Fiscal Year:	FY 2021	**Task Last Updated:**	FY 12/16/2020
PI Name:	Porada, Christopher Ph.D.		
Project Title:	Novel Microfluidic Biomarker Detection Platforms to Monitor In Vivo Effects of Solar Particle Events and Galactic Cosmic Rays Radiation, Using Mice with Human Hematopoietic Systems		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	27157	**Congressional District:**	5
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2017 HERO NNJ16ZSA001N-TRIRT. Appendix C: Translational Research Institute for Space Health (TRISH) Research Topics
Start Date:	11/01/2017	**End Date:**	07/31/2022
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	3	**No. of Master' Degrees:**	1
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	1	**Monitoring Center:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date changed to 7/31/2022 per E. Urquieta/TRISH (Ed., 10/20/2021)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Zenhausern, Frederic Ph.D. (University of Arizona) Almeida-Porada, Graca M.D., Ph.D. (Wake Forest Institute for Regenerative Medicine) Walker, Stephen Ph.D. (Wake Forest Institute for Regenerative Medicine) Langefeld, Carl Ph.D. (Wake Forest Institute for Regenerative Medicine) Wilson, Paul Ph.D. (University of California, Davis) Coleman, Matthew Ph.D. (University of California, Davis) Tepper, Clifford Ph.D. (University of California, Davis) Lacombe, Jerome Ph.D. (University of Arizona) Yang, Jianing M.D., Ph.D. (University of Arizona)		
Grant/Contract No.:	NNX16AO69A-T0103		
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Performance Goal Text:	
Task Description:	We will use humanized immunodeficient (NSG) mice (huMice) whose hematopoietic system has been repopulated with human hematopoietic stem cells (HSC) from astronaut-age M/F donors. Using these huMice (3-4 months post-repopulation) as our experimental model, we will measure space radiation-induced human and mouse blood transcriptomic and proteomic changes using our low LET (linear energy transfer) photon-validated radiation biomarker detection panel and microfluidic-based detection platform. We will also test a promising curcumin-based nanolipoprotein (NLP)-based countermeasure that we have recently shown significantly improves human HSC proliferation and differentiation in vitro following low doses of high-energy proton and iron ions delivered at NASA Space Radiation Laboratory (NSRL). The huMice will serve as avatars allowing us to study in vivo responses and leukemogenic potential of human hematopoietic systems exposed to modeled space radiation. HuMice will be developed and matured at Wake Forest Institute for Regenerative Medicine (WFIRM) and transported to Brookhaven National Laboratory (BNL), as will HuMiX gut-on-a-chip populated with intestinal cells from healthy human donors (both sexes) of typical astronaut age. At NSRL, the huMice avatars and HuMiX chips will be exposed to mission-relevant doses of high-energy protons, intermediate and high LET ions, and the GCR (galactic cosmic radiation) simulator. HuMiX chips will be monitored for short-term human gastrointestinal (GI) cell biomarker responses, and the animals will be monitored for both short and long-term human and mouse biomarker responses, using our established low LET cesium-137 gamma radiation-specific protein biomarkers coupled to an ELISA-based microfluidic device that we will further optimize for International Space Station (ISS)/in-flight use. Biomarker responses measured in these devices will be validated using qPCR, RNASeq, Western blotting, and several traditional and molecular radiobiological techniques. We will serially monitor animals for short- and long-term blood-based biomarker responses and physiological radiation effects (specifically leukemogenesis). We will submit tissue/organ samples of huMice to the NASA Human Research Program (HRP) Shared Tissue Repository, and our biomarker datasets will be deposited into the NASA GeneLab database.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	During future missions beyond low Earth orbit (LEO), such as those planned to Mars and near-Earth asteroids, astronauts will face poorly defined health risks as a result of exposure to space radiation in the form of solar energetic particles (SEP) consisting primarily of protons and light ions, and galactic cosmic rays (GCR) ranging from high-energy protons to high-energy charged (HZE) nuclei. Although the intensity of the GCR heavy ion flux is fairly low, the relative biological effectiveness (RBE) of these high charge and energy (HZE) particles can be extremely high. Long duration spaceflight can result in the accumulation of radiation