Medical countermeasures for extraterrestrial environments: Current status and future prospects with focus on acute injuries

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Abstract

In all space exploratory activities involving humans there are associated risks to health and well-being. Exposure to ionizing radiation represents one of the more serious health concerns. Although medical issues can arise shortly following the rare acute exposures, the more likely radiation-associated injuries will manifest in a delayed fashion following sub-acute exposures or protracted exposures to relatively low radiation doses. The radiological conditions that present these health risks during extraterrestrial space travel are presented in this article, along with current physical and medical countermeasures for such exposure contingencies. The aim of this article is to discuss radiation medical countermeasures that may be considered for future space exploration and travel. Biomedical advances have occurred toward the control and minimization of acute, sub-acute, and fractionated radiation exposure injuries, whether they stem from intentional application of radio-therapeutic procedures or unintentional, accidental terrestrial-based exposures. Those advances, based largely on novel bioengineering, genetic, and combinatorial pharmaceutical strategies, are briefly reviewed here, along with the promising prospects of additional, new and improved medical countering systems/agents that will be forthcoming with additional research and development efforts.

Key words: Acute/chronic radiation injuries; galactic cosmic rays; medical countermeasures; space radiation; solar particles; therapeutics

Introduction

The health risks associated with space travel are enormous, but largely manageable with proper assessments and suitable implementation of technologies designed to reduce those risks [1-3]. Considering the National Aeronautics and Space Administration's (NASA's) ambitious future mission plans (e.g., return to the lunar surface with the establishment of a semi-permanent outpost, and possible missions deep into the solar system, such as to the red planet, Mars) and the hazards they present, the agency's Human Research Program has analyzed and prioritized over thirty such unique health risks in terms of mission readiness. These risks need to be evaluated by specific analytics and to find the means to mitigate and control those risks (or simply accept those risks) [1, 2]. Three of the highest level risks, as per estimated rate of occurrence and greatest impact on health and performance during the mission and/or afterward, include that are impacted by space radiation exposure, to varying degrees, include: (1) carcinogenesis; (2) cardiovascular disease; (3) behavioral health and performance decrements [4].

Radiobiological considerations

Ionizing radiation (IR) exposures of different qualities and of sufficient doses and intensities can cause serious injury to vital cells and tissues of the exposed individual. These injuries will be expressed either early or in a delayed fashion following IR exposure, and will manifest as functionally disruptive structural, physiological, or genetic changes within select organs or organ systems of the body; i.e., pathologic processes within given organs [5, 6]. IR is indiscriminate in terms of targeting of essential biomolecules; this is due to IR's very basic nature of being highly energetic atomic particles/waves that can ionize, and in turn damage, essential cellular components either through direct or indirect means. The indirect route dominates in terms of injury induction and involves the splitting of cellular water by IR (low

linear energy transfer (LET) type such as gamma or X-rays) and the production of free hydroxyl radicals that in turn attack and damage vital cellular constituents [5, 7, 8]. It has been estimated that the major fraction (e.g., two thirds or greater) of all single-strand DNA damage is attributable to hydroxyl radical attack following radiation exposure [7]. High LET type radiation exposures (e.g., heavy ions/particles) provide the exception to the latter, in terms of a direct attack of ionization and damage to vital cellular components [5]. Extraterrestrial space environments with high LET type radiation exposures might pose significantly greater radiological risks with significantly greater health impacts than would Earth-bound terrestrial environments [2].

It has been estimated that astronauts subjected to a major solar particle event (SPE) while traveling within an aluminum (10 g/cm^2 space vehicle outside a low earth orbit would be exposed to sizable IR doses, in the range of ~0.07 Gy-Eq and 0.2 Gy to hematopoietic and cutaneous tissues, respectively [9]. However, if the astronauts were in the process of conducting extravehicular work during such a massive SPE event, the estimated radiation doses for these two vital organ systems would be much higher and clearly more hazardous (i.e., IR doses between 1.38 Gy-Eq and 28 Gy estimated for these organ systems), worst case scenario with doses acquired over 63 hours [9]. Planned extended space missions (i.e., interplanetary travel) will result in sizable cumulative exposures (e.g., 1.50-0.55 mSv/day) even under normal conditions of space-weather [10,11]. More specifically, cumulative GCR doses have been estimated at 950-1256 mSv for Mars design reference missions [11]. For beyond low earth orbit, minimal contribution from SPE to cumulative dose are expected due to shielding requirements [12].

Based on prevailing experimental and clinical evidence, chronic exposures to fluences of high LET space particles will pose significant health risks as well; in particular, those risks extend to vision, cognition and behavioral problems, along with hematologic, cardiovascular, pulmonary, gastrointestinal and urogenital syndromes [1, 13-19]. Late-arising cancers represent another major concern [20, 21].

Radiological exposure conditions of major concern within the extraterrestrial space environment certainly include, but are not limited to: (i) solar flares of varying intensities, but all uniformly rich in highly energetic protons, a type of IR generally considered to be a fairly low LET IR; and (ii) protracted streams/fluxes of galactic cosmic radiation (GCR) that are high LET heavy ions and particles [2, 22]. Further, as GCR interacts with metallic sheathing of the space craft, additional IR sources or showers of generally lower energy subatomic particles (neutrons, protons, electrons) are generated, which need to be considered and managed as well [23, 24]. Additionally, GCR or other similar high LET particles pass through soft bodily tissues and progressively tend to lose energy via ionization over the distance traversed, with lower energy spurs, or delta rays, forming penumbras of energetic particles/waves that are subsequently absorbed by tissues [25]. In sum, these radiological conditions associated with extraterrestrial environments present a fundamental problem in terms of radiological protection and need to be considered. The basic mantra of the terrestrially-based health physicist is based on three fundamentally important actions, the three pillars of radiation protection: minimize the time of radiation exposure, maximize the distance between the radiation source and the individual, and use shielding whenever possible. Clearly in the extraterrestrial setting in space, these basic strategies become problematic. First, the astronaut cannot effectively distance within the confines of the space craft; second, time of exposure is generally not adjustable by actions taken

by the astronaut, but rather fixed by the space environment; and third, effective shielding is possible, but limited in terms of basic vehicle design and the use of specially constructed, radioprotective ‘shelters’ (space radiation storm shelters) within the confines of the space vehicle that would be used specifically during intense solar particle flares and fluxes [26]. In general, the best of those shields will be able to block only a limited spectrum of IR. Aboard the space station, the use of hydrogen-rich shielding such as polyethylene in the most frequently occupied locations, such as the sleeping quarters and the galley, has reduced the crew's exposure to space radiation.

The application of safe and effective medical countermeasures for the radiological hazards of the extraterrestrial space environment might be considered to be the fourth pillar of space-associated radiation hygiene. However, this pillar still remains to be fully researched, developed and implemented, along with post-deployment testing and evaluation of effectiveness. These health hazards most prominently include, but are not limited to acute SPE-associated radiation injuries of the skin and of the major vital, internal organ systems of the body (e.g., blood forming and cutaneous systems) and delayed pathologies associated with chronic GCR-type exposures, e.g., central nervous system disturbances (cognitive deficits, motor functions, etc.) and cancer [1, 19, 26-30]

Active coordination between NASA and the United States Food and Drug Administration (FDA) in the development and authorization of space radiation medical countermeasures is required. The application of the FDA’s Animal Rule for countermeasure development and authorization applies to the extraterrestrial space environment [31]. In brief, the Animal Rule was issued by the FDA in 2002 to expedite the development of new drugs and biologics as medical countermeasures against chemical, biological, radiological, and nuclear (CBRN) threats.

This rule applies only to new countermeasures for which conclusive human efficacy investigations under phase II and III clinical trials cannot be performed due to ethical reasons. According to this rule, the FDA can approve new drugs that have been shown to be safe in humans under phase I and effective based on well-controlled animal efficacy studies.

Current status

Physical countermeasures for extraterrestrial space environments are limited relative to those that are terrestrially-based; examples include specific space craft shielding and sheltering devices, as well as in-flight dosimetry and early warning systems for solar flares and associated SPEs, as well as significant fluences of GCR [26]. In-flight radiation exposure-specific medical countermeasures are limited as well, relative to both the number and scope of effective medicinals. As a consequence, the limited nature of the on-board pharmacy has a potential negative impact on space missions, especially those of long duration and that are outside of the low-Earth orbit. Clearly, mission success hinges on the health and performance of the astronaut crew and, in turn, on having a safe and effective onboard pharmacy [32]. However, improving the 'spacecraft pharmacy' is not without challenges, whether they be scientific, logistically or regulatory by nature. Critically important inflight research studies concerning drug pharmacology (along with efficacy, safety and stability) of those drugs of interest are often limited and require the use and extrapolation of data from terrestrial-based work [32].

According to a recent report, the radiation countermeasure component of the on-board emergency medical kit consists of five basic items (Table 1) and all are designed to clinically manage the acute effects of intense SPE exposures (i.e., solar flares) [26]. There are, however, no fully approved medicinals within NASA's space medical kit designed to specifically counter late-arising pathologies associated with chronic exposure to GCR during prolonged space travel.

