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PI Name:	Kirsch, David M.D., Ph.D.		
Project Title:	Duke NSCOR: Lung Cancer Risk from HZE Ions		
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Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
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Zip Code:	27710-0001	**Congressional District:**	1
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Flight Program:			
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Key Personnel Changes/Previous PI:			
COI Name (Institution):	Bloom, Rochelle Ph.D. (Duke University) Yoshizumi, Terry Ph.D. (Duke University) Onaitis, Mark M.D. (Duke University) Stripp, Barry Ph.D. (Cedars-Sinai)		
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Task Description:	The goal of the Duke NSCOR is to understand mechanisms of high charge and energy (HZE) ion-induced lung cancer. To accomplish this goal, the Duke NSCOR has brought together experts in radiation biology, lung cancer development, lung injury and repair, radiation dosimetry, and education. We will combine sophisticated mouse genetics, in vivo lineage tracing, ex vivo isolation of lung epithelial progenitor cells, and analyses of lung cancers induced by HZE nuclei to dissect mechanisms of HZE ion-induced lung cancer. We will integrate 3 separate projects to understand how the cell of origin influences lung cancer development after HZE ion exposure, identify mechanisms of cellular response to HZE ions in different progenitor populations in the lung, and define how and when the p53 tumor suppressor, which is the most commonly mutated gene in human lung cancer, regulates HZE ion-induced carcinogenesis in the lung. We anticipate that our hypothesis-based research will ultimately lead to the development of better models for HZE ion carcinogenic risk assessment for individual astronauts and novel approaches to prevent HZE ion-induced lung cancer through biological countermeasures.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Lung cancer causes more than one million cancer deaths each year and is the leading cause of cancer death worldwide. Despite advances in the detection and treatment of lung cancer, lung cancer prevention presents a major unmet need. While many lung cancer cases are preventable as they are due to smoking, it is estimated that 25% of lung cancer cases worldwide involve never smokers. Though multiple risk factors including exposure to radiation from radon gas have been implicated, no clear-cut cause has emerged to explain the relatively high incidence of lung cancer in never smokers. Lung cancers arising in never smokers demonstrate different mutation patterns and frequencies when compared to cancers arising in smokers, suggesting that lung cancer arising in never smokers is a clinically distinct disease. Indeed, lung cancers in never smokers respond differently to targeted cancer therapies. Further research into the mechanism of lung cancer development in never smokers is needed so that more successful strategies for prevention and treatment of lung cancer can be developed. By studying the mechanisms of lung cancer initiation and development, the Duke NSCOR is generating new knowledge that can be used to develop novel approaches for the prevention and treatment of lung cancer. Lung cancer can be divided into two major forms: small-cell lung cancer and non-small cell lung cancer. Both non-small cell lung cancers and small cell lung cancers have developed in survivors of the atomic bombs in Japan. Similarly, both types of lung cancer arise in smokers. Cancers arising in never smokers preferentially develop in the distal airways and are of the adenocarcinoma histological subtype, which is a type of non-small cell lung cancer. Recently, genomic sequencing technology has been utilized to identify the most commonly mutated genes in adenocarcinomas. Based on this analysis, the two most commonly mutated genes in adenocarcinomas are Trp53, which encodes the tumor suppressor p53, and the oncogene Kras. The Duke NSCOR is utilizing sophisticated genetically engineered mouse models to study the role of p53 and Kras in non-small cell lung cancer. For example, we are studying how mutations in Kras in different kinds of cells in the lung affect lung cancer development with exposure to space radiation. We are also studying mice with an additional copy of p53 or inducible p53 suppression to investigate the timing and mechanism by which p53 suppresses Kras-driven lung adenocarcinoma progression after space radiation exposure. In addition, we are developing a mouse model of radiation-induced small-cell lung cancer. Together these studies will provide new insights into how lung cancer forms, where lung cancers develop, and how Kras and p53 mutation promote lung cancers. As we answer these questions using experiments with space radiation, we expect that our results will not only help us understand how lung cancer develops on Earth, but will also provide new insights into preventing and treating lung cancer. In addition to studying lung cancer development, the Duke NSCOR is also studying lung progenitor cell injury and repair after exposure to either terrestrial or space radiation. Injury and inflammation of the lung are key components of many diseases in people including emphysema, asthma, and lung fibrosis. Furthermore, patients receiving radiotherapy for either primary lung cancer or other neoplasms of the thoracic region (e.g. breast cancer) undergo lung tissue remodeling and declining lung function that is directly related to the dose and location of radiation exposure. By exploring which lung cells are injured by space radiation and how these injured lung cells are repaired, we anticipate that this knowledge may also lead to a better understanding of how lung diseases besides cancer develop and strategies that may be employed to moderate the effects of radiotherapy on lung tissue remodeling. This information may ultimately be used to develop novel approaches for the prevention and treatment of these lung diseases, and the improvement of public health.
	In our fourth year of funding, the Duke NASA Specialized Center of Research (NSCOR) continued to perform the experiments proposed in our application. We have developed two robust models of radiation-induced lung cancer, which we are currently using to determine the effect of space radiation exposure on lung cancer risk. Two members of the Duke NSCOR presented at the NASA Human Research Program Investigators' Workshop in Galveston, Texas from February 11-13, 2014. One of the graduate students from the Duke NSCOR attended the 2014 NASA Space Radiation Summer School from June 2-20, 2014. On December 16, 2014, we will hold an Advisory Committee meeting with external and internal Duke University experts on radiation and lung biology. Through these activities and from our research projects, members of the Duke NSCOR have gained new information about the effects of space radiation on normal lung tissue and lung cancer development. Project 1. The role of the tumor suppressor p53 in space radiation-induced lung cancer. David Kirsch, M.D., Ph.D., Lead We proposed to study the role and timing of the tumor suppressor p53 in radiation-induced lung cancer using mice with an extra copy of p53 (Aim 1) and reversible knockdown of p53 (Aim 2). In addition, we proposed to develop a model of radiation-induced small cell lung cancer (Aim 3). For Aim 1, we completed irradiation and analysis of lung tumor development in KrasLA1 mice predisposed to lung cancer bearing normal levels of p53 or an extra copy of p53. We observed that p53 suppresses lung tumor initiation in the absence of radiation, but that an extra copy of p53 does not affect the proliferation of low grade lung tumors or the expression of pERK, which is a negative prognostic marker. Exposure to neither terrestrial radiation nor space radiation impacted lung tumor initiation in mice with wildtype expression of p53. However, our results suggest that space radiation may increase the grade of lung tumors. For Aim 2, we utilized an in vivo knockdown system that enables temporal regulation of p53 expression in the lungs of mice. We have begun to use this system in combination with the model of radiation-induced lung cancer that we developed in Aim 3 to decrease p53 expression temporarily during radiation exposure or permanently during and

