# Unlocking a New Paradigm: Microgravity in Advanced Cancer Therapy

Technical Deck

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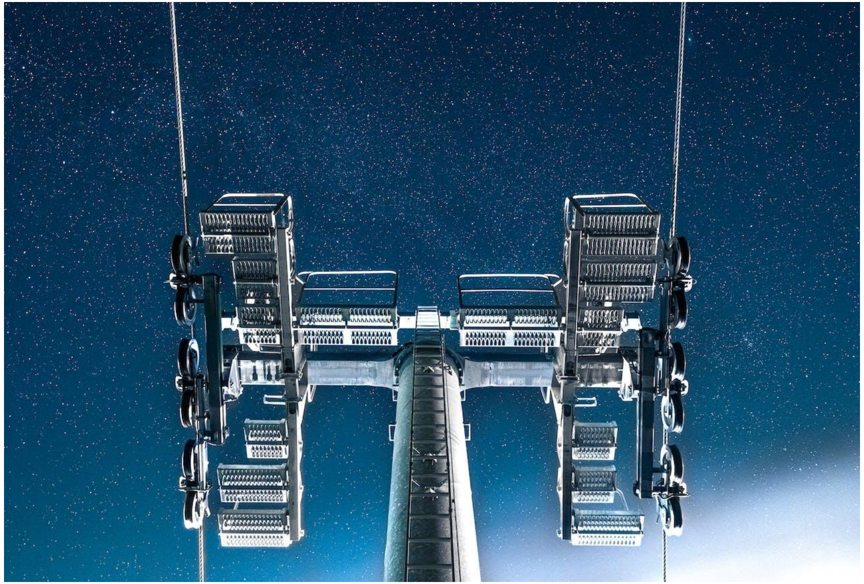
**Toronto**
**2024**

# Our Mission

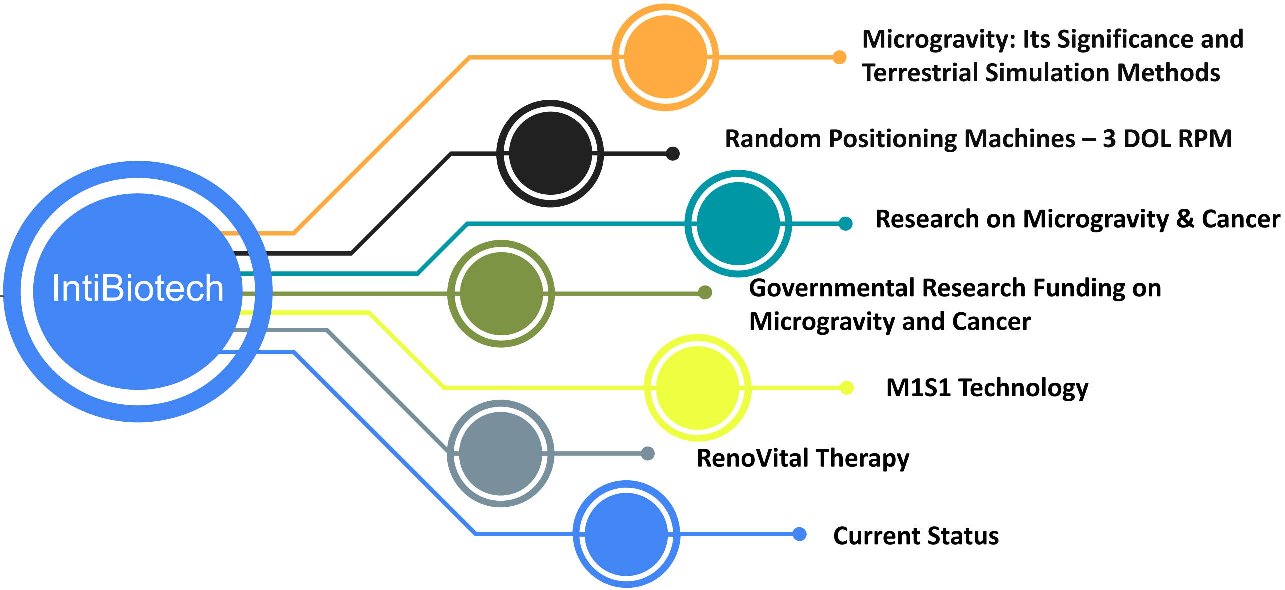


- At Inti Biotech, our mission is to revolutionize medicine by harnessing microgravity's unique environment for innovative treatments.
- With our cutting-edge M1S1 Random Positioning Machine, our aim is to contribute to novel solutions in Cancer Therapy, Regenerative Medicine, Immunology, Neuroscience, and Drug Development.

# Our journey

## RenoVital Therapy

**M1S1 Technology**



# State of the Art

# Our journey – Step 1


We initiated our investigation by comprehending the concept of microgravity, highlighting its importance in studying biological system dynamics, and outlining methods for replicating microgravity conditions on Earth.

# Microgravity and Simulated Microgravity

# What is Microgravity?

# Why is it important to carry out research on Microgravity?

- Microgravity is the condition in which people or objects appear to be weightless. The effects of microgravity can be seen when astronauts and objects float in space. (NASA)

- In Human Health this research is important because prolonged exposure to microgravity can have significant effects on the human body, such as muscle atrophy, bone density loss, and changes in cardiovascular function.  Studying these effects can lead to advancements in medical science, including treatments for osteoporosis and muscle wasting diseases, as well as countermeasures to mitigate the negative impacts of space travel on human health.

# What is Simulated Microgravity?

- Generating microgravity conditions on Earth is attainable through the implementation of random movements on a 3-axis rotating platform relative to the centre of rotation.
- This process results in an effective microgravity on the order of 10-4g (g: gravity), expressed as g* = ω2R/g, where g* denotes the gravity value arising from the random movement of the platform.
- This technological methodology enables the replication of microgravity environments akin to those encountered in space.

