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Chapter

Tumors in Space: Preparation for Spaceflight

Tricia L. Larose

Abstract

Tumors in Space is a cutting-edge cancer research experiment at the intersection of stem-cell biology and space technology selected by the United Nations Office for Outer Space Affairs and the China Manned Space Agency for a 31-day space mission on board the China Space Station. Anchored in Norway, Tumors in Space includes an international team of exceptional scientists at several European partner organizations including the University of Oslo and the Norwegian University of Science and Technology in Norway, the International Space University in France, the Belgian Nuclear Research Center in Belgium, and Vrije University Amsterdam as well as the Hubrecht Institute in The Netherlands. This chapter first presents our two novel hypotheses including the current state of scientific evidence upon which our hypotheses are based. Following, the seven main steps of our spaceflight preparation are discussed within the context of our 2025 launch date from China. Finally, some thoughts on impact, including support for the United Nation's Sustainable Development Goals and commitment to science communication in the public domain, are given. Tumors is Space is under a programme of, and funded by the European Space Agency with the support of the Norwegian Space Agency.

Keywords: China Space Station, cancer, organoids, microgravity, cosmic radiation

1. Introduction

Spaceflight is a high-risk/high-gain endeavor (**Figure 1**) that is resource intensive. For these reasons, spaceflight experiments are rare, and selection is highly competitive at the international level. Selection criteria for spaceflight experiments include scientific excellence, innovation, feasibility, impact, competency of the scientific team to achieve the proposed research goals, and timely delivery. Moreover, a selected spaceflight experiment must be novel in that it must address a critical issue for which knowledge and understanding are lacking; it must be ground-breaking in nature, both literally and figuratively. In the case of medical research in space, a selected spaceflight experiment must create value for astro-/cosmo-/taikonaut health applied to short- and long-duration space missions and solar system exploration. At the same time, space medicine must also bring value to public health on Earth. In this vein, *Tumors in Space* is a ground-breaking cancer research experiment at the intersection of space technology and stem-cell biology recently selected for a 31-day space mission on board the China Space Station (CSS).

Not only will *Tumors in Space* address cosmic radiation as a potential cause of cancer for humans in space, it will also address the side effects of radiation therapy

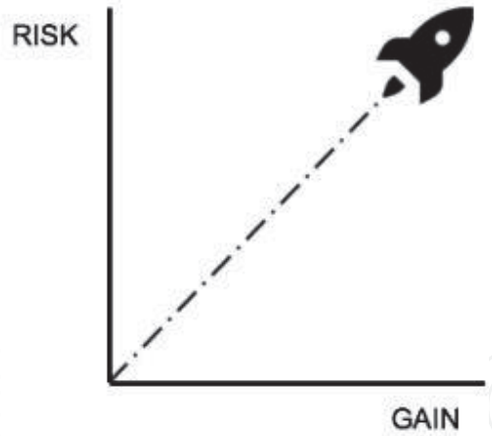

Figure 1.
Spaceflight is high risk and high gain.

for cancer patients on Earth. In addition to radiation biology experiments, *Tumors in Space* will exploit the microgravity environment on the CSS to investigate whether lack of gravity can slow or stop the growth of cancer. Like all selected spaceflight experiments, *Tumors in Space* will undergo several years of preparation before spaceflight launch. This preparation period is broken down into seven main steps over 6 years, culminating in our spaceflight experiment scheduled for a 2025 launch date out of China. This chapter will discuss the different steps of our spaceflight preparation as depicted in **Figure 2**.

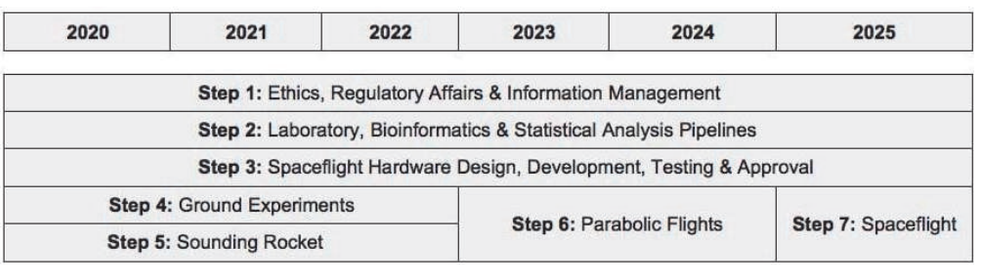

Figure 2.
Tumors in Space *project timeline over a 6-year period from 2020 to 2025 and according to seven steps of spaceflight preparation including launch to China Space Station and safe return to Earth in 2025.*

In addition to these seven steps, other important aspects of *Tumors in Space* will be presented, namely, the scientific background upon which the hypotheses are built, team structure and expertise, the importance of impact including response to the United Nations Sustainable Development Goals, and science communication in the public domain. Let us first begin with a presentation of the problem statement and the supporting evidence upon which the *Tumors in Space* hypotheses are built.

2. The problem statement and supporting evidence

As stated in Section 1, medical experiments in space must address crew health and safety while simultaneously bringing value to medicine on Earth. What follows is a short paragraph on the burden of cancer on Earth, followed by a second paragraph on space radiation risk for crewed missions. To end this section, evidence on the potential use of microgravity for cancer therapeutics will be given.

