Fiscal Year:	FY 2024	Task Last Updated:	FY 11/17/2023
PI Name:	Walsh, Kenneth Ph.D.		
Project Title:	Space Radiation Exposure and Risk M	lediated by Clonal Hematopo	piesis
Division Name:	Human Research		
Program/Discipline:			
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 Carcino	genesis	
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	kw9ar@virginia.edu	Fax:	FY
PI Organization Type:	UNIVERSITY	Phone:	434-243-8303
Organization Name:	University of Virginia, Charlottesville		
PI Address 1:	Hematovascular Biology Center		
PI Address 2:	415 Lane Rd, PO Box 801394, Suite 1	010	
PI Web Page:			
City:	Charlottesville	State:	VA
Zip Code:	22903-3390	Congressional District:	5
Comments:			
Project Type:	Ground	Solicitation / Funding Source:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D
Project Type: Start Date:	Ground 01/29/2021	Solicitation / Funding Source: End Date:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025
Project Type: Start Date: No. of Post Docs:	Ground 01/29/2021 2	Solicitation / Funding Source: End Date: No. of PhD Degrees:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025
Project Type: Start Date: No. of Post Docs: No. of PhD Candidates:	Ground 01/29/2021 2 1	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025
Project Type: Start Date: No. of Post Docs: No. of PhD Candidates: No. of Master's Candidates:	Ground 01/29/2021 2 1	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025
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Project Type: Start Date: No. of Post Docs: No. of PhD Candidates: No. of Master's Candidates: No. of Bachelor's Candidates: Contact Monitor:	Ground 01/29/2021 2 1 1 1 2 1 2 2awaski, Janice	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC
Project Type: Start Date: No. of Post Docs: No. of PhD Candidates: No. of Master's Candidates: No. of Bachelor's Candidates: Contact Monitor: Contact Email:	Ground 01/29/2021 2 1 1 Zawaski, Janice janice.zawaski@nasa.gov	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC
Project Type: Start Date: No. of Post Docs: No. of PhD Candidates: No. of Master's Candidates: No. of Bachelor's Candidates: Contact Monitor: Contact Email: Flight Program:	Ground 01/29/2021 2 1 1 2 1 2 2 3 2 3 3 4 5 4 5 4 5 4 5 5 5 5 5 5 5 5 5 5 5	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC
Project Type: Start Date: No. of Post Docs: No. of PhD Candidates: No. of Master's Candidates: No. of Bachelor's Candidates: Contact Monitor: Contact Email: Flight Program: Flight Assignment:	Ground 01/29/2021 2 1 1 Zawaski, Janice janice.zawaski@nasa.gov	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC
Project Type:Start Date:No. of Post Docs:No. of PhD Candidates:No. of Master's Candidates:No. of Master's Candidates:Contact Monitor:Contact Email:Flight Program:Flight Assignment:Key Personnel Changes/Previous PI:	Ground 01/29/2021 2 1 1 2 1 2 1 2 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC
Project Type:Start Date:No. of Post Docs:No. of PhD Candidates:No. of Master's Candidates:No. of Master's Candidates:Contact Monitor:Contact Email:Flight Program:Flight Assignment:Key Personnel Changes/Previous PI:COI Name (Institution):	Ground 01/29/2021 2 1 1 2 1 2 1 2 1 2 1 2 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone: heee Park has left the project is project. .D. (University of Virginia, y) .HN School of Medicine at P	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC t. We have 2 bachelor of science graduates and an Charlottesville) Mount Sinai)
Project Type:Start Date:No. of Post Docs:No. of PhD Candidates:No. of PhD Candidates:No. of Master's Candidates:Contact Monitor:Contact Email:Flight Program:Flight Assignment:Key Personnel Changes/Previous PI:COI Name (Institution):Grant/Contract No.:	Ground 01/29/2021 2 1 1 Zawaski, Janice janice.zawaski@nasa.gov Per the Principal Investigator (PI): Eur MD/PhD student working in part on the Garrett-Bakelman, Francine M.D., Ph Hirschi, Karen Ph.D. (Yale Universit Goukassian, David M.D., Ph.D. (ICA Evans, Megan A (University of Virgit) 80NSSC21K0549	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone: hbee Park has left the project is project. D. (University of Virginia, y) (HN School of Medicine at Minia)	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC t. We have 2 bachelor of science graduates and an Charlottesville) Mount Sinai)
Project Type:Start Date:Start Date:No. of Post Docs:No. of Post Docs:No. of PhD Candidates:No. of Master's Candidates:Contact Monitor:Contact Email:Flight Program:Flight Assignment:Key Personnel Changes/Previous PI:COI Name (Institution):Grant/Contract No.:Performance Goal No.:	Ground 01/29/2021 2 1 1 2 1 2 1 2 2 1 2 2 1 2 2 1 2 2 1 2 2 3 2 2 3 3 2 2 3 3 2 3 3 3 3 3 3 3 3 3 3 3 3 3	Solicitation / Funding Source: End Date: No. of PhD Degrees: No. of Master' Degrees: No. of Bachelor's Degrees: Monitoring Center: Contact Phone:	2019-2020 HERO 80JSC019N0001-HHCBPSR, OMNIBUS2: Human Health Countermeasures, Behavioral Performance, and Space Radiation-Appendix C; Omnibus2-Appendix D 01/28/2025 3 NASA JSC t. We have 2 bachelor of science graduates and an Charlottesville) Mount Sinai)

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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 diseases, stroke,
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 un 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 (NSRL) campaigns. In the murths 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 se

	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.
Task Progress:	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 not difference between the irradiated WT and Y* groups. At 4 weeks after the second radiation dose, the rew as no difference between the irradiated wT and Y* groups. At 4 weeks after the second radiation dose, the rew as 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 of Y* donor WBCs was higher compared to the non-irradiated of Y* donor WBCs was higher compared to the non-irradiated of Y* donor WBCs was higher compared to the non-irradiated of Y* donor WBCs was higher compared to the non-irradiated of Y* donor WBCs was higher compared to the non-irradiated of Y* donor WBCs was higher compared to the non-irradiated groups. At 2 weeks after the third radiation dose, the percentage of Y* donor WBCs was higher compared to the non-irradiated groups. At 4 weeks after the second radiation dose, there was no difference between the WT and Y* irradiated groups. At 4 weeks
	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: 05/16/2025)
Articles in Peer-reviewed Journals	Sano S, Thel MC, Walsh K. "Clonal hematopoiesis: the nonhereditary genetics of age-associated cardiovascular disease." Curr Opin Cardiol. 2023 May 1;38:201-6. <u>https://doi.org/10.1097/HCO.0000000000001032</u> ; PubMed PMID: <u>36811645</u> ; PubMed Central PMCID: PMC10079606, May-2023
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