Agents intended to be used to clinically manage (and/or prevent) acute SPE exposure-related syndromes are as follows: 1) anti-emetic agents, ondansetron (Zofran) and dexamethasone (Decadron); 2) anti-diarrheal agent, Imodium; 3) antimicrobials/anti-infectives, ciprofloxacin, cephalosporins penicillin, and macroclides; 4) skin topicals for burns/blistering, silver sulfadiazine, corticosteroid creams, topical crystalloid solution, sterile gauze and opioid analgesics; and 5) fluids to manage dehydration. [Note: all these medicinals have been evaluated previously, not only for stability, but also for storability over time [32]. Incorporating a sixth type of medicinal, or more precisely a group of recombinant hematopoietic growth factors (GFs), is currently under consideration for inclusion into the medical emergency kit as well. This group currently consists of the recombinant granulocyte colony-stimulating factor (G-CSF, Neupogen), PEGylated G-CSF (Neulasta), and granulocyte-macrophage colony-stimulating factor (GM-CSF, Leukine), and all are designed to mitigate potentially severe, life-threatening hematopathologic effects (i.e., hematopoietic component of the acute radiation syndrome, H-ARS) of intense SPE exposures during space flight [29, 30, 33-35]. A fourth recombinant GF, Nplate (romiplostim), has been recently approved by the FDA for comparable indications in treating individuals (Earth-bound patients) suffering from acute and intense, unwanted radiation exposures that are at high risk of developing severe thrombocytopenia (suppressed blood platelet levels), uncontrolled bleeding, and consequent fatal outcomes (Table 2) [36]. The clinical use of these recombinants requires prior understanding of an astronaut's hematological status, especially in the days following acute SPE exposure events: Does the blood profile of the astronaut warrant the use of these agents, i.e., is the individual sufficiently granulocytopenic or thrombocytopenic to justify medical intervention? If not, simple follow-up monitoring using on-board clinical assessment tools is essential (automated blood cell counting and cell differential devices). (Note: the

advanced preclinical large animal (nonhuman primate – NHP) studies used to evaluate the efficacy of various recombinant GFs have been conducted largely by using male animals; only in the rhuGM-CSF/Leukine studies has there been a relatively equal distribution of male and female animals employed.)

As a precautionary measure against solar flares and intense SPE exposures, an additional radioprotective agent, namely amifostine, has been carried by astronauts previously during select space (moon) missions; however, as it turned out, the radioprotector was not needed, hence not used. *(Note: The nature and utility of this agent or like agents is discussed later in the manuscript.)*

General strategies for developing and improving space associated radiation medical countermeasures

One of the major health hazards of deep space travel is the risk of excessive radiation exposures that occur both acutely and chronically. Intense solar flares and associated SPE largely contribute to the risk of acute radiation injury, whereas the ubiquitous, more chronic GCR exposure during space travel is the main concern relative to late-arising or delayed-type injuries. As indicated, basic strategies and associated current medical protocols for countering SPE-associated acute injuries have been developed and implemented, but rely heavily on post-exposure treatments, rather than on the use of preventive or mitigative medicinals. An improved medical strategy might incorporate the selective use of prophylactic/preventive agents, in combination not only with post-exposure injury mitigators, but perhaps therapeutics as well. A polypharmaceutical approach might yield significant improvements, especially if the approach is oriented toward injury prevention and bolstering native radioresistance of the individual astronaut; e.g., the selective use of synergizing combinations of radioprotective pharmaceuticals

and nutraceuticals (Figure 1) [37]. The latter approach to injury prevention would likely serve not only as an effective strategy in medically countering solar flare-associated SPE exposures, but also chronic GCR exposures and related late-arising injuries as well.

Future prospects for improving space radiation related medical countermeasures

Simply stated, the future looks promising in terms of developing and deploying safe and effective medical countermeasures for future space travelers subjected to potentially hazardous space radiation. Both high tech (newly designed, bioengineered or chemically engineered pharmaceuticals), as well as simpler, more conventional approaches (e.g., use of repurposed medicinals and the use of nutraceuticals) will no doubt come into play (Figure 1) [38]. Regardless, due to the current limited number of such medical radiation countermeasures for extended space travel, coupled with the significant recent scientific, terrestrial-based biomedical advances, there is every expectation that new and improved medicinals for the space traveler will be forthcoming. Nevertheless, there is no doubt that significant challenges lay ahead in getting such improved medicinals into the medical kits of space crafts for future exploratory space missions. Such challenges rest not only on the uncertainties surrounding the impact of various unique stressors (e.g., microgravity) of the space environment on basic drug pharmacology, storage and stability issues, but also on probable changes in the astronaut's basic health and nutritional status that would occur over a prolonged period of time in space.

High tech bio- and chemical engineering approaches

Basic discovery of new radioprotective agents via large scale production and screening technologies. This approach is based on the effective utilization of advanced synthetic and analytic systems for the production and subsequent screening of large arrays of chemical/biochemical/biologic agents with potential, radiation exposure/injury-countering

activities. In general, this approach is actively utilized by biomedical researchers, as well as by the pharmaceutical industry at large; however, in general, it has been underutilized by the radiation biology research community, but it is an approach that clearly needs to be exploited [39, 40].

Specifically, this approach is defined by the combined use of chemical, pharmaceutical and natural product libraries coupled to high throughput screening systems in order to identify brand new, novel chemical or biologic agents that have molecular or cellular targets with defined functionalities. These synthetic/analytic systems have been shown to have significant flexibility and can accommodate a wide variety of research needs and interests; e.g., the potential to conduct phenotypic screening, drug repurposing, and matrix combination screenings of appropriate libraries [41].

Chemical engineering of native or synthetic radioprotective agents. It is true that "new medicines require new molecules" and that such novel molecules often come from direct chemical synthesis that follows rationally designed procedures that are augmented by artificial intelligence (AI) by the chemical engineer, which can employ a basic set of chemical engineering tools/principles such as chemical reaction kinetics, thermodynamics, fluid mechanics, and heat and mass transfer [42-44]. The construction of the chemical library is a key component of this new molecule/new medicine discovery process [45]. The radiobiology researcher interested in searching for novel radioprotectants and/or radiomitigators need not 'reinvent-the-wheel,' for chemical libraries that are currently available within both the public and private sectors for large scale, high throughput screening for agents having specific functionalities/phenotypes (e.g., specific radioprotective characteristics).

The application of large scale screening of available chemical libraries, however, is not the only viable approach in developing useful medical countermeasures for radiation exposures. The chemical or genetic reengineering of previously well-recognized, specific radioprotective or radiomitigative agents, such as genistein (a natural soybean derived phytoestrogenic motiety), bacterial flagellin (a highly immunogenic, proteinaceous molecule), or novel sulfone-class chemical agents, have all proven to be suitable substrates for such reengineering of select natural products into tentatively useful radioprotective medicinals [46-53]. A sampling of reengineered radioprotective drugs that might be useful for use within the extraterrestrial space environment is listed in Table 3.

BIO 300, a novel formulation of genistein: Genistein, 4',5,7-trihydroxyflavone, has demonstrated radioprotective efficacy and is remarkably well-tolerated, as demonstrated in numerous preclinical studies using both small and large animal models of ARS associated injuries [50, 52, 54-57]. [Note: Although 'age' and 'gender' matching processes have been applied in some of these studies, the majority have utilized solely male animals of a standard age].

Under select conditions of prophylaxis, namely subcutaneous (*sc*) injection of genistein over a wide range of doses (25 - 400 mg/kg, ~24 h prior to exposure) provided significant levels of radioprotection (relative to vehicle-treated controls) to subsequent lethal doses of whole-body gamma irradiation in CD2F1 male mice [50, 57]. Furthermore, genistein prophylaxis was exceedingly well tolerated at doses as high as 400 mg/kg, as evidenced by assessments of locomotor activity, grip strength and motor coordination, as well as by body weight, testes weight, and histopathology [57]. Nevertheless, genistein has proven to be challenging from both manufacturing and practical-use perspectives; this is largely due to its low water solubility and

its limitation as primarily an injectable. The preferred oral route was not practicable due to the agent's relatively poor oral bioavailability. The use of synthetic processes (i.e., wet-nanomilling) of genistein to generate nanoparticles has yielded a new genistein formulation (BIO 300) that has significantly improved bioavailability while still retaining significant radioprotective activities to various organ systems regardless of the route of administration [50, 52, 54]. The new BIO 300 product when administered *sc* as single doses 24 – 12 h before total-body irradiation (TBI, 9.25 Gy ^{60}Co γ-radiation), significantly improved 30-d survival in mice [50, 52]. Further, single doses delivered by intramuscular (*im*) injections were also effective when administered up to 2 d prior to acute, lethal radiation exposures. However, BIO 300's optimal dosing regimen was found to be a dose of 150 mg/kg administered 24 h prior to radiation exposure, resulting in a calculated dose reduction factor (DRF) of 1.16 [50]. However, unlike the single *im* injection, optimal oral administrations required twice a day dosing for multiple days prior to radiation exposure in order to demonstrate significant, comparable levels of radioprotection achieved by BIO 300 injection. Interestingly, the level of BIO 300-mediated prophylactic radioprotection is comparable to that seen with a single *sc* injection of the recombinant Neulasta administered after TBI [52].

