Topical: The Need for Biological Countermeasures to Mitigate the Risk of Space Radiation-Induced Carcinogenesis.

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The Risk of Space Radiation Carcinogenesis and Enabling Prolonged Human Presence in Space

The space environment exposes astronauts to not only increased levels of ionizing radiation (IR) but also to IR that is fundamentally different than that experienced terrestrially. Space radiation is composed of high energy charged particles ranging in from protons (H^+) to heavy charged nuclei (HZE) larger than uranium. Moreover, neutrons and other small particles can be produced by the interaction of these charged particles with matter they traverse, including spacecraft shielding.

Epidemiological studies of human cohorts exposed to terrestrial IR, including the Life Span Study of atomic bomb survivors, uranium miners, medical radiation and nuclear power workers clearly demonstrate increased risks of carcinogenesis following IR exposure. However, due to fundamental differences in the way charged particles deposit energy compared to terrestrial radiation, which consists primarily of γ- and x-rays (photons), α-particles (He nuclei), and β-particles (electrons), large uncertainties exist in understanding potential consequences of space radiation exposure on human health.

Because radiation is a known health hazard, The National Aeronautics and Space Administration (NASA) has limited the amount of time astronauts can spend in space, to keep radiation exposures below agency-accepted limits, and minimize adverse health outcomes. However, long-duration exploration missions and eventual human space-habitation could result in an excess relative risk (ERR) of cancer estimate of > 3% (a value used to for terrestrial radiation protection guidance). For example, ERR estimates based on accumulated dose for a three-year mission to Mars range from 2.5 – 5%. A 30-year, space-fearing career could therefore result in an excess cancer risk of 10 times that of a single Mars mission. Therefore, identification of medical countermeasures that significantly reduce the risk of space radiation carcinogenesis is not only necessary, but imperative to enable long duration, deep space exploration, as well as to protect the long term health of human beings, as we become a space-faring species. Advancement of these approaches is essential to address a potential long duration, commercial and military human presence in space, in addition to NASA's scientific and exploratory mission.

While utilizing a traditionally therapeutic agent as a prophylactic in a population of healthy adults was traditionally been rejected by the medical community, due to the potential for unnecessary risk, this paradigm has now evolved, as these kinds of approaches have shown great potential to limit diseases and injuries. For example, for several decade, individuals at high-risk of contracting the human immunodeficiency virus (HIV) have routinely take prophylactic tenofovir with emtricitabine formulations (daily oral administration) that prevent (and treat) HIV infection. The successful development and widespread adoption of these prophylactics has been considered a

public health triumph, drastically reducing the spread of HIV, and dramatically improving quality of life[i]. Furthermore, it sets an important precedence for prophylaxis as a viable strategy to transform health outcomes that is immediately applicable to space radiation carcinogenesis, assuming a successful compound can be identified and validated.

Compound-Based Medical Countermeasures to Mitigate the Health Risks of Radiation Exposure

Traditional compound-based approaches to reduce radiation health impacts include administration of medical countermeasures prior to radiation exposure, to counter the immediate radiation damage (radiation protection), or following radiation exposure, to target and inhibit molecular processes that enhance damage or increase those pathways that promote repair (radiation mitigation). To date, the only FDA-approved compound for radioprotection during radiotherapy is Amifostine (Ethyol), which functions as a free radical scavenger and absorbs reactive oxygen species (ROS) produced by IR exposure. However, the utility of Amifostine in the space radiation environment is limited for several reasons: 1) it must be injected and present in pharmacologically-relevant concentrations during the radiation exposure (space radiation is at a chronic low-dose rate which will require constant or highly regular injections),2) it carries a unacceptable safety profile in healthy populations (Amifostine has been reported to cause severe side effects including severe anaphylactic reactions), and3) it has a short half-life (less than one minute) and an elimination half-life of approximately 8 minutes (less than 10% of Amifostine remains in the serum six minutes after drug administration)[ii]. Radiomitigators that have been approved by the U. S. Food and Drug Administration (FDA) treat the hematopoietic effects of acute radiation syndrome (ARS), such as leukocyte growth factors like granulocyte-macrophage colony stimulating factor (GC-CSF) or megakaryocyte-targeted products like romiplostim are also non-viable options, due to their mechanism of stimulating cells to divide, which may lead to the propagation of late radiation-induced damage, creating an added risk of carcinogenesis[iiiiv].