Interspersed throughout this section are some details on our scientific approach, more of which will be detailed later in this chapter.

The cancer burden on Earth is substantial. Currently, cancer is the second leading cause of death worldwide [1]. As of 2018, an estimated 18.1 million people had been diagnosed with cancer, and nearly 10 million people had died from the disease [2]. Moreover, new cancer cases are expected to increase by 70% over the next two decades [1]. As of 2009, the global cost of treating cancer patients was estimated to be $285.8 billion USD per year, and the indirect costs associated with premature death and loss of productivity was estimated at $1.16 trillion USD per year [2]. Over time, these costs will continue to rise.

The scientific community has made increasing progress toward understanding lifestyle, environmental, and genetic causes of cancer [3], yet approximately 50% of all cancer cases have no known cause. There is no cure for cancer. We must therefore adopt a broad, interdisciplinary perspective and exploit all scientific and technical capabilities to better understand this disease, including the use of orbital platforms [4]. *Tumors in Space* will exploit the natural spaceflight environment on the China Space Station to address cosmic radiation as a potential unknown cause of cancer among crew and to harness microgravity as a potential source for cancer therapy in space and on Earth.

In space, radiation risk is the most dangerous issue for crew health and safety. The spaceflight environment is characterized by high atomic number energy (HZE[1]) ions from three natural sources of radiation that could lead to crew sickness or death [5]. During space exploration missions, crew are exposed to HZE ions from trapped particle belts, solar particle events, and background galactic cosmic rays [5]. When primary HZE ions interact with spacecraft materials, they can generate harmful secondary radiation that can penetrate deeply into the human body. Cancer risk from human spaceflight and exploration is assumed to be high, but the short- and long-term effects of harmful HZE ion radiation during deep space missions are still undetermined and have large uncertainties [5]. As stated by the European Space Agency (ESA), most of this uncertainty is due to poor knowledge on the effects of galactic cosmic rays [6]. *Tumors in Space* will address some of the uncertainty associated with cosmic radiation as a potential cause of cancer among crew. At the same time, advances in space-based cancer research have the potential to improve charged particle therapy in oncology for the benefit of cancer patients on Earth.

All cancers are caused by somatic mutations, meaning an accumulation of genetic alterations that damage our deoxyribonucleic acid (DNA) at the cellular level [7]. Cell damage from exogenous exposures (e.g., HZE ion radiation), as well as endogenous exposures (e.g., circulating hormones), and modifications to DNA can lead to cancer. DNA damage in cancerous cells can be observed as distinct patterns known as mutational signatures. Just like fingerprints are unique to every individual, mutational signatures are unique to specific cancer-causing exposures. Each mutational signature is the outcome of a series of biological processes that include DNA modification or damage, DNA repair or absence of repair, and DNA replication. Using cancer genome sequencing [8], these mutational signatures can be observed, catalogued, and compared to identify unique causes of different cancer types. This was first demonstrated when mutational signatures specific to UV[2]

[1] The abbreviation HZE comes from high (H) atomic number (Z) and energy (E).

[2] UV stands for ultraviolet rays from sunlight that induces a unique mutation found specifically in skin cancer.

exposure from the sun were observed in the p53 tumor suppressor gene[3] [9]. Most cancer genomes are now characterized by multiple mutational signatures across multiple mutational processes that generate more than one somatic mutation in each cancer cell [10]. This evidence leads us to our first *Tumors in Space* hypothesis.

Hypothesis 1: We will identify a novel mutational signature of cosmic radiation in healthy and cancer human organoids after 31-days exposure to cosmic radiation in space. The mutational signature of cosmic radiation can then be used as a biological marker for early detection of cancer and cancer susceptibility among spaceflight crew.

Beyond cosmic radiation, the space environment provides unique microgravity conditions that Earth-bound laboratories can simulate but never replicate. Microgravity can be exploited to study biological mechanisms and pathways to improve our understanding of many diseases, including cancer. Previous spaceflight experiments have shown that exposure to the space environment alters immune cell growth and function [11], causes cytoskeleton and cell shape changes [12], and alters cellular gene expression including genes involved in replication and suppression of tumors [13, 14]. This brings us to our second *Tumors in Space* hypothesis.

Hypothesis 2: Exposure to microgravity during a 31-day space mission will slow or stop the growth of cancer.

We will conduct a tumor colony survival analysis which is as simple as counting the number and/or expansion of our organoids after long-term exposure to microgravity and then compare those results to our ground-based controls. It is crucial to block out cosmic radiation that may interfere with this microgravity experimental arm. For that reason, we are building radiation shielding to ensure the cancer organoids exposed to microgravity are not hit by cosmic radiation.