Task Progress:

following radiation exposure to investigate the timing of p53-mediated tumor suppression. Preliminary results suggest that permanent knockdown of p53 reduces the survival of mice following radiation.

For Aim 3, we find that fractionated exposure to terrestrial and space radiation accelerates lung tumor formation in a genetically engineered mouse model of small cell lung cancer and adenocarcinoma. This model will be valuable for determining the relative biological effectiveness with which terrestrial and space radiation cause lung cancer to develop. In the coming year, we plan to expose these mice to varying doses of terrestrial and space radiation to determine whether space radiation is more effective at causing lung cancer.

Project 2. The role of cell of origin in space radiation-induced lung cancer. Mark Onaitis, M.D., Lead

We proposed to study the cell of origin of K-RasG12D-induced lung cancer in response to space radiation. Our aims include studying the effects of 600 MeV/n 56Fe (HZE radiation) on mice in which K-RasG12D is inducibly expressed in different cell types of the lung: Club cells (formerly known as Clara cells) (Aim 1), basal cells (Aim 2), and Type II cells (Aim 3).

For Aim 1, we have irradiated CC10-CreER; lsl K-RasG12D mice with 5 fractions of 0.2 Gy 600MeV/n 56Fe and have CC10-CreER; lsl K-RasG12D controls that were sham irradiated and the lungs analyzed 8 weeks post irradiation. Our data suggest that HZE radiation increases tumor burden, but does not affect the distribution or numbers of tumors. We are currently irradiating more mice with a single fraction of 0.1 Gy 600 MeV/n 56Fe for a low dose comparison. Additionally, another cohort will be irradiated with a single fraction of 0.1Gy 300MeV/n 28Si to compare another ion species. These tumors will all be compared for relative biological effectiveness to terrestrial radiation-induced tumors using mice exposed to 320kVp X-rays at Duke University.

For Aim 2, we generated K5-CreER; lsl K-RasG12D mice and treated them with tamoxifen. Unfortunately, these mice quickly developed tumors in the forestomach and lip causing respiratory occlusions and morbidity. Therefore, we have not been able to characterize the impact of HZE radiation in this model.

