mortality were not seen in either of the other two cohorts. There was also a sex bias where the TP53 mutant males were more affected than the females. There was minimal mortality in the PPM1D cohort, and the mortality did not show a radiation effect, clonal hematopoiesis (CH) mediated effect, or a sex bias. Thus far, TET2 does not appear to have a radiation effect, but there is a CH-mediated effect and a sex bias. Mice in the TET2 knockout groups have higher mortality regardless of radiation type, and mortality in the female KO groups is greater than that in the males. We also conducted a pilot study to test the hypothesis that gamma radiation would promote the loss of Y chromosome (LOY) clone growth. Male C57Bl6 mice were adoptively transplanted with GFP-positive bone marrow cells with (WT) or without (Y*) the Y chromosome (LOY model). Mice were exposed to 3 doses of 100cGy gamma radiation at 4, 8, and 12 weeks post-adoptive transfer. A subset of mice was not exposed to radiation and served as controls. Blood was collected at 2 and 4 weeks after each radiation dose to assess the percentage of GFP-positive donor white blood (WBCs) cells by flow cytometry. Two weeks after the first radiation dose, the percentage of both WT and Y* donor WBCs was higher in the irradiated groups compared to non-irradiated controls; however, there were no differences between the two irradiated groups. At 4 weeks post-irradiation, the percentage of Y* donor WBCs remained significantly higher in the irradiated group compared to the non-irradiated group. At 2 weeks after the second radiation dose, the percentage of Y* donor WBCs was higher compared to the non-irradiated Y* group; however, there was no difference between the irradiated WT and Y* groups. At 4 weeks after the second radiation dose, there was no difference between any of the groups. At 2 weeks after the third radiation dose, the percentage of Y* donor WBCs was higher compared to the non-irradiated controls, although there was no difference between the WT and Y* irradiated groups. At 4 weeks after the third radiation dose, the percentage of WT donor WBCs was significantly higher than that of Y* in the irradiated groups. While gamma radiation exposure may promote LOY clone expansion to a small extent, this expansion appears to be limited by the number of radiation doses. Moreover, the extent of expansion of LOY cells post-radiation exposure appears to be considerably smaller than that of TP53 and PPM1D mutant clones, as observed in the parent study. In addition to in vivo experimental models, ongoing cell culture assays are investigating the direct effects of gamma-irradiation mLOY in various cell types. The percentage of mLOY cells will be quantified in primary human fibroblasts and endothelial cells before and after gamma-irradiation treatment, and treated cells will be further expanded in culture and assessed for mLOY over time. These results will determine whether gamma-irradiation has direct effects on the induction and/or expansion of mLOY in model cell types, potentially providing mechanistic evidence for the effects of irradiation on mLOY. Going forward, we plan to continue flow cytometry and whole blood analysis every 4-6 months and echocardiography every 6-9 months for the TET2 and TP53 cohorts. The mice remaining in the TET2 cohort will be sacrificed and tissues harvested in December 2023 or early January 2024. In planning for future studies, we submitted proposals to irradiate additional cohorts of mice in the Spring and Summer 2024 campaign and the Fall 2024 campaign. Most likely, these new cohorts will examine a model of DNMT3A-mediated clonal hematopoiesis, which is the most prevalent form of clonal hematopoiesis observed in humans.
Bibliography Type:	Description: (Last Updated: 01/03/2024)
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