Fiscal Year:	FY 2023	**Task Last Updated:**	FY 03/27/2023
PI Name:	Fornace, Albert M.D.		
Project Title:	Radiation Carcinogenesis by GCRsim in Animal Models for High Priority Cancer Types (NSCOR)		
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:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:	http://www9.georgetown.edu/		
Project Type:	GROUND	**Solicitation / Funding Source:**	Directed Research
Start Date:	05/25/2022	**End Date:**	05/24/2024
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date changed to 05/24/2024 per S. Mack/JSC. Original end date was 05/24/2023 (Ed., 5/22/23).		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Shubhankar, Suman Ph.D. (Georgetown University) Shay, Jerry (University of Texas Southwestern Medical Center at Dallas) Brenner, David (Columbia University)		
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Task Description:	Considering the high spontaneous incidence of gastrointestinal (GI) and lung cancer, an even modest increase by space radiation exposure could have a significant effect on astronauts' health risk estimates during and after long-duration manned space flights. However, there is substantial uncertainty for GI and lung cancer risk estimation from space radiation due to the lack of in vivo human data. The overarching goal of the current proposal is to specifically investigate cellular and molecular hypothesis-driven mechanisms in relevant mouse models that will contribute to carcinogenic risk estimates of GI and lung cancer after exposure to space radiation beams prioritized by NASA's Human Research Program (HRP). Specifically, this proposal builds on the data generated from previous NSCOR studies and seeks to determine incidence, tumor frequency, and grade as well as identify molecular perturbations in the target cells associated with GI and lung tumorigenesis through monitoring of changes in the tumor number, histology, gene/protein expression, and metabolome of the proposed model system after exposure to space radiation type beams. Having found modest effects in earlier studies with protons and considering the importance of assessing the effects of mixed beams, we have chosen high priority Galactic Cosmic Radiation (GCR) type beams for comparison with low-LET (gamma-rays) radiation in our proposed studies. The full-spectrum (33-ion) acute (single dose) and protracted (up to 6 weeks) GCRsim beams closely mimic the mixed particle radiation environment in deep space. Our overall hypothesis is that GCRsim exposure is more carcinogenic due to important qualitative differences from low-LET gamma rays. The overall objective of this proposal is to acquire quantitative and qualitative cancer data after full-spectrum GCRsim exposure to model the relative risk of GI and lung tumorigenesis and compare it to gamma radiation where human epidemiologic data are available. Our proposed Aims are: Aim 1. Quantitatively assess GI tumorigenesis in mouse models of GI cancer and collect samples for qualitative analysis. Aim 2. Understanding molecular signaling associated with space radiation-induced GI-tumorigenesis and functional alterations. Aim 3. Quantitatively and qualitatively compare effects of GCR-type irradiations on lung cancer initiation and progression in normal as well as lung cancer susceptible mice. Aim 4. Development of mathematical modeling for GI and lung cancer risk assessment.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	According to estimations from the American Cancer Society (ACS), the lifetime risk of developing colorectal (1 in 23 for men and 1 in 25 for women) and lung (1 in 16 for men and 1 in 17 for women) cancer is significantly high in the US population. Considering the high prevalence of GI and lung cancer, an even slight increase in the incidence of GI and lung cancer in astronauts due to space radiation exposure is a critical health concern and has a significant impact on the planning of upcoming manned deep space exploration missions. In addition to cancer risk estimation studies after space radiation exposure, investigations on the persistence of oxidative and inflammatory stress after space radiation and its role in GI and lung cancer development may provide insight into mutagenic processes affecting genome integrity and carcinogenesis. The significance and deliverable of this project are to improve the estimates of GI and lung cancer risk after simulated galactic cosmic radiation (GCR) exposure and to identify plausible targets for the development of medical countermeasures (MCM).