So, the China Manned Space Agency (CMSA) in collaboration with the United Nations Office for Outer Space Affairs has selected *Tumors in Space* for a 31-day space mission on board the China Space Station (**Figure 3**). *Tumors in Space* utilizes space technology and three-dimensional (3D) organoid technology (see further) to study early mutational events in human DNA due to spaceflight exposure. We

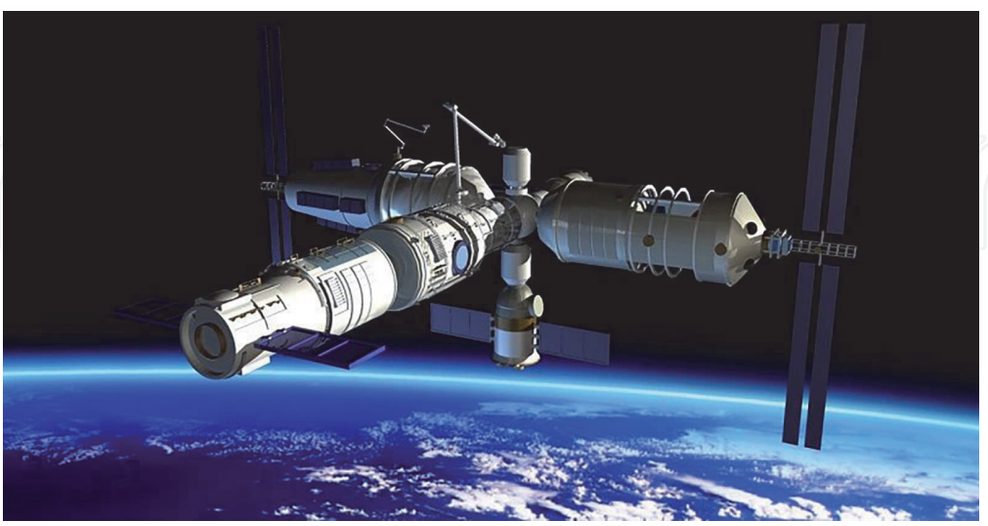

Figure 3.
Artist rendition of the China Space Station (credit: China Manned Space Agency).

[3] The p53 gene provides instructions for building a protein that acts as a tumor suppressor. Well-characterized mutations in this gene have been attributed to UV exposure and are found in many types of skin cancer.

expect to find a unique mutational signature of cosmic radiation. We also expect that cancer organoid growth will slow or stop after exposure to microgravity.

3. Going beyond state of the art

As mentioned previously, excellent science in line with the current state of evidence does not fulfill the stringent selection criteria for spaceflight experiments. Space medicine research must be novel, go beyond current scientific paradigms, and have application to crew health in space and public health on Earth. In other words, selected space medicine experiments must go beyond state of the art.

Not only does *Tumors in Space* more than double the mission length of all previous cancer experiments in space, we also use the most advanced and physiologically relevant bio-samples in our experiments—human organoids. Until now the term "organoids" has been introduced without explanation. In fact, organoid technology is so new that many scientists are unfamiliar with the term. So, what exactly is an organoid, and why is an organoid so much more advanced than a cell line that is typically used in medical research?

Human organoids are multicellular, stem-cell-derived, 3D constructs that self-organize to mimic the structure and function of the source tissue [15]. More simply, a human organoid is a 3D clump of human cells and extracellular matrix, taken as a biopsy from living human tissue, but organoids "live" in a lab. What is amazing about organoids is that they can be expanded, cloned and cared for in a way that allows them to replicate and grow while retaining the structure and function of the original source tissue or organ. For example, a colon cancer organoid derived from a tumor biopsy taken (with permission) from a colon cancer patient will not only grow into a 3D structure under laboratory conditions; it will also behave like we would expect colon cancer to behave. Same goes for healthy tissue.

Figure 4 shows an artist rendition of the growth of a colon organoid. A small sample, or biopsy, is taken from the colon tissue of a patient. Both the original colon tissue and the biopsy are 3D in nature. Once the biopsy is removed from the patient, it can be nurtured in the lab so that cell division continues, resulting in larger and

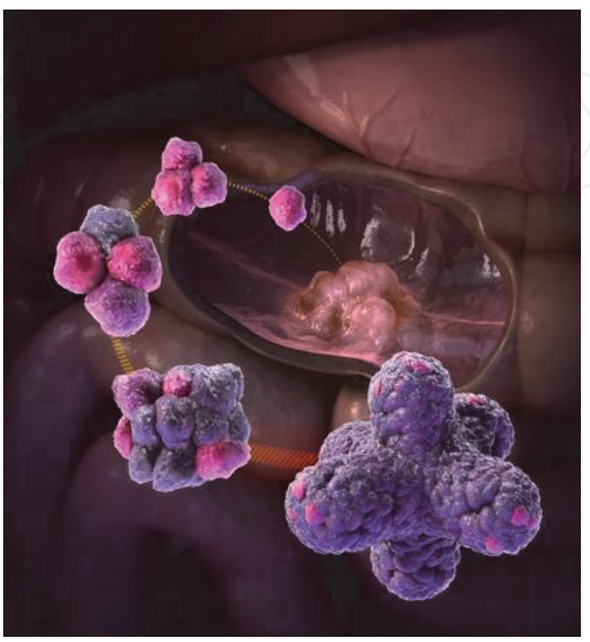

Figure 4.
Artist rendition of colon organoid growth (credit: Hans Clevers, the HUB).

more complex tissue samples that mimic the structure and function of the source tissue (in this case, the human colon). Organoids are much smaller than the organ they mimic but can be up to 5 mm in size. These organoids are the most advanced and physiologically relevant bio-samples available for medical research today. This means that we can conduct cutting-edge cancer research using human organoids rather than cell lines.