For Aim 3, we generated SPC-CreER; lsl K-RasG12D mice and treated them with tamoxifen. Unfortunately, we found that this model was leaky in that many of the mice developed confluent tumors in the lung even before radiation exposure. Therefore, we have not been able to characterize the impact of HZE radiation in this model.

Because the K-RasG12D mutant mice develop widespread tumors causing death of the mouse within 24 weeks after tamoxifen administration, as an alternative approach, we have begun irradiating an Rbflox/flox; p53flox/+ model with the CC10-CreER (Aim 1); K5-CreER (Aim 2); and SPC-CreER (Aim 3) genes in order to assess the effects of radiation in a less penetrant model.

Project 3. Effects of space radiation and p53 signaling on lung progenitor cells. Barry Stripp, Ph.D., Lead

The focus of this project is to compare direct and non-targeted effects of X-rays and HZE radiation on the clonogenic and repair capacity of lung epithelial progenitor cells, and to determine the impact of p53 deficiency on these responses. We have shown that region-specific progenitor cells maintain the specialized epithelium of mouse and human airways and have developed novel mouse models to functionally investigate their behavior in vivo and in vitro. An important feature of our in vitro model used to assess the clonogenic behavior of epithelial progenitor cells is the use of a three-dimensional culture environment in which epithelial cells are co-cultured with stromal support cells.

For Aim 1 we have used in vivo lineage tracing and novel in vitro models that recapitulate epithelial-stromal interactions seen in small airways, to determine how either 320 kVp X-ray or 600 MeV/n 56Fe particles (HZE) impact clonal expansion of epithelial progenitor cells. Lineage tracing coupled with morphometry was used to establish that whole body exposures to either X-ray or HZE were associated with dose-dependent increases in the probability that epithelial progenitor cells expanded to yield large clone sizes within airways. However, in vivo clonal expansion of epithelial progenitor cells was not associated with a significant change in the epithelial proliferative index. Ongoing experiments are using double labeling methods to define the effects of radiation dose and type on the pool size of epithelial progenitor cells in vivo. Moreover, we have initiated pilot experiments to determine how lung injury from either ozone or influenza virus impacts the rate of progenitor cell expansion following radiation exposure. We predict that the range of radiation effects on epithelial progenitor cells from different radiation doses and qualities of radiation will be amplified by environmental triggers that cause epithelial cell injury.

In vitro experiments performed over the past funding period have revealed direct effects of either X-ray or HZE exposure on lung progenitor cells following whole-body exposures. Our ability to couple lineage tracing of epithelial progenitor cells with in vitro clonal behavior has provided a sensitive measure of moderate to low-dose effects. We found that both X-ray and HZE exposures caused a dose-dependent decrease on freshly isolated airway epithelial cells immediately following radiation. Interestingly, when we isolate airway epithelial cells two months after HZE exposure, we see that colony forming efficiency is still significantly decreased. We are currently investigating if the sustained decrease in colony forming efficiency occurs following low-LET radiation exposure as well.

For Aim 2, we used both in vitro and in vivo experiments to assess the role of p53 airway epithelial repair following radiation exposure. We used lineage tracing methods in mice deficient for p53 and assessed clonal expansion following HZE exposure. We found that, while wild type mice had significantly increased clone sizes post-radiation, the p53 deficient mice did not undergo clonal expansion. We are working to uncover the mechanism by which clonal expansion is triggered following radiation exposure and how p53 modulates this response. Additionally, we exposed p53 deficient cells to low-LET radiation, plated them in our in vitro 3-D co-culture assay, and assessed colony forming ability as compared to wild type controls. p53 null cells had a higher colony forming ability at baseline as well as following radiation exposure. We are currently performing live imaging of these cells to determine the mechanism by which colony forming ability is increased when p53 is lost.

Core A: Administrative Core. David Kirsch, M.D., Ph.D., Lead ; Duke NSCOR Administrators: Michelle Cooley, Lisa Hall, Marcia Painter The Administrative Core (Core A) provides overall management of the NSCOR award by ensuring that projects make satisfactory progress. During the fourth year of funding, the Administrative Core has monitored project progress by conducting Duke NSCOR meetings once a month and multiple teleconferences with NASA funded investigators. Minutes were recorded at these meetings in order to ensure that tasks were completed in a timely manner. In addition, we have scheduled our annual Internal Advisory Committee Meeting for December 16th, 2014, which will include two outside experts in lung biology and space radiation. During this meeting, our Project leads will present their current work and the Advisory Committee will provide feedback. Ideas for our NSCOR renewal will also be discussed

at this meeting.