# Alternative approaches to simulating Microgravity

- **Drop Towers:** A drop tower constitutes a vertical structure for releasing experiments, inducing a brief microgravity episode as objects fall freely within the tower. These towers offer concise microgravity environments, typically lasting from 2 to 9 seconds.
- **Parabolic Flight:** Aircraft, exemplified by the "vomit comet" like NASA's KC-135, follow parabolic trajectories. Occupants within the aircraft undergo brief microgravity periods during the free-fall phase, providing approximately 20-30 seconds of uninterrupted microgravity.
- **Neutral Buoyancy Laboratory (NBL):** Utilizing large water tanks, such as NASA's Johnson Space Center's NBL, facilitates the simulation of microgravity for underwater astronaut training.
- **Drop Capsules:** Specialized capsules or containers can be released from high altitudes using balloons or other means, generating a short-term microgravity environment.
- **Centrifuges:** Simulating gravity, centrifuges spin objects or organisms at high speeds. Adjusting the rotation speed allows researchers to create environments with gravitational forces resembling those on celestial bodies.
- **Space-based Platforms:** The ultimate microgravity setting is, unequivocally, space itself. Experiments conducted on spacecraft or space stations encounter genuine weightlessness. **The International Space Station (ISS)** stands out as an example.
- **Random Positioning Machines (RPM):** Simulate microgravity conditions by continuously and randomly changing the orientation of samples placed within it.

# Our journey – Step 2


The Random Positioning Machine (RPM) effectively replicates microgravity conditions on Earth, providing flexibility to accommodate different sample types and experimental setups, facilitating a wide range of research endeavors across various scientific fields. Dr. Lavan's 3 Degrees of Liberty (3 DOL) RPM represents a significant advancement in this technology.

# Random Positioning Machines (RPMs)

A Random Positioning Machine (RPM) offers distinct advantages over alternative devices for simulating microgravity conditions.

- An RPM provides more precise and consistent control over the orientation and motion of samples.
- An RPM offers extended durations (hours) for conducting experiments under simulated microgravity conditions.
- RPMs continuously and randomly change the orientation of samples, effectively cancelling out the effects of gravity in all directions.
- This dynamic motion closely mimics the erratic nature of microgravity experienced in space, resulting in a more accurate simulation for scientific research.
- An RPM is capable of accommodating a wide range of sample sizes and types, allowing for greater flexibility in experimental design and facilitating diverse research applications.

# One degree of Liberty

Image source: [NASA]

## Bioreactor

- Single-axis rotational microgravity machine suitable for cellular cultivation experiments.
- Cultures are placed in the center of the rotating platform.
- It is versatile and manageable due to its size, and can be easily adapted into a CO2 incubator.

# Two degrees of Liberty

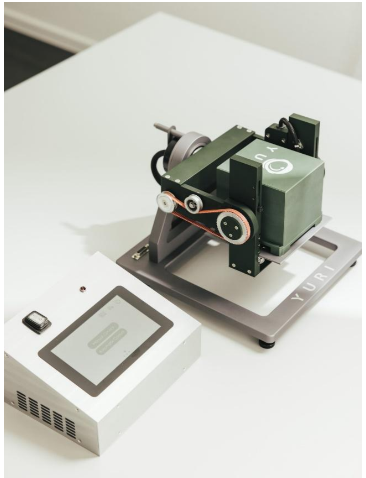
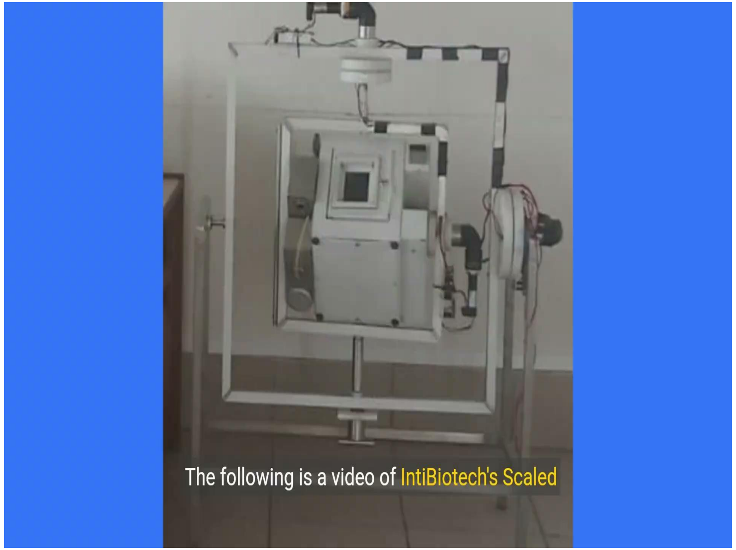



Image source: [Yuri]

## Random Positioning Machine

- Microgravity machine with two degrees of liberty, where the rotational axis of each rectangular ring is connected to a motor through a belt.
- Suitable for cellular cultivation experiments, where cultures are placed in the center of the rotating platform.
- It is versatile and manageable due to its size and can be easily adapted into a $CO_2$ incubator.