Prophylaxis with the BIO 300 nanosuspension appears to attenuate irradiation-associated induction of a proinflammatory cytokine storm; namely, the attenuation of interleukin-1β (IL-1β), IL-6, and cyclooxygenase-2 (COX-2) in mouse bone marrow and spleen. The latter may well serve to protect vital stem and progenitor cell populations within hematopoietic tissues, augmenting subsequent recovery of blood neutrophil and platelet levels [50, 52].

It is important to note that genistein has been shown to decrease adverse effects of radiotherapy and chemotherapy in clinical trials with cancer patients [46]. The oral form of BIO 300 is currently being evaluated in cancer patients undergoing chemotherapy (carboplatin and

paclitaxel) and radiotherapy (radiation exposure of 1.8 – 2 Gy fractions for a total of 60 -70 Gy) for non-small cell lung cancer (NSCLC). Patients received BIO 300 oral formulation daily at doses of 500 mg, 1,000 mg, or 1,500 mg for up to eight weeks. This study is being conducted at multiple medical centers [46]. The oral form of BIO 300 is currently also being evaluated in a phase 2 trial in COVID-19 patients recently discharged from the hospital to determine its effectiveness to mitigate long-term pulmonary injury. In both oncology and COVID-19, the drug's ability to attenuate the inflammatory response resulting from radiation exposure or viral infection is central to therapeutic potential [46].

Bacterial flagellin and its reengineered analog entolimod (CBLB502). The second example of a successfully reengineered, natural product is entolimod, a laboratory modified protein extracted from the flagella of select bacterial species. Similar to native flagellin of Salmonella species, entolimod has been shown to elicit strong radioprotective effects by stimulating natural killer cells and T lymphocytes through interactions with specific Toll ligand receptor-5 (TLR5) and the subsequent activation of nuclear factors- κB (NF-κB) [51, 58-60].

Although native flagellin is less than ideal as a potential radioprotectant due to strong immunogenicity, a more useful product was produced by selective chemical/genetic engineering. A truncated form of the flagellin protein with markedly improved characteristics was synthesized, researched and developed as a potential radiation countermeasure [51, 60]. Relative to the lab-engineered protein, the stability and radioprotectiveness of the native protein was retained, while immunogenicity of the protein was substantially reduced. Since its development, entolimod demonstrated significant potential as a medical countermeasure, especially in terms of its capacity to counter the development of two major ARS sub-syndromes, H-ARS and GI-ARS, in murine and NHP models [51, 58-60]. [Note: In early radioprotection studies, female NIH

Swiss/ICR mice were utilized, whereas both male and female NHPs were used for the large animal studies]. However, the lingering immunogenicity of the agent has proven to be problematic, as evidenced by the results of an initial, Phase 1 clinical trial of entolimod's safety and efficacy [61]. Entolimod was found to induce immune response due to memory from earlier exposure to flagellated Enterobacteria [61]. Subsequent development of this agent, however, provides testimony to the utility of current genetic engineering and synthetic biochemical technologies in the research and development (R&D) of ARS countermeasures. A structure-guided reengineering of entolimod was pursued and successfully yielded the next-generation variant, GP532, that was substantially deimmunized, but that retained capacity to induce TLR5-dependent NF-κB activation. The GP532 variant is smaller than the parent agent and has selective mutations that effectively eliminate B and T-cell epitopes and an inflammasome-activating domain [61]. The variant is resistant to human entolimod-neutralizing antibodies, with improved bioavailability and a longer-lasting effect on NF-κB. Most importantly, the new variant, much like the parent, retains potent prophylactic and therapeutic efficacies for irradiation-induced injuries (as per results of mouse model study) [61]. The recent report now suggests that the new and improved entolimod variant has been optimized as a TLR5 agonist and is suitable for patients with high titers of pre-existing flagellin antibodies. Nevertheless, additional assessments of the efficacy and safety of the new entolimod variant is not only warranted, but essential in order to move this agent forward in the process of securing FDA approval for use in treating and managing ARS. In this regard, it should be noted that at least one other 'entolimod-like' agent (KMRCO11) has been bioengineered and fully tested for its radioprotective attributes [62-64].

Biochemical engineering using recombinant DNA technologies. Recombinant DNA technologies permit the novel synthesis of a variety of biologics from living cells (commonly yeast, bacteria, or mammalian cells) that have been artificially redesigned to produce agents not naturally produced by those cells. The technology is now decades old, having initially produced a number of seminal pharmaceuticals and industrial products for global markets [65]. As in the above mentioned situations, the use of these cutting edge technologies has been quite limited in terms of developing novel medicinals for the specific use of countering IR exposures and associated radiation injuries, regardless of whether those radiation exposures are terrestrially or extra-terrestrially based. Nevertheless, the application of these technologies has yielded a number of widely used recombinant GFs that have proven useful in treating a variety of blood disorders (e.g., cancer treatment-induced acute cytopenias) and with a select few have repurposed for treating individuals exposed acutely to unwanted IR (i.e., accidental IR exposures, etc.) who are suffering from H-ARS. This group of recombinants include filgrastim (rhuG-CSF, neupogen), PEGfilgrastim (rhuPEG-CSF, neulasta), sargramostim (rhuGM-CSF, leukine), and romiplastim (rhuTPO, Nplate) and all have been authorized for use by the FDA for H-ARS (as radiomitigators) [40, 66-75]. Additional details on these agents are provided below and in Table 2.

Molecular engineering and the production of chimeric molecules. As pointed out in a previous report "Growth factors and cytokines are integral to tissue microenvironments and the important roles they play in both health and disease, especially in terms of modulating pathogenic processes [40]." They are key components associated with infection, inflammation, and immunity and are double-edged in terms of their capacity to help or to harm the individual. Reengineered (both genetically and chemically) forms of GFs/cytokines have been developed

and investigated over many decades for their therapeutic potentials, including the potential to mitigate and treat acute radiation injuries. New types of recombinant GFs/cytokines with extended therapeutic potentials have been produced; one of these new types are chimeric molecules and are commonly referred to as superkines [76]. The following two chimeric recombinants, namely myelopoietin (MPO) and stable chimeric fibroblast GF (FGF-C), have been tested in a preclinical research setting for their capacity to mitigate acute, potentially fatal radiation injuries in NHPs and rodents, respectively. MPO is a genetically engineered chimeric agent which binds to the G-CSF and IL-3 receptors on hematopoietic cells. The chimeric recombinant has been assessed for its capacity to mitigate potentially fatal hematopoietic injury of male rhesus macaques following acute, whole body γ-irradiation (either 7 or 6 Gy of gamma rayGy). MPO treatments limited both the depth and the duration of the acute irradiation-induced neutropenia and thrombocytopenia, as well as apparently sparing (and promoting recovery) vital bone marrow compartments (e.g., multilineage progenitors) [77-79].

Beyond the design and development of chimeric GFs that solely target hematopoietic tissues, other types of chimeric GFs for other ARS sub-syndromes (e.g., gastrointestinal sub-syndrome or GI-ARS) have been developed and proven to be promisingly reparative in terms of irradiation-associated gastrointestinal tissue injuries. For example, FGF-C is one of such chimerics that is formed by the union of FGF-1 and FGF-2, which strongly stimulates epithelial cell proliferation and, consequently, is endowed with significant reparative activity. When the FGF-C is administered either prophylactically or during the early period following radiation exposure, the agent appears to be efficacious (rodent model of radiation injury) in terms of both protecting and/or repairing epithelia when administered either prophylactically or in the early period following radiation exposure. FGF-C treatments appeared to limit irradiation-induced

apoptosis (cell death), promote crypt cell cycling and the depth of crypt cells, and increase epithelial differentiation in general [80].

Radioprotective gene transfer. Basic elements of radioprotective gene transfer technologies and platforms have been pursued experimentally for several decades and with some success clearly being achieved [81-99]. The superoxide dismutase (SOD) gene family and its three principal metalloprotein gene products have been a favorite molecular target for attempting to mitigate the adverse, injurious effects of IR-generated superoxide ($O2^-$) and related reactive oxygen species (ROS) within sensitive, vital cells and tissues of the body.

In an early study by Suresh *et al*., several neoplastic human cell lines (i.e., erythroleukemic cells - K562 and melanoma cells - A375) were transfected via electroporation with retroviral constructs bearing the human manganese superoxide dismutase (MnSOD) gene in both the sense and anti-sense orientations, and these different genic constructs were able to modulate the expression of radioprotective MnSOD protein within the transfected cells [81]. In yet another in vitro study, pre-neoplastic murine hematopoietic progenitors (32D cl3) gained radioresistance as they overexpressed MnSOD following transfection with a plasmid containing the human MnSOD gene [91].