Core A made travel arrangements for the Duke NSCOR team to travel to Brookhaven National Laboratory in Spring and Fall 2014 in order to expose mice to 56Fe and 28Si ions. Travel arrangements were also made for the annual Radiation Research Society Meeting in September. Moreover, the Administrative Core arranged for the two day NASA HRP Investigators' Workshop in Galveston, Texas in February.

Duke NSCOR administrators served as liaisons between the project groups to guide BNL and Duke training and credentialing of new investigators, ensure timely and accurate submission and renewal of IACUC protocols, NSCOR progress reports, as well as application for NSRL Beam Time Request for 2015. Core A provided budget oversight for the Duke NSCOR. Lisa Hall monitored project expenditures. Mrs. Hall met monthly with Dr. Kirsch to review spending and fiscal matters for each NSCOR project and Core. Marcia Painter assisted with the financial accounting for the Duke NSCOR.

Core B: Physics Core. Terry Yoshizumi, Ph.D., Lead

The Physics Core (Core B) provides comprehensive measurements of radiation dose (dosimetry) and oversees the radiation safety of experiments performed by investigators in the Duke NSCOR for experiments with X-rays. By performing routine dosimetry measurements on the standard small animal X-Ray irradiator, the Physics Core provided quality control for radiation exposure experiments. Members of the physics core participate and present physics reports at regularly-scheduled NSCOR meetings. The Core ensures the timely incorporation of new dosimetry technology to provide state-of-the-art dosimetry support. The Physics Core has established collaboration arrangements with X-ray Irradiator manufacturers in developing industry standards for quality assurance and performance improvement.

Core C: Education Core. Rochelle Schwartz-Bloom, Ph.D., Lead

The Education Core (Core C) has developed a problem-based unit to teach high school students about radiation in space by incorporating principles of physics, chemistry, and biology. The unit contains a hypothetical scenario in which a group of young astronauts are selected to travel to Mars in the year of 2040. The astronauts must learn about the types of radiation they will encounter in space (compared to on Earth), the damage these high energy particles and cosmic rays can cause to their DNA molecules, how their bodies can deal with the damage using a protein called p53, and what would happen if their p53 gene has a mutation. They also learn how mutations in p53 genes can increase the risk of cancer, especially of the lung. The astronauts will meet some “virtual” scientists (the PIs of projects 1-3) who study these topics and whose research findings are crucial to the development of a successful space program that includes a trip to Mars. This past year after completion of the unit, it was field-tested in a local high school AP biology class for impact on content knowledge. Students demonstrated significantly increased knowledge of biology and physics principles after working with the unit.