Although monolayer cancer cell lines are easily accessible, well understood, and used in all previous cancer experiments in space, they lack spatial cellular organization and physiological relevance [16]. Cell lines cannot mimic conditions found in real humans, whereas organoids can. Using human organoids ensures that our results are applicable to patient diagnosis and treatment. This is crucial since we need to get as close as possible to studying the real human body in space. Organoid technology bridges the gap between cell lines and humans, or in vitro and in vivo. In 2017, organoids were named Method of the Year by *Nature Methods* for their unparalleled potential to advance our understanding of human biology, especially cancer [17]. We are the first team to send healthy and cancer human organoids into space.

4. International collaboration

Before we dig into the seven steps of preparation for our spaceflight experiment, a quick note on international collaboration is warranted. Success of such a complex space medicine experiment requires world experts across many different fields. **Figure 5** is color coded to show collaborating countries, not only according to levels of responsibility with regard to the project but also according to geopolitical boundaries. You can see from **Figure 5** that Norway (in light gray) and China (in dark green) stand apart from the other collaborating countries like the Netherlands, Belgium, and France (in dark blue).

Norway, the center of the figure, is the coordinating country because this is where the principal investigator is located, along with the several members of the *Tumors in Space* team. We also have the support of the Norwegian Space Agency. Furthermore, Norway has identified China as a priority country for technical and scientific collaboration—for example, in satellite and broadband communication, as well as medicine. Norway and China are united by an underlying cooperative effort for the *Tumors in Space* collaboration. China is the host country for the Norwegian *Tumors in Space* experiment that has been selected for a 31-day space mission on board the China Space Station.

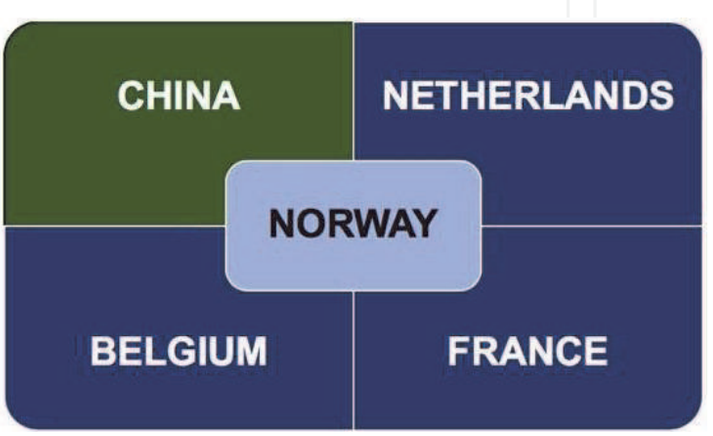

Figure 5.
Tumors in Space *collaborating countries.*

Our additional collaborators are based in Europe and are part of the European Union. The Netherlands is a collaborating country where two critical organizations are located. Firstly, our patient-derived organoids come from Hubrecht Organoid Technology (the HUB) in Utrecht, and some of our ground-based experiments will take place at the European Space Research and Technology Center of ESA in Noordwijk. Our radiation biology experts are located in Belgium at the Belgian Nuclear Research Center where our ground radiation experiments in preparation for spaceflight will be conducted. Not only do they house safe infrastructure for radiation biology experiments, our Belgian colleagues have years of space experience and substantial experience in the safe design of nuclear reactors. For the latter reason, our Belgian colleagues will also participate in the design and development of our spaceflight hardware in close collaboration with Norway and China. Finally, France is the location of the International Space University, to which the principal investigator and other *Tumors in Space* team members are affiliated. Our aircraft parabolic flight campaigns will also be flown from France.

This brief summary shows how important international collaboration is. Without all of these partners, realizing the most advanced cancer experiment ever conducted in space would not be possible. It is because of the unique strengths and expertise of all collaborating partners that the *Tumors in Space* project has become a reality.

5. The *Tumors in Space* experiment in seven steps

The step-by-step preparation of our spaceflight experiment is now described. Each one of these steps could be a chapter in itself. Certainly, some details must be omitted. The success of this entire project relies heavily on the success of each step, both independently and collectively.

5.1 Ethics, regulatory affairs, and information management: step 1

This step ensures proper procedures for the selection, release, and use of intraindividual healthy and cancer organoids according to biobanking protocols and informed consent procedures. Procedures and consents are subject not only to the approval of the HUB biobank in the Netherlands but also from the host institute in Norway, as well as our collaborating institutes where our organoid research will be conducted (the European Space Agency in the Netherlands, the Belgian Nuclear Research Center in Belgium, parabolic flight laboratory in France, and the China Manned Space Agency in China for our spaceflight). Each institute has its own procedure, and each country or set of countries have their own legislations. Shipping bio-samples involves material transfer agreements and customs paperwork, and since human organoids contain human DNA, special ethical requirements apply.