The capacity of transfected MnSOD to exert increased radioresistance in intact animals was subsequently demonstrated: in studies with experimental female mice (C3H/HeNHsd) receiving either single or fractionated doses of local (head and neck) irradiation that had been treated orally with liposome-encased MnSOD-plasmid preparations fared better than did placebo-treated control animals. Clinical improvements were noted in terms of decreased mucosal ulceration, lessening of weight loss and overall improved survival [96]. Additional gene transfer platforms for the SODs have been developed for several different tissues/organ systems,

including adenoviral and lentiviral vectors for both acute and delayed, late-arising radiation injuries of pulmonary, vascular and cutaneous tissues [92, 95, 100]. These gene transfer platforms have encompassed not only recombinant viral vectors, but also plasmid liposomes, including minicircle/plasmid liposomes, that have used different routes of delivery (e.g., direct injections/infusions, oral administrations using inhalers, etc.) [101]. It has been demonstrated that the effectiveness of these SOD transgenes are limited, both in time and location. Optimal SOD expression, hence radioprotectiveness, requires specific mitochondrial or cytoplasmic cellular locales, while being constrained to a time frame of ~12 – 28 h [88].

Recent strategies to enhance radioprotection of the individual either by direct gene editing (CRISPR/Cas9) or by 'vaccination' using gene-specific mRNA/liposomes have been discussed in reports, but not yet tested experimentally [102-104].

Simpler protective strategies: Employing nutraceuticals and/or repurposed pharmaceuticals

Nutraceuticals. Nutrition and physiology clearly intersect when it comes to the health and well-being of the individual, whether the individual is earth-bound or in outer space. It is well recognized that the extraterrestrial space environment is unique in terms of its microgravity and radiation, and that these natural space conditions can interrupt basic nutritional/physiological interactions (e.g., risk to infections due to IR/nutrition elicited suppression of immune response). The application of select types of nutraceuticals could well serve to mitigate adverse molecular events, physiological, and/or behavioral decrements arising from either acute solar flares or chronic galactic particle exposures [19, 26, 27, 105-109]. Various forms of tocopherols (i.e., alpha, delta and gamma tocopherols), vitamin A (retinyl acetate), isoflavones (genistein), and a variety of natural fruit extracts have been documented experimentally to provide either significantly increased resistance to the acute, potentially lethal effects of TBI or to exert

radioprotective genomic responses within cells of vital bodily tissues [19, 27, 39, 57, 105-108, 110-112]. All of these agents can be easily administered by various delivery routes (as injectables or as oral supplements), are exceedingly well tolerated with fairly broad time windows of effectiveness, and are quite stable and storable over prolonged periods. For example, prophylaxis of male mice (CD2F1) prior to lethal radiation exposures with vitamin E injections (*sc* doses of 400 mg/kg) yielded substantially increased levels of radioprotection, as evidenced by the estimated DRFs of ~1.23 [105, 113]. Other vitamin E family members, in particular the gamma and delta tocotrienols, are strongly anti-oxidative, with significant levels of demonstrated radioprotectiveness within both small and large animal models [112, 114-116]. A relatively recent report of an advanced preclinical assessment study of gamma-tocotrienol (GT3) efficacy was reported which highlighted the protection from potentially lethal radiation injuries in exposed male and female NHPs [117, 118]. For this study, two different doses of GT3 (37.5 and 75 mg/kg) were tested in animals irradiated with lethal and supra-lethal doses. Specifically, these studies demonstrated that GT3 significantly mitigated acute irradiation-induced H-ARS and fostered an enhanced recovery of blood cell counts (i.e., blood neutrophils and platelets). Most importantly, GT3 prophylaxis elicited recovery was largely independent of supportive care or of the need of multiple doses of recombinant GF treatments (i.e., Neupogen) that would normally accompany the application of the 'standard of care' treatments [118]. [Note: There was little difference noted in the responses of the male and female animals, but the small number of animals of each gender utilized limited the statistical power of this study].

While intense exposure(s), whether they be single or multiple exposures, or intense solar flares, will result in acute injuries, more chronic exposures to fluences of highly energetic, heavy galactic particles would elevate the risk of developing significant 'late arising' pathologies, the

majority of which have serious health and/or behavioral consequences. The latter most certainly includes both malignant and benign cancers of both solid tissues and of blood-forming tissues (e.g., bone marrow and leukemias), and non-cancerous, late-arising pathologies of virtually every organ system of the body; e.g., ocular, vascular, and nervous systems. These space-associated health risks are ill-defined, as are the ways and means to effectively reduce and counter those risks.

The potential adverse health effects associated with space travel clearly are real and problematic, not only in terms of the basic relationships between conditions of irradiation (dose, dose-rate, radiation quality), but also relative to developing and implementing medical countermeasures. For example, possible irradiation-induced damage to the central nervous system that manifests inflight decrements of cognition, reduced motor function, and behavioral changes, all of which may affect performance and human health [28]. There are, however, reports of preclinical studies that might be instructive, in terms of achieving progress on this daunting space-associated problem. Take for example, the studies by Rabin and colleagues documenting the disruptive effects of neural systems and behavioral patterns in experimental rats exposed (whole-body) fluences of highly energetic charged iron particles (e.g., 1.0 Gy of 1 GeV/n ^{56}Fe from a dedicated port of a gradient synchrotron at Brookhaven National Laboratory) [17, 119]. Experimental male rats (Sprague-Dawley) were initially pre-conditioned (i.e., pre-trained Morris water maze spatial learning/cognitive performance), then exposed to simulated galactic space IR and subsequently re-evaluated by the performance test nine months following initial exposure. Not surprisingly, the radiation-exposed rats were adversely affected in terms of their cognitive/learning performance with performance decrements that were analogous to a pre-mature aging syndrome [17, 119]. However, by contrast, in another report by the same

researchers that focused on a variety of preconditioning behavioral, learning and memory tasks (i.e., behavioral and cognitive endpoints) in comparably irradiated rats (1.5 and 2.0 Gy doses) that were maintained on strongly antioxidant diets (e.g., ~2% blueberry extract) for weeks prior to heavy particle exposures generally fared better than the irradiated controls (i.e., those IR exposed animals not provided antioxidant-rich diets) in the final, delayed post-exposure cognitive/behavioral tests [107]. Although the effectiveness of the diets clearly served to at least mitigate in part the IR exposure-induced deterioration in performance, the degree of mitigation varied as a function of both specific diets tested and the specific endpoint examined. The study also revealed that animals fed antioxidant diets prior to radiation exposure showed reduced incidence of solid tumors (i.e., at ~1 year post-exposure) as compared to the animals fed the control diet. The authors conclude that high-energy particle irradiation can produce not only age-like decrements in cognitive behavior within standard laboratory animals, but it can also increase tumor formation, and that these radiation-elicited late arising syndromes can be mitigated, at least in part, by specific dietary supplements [107]. As the report concludes, there is little doubt that there are comparable health risks for astronauts during long-term space travel beyond the magnetosphere and that specific dietary regimens might prove useful in countering these adverse health effects [26, 27, 105, 106, 108].

Probiotics. As defined by well-characterized microbial preparations (e.g., *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and other related *Lactobacillus* species) and endowed with the capacity to alter the makeup of microflora of the individuals' gut microbiome, represent a quite different, but nevertheless important type of nutraceutical strategy for the promotion of gastrointestinal health of the individual, independent of age and gender, and regardless of whether the individual is earth-bound or in deep space [120, 121]. Both preclinical and clinical

observations tend to support the contention that probiotics might have significant medicinal value, especially in terms of mitigating irradiation-induced gastrointestinal injury and associated functional impairments (e.g., reversing the IR effects of microbial dysbiosis, anti-oxidant depletion, loss of intestinal crypt/stem cells and overall mucosal wall integrity, diarrhea, etc.) [122-125]. The underlying basis of probiotics' radioprotective and mitigative actions appear complex, and need to be better characterized. Nevertheless, work in this area continues to yield interesting, informative results; e.g., a rationally designed probiotic, *L. rhamnosu*s GG (LGG), is radioprotective when administered via gavage to experimental mice subjected to acute intestinal irradiation and that this radioprotection is "TLR2 and COX-2 dependent and is associated with the migration of COX-2^{+} mesenchymal stem cells (MSCs) from the lamina propria of the villus to the lamina propria near the crypt epithelial stem cells" [126]. Further, the authors suggest that the probiotic treatment orchestrates processes involving a timed release of the radioprotective TLR2 agonist, lipoteichoic acid (LTA), followed by a priming of epithelial stem cells and the subsequent triggering of a 'multicellular adaptive immune signaling cascade' [126].

Still another, quite different strategy employed immune gene (IL-22) transgenes being established in gut-associated microbial vectors (e.g., *Lactobacillus reuteri* or *Escherichia coli*) and orally delivered as a dietary probiotic to experimental mice, with the overall intent to bioengineer a more radiation-resilient microbiome of gastrointestines of experimental mice [127]. The recombinant rhuIL-22 directly targets intestinal stem cells (ISCs) and augments growth and expansion of both mouse and human ISCs and is, therefore, inherently reparative for radiation-injured intestinal crypt stem cells [128]. In a proof-of-principle study, experimental mice (C57BL/6) received an IL-22-transgene probiotic at 24 h following acute TBI (9.25 Gy), and the number of recombinant IL-22 positive bacteria within the intestine increased

proportionally to the rise in number of G-protein-coupled receptor 5-expressing intestinal stem cells; a relationship that corresponded to a noted increase in survival (30 d) of the irradiated mice [127].