	Pointwise progress made for Aim/Projects 1–4 is summarized below. 1) Ionizing radiation dose, dose rate, and gender effects are known variables that need to be addressed for an accurate estimation of space radiation-induced tumorigenesis risk. In order to address this issue, we participated in the NSRL-22B (summer) beam campaign and conducted a comprehensive GI-tumorigenesis experiment with male and female Apc1638N/+ mice exposed to acute and chronic full-spectrum (33-ion) GCR beams. Additionally, to investigate heavy-ion (HZE) contributions in GCRsim-induced GI tumorigenesis, male Apc1638N/+ mice were exposed to p+He (45.7 cGy, i.e., HZE omitted) and HZE only (4.3 cGy, i.e., p+He omitted) beams. In addition to tumorigenesis studies, we also exposed C57BL6 and Lgr5+ mice to full-spectrum GCR beams, and samples obtained from these animals are being used for molecular studies focused on analyzing GCRsim-induced senescent cell signaling, and GI-functional changes, relative to low-LET gamma-ray exposure. 2) Dose response studies to develop quantitative GI-tumorigenesis data after acute GCRsim (10-75 cGy) exposures were completed in male and female Apc1638N/+ mice. A significant induction in GI-tumorigenesis was noted at all tested GCRsim doses, relative to the control group, which suggested a good signal-to-noise ratio for GI-tumorigenesis in both male and female Apc1638N/+ mice at 150 days post-exposure. In male Apc1638N/+ mice, a GCRsim dose-dependent increase in GI-tumorigenesis was observed up to the 50 cGy dose, while in the case of female mice, a dose-dependent increase in GI-tumorigenesis was observed up to the 25 cGy dose. Additionally, male preponderance for GCRsim-induced GI-tumorigenesis was also observed. 3) The dose rate effect was analyzed using acute and chronic GCRsim exposures. We completed 25, 50, and 75 cGy of exposures in acute and chronic exposure settings. To simulate chronic GCRsim exposure, mice were exposed to a fractionated (2.08 cGy/day) GCRsim beam, i.e., 25 cGy (6 days/week for two weeks), 50 cGy (6 days/week for four weeks), and 75 cGy (6 days/week for six weeks). Both acute and chronic exposure resulted in significantly higher GI tumorigenesis in male and female mice, relative to the control group. No significant difference in GI tumorigenesis was observed between acute and chronic GCRsim exposed male and female Apc1638N/+ mice. Our findings suggest that GI tumorigenesis after GCR exposure is independent of dose rate. 4) Current risk predictions are based on RBE values derived from in vivo studies using single-ion beams, while GCR is essentially a mixed radiation field composed of protons (p), helium (He), and heavy ions (HZE). Therefore, Apc1638N/+ mice were total-body irradiated with 33-ion GCRsim beam components, i.e., p+He (HZE omitted, i.e., LET up to 10 keV/micron) and HZE only (p+He omitted, i.e., LET >10 keV/micron) beams. Further, GI-tumor data from equivalent doses of p+He (i.e., 45.7 cGy), HZE (i.e., 4.37 cGy), and GCRsim (i.e., 50 cGy) were compared to understand the contributions of heavy ions in GI-tumorigenesis. A comparison of tumor data from GCRsim and equivalent doses of heavy ions revealed an association between higher GI-tumorigenesis and dose contributed from the HZE fraction, as the induced tumor per unit (cGy) dose was remarkably higher for HZE compared to p+He and GCRsim exposures. 5) Male C57BL6J mice (8 weeks old) were exposed to either sham, gamma-rays (50 cGy), or acute GCRsim (50 cGy), and intestinal samples were obtained 150 days post exposure. Intestinal tissue sections stained for 4-hydroxyneonenal (4-HNE), a marker of oxidative stress, indicated a significant increase in lipid peroxidation after GCRsim exposure, which was significantly higher than that in the gamma-rays and control groups. Further, DNA double-strand breaks (DSBs) were detected and quantified using gammaH2Ax. Approximately a 2-fold increase in gammaH2Ax foci was noted in the GCRsim-exposed group, compared to a 1.5-fold increase after gamma-ray exposure. In conclusion, a radiation quality-dependent increase in persistent oxidative stress and DNA damage was observed in mouse intestinal epithelial cells (IECs).