Bibliography Type:	Description: (Last Updated: 03/11/2021)
Abstracts for Journals and Proceedings	Belley MD, Stanton IN, Nguyen G, Yoshizumi TT, Therien MJ. "A nano-particle based fiber-optic detector for in-vivo radiation detection and measurement in medicine." Presented at the Council on Ionizing Radiation Measurements and Standards (CIRMS) Annual Meeting, Gaithersburg, MD, March 10-12, 2014. Council on Ionizing Radiation Measurements and Standards (CIRMS) Annual Meeting, Gaithersburg, MD, March 10-12, 2014. http://www.cirms.org/conference_agenda_2014.html ; accessed 11/14/14. , Mar-2014
Abstracts for Journals and Proceedings	Belley MD, Stanton IN, Langloss BW, Nguyen G, Wang C, Dewhirst MW, Therien MJ, Yoshizumi TT. "Overview of an inorganic nano-crystalline scintillator based fiber optic detector for real-time, in-vivo, small animal therapy dosimetry." Presented at the Second Symposium on Precision Image-Guided Small Animal RadioTherapy, Vancouver, Canada, August 11-13, 2014. Conference proceedings, in press. Second Symposium on Precision Image-Guided Small Animal RadioTherapy, Vancouver, Canada, August 11-13, 2014. , Aug-2014
Abstracts for Journals and Proceedings	Belley MD, Bache ST, Stanton IN, Min H, Kirsch DG, Adamovics J, Oldham M, Therien MJ, Yoshizumi TT. "Real time verification of planned dose during treatment on a small animal image guided radiation therapy treatment system using a nano-scintillator fiber optic detector." Presented at the Second Symposium on Precision Image-Guided Small Animal RadioTherapy, Vancouver, Canada, August 11-13, 2014. Conference proceedings, in press. Second Symposium on Precision Image-Guided Small Animal RadioTherapy, Vancouver, Canada, August 11-13, 2014. , Aug-2014
Abstracts for Journals and Proceedings	Yoshizumi T, Wang C, Belly M, Nguyen G, Chao N, Kirsch D, Dewhirst M. "Small Animal Irradiator Physics Program at a Large Academic Medical Center." Presented at the Second Symposium on Precision Image-Guided Small Animal RadioTherapy, Vancouver, Canada, August 11-13, 2014. Conference proceedings, in press. Second Symposium on Precision Image-Guided Small Animal RadioTherapy, Vancouver, Canada, August 11-13, 2014. , Aug-2014
Abstracts for Journals and Proceedings	Moding EJ, Min HD, Castle KD, Lee CL, Kirsch DG. "An Extra Copy of p53 Suppresses Initiation of Kras-Driven Tumors but Not Radiation-Induced Lymphomas." Presented at the 2014 NASA Human Research Program Investigators' Workshop, Galveston, TX, February 12-13, 2014. 2014 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 12-13, 2014. http://www.hou.usra.edu/meetings/hrp2014/pdf/3288.pdf , Feb-2014
Abstracts for Journals and Proceedings	Lee CL, Moding EJ, Castle KD, Blum JM, Min H, Rodrigues RC, Williams N, Ma Y, Borst L, Kim Y, Kirsch DG. "The Tumor Suppressor p53 Acts During Total-Body Irradiation to Decrease Hematopoietic Stem/Progenitor Cell Fitness and to Promote Lymphoma Development." Presented at the 2014 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 12-13, 2014. 2014 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 12-13, 2014. http://www.hou.usra.edu/meetings/hrp2014/pdf/3313.pdf , Feb-2014

Abstracts for Journals and Proceedings	Rampersad RR, Onaitis MW. "Fractionated High LET Iron Irradiation Effects on K-RasG12D-Induced Tumor Progression." Presented at the 2014 NASA Human Research Program Investigators' Workshop, Galveston, TX, February 12-13, 2014. 2014 NASA Human Research Program Investigators' Workshop, Galveston, TX, February 12-13, 2014. http://www.hou.usra.edu/meetings/hrp2014/pdf/3317.pdf , Feb-2014
Abstracts for Journals and Proceedings	Farin A, Manzo N, Stripp B. "p53 is required for radiation-induced expansion of airway epithelial progenitor cells." Presented at the 2014 Annual Lung Regeneration and Repair Consortium Meeting, Bethesda, MD., September 15-16, 2014. Conference proceedings, in press. 2014 Annual Lung Regeneration and Repair Consortium Meeting, Bethesda, MD., September 15-16, 2014. , Sep-2014
Abstracts for Journals and Proceedings	Farin A, Manzo N, Stripp B. "Trp53 dependent clonal expansion of airway epithelial progenitor cells following high-LET radiation exposure." Presented at the 60th Annual Meeting of the Radiation Research Society, Las Vegas, Nevada, September 21-24, 2014. Conference proceedings, in press. 60th Annual Meeting of the Radiation Research Society, Las Vegas, Nevada, September 21-24, 2014. , Sep-2014
Articles in Peer-reviewed Journals	Farin AM, Manzo ND, Terry KL, Kirsch DG, Stripp BR. "Low-and high-LET radiation drives clonal expansion of lung progenitor cells in vivo." Radiation Research. 2014 In Press, as of November 2014. , Nov-2014
Articles in Peer-reviewed Journals	Stanton IN, Belley MD, Nguyen G, Rodrigues A, Li Y, Kirsch DG, Yoshizumi TT, Therien MJ. "Europium- and lithium-doped yttrium oxide nanocrystals that provide a linear emissive response with X-ray radiation exposure." Nanoscale. 2014 May 21;6(10):5284-8. http://dx.doi.org/10.1039/c4nr00497c ; PubMed PMID: 24696056 , May-2014
Articles in Peer-reviewed Journals	Belley MD, Wang C, Nguyen G, Gunasingha R, Chao NJ, Chen BJ, Dewhirst MW, Yoshizumi TT. "Toward an organ based dose prescription method for the improved accuracy of murine dose in orthovoltage x-ray irradiators." Med Phys. 2014 Mar;41(3):034101. http://dx.doi.org/10.1118/1.4864237 ; PubMed PMID: 24593746; PubMed Central PMCID: PMC3987731 , Mar-2014