First and foremost, all patients provide written, informed consent to the HUB for future use of their bio-samples in medical research according to very specific guidelines. Research ethics approval for each experiment is required from the HUB and from the host institute in Norway. No experimentation can begin without these ethics approvals that assess our intended use of the samples across all of our collaborating sites in Europe and China. Since the HUB biobank is located in Europe, many of the intricacies with regard to ethics and legislation across our European collaborating sites are already in line. However, human DNA can be imported, but not exported from China. This means that we can ship our organoid samples into China in preparation for our spaceflight launch, but after our

spaceflight experiment has been conducted and safely returned to Earth, those same samples cannot be exported to our genomics laboratory in Norway. Consequently, laboratory analysis of our spaceflight samples must be conducted in China, after which time the organoid samples will need to be destroyed and the data will need to be transferred to Norway for bioinformatic and statistical analysis.

5.2 Laboratory, bioinformatics, and statistical analysis pipelines: step 2

In this step, we go from human organoids in a lab that have been exposed to radiation (ground and space) and/or (micro)gravity (ground and space) to laboratory methods at the Genomics Core Facility (GCF) housed in Norway. We then treat the "big data" with bioinformatics tools to break down the noise and scale down the data for statistical analysis. It is important to note that the details in this step are given at the time of writing in March 2020. Genomics as a field is constantly advancing, sometimes exponentially, and so are the tools used to work with the samples and analyze the data. That being said, our *Tumors in Space* experimental methods are still being finalized, but this at least gives you a glimpse into our preparation for spaceflight.

Organoid sample selection: To account for tissue heterogeneity and differences in mutational signatures, we will use paired samples from three different patients. Assuming 7–10 subclones from each starting culture, we would have 21–30 paired samples which will give sufficient statistical power to detect consistent differences in mutational signatures. Subcloning is an essential step for this experimental strategy, as subcloning ensures that mutations occurring prior to subcloning will be fixed in subsequent culture, allowing these mutations to be detected by whole genome sequencing and somatic genotyping.

Organoid cell culture and subcloning: Laboratory procedures will be handled by the Genomics Core Facility at the Norwegian University of Science and Technology (NTNU) in accordance with previously published methods [18]. The CGF provides state-of-the-art high-throughput genomics technology including sequencing and genotyping microarray analysis. The major advantages of using the CGF include highly experienced staff and the use of robotics. Tumor organoids must be cultured in specifically designed medium [18]. After a fixed exposure time, subclones from each source organoid will be cultured for an additional fixed number of cell divisions to allow for the occurrence of random mutations before whole genome sequencing and somatic genotyping [19].

Whole genome sequencing and somatic genotyping: Classic sequencing-based whole genome analyses of mutational signatures (healthy vs tumor tissue) rely on mutations being clonally expanded due to tumor growth, i.e., the mutation occurred in an ancestor cell so that all daughter cells will have this mutation, and the mutation is detectable if a sufficiently large percentage of the tumor cells share this mutational ancestry. Clonal expansion ensures that within an organoid sample, there will be several DNA fragments and sequencing reads that share the same mutation. In this manner, mutations can be distinguished from sequencing errors. This also means that the mutations occurring within the tumor organoid source cells will not be detectable, as these mutations have not yet been amplified by cell division (unless the same mutation occurs at the exact same nucleotide within a sufficiently large subset of the current cells). The sequencing depth determines the percentage of cells that need to share mutations in order to be detected. For example, 100x sequencing depth can detect mutations if the mutations are present in 5% of cells, whereas 1000× sequencing depth can detect mutations present in only 0.5% of cells.

Although polymerase chain reaction (PCR[4]) is a widely used approach, we cannot use this approach for the current study. Even if we increase the sequencing depth to 5x the number of cells, we would get PCR-amplified copies of the DNA in a single organoid sample and would not be able to distinguish PCR errors from mutations. However, with rolling circle amplification, we would always use the same original (circularized) DNA fragment as a template for amplification. In this way, we will have a long DNA fragment with multiple copies of the original DNA fragment, and by sequencing these copies in a single read, we will detect mutations as consistent changes within each copy.

Bioinformatics and statistical analysis pipeline: All somatic changes in whole genome data will be analyzed with mutation calling pipelines developed in Norway. Healthy tissue more than 5 cm from the tumor and consisting of epithelial and connective tissue [18] will be used as the "germline" reference. Mutations considered to be germline will be removed. Mutational signature extraction using non-negative matrix factorization will be performed [20]. Signature attribution in reference to the Catalogue of Somatic Mutations in Cancer [10] and the Pan-Cancer Analysis of Whole Genome [21] will be conducted.

5.3 Spaceflight hardware readiness: step 3

In this step, we are designing, developing, testing, and validating an automated cell culture experimental unit and six-paneled tantalum cover for cosmic radiation protection. Several characteristics make tantalum a good choice for radiation protection, all of which will be presented below. We will use our ground-based platforms, a sounding rocket, and aircraft parabolic flights to test the impact of environmental stressors and operational constraints (e.g., vibration, changes in gravity, changes in temperature, experimental lag time) on the fidelity of our organoid culture technique and the readiness of our spaceflight hardware. Our spaceflight hardware must fit according to requirements and constraints of the biological research module on the China Space Station. Other experimental equipment that we need for our experiment will be hosted by the China Space Station including a temperature-controlled incubator/cooler and a 1 g centrifuge or variable gravity rack. We also need dosimeters to measure radiation levels for the duration of our experiment.