# Three degrees of Liberty

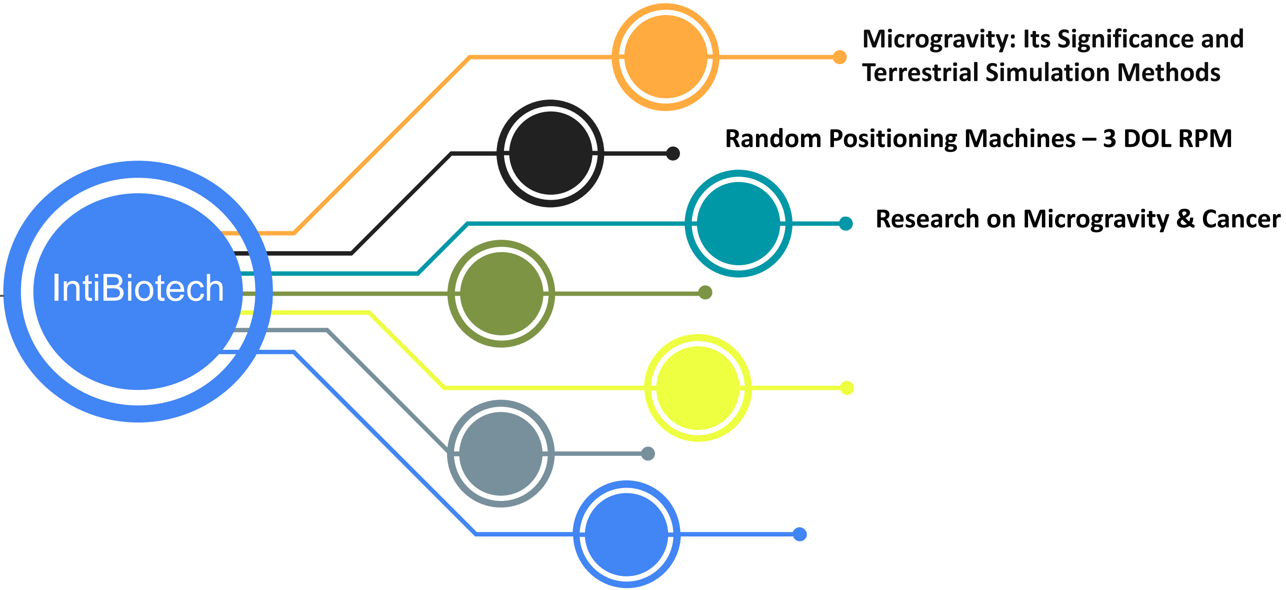



Video source: IntiBiotech

## IntiBiotech Scaled Model RPM

- Microgravity machine with three degrees of liberty, where the rotational axis of each rectangular ring is connected to a motor.
- It has a load capacity of 10 kilograms and can accommodate a 15 cm3 $CO_2$ incubator for cellular cultivation experiments.
- Presently, all microgravity machines consist of interconnected rectangular frames, where the rotation axes of these frames are directly or indirectly linked to an electric motor.
- Click here or on the picture to the left to watch video

# Our journey – Step 3




The rationale behind incorporating microgravity into cancer research arises from two primary factors. Firstly, microgravity affects human biological systems. Secondly, cancer stands as the leading cause of mortality. This prompts investigation into microgravity's potential for cancer treatment. David Lavan initiated this groundbreaking research in microgravity in 2007 (with support from the European Space Agency - ESA).

# Research on Microgravity and Cancer

# Why Cancer?

- Carcinoma denotes a malignant neoplasm originating from epithelial tissues, representing cancer affecting the internal or external linings of the body. **These malignancies, constituting 80 to 90 percent of all cancer cases**, encompass various organs, including the prostate, breasts, liver, blood vessels, pancreas, colorectal, among others. (NIH – National Cancer Institute)

- The regulatory genes of these epithelial tumors are mostly transmembrane proteins, where cell-cell interaction is regulated by these transmembrane proteins.

- The regulatory genes inherent to epithelial tumors predominantly encode transmembrane proteins responsible for governing cell-cell interactions. **These proteins, exhibiting a mass exceeding 36 kDa (kilodalton), experience inhibition under microgravity conditions**, leading to a consequential hindrance in the cellular proliferation process and, consequently, impeding tumor growth. (Lavan 2023)

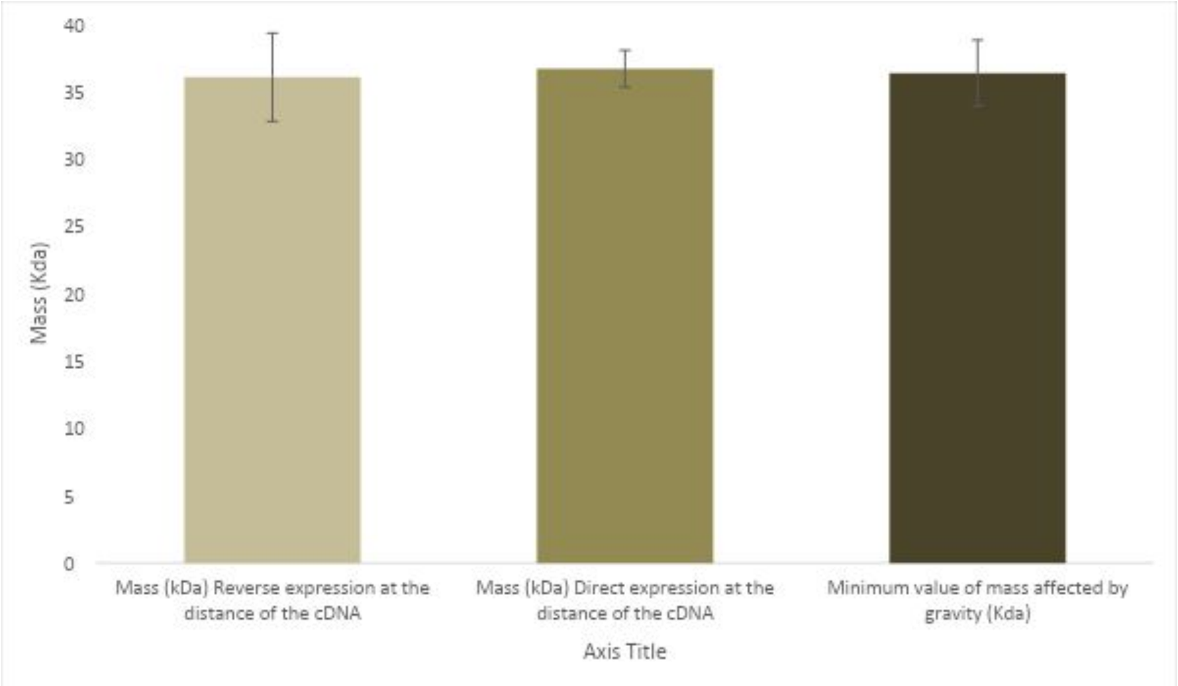
# Possible critical mass (Kd) value limiting expression of Drosophila melanogaster pupae under microgravity conditions.

