WHITE PAPER

TECHNOLOGIES TO ENHANCE CELL THERAPY POTENCY IN SOLID TUMORS

xcellbio

Dr. Steven Rosenberg with Linda Taylor, a former melanoma patient he cured using her own T cells. Photos were taken 30 years apart (1984 - 2014)

THE BRIEF HISTORY OF ADOPTIVE CELL THERAPIES: ROSENBERG'S INSIGHT

Adoptive cell therapies, also known as immunotherapies, have delivered extraordinary results for cancer patients with grim prognoses — often, patients whose cancer did not respond to traditional treatments. Scientists around the world are eager to expand the array of adoptive cell therapies, making them available to more patients with a broader range of cancer types. These innovative therapies emerged from advances in our understanding of the interplay between cancer cells, the immune system, and the tumor microenvironment (TME).

Some of the earliest progress came from Steven Rosenberg, now Chief of Surgery and Head of Tumor Immunology at the National Cancer Institute. Back in the 1970s, he was a surgical resident at Peter Bent Brigham Hospital in Boston when an unusual case caught his attention. A kidney transplant patient had developed cancer, and doctors discovered that the cancer came from the transplant — it had ridden along with the kidney into its new host. They took the patient off the immunosuppressant drugs needed to keep the kidney from being rejected. Amazingly, the patient's immune system roared back to life and wiped out the transplanted tumor cells.

That case influenced the direction of Rosenberg's career, leading him to study how immune cells can be trained to kill cancer cells. He identified immune cells with anti-tumorigenic properties and pioneered immunotherapy by infusing them back into a patient, essentially fighting the cancer in the process. The first adoptive cell therapies followed Rosenberg's approach. Since then, advances have made newer versions of these therapies more effective for a broader group of patients.

These developments have been based on improving our view of the tumor microenvironment, and the sophisticated interplay among tumor cells, immune cells, and supporting stromal cells. And while many adoptive cell therapies are now on the market, more innovation is urgently needed: current therapies have been successful in targeting blood-based cancers. However, no successful treatment has yet been developed for solid tumors, which represent the vast majority of cancers diagnosed each year.

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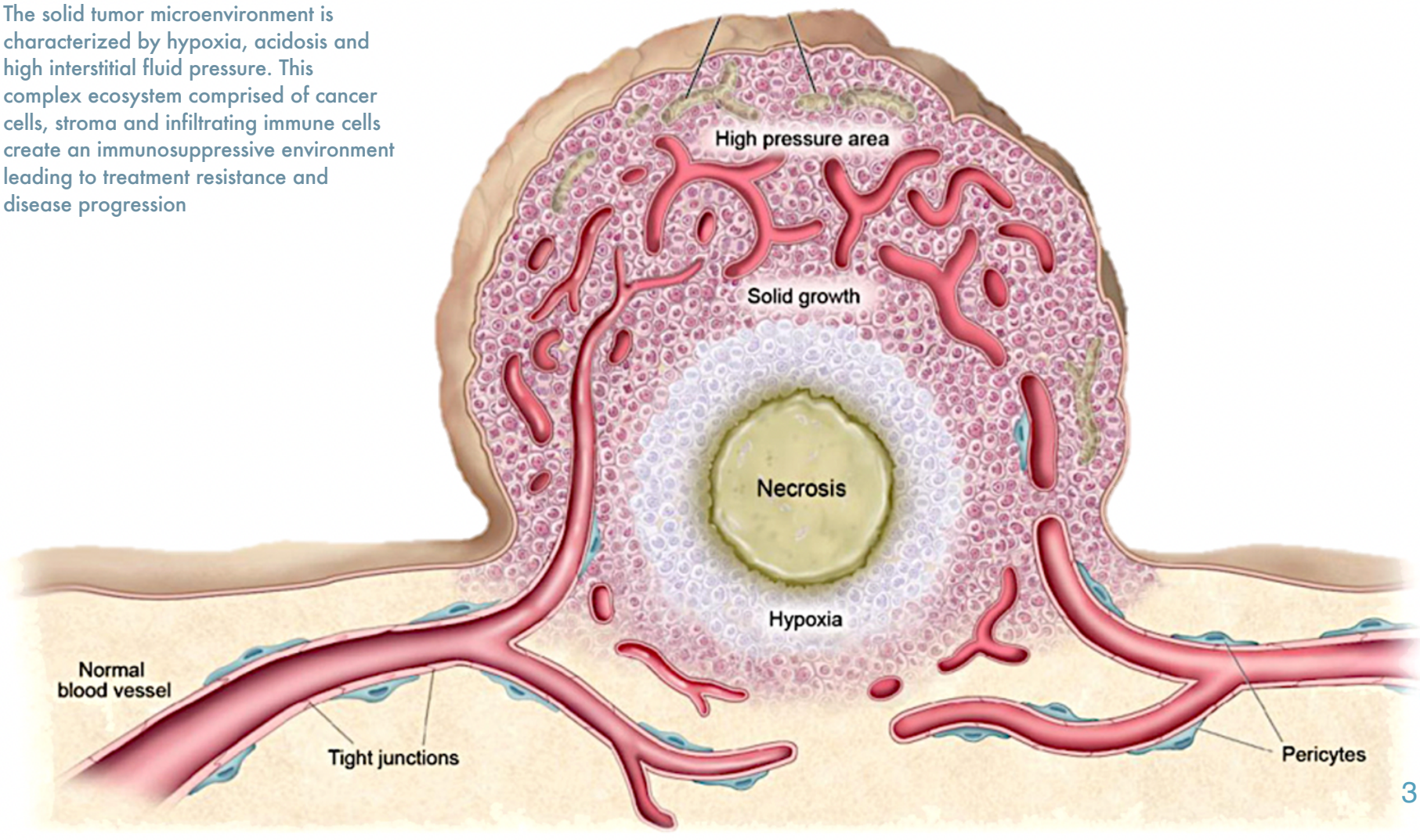
OVERCOMING THE SOLID TUMOR MICROENVIRONMENT