Experimental unit: We are designing and developing a biocompatible-automated organoid culture experimental unit. An essential first step is to complete biocompatibility testing for each material required for the construction of the unit. Direct contact between organoid culture medium and experimental unit materials must not attenuate organoid growth. More specifically, for our experimental unit to be deemed biocompatible, 75% of all organoid samples must survive. Our unit is required to be operational for the full duration of the 31-day experiment, including automated nutrient solution exchange on days 6, 12, 18, and 24. Our unit must adhere to a standard temperature profile (18–38°C for organoid growth, 4°C for fixed samples) and CO_2 saturation level (5%). Finally, our unit must be capable of enduring physical stressors (vibration, g-forces) while maintaining organoid culture and survival.

We cannot procure market-ready experimental units because all previous cancer experiments in space have used 2D monolayer cancer cell lines and *Tumors in Space* uses 3D human organoids. However, units used for previous cancer experiments in

[4] PCR is a widely used molecular biology method that makes millions to billions of copies from a DNA sample, allowing amplification of a very small amount of DNA to a larger amount of DNA for a detailed study.

space do provide us with a good foundation for our own design and development. For example, Grimm's lab first tested their own cancer cell line experimental unit during the Shenzhou-8 mission [22], which was then further improved for the SpaceX CRS-8 ISS mission [23]. Grimm's unit sets the standard for safe cell culture and cell nourishment followed by fixation according to a pre-programmed timeframe. From this foundation, we are designing our experimental unit with built-in pre-programmed electronics to control temperature gradients for organoid cell culture medium, as well as automated oxygen, carbon dioxide, and nutrition cycles to keep our organoids alive for the duration of the 31-day space mission.

A small temperature-controlled incubator/cooler with a rack insert: Our experimental units will be fully automated removable inserts designed to interface with a small temperature-controlled incubator/cooler that serves as a miniature laboratory for self-contained, automatic microgravity experiments on cells/organoids—similar to ESA's Kubik[5] on the International Space Station (**Figure 6**) [24]. This equipment will be hosted by the China Space Station. The rack insert of the incubator/cooler will provide passive structure to house our experimental unit. The incubator/cooler will operate between 4°C and 38°C and will permit a 1 g centrifuge insert to allow for simultaneous experiments with 1 g control samples and microgravity samples.

Onboard 1 g centrifuge or variable gravity rack: To thoroughly test our hypotheses and delineate compound spaceflight exposures (microgravity and cosmic radiation), we also require an onboard 1 g centrifuge or variable gravity rack that will allow us to expose our organoids to only cosmic radiation while maintaining the standard gravitational force felt on Earth (1 g). Our organoid medium can stay at 37°C, while in the centrifuge, our experimental unit will fit the standard payload boxes hosted by the China Space Station. A variable gravity rack which contains a centrifuge with rotating containers for biological and fluid experiments [25] may be larger than the standard 1 g centrifuge available within the incubator/cooler insert rack. Either the smaller 1 g centrifuge or the larger variable gravity rack will be used for our experiment, and this decision will be taken in close collaboration with the China Manned Space Agency during our hardware development phase.

Tantalum cover for experimental unit: In order to test whether exposure to microgravity slows or stops the growth of cancer, we need to protect our organoids from cosmic radiation exposure. To do so, we are designing a six-sided radiation shield to cover all sides of our organoid cultivation chamber in order to shield the organoids from cosmic radiation. The size, shape, and mass of the tantalum cover

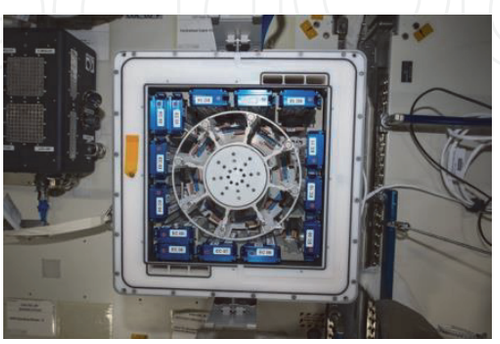

Figure 6.
Kubik on the ISS (credit: ESA).

[5] Kubik (from Russian for cube) is a miniaturized laboratory in a 40 cm cube, installed in the ESA orbital laboratory Columbus module on board the ISS, and is used for several kinds of biology experiments in space.

are being designed according to the size and shape of our experimental unit and the mass constraints of the payload. Several characteristics make tantalum a good choice for radiation shielding of biological experiments in space including very good biocompatibility, excellent corrosion resistance, and very high melting point. Tantalum can also be easily welded into the required shape and size.

Dosimeters: We also need electronic dosimeters to provide passive reading of radiation dosage for the duration of the 31-day experiment. The dosimeters must be sensitive enough to measure high-energy cosmic radiation, lightweight, and compact yet durable enough to be operational in the spaceflight environment [26]. The dosimeters must provide tissue equivalent readings. This means that the electronic data must provide the same information about cosmic radiation dose as we would expect if human tissue, rather than the dosimeter, was directly exposed.