Repurposed medicinals. This basic strategy has been in play regarding space medicine for some time. Several of these candidate drugs are currently under consideration and will most likely be repurposed in the near future for the extraterrestrial space environment, which include not only the FDA approved recombinant hematopoietic GFs (Neupogen, Neulasta, and Leukine) for treating and mitigating the ARS that arise from intense and unwanted radiation exposures, but also latest of the FDA approved reparative recombinants, romiplostim (Table 2). The intent of incorporating these agents into the space craft's medical kit and pharmacy would be to mitigate the risk of developing life-threatening injuries to the blood forming tissues of astronauts following intense solar flare exposures. An additional word or two on romiplostim is warranted, as this drug might well prove to be exceptionally useful as a space radiation countermeasure. This drug is not only reparative in terms of its actions on select compartments of radiation-injured bone marrow (blood platelet producing megakaryocytes), but it also has a documented survival-sparing effect [73-75]. The drug was developed decades ago by Amgen and was indicated for the fairly rare condition, chronic idiopathic thrombocytopenic purpura (ITP). The drug was FDA approved for marketing in 2003. Romiplostim has been demonstrated to be efficacious in ITP patients in relieving thrombocytopenia via the stimulation of growth and development of marrow megakaryocytes and the associated sustained platelet responses [75, 129, 130]. Based on these attributes, romiplostim was developed as a radiomitigator for H-ARS. Efficacy assessments of the drug using preclinical animal models have continued to support the contention that the drug increases survival within potentially lethally irradiated animals by

supporting and promoting hematopoiesis in bone marrow [40, 73, 74]. [Note: Although these preclinical studies clearly demonstrated an overall, gender-independent survival benefit of romiplostim treatments of acutely irradiated NHPs, female animals were reported to be more sensitive than males to acute, high doses of TBI [73, 74, 131]]

Another FDA-approved recombinant that might be considered for repurposing is palifermin (Kepivance), a recombinant GF (rhuKGF) with reparative capacities for epithelial cells of the body (i.e., cells lining the oral cavity, liver hepatocytes, gastrointestinal tissue, pulmonary cells (type II pneumocytes), hair follicle cells, transitional urothelial cells, and keratinocytes in stratified squamous epithelial cells) [132-134]. Much like the natural keratinocyte GF, palifermin functions in protecting epithelial tissues and repairing their injuries [133-136].

An additional recombinant with considerable therapeutic potential that might be considered for repurposing is oprelvekin (Neumega). This agent was originally licensed by the FDA and marketed for the treatment of chemotherapy-induced acute thrombocytopenia [137] and has a long history of clearly, experimentally demonstrated reparative and survival promoting properties following acute irradiation in experimental animals [138-142]. However, as a radioprotectant, clinical transitioning of this agent has proved to be difficult, largely due to the recombinant's toxicity when delivered systemically [142]. Successful repurposing of this agent will require either a reengineering, or a change in the mode of delivery, or perhaps a narrowing of the range of tissue/organ system targets. Regardless, it has already been demonstrated experimentally that a simple reengineering by PEGylating the recombinant works to improve its overall efficacy (i.e., increasing the time-window of effectiveness, enhancing repair of critically radiation-injured hematopoietic function(s), while reducing the incidence of fatal outcomes)

[143]. Similarly, a change in drug delivery (i.e., from an injectable to either an oral or a direct intraluminal administration) has been shown to be beneficial in terms of reducing the recombinants' systemic toxicity, while still retaining reparative efficacy [140, 142]. [Note: Oprelvikin, as well as palifermin, have strong, well-documented reparative effects on injured epithelia of the body, regardless of species, age or gender].

The aminothiols represent still another important class of radioprotective agents that need to be seriously considered for use in extraterrestrial space flights [144]. Amifostine represents the class's arch-type molecule. Despite amifostine's long history as a well-documented radioprotectant that provides significant levels of radioprotection systemically to properly prophylaxed individuals, the drug still has very limited regulatory approval and only for specific clinical conditions [145]. Due to the toxic 'side-effects' (i.e., nausea, vomiting, general gastrointestinal disturbances, etc.) elicited by the drug when delivered at optimally high radioprotective doses, the agent has not received FDA approval for general public use during nuclear/radiological emergencies, or for space missions. There are, however, anecdotal reports that amifostine was carried by astronauts as a safety precaution during early Apollo moon missions. Regardless, the drug has not been approved by the FDA or NASA for such space mission related purposes. In spite of the drug's limitations (medical and regulatory), research efforts continue to make better use of this very powerful radioprotectant, largely by attempting to limit its toxic side effects [39, 145, 146]. One such research effort (preclinical studies performed largely using male mice) has indicated that by simply reducing the dose of amifostine administered below the threshold for these toxic side effects, some level of radioprotection would remain and might be effectively leveraged by subsequent preventive treatments (e.g., post-exposure GFs and cytokines) [19, 39, 145, 147].

Angiotensin converting enzyme inhibitors (ACEi) function by helping to relax veins and arteries and to lower blood pressure. A large number of these agents are commercially available and are widely used to treat hypertension and heart related problems, but there are also other approved indications as well (e.g., prevention of heart attacks, chronic kidney disease, migraines, and scleroderma). Relative to the problem at hand, it is well documented experimentally that ACEi drugs have the potential to mitigate both early and late-arising irradiation-induced pathologies of several organ systems; most prominently late-arising pathologies of the lung and kidneys of heavily irradiated animals, but also early arising hematopoietic syndromes as well [148-150]. For example, captopril given to experimental rats (Wistar) in drinking water (140 – 180 mg/kg/d) following single, high doses (11 – 15 Gy) of thoracic irradiation clearly spares select lung functions and reduces the incidence of pneumonitis while promoting survival. These measures of ACEi's effectiveness in mitigating irradiation-induced lung injury were utilized to calculate the DRF for specific endpoints related to irradiation-induced lung disease. For overall lung associated morbidity, the dose modifying factors (DMF) were estimated to be 1.07 – 1.17, while for pneumonitis associated tachypnea, estimated DMFs ranged from 1.21 – 1.35 [150].

Relative to the irradiation-induced early arising hematopoietic tissue injury, it was shown experimentally (in a standard acute irradiation-based rodent model) that daily low-dose captopril regimens initiated as late as two days following acute irradiation significantly enhanced overall survival, as did higher drug doses [148]. The drug's capacity to promote survival of acutely irradiated rodents was temporal, perhaps causally related to a promoted hematopoietic recovery phase (i.e., of bone marrow cellularity and blood cells counts) ~3 – 4 weeks following irradiation. Furthermore, captopril treatments did not appear to affect irradiation-induced cell cycle arrest genes or the immediate loss of hematopoietic precursors, but it did reduce the

expression of select hematopoietic GFs/cytokines such as erythropoietin (EPO) and G-CSF in blood plasma. Interestingly, this study reported that captopril treatments, even when delayed (i.e., up to ~48 h post irradiation), appeared to mitigate micro-hemorrhages within brains of radiation-exposed mice [148].

The efficacy of captopril to ward off significant radiation injuries of several major organ systems of the body was demonstrated not only in small experimental animals but also in large animal models as well. For example, in one such study with a mini-pig model of H-ARS was employed and demonstrated that captopril treatments (oral administrations 0.96 mg/kg twice a day for 2 weeks) following TBI (^{60}Co 1.79 Gy, 0.42 – 0.48 Gy/min) improved the chance of survival of the drug-treated animals (i.e., survival rates of 62.5% in the vehicle-treated control animals compared to 87.5% survival in the captopril-treated group) [148, 151]. Additionally, captopril significantly improved the recovery of peripheral blood mononuclear cells, and trended towards improving the recovery of red cells and platelets. Similar to the results found in the small animal study mentioned earlier, captopril significantly reduced irradiation-induced expression of the cytokines EPO and GM-CSF and an acute-phase inflammatory response cytokine serum amyloid protein A. The latter observation was consistent with a significant suppression of irradiation-induced expression of redox stress genes and improved expression of select hematopoietic cytokines [148].

Statins-3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors are widely prescribed medicinals that are used for the primary purpose of limiting or reducing blood levels of cholesterol, or more specifically low density lipoprotein fraction of cholesterol and in turn, lowering the risk of cardiovascular disease [152]. As a pharmaceutical group, the statins are considered both safe and effective, especially when appropriate changes in diet and exercise are

applied. The statin group is large with numerous members that include: atorvastatin, rosuvastatin, simvastatin, pravastatin, lovastatin, fluvastatin and pitavastatin [153]. Despite the benefits of statin medications, they are not without side effects. The more common of these include: muscle pain (~10% of the population), headaches, dizziness, tiredness, sickness or weakness, sleep problems, and low platelet counts.