A promising new approach to help scientists characterize the tumor microenvironment, condition immune cells to fight cancer, and manufacture cell therapies has come from an innovative cell incubation technology. By more accurately recreating the native conditions of these cells, this smart technology has already demonstrated value in basic and clinical research for developing therapies that work effectively in solid tumors. Scientists are working feverishly to develop adoptive cell therapies for solid tumors. Recent progress has focused on genetically engineering immune cells to target multiple antigens at once, rather than just a single target as most adoptive therapies do. It is hoped that this would make immune cells more potent killers for solid tumors, which are marked by cellular heterogeneity that has made them more resistant to treatment.

Another challenge comes in the form of cell exhaustion. Therapies based on chimeric antigen receptor (CAR) T cells have performed very well in blood-based cancers; scientists believe that the confines of the circulatory system give infused immune cells ready access to their cancer targets. With solid tumors, though, delivery is a real problem. Experiments have shown that few infused cells ever reach their destination, and those that do arrive depleted, having spent their energy just traveling to the tumor site. By the time they get there, they encounter a hostile environment, bereft of nutrients and oxygen required to sustain the little cancer-killing energy that remains.

Addressing both of these issues is a huge priority for cancer researchers, and progress is already being made by deploying a new incubator technology that carefully recreates the *in vivo* environment of both cancer and immune cells. This tool is helping scientists learn more about how immune cells behave in TME, while reproducing biological mechanisms such as cell exhaustion that are not captured with traditional incubators.

The solid tumor microenvironment is characterized by hypoxia, acidosis and high interstitial fluid pressure. This complex ecosystem comprised of cancer cells, stroma and infiltrating immune cells create an immunosuppressive environment leading to treatment resistance and disease progression



Sun et al., Acta Pharmacol Sin, 2020

MIMICKING THE TUMOR MICROENVIRONMENT *EX VIVO*

Cell incubators found in most labs today aren't significantly different from what was used to house the first successfully established human cell line back in 1951. Cell incubation is a fundamental, and remarkably unchanged technology at biology labs in academic and biotech organizations around the world.

But recently, in-depth studies have revealed important limitations with current incubation technologies. Perhaps the most alarming is that because incubators do not accurately represent the native microenvironment of most cells, the results generated from these studies do not accurately reflect the natural biology of cells. Indeed, research has now shown that growing cells in traditional CO_2 incubators dramatically alters their gene and protein expression, metabolic processes, and other important biological factors.[1]

There is increasing awareness among biologists that cells adapt to new environments within a matter of hours. When cells move to a new environment — whether in the body or to a traditional incubator — it alters their behavior. Tumor cells stored in incubators have different signaling patterns than those taken directly from a patient. As a result, scientists interrogating those cells lose the ability to make meaningful insights, since the cells no longer reflect their native biology.