**Source: (Forthcoming) Laván, D. A.** (2023). Study 1: Possible critical mass (Kd) value limiting expression of Drosophila melanogaster pupae under microgravity conditions. Journal of Applied Developmental Biology, 78, 19-25. doi: 10.1016/j.jadb.2023.01.002.

- The goal of this study was to identify genes involved in regulating the response to microgravity in Drosophila melanogaster using microarray analysis. Unexpectedly, it was found that the regulatory elements did not display any significant response to microgravity exposure. This observation prompted the team to investigate further into the mechanism underlying the regulation of gene expression under microgravity conditions.
- Specifically, the research aimed to determine whether the lack of response in regulatory elements was due to the constancy of experimental conditions and initial gene expression levels, or if it was attributed to differences in the timing of polymerase activity during transcription. The team's findings suggest that the latter may be the case, highlighting the importance of considering the kinetic aspects of gene expression in the study of microgravity effects on living organisms.

# Fruit flies and gene expression in microgravity

- Lavan's research on fruit flies has shown that gene expression in microgravity conditions depends on the size of mRNA.
- Extrapolating this result to humans, we can hypothesize that glandular epithelial tumors are inhibited in microgravity conditions due to their larger mass exceeding 36 kD.




**Note:** Slide 19 provides an overview of our research findings. The complete study is in print and will be published shortly.

# Fruit flies and gene expression in microgravity

Results from our research on fruit flies and microgravity.

**Note:** Slide 19 provides an overview of our research findings. The complete study is in print and will be published shortly.

| Name of Gene | Mass(kDa) Reverse expression at the distance of the cDNA |
|---|---|
| CG31743 | 37.8 |
| CG5567 | 36.4 |
| Gapdh1 | 35.4 |
| Dnaj-1 | 37 |
| CG6906 | 28.3 |
| H | 37 |
| CG4778 | 38.2 |
| Impl1 | 38.3 |
| **Mass average** | **36.05** |
| **Standard deviation** | **3.27675798** |

| Name of Gene | Mass(kDa) Reverse expression at the distance of the cDNA |
|---|---|
| CG7998 | 35.3 |
| CG3597 | 37.8 |
| NUDC | 37.8 |
| Impl3 | 35.5 |
| CG7860 | 34.9 |
| Scf | 38 |
| CG30101 | 38.1 |
| CG3957 | 36.1 |
| **Mass average** | **36.6875** |
| **Standard deviation** | **1.366369015** |

# Testing 3D printed biological platform for advancing simulated microgravity and space mechanobiology research

Source: Silvani, G., Bradbury, P., Basirun, C., Mehner, C., Zalli, D., Poole, K., & Chou, J. (2022). Testing 3D printed biological platform for advancing simulated microgravity and space mechanobiology research. npj Microgravity, 8(1), 19. doi: 10.1038/s41526-022-00207-6.

- The development of microgravity simulators has enabled researchers to investigate the effects of the mechanically unloaded space environment on cellular function and dysfunction. However, conducting microgravity experiments on Earth using simulators like the Random Positioning Machine can present several practical challenges, including air bubble formation and leakage of growth medium from tissue culture flasks and plates, which can hinder research progress.

- To address these limitations, this team developed a novel, user-friendly hybrid biological platform that combines 3D printing technologies with PDMS microfluidic fabrication techniques to facilitate reliable and reproducible microgravity cellular experiments. The system has been characterized for applications in brain cancer research by exposing glioblastoma and endothelial cells to 24 hours of simulated microgravity conditions to study the activated mechanosensing pathways involved in cellular adaptation to the new environment.

- The team's platform has demonstrated compatibility with various biological assays, including proliferation, viability, morphology, protein expression, and imaging of molecular structures, offering advantages over traditional culture flasks. Their results showed that both cell types are affected when the gravitational vector is disrupted, confirming the impact of microgravity on both cancerous and healthy cells. Specifically, the team observed deactivation of Yap-1 in glioblastoma cells and remodeling of VE-Cadherin junctional protein in endothelial cells.

- The study supports the application of the proposed biological platform for advancing space mechanobiology research and highlights perspectives and strategies for developing next-generation brain cancer molecular therapies, including targeted drug delivery strategies.

# Influence of microgravity on apoptosis in cells, tissues, and other systems in vivo and in vitro

Source: Prasad, B., Grimm, D., Strauch, S. M., Erzinger, G. S., Corydon, T. J., Lebert, M., Magnusson, N. E., Infanger, M., Richter, P., & Krüger, M. (2020). Influence of microgravity on apoptosis in cells, tissues, and other systems in vivo and in vitro. International Journal of Molecular Sciences, 21(24), 9373. doi: 10.3390/ijms21249373.