Beyond statin's most positive effect in reducing risks of cardiovascular accidents, there are also several additional medical advantages for using statins; these advantages include, but are not limited to, radioprotection of select types of normal tissues, anti-inflammatory and anti-fibrotic effects, along with the added bonus of being anti-neoplastic under certain circumstances [154]. Although, the precise nature of the statins' anti-neoplastic action is uncertain, it is generally believed that the effect is mediated, in part, by the capacity to delay DNA repair process(es) and by the promotion of neoplastic cell death (apoptosis) [155]. It has been suggested that these drug effects are founded on protein prenylation, rather than its better recognized HMG-coenzyme A reductase inhibitor effect(s) on cholesterol metabolism [155].

Regardless of the uncertainties surrounding the mechanisms of statins' anti-cancer effect(s), it is fairly clear from several epidemiological studies that the extended use of statins serves to ward off irradiation-associated cancers. One report indicated that the daily use of statins (i.e., $\geq$ 4 years) significantly reduced the risk of developing radiotherapy-associated cancer; e.g., the calculated odds ratios dropped from 0.80 (95% CI 0.66 – 0.96) to 0.64 (95% CI 0.44 – 0.93) [156]. Another retrospective radiotherapy patient cohort study found that the overall cancer mortality rate dropped by approximately 15% in statin-medicated patients [157]. In yet another retrospective cohort study, statin use after a diagnosis of esophageal adenocarcinoma and

subsequent radiotherapy was associated with reduced cancer (esophageal cancers) incidence and overall mortality [158].

By contrast to the statins' apparent suppressive effects on irradiation-associated neoplasia, normal, but injured cells and tissues respond to these drugs in quite the opposite fashion; i.e., in a protective way, as evidenced by enhanced DNA double strand break repair and by limiting the injured cell's overall response to genomic damage, while suppressing subsequent, apoptotic pathways [159]. In addition, statins' anti-inflammatory actions also play a role in the noted protective/tissue reparative processes by largely limiting irradiation-associated inflammation and tissue fibrosis. Specifically, statin treatments appear to suppress not only mRNA expression of both pro-inflammatory and pro-fibrotic cytokine genes, but also dampen neutrophil trafficking to sites of tissue injury, as well as endothelial activation and down-stream inflammatory associated thrombotic responses [160-162].

As mentioned above, the therapeutic attributes of statins have been amply demonstrated both in preclinical work involving experimental animals, as well as in patients enrolled in clinical studies. Select statins have been shown to mitigate significant, life-threatening radiation injuries (or other physicochemical toxicants) of several vital organ systems of the body; e.g., pulmonary, cardiovascular, gastrointestinal and hematopoietic systems [141, 163-166]. For example, in thoracic irradiated rodents, post-exposure treatments of lovastatin limited the buildup of irradiation-induced pro-inflammatory cytokines and presumably, in turn, down-stream tissue fibrosis, while daily, post-irradiation treatments with simvastatin attenuated acute radiation injury of both gastrointestinal and bone marrow tissues [165]. These radiation injury-mitigating effects have been causally linked at the molecular level to statins' targeting of a key protooncogene (i.e., Ras-homologous or Rho) involved in the initial molecular signaling of cell

proliferation and in subsequent p53 regulation of the caspase-dependent apoptosis [159]. Reports of statins' efficacy to mitigate radiation injuries have been extended to the protection of gastrointestinal tissues within cancer patients undergoing radiotherapy as well [141, 167].

Considering the totality of the above reports of statin's positive medicinal effects, it has been rightfully pointed out that these medicinals might be ideally suited for use by astronauts during long space missions, especially under the daily assault of space weather (i.e., space radiation) [144]. In addition to the ACE inhibitors and the statins, a number of other commonly used medicinals have been or are currently being considered for repurposing for the space environment. These include, but not limited to the following: nonsteroidal anti-inflammatory drugs (NSAIDs), N-acetylcysteine, melatonin, metformin, calcium channel blockers, pentoxifylline, β-adrenergic receptor blockers and entolimod [144]. A partial list of medicinals that have been considered for repurposing is shown in Table 4.

Polypharmaceutical approaches/combinations of effective agents

Sufficiently intense radiation exposures, acquired either acutely or chronically, either terrestrially or extraterrestrially, have the potential to elicit pathologies within various tissues and organ systems of the body; the very definition of ARS embodies this concept by including an entire complex of often interacting pathologies of different organ systems of the body [168]. As such, it is unlikely that any single drug or medicinal will be sufficient to either prevent, mitigate, or to effectively treat all pathologies within the overall disease complex. Most medical researchers who are involved in this area of study are fully cognizant of the situation and accept the fact that such an optimal, single effective drug (i.e., the silver bullet for ARS) is not close at hand. In lieu of this situation, a polypharmaceutical approach to the problem might represent a reasonable and prudent option. In this regard, there have been a significant number of preclinical

studies that have sought to and have demonstrated the utility of this approach [37, 169, 170]. Further, the overall effectiveness of this approach would be substantially improved by the application of AI and the related decisions concerning the best combination of agents for radioprotection purposes. Briefly, several of the more promising of these different drug combinations are briefly described below, while additional combinations are also listed in Table 5.

Combinations of recombinant GFs and cytokines. The R&D of selecting combinations of recombinant GFs/cytokines for the treatment of H-ARS (terrestrially-based or otherwise) is ongoing and needs to continue in order to improve the efficacy of the current treatment regimens for the ARS. A number of reported preclinical studies using both small and large animal models have demonstrated significant life-sparing effects when select drug combinations have been administered over the early period following acute irradiation [73, 74, 171, 172]. For example, a drug combination consisting of EPO, G-CSF, romiplostim, and nandrolone decanoate was shown to remarkably improve the clinical outcome of acutely irradiated animals receiving the combined treatments; i.e., complete hematological recovery coupled with elimination of early irradiation-associated deaths [171]. The therapeutic utility of using select combinations of GFs/cytokines in treating ARS has been demonstrated pre-clinically using large animal models as well. For example, recent reports by Wong and colleagues showed that the administration of a combination of PEGfilgrastim and romiplostim resulted in significantly improved hematological profiles within acutely irradiated NHPs (as compared to control animals) [40, 73-75] (Table 5).

Combinations of radioprotective and radiomitigative agents. One possible combination would be genistein prophylaxis coupled with post-irradiation captopril treatment (Table 5). Enhanced protection from acute irradiation-associated injury has been demonstrated in a small rodent

model of acute radiation injury (i.e., C57BL/6J mice exposed to lethal doses of 8.25 Gy ^{60}Co gamma-rays TBI) by combining a prophylactic injection of genistein 24 h prior to exposure and then subsequently following radiation exposure treatment with an ACEi (captopril) for 30 days via oral route of delivery (in drinking water). Results showed that the combined treatment yielded a 95% survival, where genistein prophylaxis alone or post-irradiation treatments with captopril provided far lower survivors, namely 72% and 55%, respectively [173]. As the latter report indicates, the enhanced rates of survival with the combined treatment were directly related to a sparing and recovery of irradiation-associated hematopoietic tissue injury; notably, there was a reduction in irradiation-induced anemia and enhanced recovery of blood cell counts and bone marrow cellularity. A noted early recovery of select progenitor compartments within the marrow was also observed. Interestingly, genistein prophylaxis alone or in combination with captopril post-irradiation treatments seemed to protect marrow progenitors from irradiation-induced micronuclei formation, while captopril alone had no such effect. As other reports have indicated, the inclusion of captopril in the treatment regimen appeared to suppress expression of irradiation-induced EPO [50, 173].

A second possibility for a space-useful drug combination would be prophylaxis with one of the more efficacious aminothiols (e.g., amifostine), coupled with post-irradiation recombinant GFs/cytokine treatments. The radioprotective power of this drug coupling process was clearly demonstrated by several founding proof-of-principal studies [174, 175]. In one such study, a large animal (canine) model of acute radiation injury was employed and used to evaluate the effectiveness of a combined drug regimen (amifostine prophylaxis, plus recombinant hematopoietic growth factor (rhuG-CSF) treatments) to promote survival in irradiated animals over a range of near-lethal to supralethal radiation doses [175]. Groups of animals were

prophylaxed separately with either amifostine alone (150 mg/kg/kg, *iv*), treated post-irradiation with the recombinant hematopoietic GF; G-CSF (10 μg/kg, *sc*), or treated with both amifostine and G-CSF. Administration of a standard clinical support also served as an additional variable. Sixty day survival was used as the primary endpoint and was used to calculate LD_{50} values and in turn, DRFs for the different treatment groups. The results were remarkable: the estimated LD_{50} value for the untreated irradiated controls was 260 cGy, while for the other treatment groups, the values rose to 340 cGy (for clinical support only), 510 cGy (clinical support, plus G-CSF treatment), 607 cGy (amifostine, without clinical support), 790 cGy (amifostine, plus clinical support), and finally ~1150 cGy (for the combined treatment regimen of amifostine, plus G-CSF, plus clinical support). The DRFs for the latter treatment groups were estimated to be 1.0 (control group), 1.3 (clinical support only), 2.0 (G-CSF, plus clinical support), 2.3 (amifostine prophylaxis, without clinical support), 3.0 (amifostine, plus clinical support), and ~3.9 (full treatment regimen: amifostine, G-CSF and clinical support) [175]. Comparable results were reported as well using a small rodent model of acute radiation injury [174].