We will work in close collaboration with the China Manned Space Agency to develop, assemble, and test spaceflight hardware according to CMSA standards and quality controls with particular attention to interface compatibility, mass, size, power requirements, and duration of experiment. We will also test our spaceflight hardware during our ground, sounding rocket, and parabolic flight experimental arms.

In preparation for spaceflight, we are running several experiments on research platforms including ground-based space analogues that will produce effects on our organoid models similar to those experienced in space. This includes radiation facilities for ionizing and heavy ion irradiation and the random positioning machine (RPM) to simulate microgravity. It is important to remember that the spaceflight environment can be simulated but never replicated on the ground. We are also preparing for experiments on a sounding rocket and during aircraft parabolic flights. These preparatory experiments are essential to ensure a successful experiment in space. Not only will our organoid cultures and hardware development be tested, but we will also collect important baseline data for later comparison to our spaceflight data. In addition, we will have the opportunity to standardize our shipping, handling, and storage procedures for the organoids, as well as define our laboratory, bioinformatics, and statistical analysis pipelines. Finally, our ground radiation work does not only provide us with a mutational signature of different types of radiation for later comparison with cosmic radiation; it is designed to better understand the side effects of radiation therapy for cancer patients on Earth.

5.4 Ground experiments: step 4

We are using ground-based facilities at the Belgian Nuclear Research Center and ESA's European Space Research and Technology Center to test our hypotheses and theoretical approach and to refine our methodology and operational procedures in preparation for spaceflight. We will collect baseline data on the mutational signature of ionizing and heavy ion radiation and use the random position machine (RPM) to test whether simulated microgravity stops or slows the growth of cancer.

Ionizing radiation: To identify a unique mutational signature of cosmic radiation, we must verify the mutational signature of ionizing and heavy ion radiation for comparison. Using the gamma beam irradiator at the Belgian Nuclear Research Center, we will irradiate the following:

i. Our 1 g control samples

ii. Our samples exposed to simulated microgravity in the RPM

iii. Our experimental unit and tantalum cover

Our 1 g control samples can be placed in direct line of the gamma ray to capture the mutational signature of ionizing radiation. By varying the dose and time of the beam, we will simulate radiation therapy given to cancer patients on Earth and examine whether healthy organoids from cancer patients turn cancerous after dose- and time-dependent exposure to radiation. Next, our organoids will be contained in the RPM, and the RPM will then be placed in front of the irradiator. This will allow us to simultaneously expose our organoids to radiation and simulated microgravity. Finally, we will test the fidelity of our spaceflight hardware by exposing our organoids housed within the experimental unit and protected by the tantalum shielding to a predetermined dose rate of ionizing radiation.

The random position machine: We are using the RPM at ESA-ESTEC to collect baseline data on the effect of simulated microgravity on the growth of cancer organoids. The RPM (**Figure 7**) uses two rotating axes, each with independent motor drives running at random speeds and generating random three-dimensional movements [27, 28] that change the direction of the gravitational vector felt by the organoids such that cumulative gravitational effects are cancelled over time. The maximum angular speed of the inner and outer frame of the RPM generally ranges from 20°/s to 120°/s. Thus, the geometry and size of the container within which the biological system is placed must be carefully considered [29]. The sample container will then be mounted to the inner axis. The RPM can run for the 31-day duration of the experiment, and cultural medium can be replaced when needed.

5.5 Sounding rocket: step 5

We will use a sounding rocket to test the impact of environmental and operational factors (e.g., vibration, changes in gravity, changes in temperature, experimental lag time) on the fidelity of our models and readiness of our hardware. Sounding rockets carry experiments up to 750 km above the Earth's surface and offer up to 13 min of microgravity. After launch, the sounding rocket motor is shut down, and our experiment will be in free fall. Parachutes are deployed on the downward arc to return the rocket and experiments safely to the ground. The rocket and experimental unit will reach a peak gravitational force of 12 g which could impact our organoids, as can launch vibrations and other environmental factors. This will be our first microgravity experiment. Not only will we collect valuable data on the underlying gene expression of our organoids after exposure to microgravity, we will also have the opportunity to test our hardware as part of a rocket launch. At the end of this step, we will use the data to improve our logistics and operations, our organoid models, and the fidelity of our spaceflight hardware.

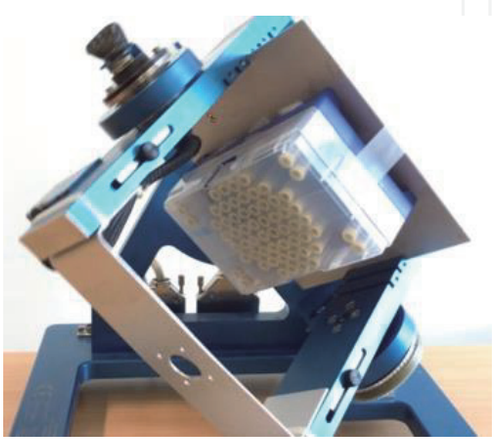

Figure 7.
The RPM at ESA-ESTEC (credit: ESA).