- All living organisms have evolved on Earth under the constant influence of gravity, developing mechanisms to counteract its effects. However, in space, gravitational forces are significantly diminished or absent, leading to changes in the balance between cellular architecture and external forces. This disruption can occur at multiple levels, including the cytoskeleton, signal transduction pathways, membrane permeability, and others. Additionally, exposure to cosmic radiation poses a significant threat to astronauts' health due to its high linear energy transfer values, which can cause complex DNA and cellular damage. The unique environment of space has been shown to modulate apoptosis in various cell types, a process crucial for morphogenesis, organ development, and wound healing.

- This review provides an overview of microgravity research platforms and their applications in studying apoptosis. It summarizes the current understanding of how microgravity and cosmic radiation affect cells, with a focus on apoptosis. The article presents experiments conducted using different mammalian model systems, examining the impact of microgravity on immune cells, cardiovascular cells, neurons, eye cells, cartilage, bone, gastrointestinal cells, liver cells, pancreatic cells, and cancer cells. These studies demonstrate the potential of the space environment in advancing biomedical research.

# Using space-based investigations to inform cancer research on Earth.

- Cancer researchers rarely consider the microgravity environment of space when designing experiments. However, space offers unique physical conditions that cannot be replicated on Earth, as well as opportunities to investigate cellular mechanisms and pathways that govern cell growth and behavior. Over the past four decades, research has demonstrated that exposure to microgravity affects various biological processes relevant to cancer.
- For this research the team examined the impact of microgravity on cellular biology, specifically discussing tumor cells cultivated in space alongside ground-based studies employing analogous models.

Source: Becker, J. L., & Souza, G. R. (2013). Using space-based investigations to inform cancer research on Earth. Nature Reviews Cancer, 13(5), 315-327. doi: 10.1038/nrc3507.

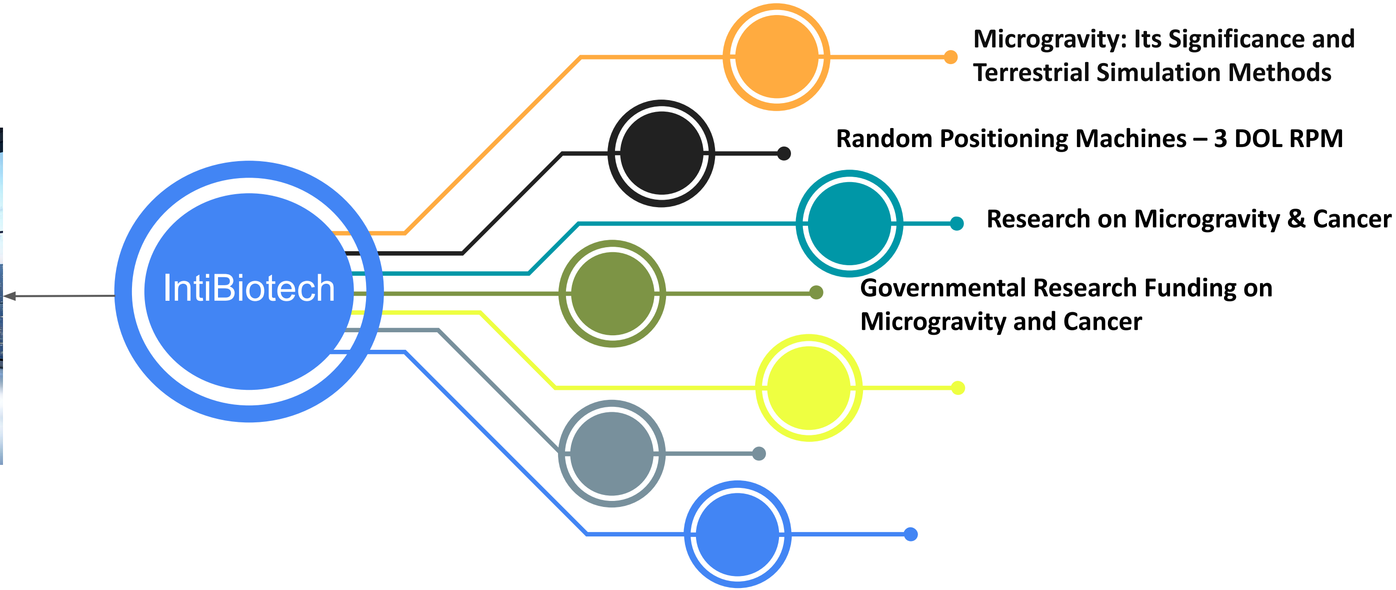
# Spaceflight-related suboptimal conditions can accentuate the altered gravity response of Drosophila transcriptome

Source: Herranz, R., Benguria, A., Laván, D. A., López-Vidriero, I., Gasset, G., Medina, F. J., van Loon, J. J. W. A., & Marco, R. (2010). Spaceflight-related suboptimal conditions can accentuate the altered gravity response of Drosophila transcriptome. Molecular Ecology, 19(19), 4255-4264. doi: 10.1111/j.1365-294X.2010.04795.x.

- Reducing gravity levels during Drosophila metamorphosis in the International Space Station (ISS) leads to significant alterations in gene expression, as revealed by genome-wide transcriptional profiling. However, it is important to note that the preparation procedures for spaceflight and the non-ideal environmental conditions on board the ISS can also impact gene expression. To better understand the effects of microgravity on gene expression, this team performed simulated microgravity experiments on the ground using a random position machine (RPM). While these experiments showed more subtle effects on gene expression, repeating the experiments under conditions that reproduced the additional environmental stresses imposed by spaceflight procedures resulted in 79% of the differentially expressed genes (DEGs) detected in the ISS being reproduced by the RPM experiment. Gene Ontology analysis of these DEGs revealed that they are primarily involved in respiratory activity, developmental processes, and stress-related changes.