exposures that may produce significant short- and long-term untoward effects on human physiology, and that could potentially increase cancer morbidity/mortality in astronauts. Unfortunately, an incomplete understanding of biological effects resulting from exposures to this unique/complex radiation environment and the paucity of human epidemiological studies for these radiation types make it difficult to accurately estimate risks of carcinogenesis for various organ sites due to exposure to space radiation. It is well appreciated that the stem cell compartments of the hematopoietic and gastrointestinal (GI) systems constitute some of the most radiosensitive tissues of the body. Leukemias represent one of the most frequent radiogenic cancers and also exhibit the shortest latency periods. Ionizing radiation is also an established risk factor for colorectal cancer, and the Fornace group demonstrated exposure to HZE ions (1 GeV/n 56Fe ions) significantly enhanced the development and progression of intestinal tumors in Adenomatous polyposis coli (APC) mouse models. Compounding the carcinogenic risks that could arise from low to intermediate dose SEP/GCR exposures are numerous studies collectively demonstrating that extended spaceflight conditions deleteriously affect the immune system at multiple levels and impair astronauts' ability to respond to infection or immune challenge. Collectively, these findings illustrate an important need to use appropriate human hematopoietic and GI experimental models to precisely identify SEP/GCR radiation-induced effects, namely to better understand the genomic and epigenomic alterations responsible for low or high LET charged particle-induced carcinogenesis; identify appropriate molecular targets for effective countermeasure development; and provide more refined datasets for NASA's risk estimation modeling efforts. Herein, we are performing studies in humanized mice to enable omics capability for in-flight measurements of radiation/stress blood biomarkers (human) using RNASeq and microfluidic-based transcriptomic/proteomic biomarker detection platforms. We are also utilizing the Human-Microbial Cross-Talk model (HuMiX) gut-on-a-chip to perform the 1st studies monitoring the effects of simulated space radiation on the human GI tract. Based on exciting preliminary data, we will also examine the ability of the dietary supplement curcumin to prevent and/or mitigate the effects of space radiation on the hematopoietic and GI systems, and to determine the optimal working concentration for maximal radioprotective/radiomitigating effects. Given curcumin's poor water solubility, we are also performing studies to validate the ability of nanolipoprotein particles (NLP) loaded with curcumin (cNLPs) to serve as an effective countermeasure against the effects of SEP/GCR radiation in both the human hematopoietic and GI systems. Once validated as an effective countermeasure, we will also assess the stability of these cNLPs for long-term storage aboard ISS/long duration missions, suitability for lyophilization/resuspension for oral delivery. If successful, these cNLPs could readily be implemented as a dietary supplement during prolonged missions, protecting astronauts from the deleterious effects of space radiation on the hematopoietic and GI systems.
	The original aims of this project were to: 1) use mice with humanized hematopoiesis to define changes in radiation/stress blood biomarkers in response to mission-relevant doses of simulated space radiation; 2) use the innovative Human-Microbial Cross-Talk human gut-on-a-chip model (HuMiX) to perform studies defining the effects of mission-relevant doses of simulated space radiation on the human gastrointestinal (GI) tract; 3) validate the ability of nanolipoprotein particles (NLP) loaded with curcumin to serve as an effective countermeasure against the effects of simulated space radiation in both the human hematopoietic and GI models; and 4) assess the suitability of curcumin-NLPs as space radiation countermeasures. During this third year of funding, we have made significant progress towards achieving these goals. During this time, we have: • Performed H+, 56Fe ion, 16O, ?28Si, ?-ray, and simplified 5-ion galactic cosmic ray (GCR) simulator irradiations on both wild-type mice, as requested by TRISH (Translational Research Institute for Space Health), to provide an in vivo comparator/validation of the in vitro HuMiX human gut-on-a-chip system, and on NSG mice whose hematopoietic systems were first humanized by repopulation with human hematopoietic stem cells (HSC) isolated from the bone marrow of healthy, astronaut-age donors.