[Note: It needs to be pointed out however, that the doses of amifostine used to prophylax the animals in this study were sufficiently high cause of emesis, and therefore inappropriate for consideration for use during space travel. Nevertheless, the study is clearly instructive in terms of demonstrating what might be possible, what might be achievable by use of the 'polypharmaceutical approach' to radioprotection.]

A listing of additional promising radiation protective agents that might be used in combination for radioprotection is given in Table 5.

Conclusions

Relative to the current status, the systems, processes, and tools needed for prevention and treatment of extraterrestrial radiation exposures are indeed available, but limited in scope and capacity to manage such health risks. The general strategic approach currently being taken by the various US federal agencies, including NASA, for the development and improvement of radiation countermeasures is multipronged, involving a mix of both basic and applied physical and biomedical research. We have highlighted a number of these approaches in this report, including high tech bio- and chemical engineering approaches, as well as other approaches that are perhaps somewhat less innovative and cutting edge by nature, but exceedingly useful nonetheless. The latter includes approaches that seek to exploit widely used, over-the-counter nutraceuticals, along with the reexamination of select pharmaceuticals that might be repurposed for the astronaut for use during extraterrestrial space voyages. The repurposing strategy has already paid dividends, as evidenced by the current medicinals already contained within the space traveler's medical kit for the express purpose of clinically managing irradiation-associated overexposure (e.g., anti-nausea, anti-diarrhea drugs, broad spectrum antibiotics, topicals and inflammatories for solar burns, etc.). There is every expectation that the recombinant GFs/cytokines, so prevalent in terrestrial-based medicine, will find their way shortly into medical kits of space vehicles as 'repurposed' medicinals. There is little doubt that a number of ACEi drugs, or perhaps the statins, will be following shortly; as they currently are widely dispensed medicinals that are easily delivered (via the oral route), exceedingly well tolerated, and particularly effective in warding off not only cardiovascular problems, but also under select conditions, both acute and chronic radiation injuries of various vital tissues and organ systems that might manifest under extended times within the space environment.

In the same light, the inclusion of radioprotective nutraceuticals might serve as a ‘simple fix’ in attempting to counter and to manage some of the health risks associated with extended space travel and the inherent exposures to potentially hazardous solar/galactic radiations. This is not simply wishful thinking, but based on well-documented preclinical studies that employed both small and large animal models of radiation injury and that were previously cited regarding the radioprotective/radiomitigative attributes of such nutraceuticals as genistein, gamma tocotrienol, etc. Considering the upside, which includes medical benefits to the space traveler and making use of select types or combinations of nutraceuticals: e.g., ease of oral delivery, effectiveness lasting days to weeks, general lack of toxicity and/or substantial side-effects, easy storage, extended shelf life and stability. NASA has already invested heavily into improving the nutritional health of astronauts, and this effort will most certainly continue into the foreseeable future [176-178]. Any additional nutritional research that impacts space-associated radiation protection will prove to be beneficial from a health standpoint. By comparison, very few, if any, of the more conventional radioprotective pharmaceuticals have all of these positive features. However, the real power of the nutraceutical approach comes into play when selected nutraceuticals (e.g., vitamin E family members) with admittedly modest effectiveness, are combined with other, more potent, radioprotective pharmaceuticals (e.g., aminothiols with sustained release formulations): we refer to this combinatorial approach as the ‘polypharmaceutical strategy’ for improving the quality of radiation medical countermeasures for the extraterrestrial space environment.

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Countermeasures for extraterrestrial radiation exposures and related injuries

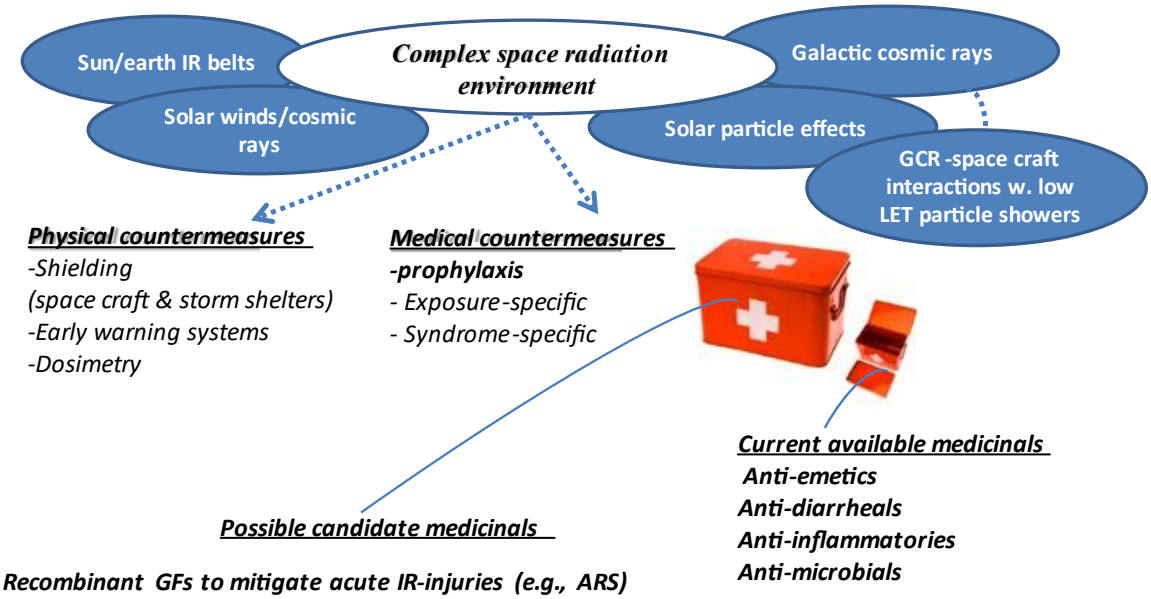

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Figure 1

Table 1. Current radiation countering medicinals within space vehicle's emergency medical kit

#	Drug type/ class	Mechanism of action	Intended use	Drug name	Drug maker	Route/ dose
1a	Anti-emetics	5-HT3 receptor antagonist	Prevent nausea/vomiting	Ondansetron (Zofran) (or Gransietron - Kytril)	Generic	Oral/1 tablet/d (8 – 24 mg)
1b	Anti-inflammatories	Glucocorticoid receptor agonist	Minimize inflammation associated pain	Dexamethosone (Decadron®)	Generic	Oral-liquid or tablet – 1 d
1c	Anti-diarrheals	Opioid μ receptor agonist	Diarrhea	Loperamide hydrochloride Imodium®	Generic	Oral/1 – 2 tablets (2 mg/tab); as required
2a	Antimicrobials - floroquinolone	Inhibits bacterial DNA replication	Broad spectrum-prevention/control of mixed infections - Gram negative microbes	Ciprofloxacin	Generic	Oral or injection 250 – 500 mg/12 h/d
2b	Antimicrobials - beta lactams	Inhibits mainly gram positive microbes	Inhibit microbial cell wall synthesis	Cephalosporin Cephradine or Ceftoaroline 1st & 5th generation	Generic	Orally or injection
2c	Antimicrobials - beta lactams	Inhibits gram positive microbes	Inhibits microbial cell wall synthesis	Penicillin e.g., penicillin V potassium	Generic	Oral tablets/250 mg/tablet
2d	Antimicrobials - macrolide	Inhibits gram positive microbes, with wider range than penicillin	Inhibits microbial protein synthesis via binding to ribosomal binding & limiting	Macroclides e.g., erythromycin, clarithromycin, azithromycin	Generic	Oral tablets/333 mg/8 h
3a	Skin treatments - Sulfa drugs	Bactericidal	radiation burns & blisters anti-infectives	Silver sulfadiazine Silvadene	Generic	Topical
3b	Skin treatments - anti-inflammatories	Suppression of immunologic mediators of inflammation	Irradiation blisters/burns anti-inflammatory	Corticosteroids	Generic	Topical
3c	Skin treatments - analgesics	Suppression of pain via neutral mediators	radiation blisters/burns analgesic	Opioids	Generic	Topical/oral
3d	Skin treatments - topicals	Control of skin barrier damage	irradiation blisters/burns lesion treatment(s)	Crystalloid solutions Sterile gauze	Generic	Topical
4	Dehydration	Cutaneous water loss	radiation associated dehydration	*iv* provided physiological saline solutions	Generic	*iv* infusions

Inclusion of the hematopoietic growth factor, rhuG-CSF, is under consideration.
5-HT3, 5-hydroxytryptamine (serotonin)

Table 2. Select recombinant growth factors: Possible candidates for inclusion in the space radiation medical emergency kit