- To further analyze the effects of microgravity on gene expression,'gene expression dynamics inspector' (GEDI), the team used self-organizing maps. These maps revealed a subtle response of the transcriptome to microgravity, with hypergravity simulation inducing a similar response in the opposite direction. That is, the genes promoted under microgravity are generally suppressed under hypergravity. These findings suggest that the transcriptome is finely tuned to normal gravity and that microgravity, along with environmental constraints associated with space experiments, can have profound effects on gene expression.

# Our journey – Step 4




What are the sources of funding for cancer research utilizing microgravity? It commenced with ESA in 2003, followed by NIC (USA) in 2016; CSA in 2019; and NASA in 2022. The financial commitments from these agencies amount to billions of dollars.

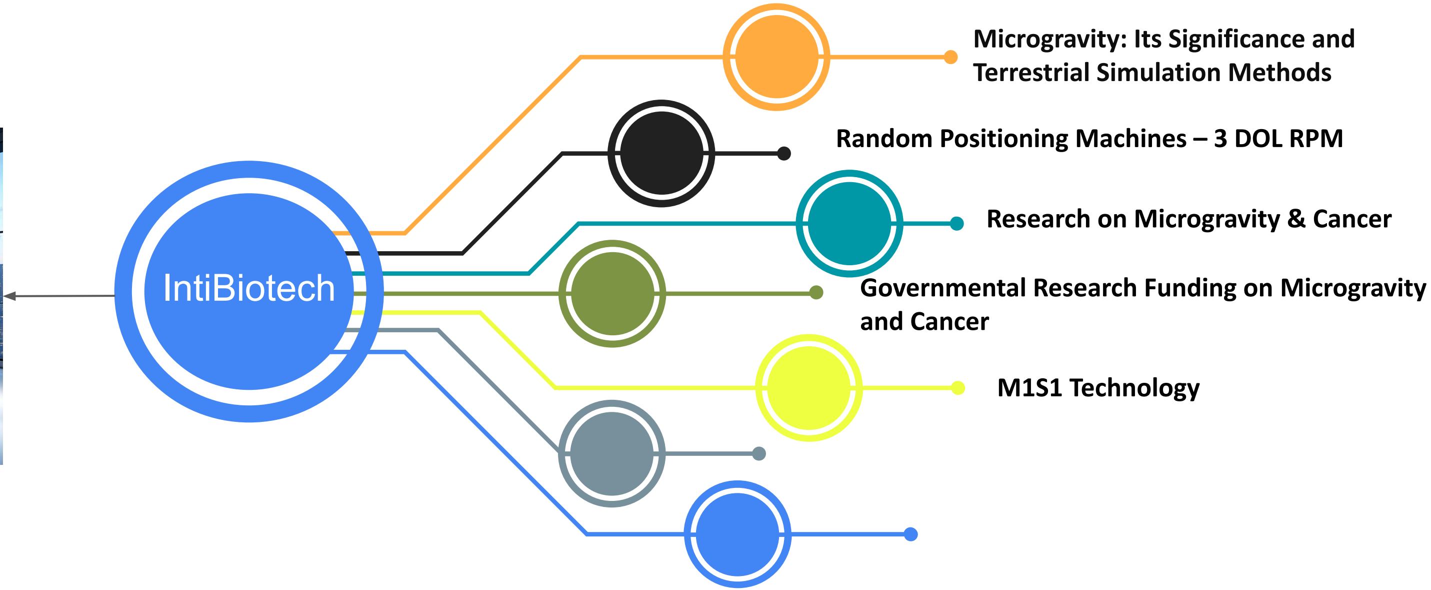
# Government Research Funding on Microgravity and Cancer

# What are governments currently investing in?

- NASA (2022): Space Station Provides a Platform for Seeking Better Cancer Treatments.
- Canadian Space Agency (2019): Health Beyond Initiative.
- National Cancer Institute (2016): Cancer Moonshot.
- European Space Agency (2003): Space science joins battle against cancer.

# Our journey – Step 5

**M1S1 Technology**




The development of our M1S1 Technology, including its distinctive attributes and the proprietary algorithm behind the M1S1, stems from Dr. Lavan's extensive experience spanning over 15 years in pioneering simulated gravity conditions on Earth.

# M1S1 Technology

# The beginning of the **M1S1 Technology**

- In 2007, David Lavan initiated his doctoral research on the inhibition of gene expression levels in Drosophila melanogaster subjected to microgravity conditions.
- This experimentation involved the sending organisms to the **International Space Station (ISS)** via the **European Space Agency (ESA)** and employing microgravity simulators on Earth.
- Successfully designed and tested algorithm for use in microgravity simulator.
- **In 2013, David Lavan (and his team):**
  - Successfully developed and implemented a second iteration of our microgravity simulation device, which enabled us to investigate the effects of microgravity on gene expression and cellular behavior.
  - Subsequently, initiated a research program in the Instituto Nacional de Investigación y Capacitación de Telecomunicaciones de la Universidad Nacional de Ingeniería (INICTEL-UNI) - utilizing this novel tool to explore the genetic aspects of microgravity and its impact on various biological systems.

# The beginning of the **M1S1 Technology**

From 2012 to 2022, David Lavan and his team conducted the following research:

- Microgravedad y cáncer
- David Laván contra el cáncer: los efectos de la microgravedad
- Estudian cómo vencer al cáncer utilizando moscas sometidas a microgravedad

In 2023, David Lavan and his team secured additional funding to build the first microgravity machine with an internal incubator:

- Pro Innovative Perú 2023 - Programa Nacional de Desarrollo Tecnológico e Innovación.