Task Progress:	• Collected tissues (at multiple times post-irradiation) and are analyzing GI system, blood for biomarker identification by omics, and hematopoietic system for lineage alterations and DNA damage. • Found marked histological alterations in small intestine in response to protons and both 16O and 56Fe ions. • Discovered that exposure to Mars mission-relevant doses of protons and 56Fe ions leads to disruption of the epithelial barrier of the small intestine, as assessed by immunofluorescent staining for Claudin-3. • Humanized the hematopoietic system of over 200 NSG mice, installed RFI-tracking p-Chips, and irradiated these mice during NASA Space Radiation Laboratory (NSRL)-19C and the abbreviated 20A/20B run. All mice from NSRL19A have now been sacrificed and tissues are being analyzed. Mice from NSRL 19C are being sacrificed and analysis has begun. • Have begun analyzing the various tissues from the 96 wild-type C57Bl/6 mice we irradiated during NSRL-19B by both immunohistochemistry and multi-omics approaches to define the effects that solar energetic particles (SEP) and GCR radiation exert on the various tissues/organ systems of the body and the ability of our curcumin-laden NLPs to protect against these effects. • We have found that pre-treatment of the wild-type mice with cNLPs appears to reduce the amount of apoptosis seen in the small intestine following exposure to SEP/GCR radiation. • The HuMix gut-on-a-chip platform has been further optimized and limited bacterial species have been incorporated into the HuMiX chip. This new optimized system with a simplified microbiome was exposed to various ions during NSRL-19C and will be returning for NSRL 20C. • Have now shown that the presence of representative species from the microbiome in the HuMiX system appears to provide some degree of protection against the deleterious effects of space radiation. Further studies are now underway to define the mechanistic basis for these observations. • Have shown cNLPs can mediate protection against SEP and GCR radiation in vivo, at omics level. • We have shown that the cNLPs provide markedly better protection against all varieties of ions tested than simply giving curcumin in DMSO (dimethylsulfoxide), using human fibroblasts. • Have generated a wealth of data on the cellular/molecular pathways that are altered by both SEP and GCR radiation and the impact that cNLPs (and DMSO-curcumin) have on these alterations, and have shown that curcumin delivered packaged in NLPs is far more effective, and induces transcriptomic changes that differ markedly from, than curcumin suspended in DMSO. • We have shown, for the first time ever, that the exposure of mice harboring human hematopoietic systems to GCR radiation (56Fe ions, thus far, other ions now being tested) leads to enlargement of the spleens to roughly 30 times the normal size (in several mice; not all). Immunohistology exam by clinical hematopathologists has preliminarily concluded this is due to induction of human hematological malignancy, as we observed in our prior studies in which human HSC were exposed to these same ions in vitro and then used to repopulate NSG mice. Extensive analyses are underway to define the nature of the malignancy. Our progress to-date has enabled us to begin rigorously testing our hypotheses regarding the effects of space radiation (SEP and GCR) on the human hematopoietic and GI systems, and has provided compelling evidence to support our hypothesis that exposure to space radiation at Mars mission-relevant doses, produces marked deleterious effects on both of these human systems. These data have also supported our premise that a novel formulation of a readily available dietary supplement (curcumin) may have the ability to serve as an easy to administer countermeasure that can protect astronauts from at least some of the deleterious effects of space radiation during prolonged missions beyond Low Earth Orbit (LEO). Our omics work thus far has also begun to shed light on the molecular/ biological pathways that are altered within multiple tissues as a result of exposure of a living organism to ?-ray radiation and to simulated SEP and GCR radiation, and it is beginning to provide mechanistic clues regarding the means by which curcumin-NLPs mediate their protective effects. Our newly formulated HuMiX chip is performing well, and it has begun providing a wealth of information (the first of its kind) on the response of the human GI tract to space radiation and the role played by the gut microbiota in this response.
Bibliography Type:	Description: (Last Updated: 01/30/2023)
Articles in Peer-reviewed Journals	Almeida-Porada G, Atala AJ, Porada CD. "Therapeutic mesenchymal stromal cells for immunotherapy and for gene and drug delivery." Mol Ther Methods Clin Dev. 2020 Mar 13;16:204-24. https://doi.org/10.1016/j.omtm.2020.01.005 ; PMID: 32071924; PMCID: PMC7012781 , Mar-2020
Articles in Peer-reviewed Journals	Bisserier M, Saffran N, Brojakowska A, Sebastian A, Evans AC, Coleman MA, Walsh K, Mills PJ, Garikipati VNS, Arakelyan A, Hadri L, Goukassian DA. "Emerging role of exosomal long non-coding RNAs in spaceflight-associated risks in astronauts." Front Genet. 2022 Jan 17;12:812188. https://doi.org/10.3389/fgene.2021.812188 · PMID: 35111205; PMCID: PMC8803151 , Jan-2022
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Awards	Kuhlman B. "36th Annual Meeting of the American Society for Gravitational and Space Research, Virtual Meeting, November 5-6, 2020. Lightning talk." Nov-2020
Awards	Porada C. "Invited to join the Scientific Advisory Board of Orbital Transports Inc., November 2019." Nov-2019

Awards	Diaz J. "36th Annual Meeting of the American Society for Gravitational and Space Research, Virtual Meeting, November 5-6, 2020. Lightning talk." Nov-2020
Awards	WFIRM and UC Davis teams. "Our TRISH project was selected for 4 of the 8 posters to be presented at the 66th Annual Meeting of Radiation Research Society, Virtual Meeting, October 18-21, 2020." Oct-2020
Dissertations and Theses	Diaz J. "Characterizing the effects of Mars mission-relevant doses of space radiation on the gastro-intestinal tract." Thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science in “Biomedical Engineering” at Wake Forest University/Virginia Tech. Supervisors: Christopher D. Porada and Graça Almeida-Porada at Wake Forest Institute for Regenerative Medicine (WFIRM), May 2021. , Jan-2023