Therefore, the nature of the space radiation environment leads to a unique and complex question: how can a yearly carcinogenic event resulting from space radiation exposure be reduced or eliminated, to mitigate the long-term risk of radiation-induced carcinogenesis? This question is not a trivial and highlights the immense effort and resources necessary to tackle the challenges it presents.

Current Strategies to Identify Medical Countermeasures and Their Limitations

The Human Research Program (HRP) Space Radiation Element has been evaluating compound-based medical countermeasures for several years on a targeted basis. Due to budget and timeline constraints, this approach has sought to evaluate drugs that are either already FDA approved, or in late stages of the FDA Investigational New Drug (IND) pathway as radioprotectors or radiomitigators for carcinogenic endpoints following space radiation exposure(e.g., aspirin[v], metformin (currently unpublished), 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid, methyl-ester (CDDO-Me - a synthetic triterpenoid compound with anti-inflammatory, anti-tumor and cytoprotective properties)[vi], and avasopasem manganese[vii]. These types of targeted evaluations rely on assessing long-term carcinogenic outcomes in *in vivo* animal models, which are both

expensive and time-consuming, due to the need to use of hundreds of animals, and age them appropriately prior to and following radiation exposure. While these efforts have the potential to identify a single, effective medical countermeasure per study, success is not guaranteed. Furthermore, reduction in carcinogenesis in an animal model may not effectively translate to humans.

A Better Approach: Utilizing Modern Drug Screening and Big Data Techniques to Perform Large-Scale Compound Screening and Targeted Compound Development

Two approaches could potentially be leveraged to identify a compound, or suite of compound-based medical countermeasures that effectively reduce the risk of radiation carcinogenesis. While both approaches individually offer a pathway to compound discovery, an ideal strategy would be to invest in the labor, capital, and time required to perform both unbiased screening and data-driven target discovery in parallel.

The first is an "unbiased" approach, leveraging existing, high-throughput screening facilities located within U. S. institutions to evaluate existing compound libraries consisting of FDA approved, drugs for which INDs are being pursued,, non-FDA approved but previously characterized, and novel compounds, for efficacy against radiation carcinogenesis. Some of these compound libraries number in the hundreds of thousands and target molecular and cellular processes across many human biological endpoints. The timeline for such a strategy would likely range from 5-15 years, depending on the current FDA approval status and preliminary information available for candidate compounds. The first two to five years would require development and implementation of reproducible techniques to perform high-throughput screenings to identify compounds capable of reducing the risk of carcinogenesis. These approaches could utilize classical techniques like anchorage-independent growth in soft agar, or cytogenetic analysis of surrogate markers such as DNA damage repair foci, oxidative stress probes, or clonogenic cell viability assays. Novel techniques and/or assays may need to be developed to identify appropriate candidate compounds, a process that could potentially go beyond five years. Implementing a battery of assays may be needed to adequately capture the unique challenge of preventing early carcinogenic events from occurring, without promoting adverse side effects. A few notable compounds that have previously been evaluated (e.g., metformin and CDDO-Me) could be used as positive controls to support selection and development of assays. Once candidate compounds have been identified, validation of efficacy can be conducted in rodent lifetime follow-up studies and/or advanced, humanized systems (e.g., tissue chips), which can take up to an additional five years to complete. While labor-intensive and costly, this approach offers a high likelihood of success in identifying candidate medical countermeasures to reduce the carcinogenic risk following space radiation exposure, by providing an unbiased approach that leverages large, existing libraries. Additionally, once successful compounds are identified through screening, they could be modified to improve safety and/or efficacy. This approach has led to a rapid explosion of drug discovery in disease states as varied as cancer, Alzheimer's, and heart disease[viii, ix].

The second approach would be a targeted or informed one, to identify target pathways that lead to radiation carcinogenesis. This strategy would take advantage of cutting edge "-omics" analyses, including RNA and whole genome sequencing, proteomics, metabolomics, etc. to generate large

data sets to identify critical pathways that could be selectively targeted to inhibit carcinogenesis. Leveraged models could include, but are not limited to, *in vitro* primary cell culture systems, induced human pluripotent stem cells, human tissue-on-a-chip technologies, genetically inbred or outbred mouse strains, , larger animals such as minipigs or nonhuman primates, or potentially, existing human tissue repositories to explore target pathways. Utilizing these kinds of laboratory models could quickly result in the generation of large data sets across a variety of tissues that could be leveraged with emerging "big data" technologies. These studies could also accelerate fundamental understanding of the interaction of space radiation with human tissues, as well as identify pathways for medical countermeasure targeting and potential biomarkers that could be monitored post-flight to aid in the early detection of space radiation-induced cancers. Advanced computing resources, including machine learning, deep learning, and neural networks can accelerate exploration of both fundamental mechanisms of space radiation exposure and medical countermeasure identification. In addition to providing candidate targets for medical countermeasure efficacy, this approach would also expand the understanding of space radiation carcinogenesis across a broad array of endpoints. Dataset generation would likely take one to three years for *in vitro* and *in vivo* rodent models, while big data technologies could be applied within one to two years, once appropriate datasets have been generated.