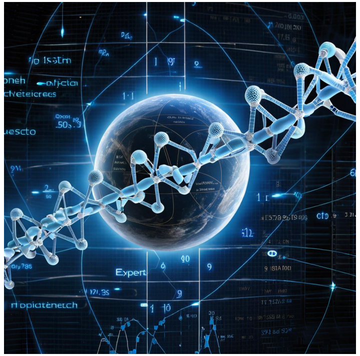
# The M1S1

- The M1S1 consists of two spherical caps connected by an innovative interface.
- This design allows for the accurate replication of space microgravity conditions, enabling scientists to conduct groundbreaking research and testing without leaving Earth's atmosphere.

# M1S1 Characteristics

- Ergonomic space, equipped with O2 and Ozone Therapy capabilities.
- Individualized cabin for each patient.
- Specialized Work Hub Dedicated adjacent to the cabin.
- Integrated Vital Parameter Sensor.
- Sound & Visual Proficiencies.
- Incorporated IMU Motion Sensor.
- Motion in Six Distinct Directions.

# M1S1 Algorithm



**TRADE SECRET**

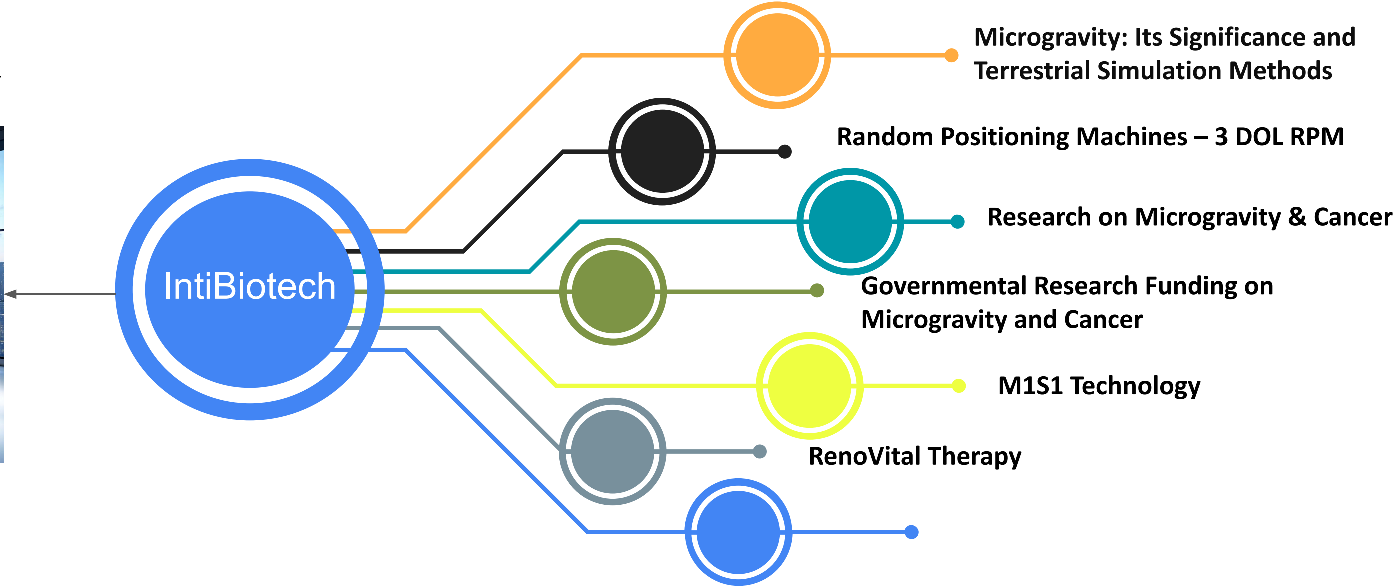
- Microgravity is not exclusively related to the structural properties of a platform, but instead, it depends on the random angular velocity and radius of curvature at the platform's center. Consequently, any platform capable of displaying random motion in all three axes of freedom can produce a change in gravity, rendering it appropriate for microgravity simulations.

- Nevertheless, to achieve this on Earth, a **specialized algorithm** must be created.
    - David Lavan has designed a **proprietary algorithm** that has been effectively tested on all prototype models, enabling microgravity research to be conducted.

# Our journey – Step 6

## RenoVital Therapy

**M1S1 Technology**




How does RenoVital therapy set itself apart from conventional cancer treatments, and what unique attributes does it possess? The differentiating factor lies in Dr. Lavan's groundbreaking cancer research.

# RenoVital Therapy

# What is RenoVital Therapy?

- A Non-invasive Cancer Therapy.
- How does it work?
    - The patient lays in a ergonomic bed for a period of two (2) hours per session
    - During each session, the patient is exposed to simulated microgravity
    - The total number of sessions is 40, carried out for a total of 8 weeks (80 hours in total)
- RenoVital therapy is done using our M1S1 microgravity simulator.