5.6 Parabolic flights: step 6

Similar to the sounding rocket, we will use aircraft parabolic flights to test the impact of environmental and operational factors on our experiment. The parabolic flight takes place on a specially designed commercial aircraft [30] that flies through a series of parabolas and gives 20–22 s of microgravity on each parabola. The aircraft flies up and down at 45° angles, and at the top of the curve, 20–22 s of microgravity is experienced. About 2 g is experienced during ascent and descent. A typical parabolic flight campaign involves 30 parabolas per flight and 3 flights over a 1-week period. This will be our final step before spaceflight and our last opportunity to make any and all necessary improvements to our experiment.

5.7 Spaceflight experiment: step 7

According to standard operating procedures and in close collaboration with the China Manned Space Agency, we will finalize our preparation for spaceflight in the following manner. Our experimental units will be assembled prior to launch, including several backup units. All hardware will be sterilized and approved for launch. Our samples will be transported to the launch site on dry ice from our biobank in the Netherlands. Our organoids will arrive a minimum 7 days before the launch. After the initial phase of growth, our organoids will be placed into the validated and approved units, and the units will be inserted into the incubator/cooler. The incubator/cooler housing our units will be loaded into the rocket prior to launch (-1 hour to -2 days). Once the payload is ready for flight, no additional handling of the experiment will be required before launch.

The experiment will start and end under constant environmental conditions (e.g., temperature, pressure, humidity) after our experimental units have been installed in the incubator/cooler on the China Space Station. The duration of our experiment is 31 days for organoids exposed to the natural spaceflight environment (microgravity and cosmic radiation), 31 days for organoids exposed to microgravity under the tantalum cover designed to protect our samples from cosmic radiation, and minimum 10 days for organoids exposed to cosmic radiation and Earth gravity with the use of the 1 g centrifuge. Dosimeters will be used to read the radiation level for the duration of the experiment.

The cell nourishment and fixation will be automated and pre-programmed according to experimental needs. Subsequent cold stowage of the fixed samples will be required at the end of the experiment. After completion of the mission and successful environmentally controlled return to the ground, our units will be transported to our laboratories for organoid subcloning, sequencing, and genotyping as described above.

6. Impact

Tumors in Space is a cancer research project at the intersection of stem-cell biology and space technology that will acquire new knowledge on cancer etiology due to the influence of the spaceflight environment. By conducting cutting-edge laboratory-based research in orbit and testing our two novel hypotheses, we are challenging the current scientific paradigm. We aim to spark curiosity and inspire the public by conducting excellent science at the forefront of international research in collaboration with the United Nations Office for Outer Space Affairs (UNOOSA) and the China Manned Space Agency.

Figure 8.
The 17 Sustainable Development Goals (credit: United Nations).

The Sustainable Development Goals: The UNOOSA works to promote the peaceful use of outer space for the benefit of humankind. This includes a collaboration with the CMSA to fly international experiments on board the China Space Station. The 17 Sustainable Development Goals (**Figure 8**) were adopted by the United Nations in 2015 [31] as a global call for action to improve environmental conditions on Earth and socioeconomic as well as health conditions for all humanity. *Tumors in Space* has a role to play in the attainment of the Sustainable Development Goals by 2030 through the peaceful and collaborative use of outer space to ensure healthy lives and promote well-being for all people of all ages.

Science communication in the public domain: Science funded by the people is meant to be delivered to the people, and this is one of the goals of *Tumors in Space.* We have designed a comprehensive science communication and research dissemination plan. The main feature of our plan is our project website that will include open access to our research results and scientific publications, research highlights, a color photo library, team bio-sketches, blog posts, and social media feeds (e.g., Twitter), as well as an up-to-date list of all interviews and feature articles for the *Tumors in Space* project. Our efforts also include educational outreach focused on girls and marginalized youth.

7. Conclusions

This chapter provides an overview of the *Tumors in Space* experiment beginning with the scientific evidence upon which the hypotheses are based. The seven main steps in our spaceflight preparation have been presented including ethics and regulatory affairs, laboratory methods, spaceflight hardware design, ground-based experiments (radiation and simulated microgravity), as well as sounding rocket and aircraft parabolic flight experiments. This takes us to the final preparations for our spaceflight experiment set to be launched to the China Space Station in 2025. Finally, some brief notes on the importance of the Sustainable Development Goals and communication of science in the public domain have been provided. To end this chapter, we provide a note of encouragement. Do share this chapter widely in the spirit of open access to scientific knowledge. We welcome you to follow our journey into orbit as we reach for our ultimate goal: to make novel contributions to cancer risk prediction, diagnostics, and therapeutics for human health during short- and long-duration space missions and public health on Earth.

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Author note

"As a final outcome of the application and selection process in response to the first cycle of Announcement of Opportunity under the United Nations/China Cooperation on the Utilization of the China Space Station (CSS) initiative, being implemented by the Office for Outer Space Affairs (OOSA) and the China Manned Space Agency (CMSA) respectively, your proposal entitled "Tumors in Space: Signatures of early mutational events due to spaceflight conditions on 3D organoid cultures derived from intra-individual healthy and tumor tissue", has been fully accepted for implementation on board the CSS."

As announced on 12 June 2019 in Vienna, Austria, during the 62† Session of the Committee on the Peaceful Uses Of Outer Space and signed on 9 July 2019 by Director of United Nations Office for Outer Space Affairs (UNOOSA), Ms. Simonetta Di Pippo.

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