An additional five to ten years may be needed if FDA approval through the Animal Rule pathway[x] is required for any novel candidate compounds identified through these approaches. In parallel, specific processes will need to be developed to ensure appropriate transition of research results to successful spaceflight operations, including development of measurable thresholds for medical countermeasure implementation, health surveillance strategies, and risk trade space analysis. Thus, identification, validation, and implementation of compound-based medical countermeasures for long-duration spaceflight could take nearly twenty years complete. With Mars missions currently scheduled to take place starting in 2039, it is imperative that the foundations for these studies are put into place immediately to ensure that risks associated with these missions do no exceed acceptable limits, and to protect astronaut health and well-being beyond their spaceflight career.

The Payoff at Home and Beyond

Identification and validation of effective compounds for mitigating the risk of space radiation carcinogenesis will likely have the added benefit of providing mitigation for the risk of carcinogenesis due to a number of other factors, including genetic predisposition, and exposures to terrestrial radiation or other carcinogens. Such a compound could potentially be administered to radiation workers, individuals, or populations at high risk for developing genetically driven tumors, individuals or populations exposed to radiation from a nuclear explosion, and radiotherapy patients. Not only would quality of life improve, but also the economic cost of cancer could be drastically reduced by providing a viable avenue for broad cancer prevention. Thus, the benefits of a space radiation anti-carcinogenesis medical countermeasure could apply both in space and back on Earth – extending the potential life-saving capability from a few dozen to millions.

Recommendations:

- Invest in both physical and human infrastructure to leverage advanced human models (induced pluripotent stem cells, tissue chips, humanized rodent models, and large

laboratory animal species) to improve mechanistic understanding of space radiation-induced carcinogenesis, identify potential early biomarkers of late disease, and establish viable medical countermeasure targets.

- Large -omics data sets derived from human samples should be generated utilizing the NASA Space Radiation Laboratory at the Brookhaven National Laboratory that can be leveraged, in conjunction with cutting edge big data and informatics techniques, to expand the understanding of space radiation carcinogenic processes and identify pathways for targeted medical countermeasure development.
- Perform high-throughput compound library screening to determine if existing, FDA-approved, IND in process, or exploratory compounds can be repurposed to mitigate the risk of space radiation carcinogenesis.

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[i] Mayer et al., "Emtricitabine and Tenofovir Alafenamide vs Emtricitabine and Tenofovir Disoproxil Fumarate for HIV Pre-Exposure Prophylaxis (DISCOVER)."

[ii] Singh and Seed, "The Efficacy and Safety of Amifostine for the Acute Radiation Syndrome."

[iii] Singh, Newman, and Seed, "Colony-Stimulating Factors for the Treatment of the Hematopoietic Component of the Acute Radiation Syndrome (H-ARS)."

[iv] Singh et al., "Animal Models for Acute Radiation Syndrome Drug Discovery."

[v] Suman et al., "Effects of Dietary Aspirin on High-LET Radiation-Induced Prostaglandin E2 Levels and Gastrointestinal Tumorigenesis in Apc1638N/+ Mice."

[vi] Eskiocak et al., "CDDO-Me Protects against Space Radiation-Induced Transformation of Human Colon Epithelial Cells."

[vii] Sishc et al., "Avasopasem Manganese Synergizes with Hypofractionated Radiation to Ablate Tumors through the Generation of Hydrogen Peroxide."

[viii] Paik, Chandy, and Wu, "Patient and Disease-Specific Induced Pluripotent Stem Cells for Discovery of Personalized Cardiovascular Drugs and Therapeutics."

[ix] Qian and Tcw, "Human IPSC-Based Modeling of Central Nerve System Disorders for Drug Discovery."

[x] (21 CFR 314.600-650 for drugs; 21 CFR 601.90-95 for biologics; effective July 1, 2002)