# What is the outcome of RenoVital Therapy?

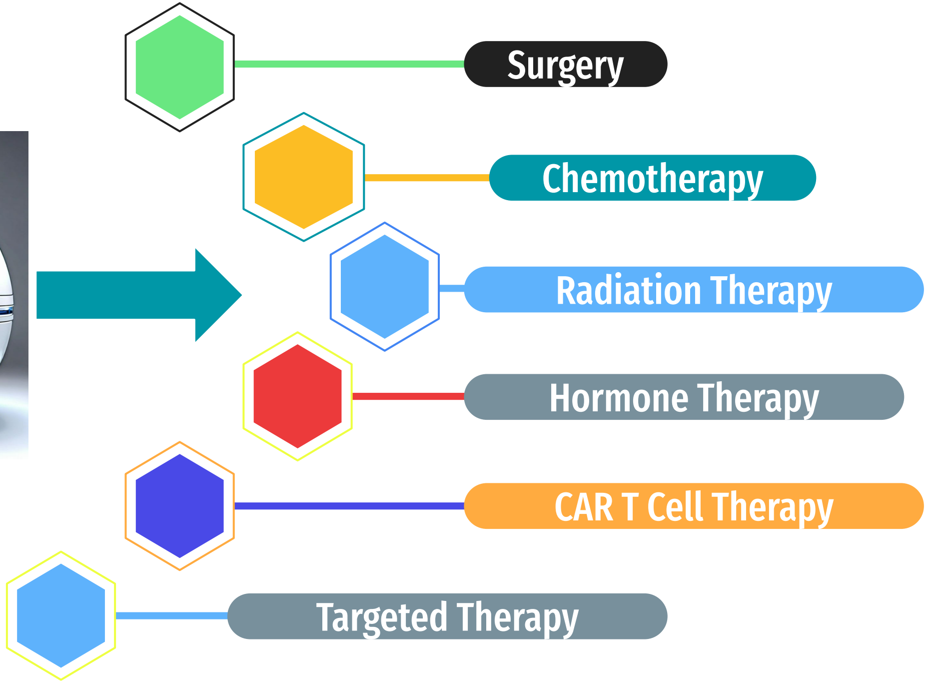
- Therapy results include:
  - Impeding the growth and expansion of Cancerous tumors in Humans.
  - Inhibiting the growth of Cancerous cells.
  - Preventing the proliferation of Cancer cells and tumors when the patient returns to gravity.

# How RenoVital Therapy success is measured?

- Results are measured using the following criteria:
  - **Frequency:** Evaluation commences after the initial five (5) sessions, with subsequent assessments conducted every five (5) sessions thereafter.
  - **What is measured:** Gene expression levels of cancer-activating regulators using molecular PCR techniques.
  - **Tool is utilized:** PCR Testing – Recognized as the Gold Standard

# Competition

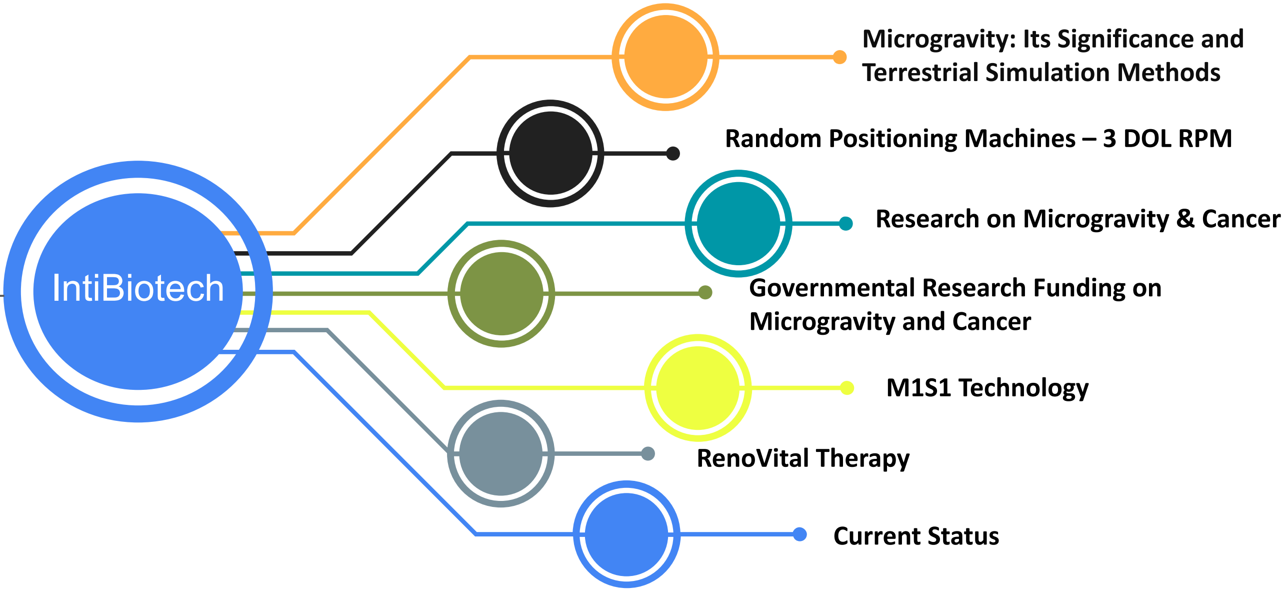
- RenoVital is a **non-invasive therapy** that **inhibits, and prevents** the growth and expansion of Cancerous tumors in Humans.
- **RenoVital** will serve as the **first line of defense**, precisely targeting the disease.
- **RenoVital lays the groundwork for improved outcomes**. And if necessary, allows subsequent invasive procedures to precisely target any remnants.



# Our journey – Step 7

## RenoVital Therapy

**M1S1 Technology**




We are ready to commence construction of our first Microgravity Simulator (M1S1 Technology) and make RenoVital Therapy (a non-invasive cancer therapy) accessible to all.

# Current State

# Current State

**RenoVital Therapy**

- Therapy Design √
- Therapy Validation X
- Therapy Effectiveness X

**M1S1**

- Architectural Design √
- Algorithm Validation √
- Ergonomic Design √
- Ambiance Element Definitions √

# Leadership

**David Laván, PhD**

Co-founder & CEO
Molecular Biologist & Physicist

UAM Universidad Autónoma de Madrid

**Arturo Ruiz, MBA**

Co-founder & CFO
Mechanical Engineer

Durham University

**Felipe Rubio, PhD**

Co-founder & COO
Social Scientist

Freie Universität Berlin

# Thank you

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**Toronto**

**2024**