#	Drug class	Intended use	Drug name	Manufacturer	Dosage/dosing
1	Recombinant GF rhuG-CSF	Mitigate acute granulocytopenia Stimulate granulocytopoiesis	Filgrastim - Neupogen	Amgen	Injection - 10 μg/kg/d Initiate - 1 d post irradiation Multiple injections until ANCs > 1,000/μl for 3 consec d
2	Recombinant GF rhuPEGG-CSF	Mitigate acute granulocytopenia Stimulate granulocytopoiesis	PEG-filgrastim - Neulasta	Amgen	Injection - 6 mg x 2 Initiate - 1 d post-irradiation 2 injections at 1 & 8 d
3	Recombinant GF rhuGM-CSF	Mitigate granulo/monocytopoienia Stimulate granulo/moncytopoiesis	Sargograstim - Leukine	Sanofi-Aventis	Injection - 7 μg/kg/d Initiate - 2 d post-irradiation Multiple injections
4	GF receptor agonist TPO	Mitigate thrombocytopenia, stimulate thrombocytopoiesis	Romboplastim - Nplate	Amgen	Injection - 10 μg/kg once Initiate - 1 d post-irradiation Single injection
5	Recombinant GF rhuEPO	Mitigate anemia, stimulate erythropoiesis	EPO - Epogen	Amgen	Injection/infusion - 150 U/kg/3x weekly Initiate- post-irradiation with presentation of severe anemia (e.g., Hg < 9-10 g/dl) Multiple injections
6	Recombinant GF rhuKGF	Prevent/mitigate chemo/radio-pathology of epithelium, stimulate epithelial repair/regrowth	Palifermin - Kepivance	Amgen	Infusions - 60 μg/kg/6x, multiple infusions - 3 prior, plus 3 following exposure to cytotoxic agent.

Table 3. A representative sampling of reengineered radioprotective drugs for possible use within the extraterrestrial space environment

#	Drug class	Drug name	Modification	Intended use	Manufacturer
1	Recombinant interleukin	BBT-059 - PEG-IL-11	PEGylation	Mitigation-acute radiation-induced GIS-ARS	Boulder Pharmaceuticals
2	Nutraceutical	BIO 300	Nanoparticulization via Wet-milling	Prevention/mitigation – radiation injury	Neumedicines
3	Toll like receptor agonist	Entolmolid - CBLB502	Recombinant molecular reengineering	Prevention/mitigation – radiation-induced acute hematopoietic injury	Cleveland BioLabs
4	Aminothiols	PEG-amifostine - encap-amifostine	PEGylation, encapsulation	Systemic radioprotection /mitigation of acute, early and delayed or late-arising radiation injuries	None - reported by research institutes, Chinese Mil Institute, AFRRI

Table 4. A partial list of commonly used and marketed pharmaceuticals with documented radioprotective attributes and with potential to be repurposed for the space environment

	Drug class	Generic name	Primary use	Repurposed use	Delivery route/ Dosing[5]
1	ACE inhibitors	e.g., Captopril (~1/9 alike drugs)	Hypertension/cardiovascular disease	Mitigate acute/chronic radiation injuries of blood/marrow, heart & vasculature, lung & kidney	Oral/daily dosing/TBD (e.g., 10 mg/d each day as needed)
2	Statins	e.g., Atorvastatin (~1/ 'alike' drugs)	Regulate cholesterol metabolism/mitigate hypertension & cardiovascular disease	Mitigate- acute/chronic radiation injuries- blood/marrow, gastrointestinal & heart, mitigate radiation cancer risks	Oral/daily dosing/TBD (e.g., 20 mg/d each day as needed)
3	Anti-inflammatories	NSAIDs e.g., Aceta-minophen	Reduce inflammation/reduce pain	Reduce radiation associated inflammation/cytokine storm	Oral, injection, or topical dosing/TBD (e.g., oral dosing - 500 mg/6 – 12 h each day as needed)
4	Aminothiols	NAC e.g., NAC	Nutritional supplement - anti-oxidant/detoxifier /glutathione regulator/ alleviates pulmonary inflammation	Prevent/mitigate acute radiation associated injuries via free radical quenching, possible mitigation of radiation associated cancer risks	Oral dosing/TBD (e.g., oral daily dosing of 600 – 1200 mg/d
5	Hemorheological agents	Pentoxifylline	Improves arterial blood flow/ improves tissue oxygenation / alleviates muscle cramps	Prevent acute radiation injuries of select vital organ systems; pulmonary, vascular, hematopoietic and gastrointestinal via anti-oxidant, anti-inflammatory, immune-modulating effects with improved blood flow	Oral dosing/TBD (e.g., oral dosing of ~400 mg/kg)
6	Nutraceuticals	Tocotrienols e.g., gamma tocotrienol	Nutritional supplement-antioxidant/anti-inflammatory effects	Prevent/mitigate acute radiation associated injuries via anti-inflammatory & anti-oxidant properties	Oral or injection dosing/TBD (e.g., Oral dosing - 50 mg/tablet/d
7	Nutraceuticals	Genistein	Nutritional supplement-immune support/ bone health	Prevent/mitigate acute radiation associated injuries via free radical quenching. Mitigate long-term radiation cancer risks	Oral or injection dosing/TBD (e.g., oral dosing
8	Nutraceuticals	Melatonin [6]	Nutritional supplement-immune system support /sleep aide	Prevent/mitigate acute radiation associated injuries, immune system support/sleep aide	Oral dosing/TBD (e.g., oral dose of 1 – 5 mg/tablet/ dose/d as needed

Listed dosing/dosing regimens are all based on those commonly used terrestrially: extraterrestrial dosing levels that are both safe and effective will need to be determined.

Table 5. A selection of potentially useful drug combinations that might serve as effective radiation countermeasures for the extraterrestrial environment

#	Drug class combination	1st drug- MCM effect/target	2nd drug- MCM effect/target	Expected clinical outcome/advantage
1	Granulopoietic GF plus thrombopoietic GF	PEGfilgrastim sparing hematopoietic function(s)	Romiplostim - Sparing hematopoietic function(s)	Enhanced recovery from acute radiation-induced (*SPEs*) granulocytopenia and thrombocytopenia, with enhanced survival
2	Granulopoietic GF and/or granulomono-poietic GF	PEGfilgrastim Sparing hematopoietic function(s)	Sargramostim - Sparing hematopoietic function(s)	Enhanced recovery from acute radiation-induced (*SPEs*) granulocytopenia, monocytopenia, and thrombocytopenia with enhance survival
3	Granulopoietic GF plus thrombopoietic GF	PEGfilgrastim Sparing hematopoietic function(s)	IL-11/Neumega - Sparing marrow & GI functions	Enhanced marrow and gastrointestinal tissue recovery from acute radiation-induced (*SPEs*)
4	ACEi plus recombinant GF	Captopril (or alike ACEi)	Romiplostim (or alike - e.g., *Eltrombopag*[1]	Enhanced protection/recovery of hematopoietic tissues from acute irradiation (*SPEs*) exposures and chronic GCR exposures
5	ACEi plus radioprotective nutraceutical	Captopril (or alike ACEi)	BIO300 genistein	Enhanced protection/recovery of hematopoietic tissues from acute radiation (*SPEs)* exposures and chronic *GCR* exposures
6	ACEi plus radioprotective aminothiol	Captopril (or alike ACEi)	Amifostine (or alike aminothiol)	Mitigation of space radiation-elicited acute/chronic morbidities (cancers, tissues fibrosis) and associated mortality risks.
7	ACEi plus statin	Captopril (or alike ACEi)	Atorvastatin	Mitigation of space radiation-elicited chronic morbidities (e.g., cancers; tissue fibrosis) and reduced mortality risks.
8	Tocol nutraceutical plus pentoxyfylline	Gamma tocotrienol (or alike tocol)	Pentoxymfylline (or alike phosphor-diesterase inhibitor)	Enhanced radioprotective/ radiomitigating actions of tocol nutraceutical. Ameliorates radiation-associate vascular and gastrointestinal injuries
9	Tocol nutraceutical plus ACEi	Gamma tocotrienol (or alike tocol)	Captopril (or alike ACEi)	Extended, enhanced radioprotection of coverage. Vital organ systems (marrow, gut, lung, kidney) against both acute *(SPEs)* and chronic (*GCRs*) space irradiation associated syndromes
10	Tocol nutraceutical plus benzylsulfone	Gamma tocotrienol (or alike tocol)	Recilisib (ExRad)[2] Chlorobenzylsuflone	Promotes radioresistance of hematopoietic and gastrointestinal tissues and limits long-terms pathologically risks of ionizing radiation exposures.
11	Tocol nutraceutical plus statin	Gamma tocotrieniol (or alike tocol)	Atvorastatin (or alike statin)	Promotes protection and function of vital organ systems. Limits 'late-effects' of radiation exposures (cancers) & associated mortality risks.
12	Gene/gene product transfer plus dietary supplements	MnSOD plasmid/liposome gene transfer	Antioxidant dietary supplements (e.g., blueberry/fruit extracts or NASA /AmeriScience dietary supplement [3]	Protection/mitigation of both intense (*SPEs*) space ionizing radiation exposures and associated acute morbidities, as well as chronic (*GCRs*) ionizing radiation exposures that elicit late arising pathologies (e.g., tissue fibrosis, cancer, behavioral and cognitive dysfunctions)