Task Progress:	6) Male C57BL6J mice (8 weeks old) were exposed to either sham, gamma-rays (50 cGy), or acute GCRsim (50 cGy), and intestinal samples were obtained at 150 days post exposure. We analyzed SA-b-gal activity (a senescence marker) in isolated IEC using C12FDG as substrate and flow cytometry. The data show a significant increase in SA-b-gal activity in GCRsim, relative to gamma-rays and control groups. The mRNA expression analysis of cellular senescence-associated genes, i.e., p16 (upregulated during senescence) and lamin-B1 (downregulated in senescent cells), also indicated a radiation quality-dependent onset of cellular senescence in the mouse intestine. Further, decreased lamin-B1, increased p21, and IL1 protein expression was also noted in GCRsim-exposed mice, relative to gamma-rays and control groups. In conclusion, a radiation quality-dependent increase in the senescent cell population was observed in the mouse intestine. 7) Male C57BL6J mice (8 weeks old) were exposed to either sham, gamma-rays (50 cGy) or acute GCRsim (50 cGy), and intestinal samples were obtained after 150 days of radiation exposure. We investigated various senescence-associated-secretory-phenotype (SASP) molecular markers using qPCR and immunostaining. The qPCR data show at least a 3-fold increase in Cxcl10, Il6, Il1, and Icam1 mRNA levels in GCRsim-exposed mice, relative to gamma-rays and control groups. Immunostaining analysis indicated a significant increase in CXCL10 protein expression in GCRsim-exposed mice, relative to gamma-rays and control groups. In conclusion, a radiation quality-dependent increase in the SASP cell population was observed in the mouse intestine. 8) We exposed lung cancer susceptible mouse models (K-rasLA-1) at the NSRL with fast switching three ion beams: Proton (H) (120 MeV/n) 20cGy, Helium (He) (250 MeV/n) 5cGy, and Silicon (Si) (300MeV/n) 5cGy with a dose rate of 0.5 cGy/min. In these studies, we observed an increase in the incidence of lung cancer initiation and progression. Additionally, when we titrated the dose of HZE ion in the above irradiation protocol, we observed a dose-dependent effect of silicon ions delivered and observed reducing the total dose of silicon from 5cGy to 2cGy and 0.5cGy, progressively reduced cancer progression back to the background rates. 9) To more closely simulate the deep space environment, we exposed lung cancer susceptible mouse models (K-rasLA-1) to acute GCRsim lasting 1-2 hours, more prolonged exposures lasting 10-15 hours, and chronic exposure experiments up to 4-6 weeks with a total dose of 50cGy and 75cGy. We also conducted 25 and 100cGy GCRsim acute experiments. We found a non-statistically significant trend in the increase of malignant adenocarcinomas for an acute 50cGy and 75cGy total dose. 10) We developed and implemented a mechanistically-motivated mathematical carcinogenesis model that includes both TE and NTE components, and applied it to the data on intestinal tumorigenesis in Apc1638N/+ tumor-prone mice. Importantly, when mixtures of several space radiation types, such as protons and different species of heavy ions, bombard the same biological target, it is not a trivial task to quantify the contribution of each radiation type and predict the overall effect of the mixture. This is the case because, when dose response shapes for the mixture components are not linear (e.g., due to NTE contributions), simply adding the predicted cancer risk contributions of each radiation type together will not correctly predict the risk from the entire mixture. In other words, the seemingly intuitive simple effect additivity (SEA) approach is known to be incorrect for curved dose responses, and more specialized alternative synergy theories such as incremental effect additivity (IEA) are required. 11) We employed the incremental effect additivity (IEA) methodology to predict the tumorigenic effectiveness of mixtures of space radiation types, based on the fits of our model to data for individual radiation types: protons (1000 MeV/n; 50 to 120 cGy; 0.22 keV/µm, 40 mice), 4He (250 MeV/n; 5 to 50 cGy; 1.6 keV/µm, 92 mice), 12C (290 MeV/n; 10 to 200 cGy; 13 keV/µm, 60 mice), 16O (325 MeV/n; 5 to 50 cGy; 22 keV/µm, 66 mice), 28Si (300 MeV/n; 5 to 140 cGy; 69 keV/µm, 136 mice), 56Fe (1000 MeV/n; 5 to 160 cGy; 148 keV/µm, 90 mice), gamma-rays (5-200 cGy, 127 mice). At the current stage of the project, a new data set on tumorigenesis after 33-ion GCRsim mixture exposures (25, 50 and 75 cGy; acute and chronic) is being assembled. This type of mixture is more detailed and more realistic of the space exposure environment. We are working on implementing our TE+NTE model formalism and the IEA synergy theory approach on this data set to provide a more accurate assessment and mechanistic explanation for the overall mixture effect, and for the contributions of its individual components.
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Abstracts for Journals and Proceedings	Suman S. "Role of oxidative stress, persistent DNA damage, premature senescence and acquisition of senescence-associated secretory phenotype (SASP) in space radiation-induced carcinogenesis." International Conference on Environment, Water, Agriculture, Sustainability and Health (E-WASH 2022): Strategizing a greener Future and 4th Annual Meet of Save the Environment (STE), Hindu College, Delhi University, Delhi, India, January 12-13, 2023. Abstracts. International Conference on Environment, Water, Agriculture, Sustainability and Health (E-WASH 2022): Strategizing a greener Future and 4th Annual Meet of Save the Environment (STE), Hindu College, Delhi University, Delhi, India, January 12-13, 2023. , Jan-2023
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