A novel incubation platform more closely mimics the cells' native microenvironments and has been shown to produce more physiologically relevant results from the cells it houses. It accomplishes this by giving scientists greater control over more environmental conditions, including pressure and hypoxia, instead of the one-size-fits-all approach used by traditional incubators that only allow for adjustments to temperature, CO_2, and humidity.

The AVATAR System lets you fine-tune oxygen and pressure levels to cater culture conditions to your cell type of interest. Customizing settings based on tumor type or native microenvironment allows cells to behave as they would *in vivo*

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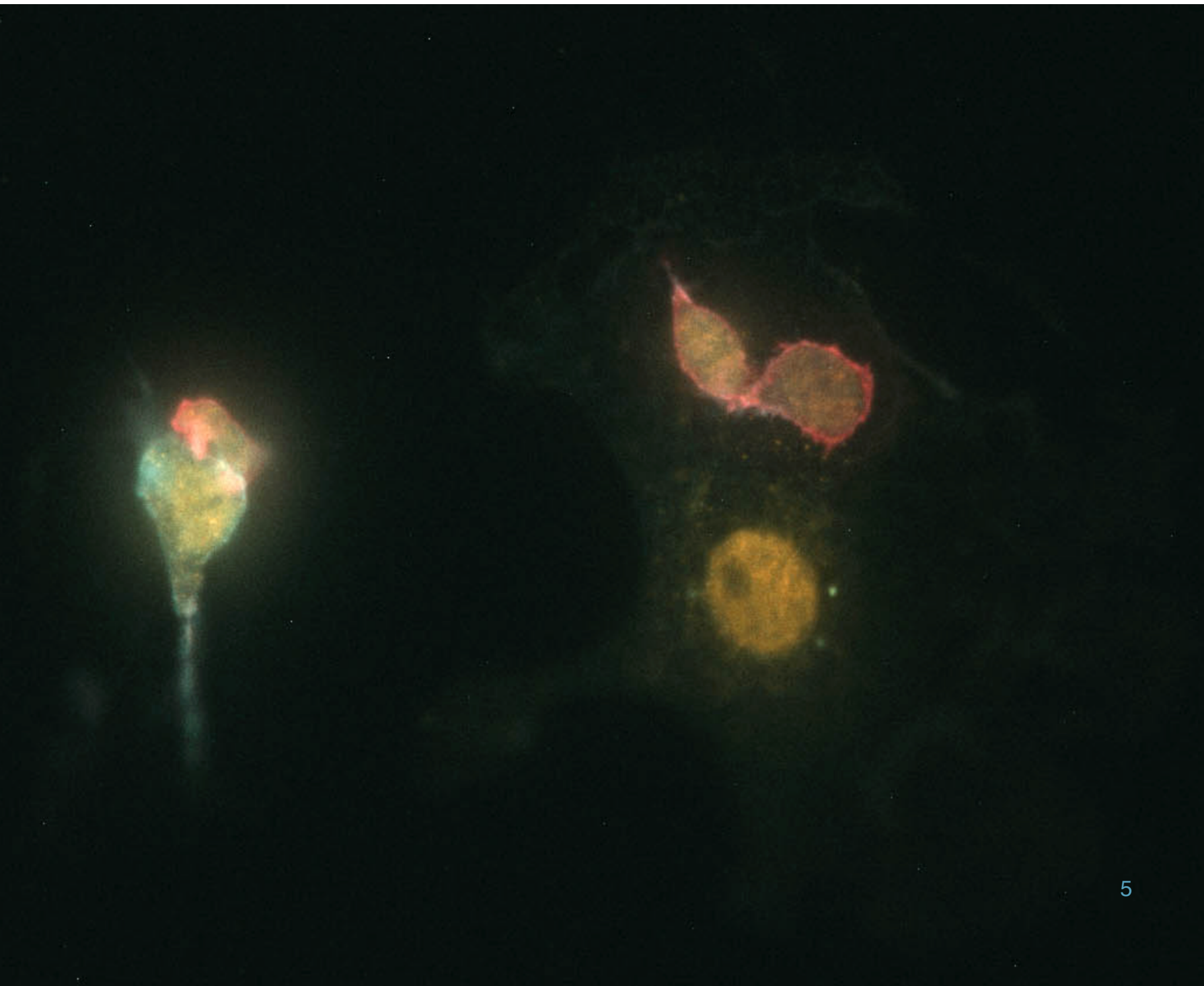
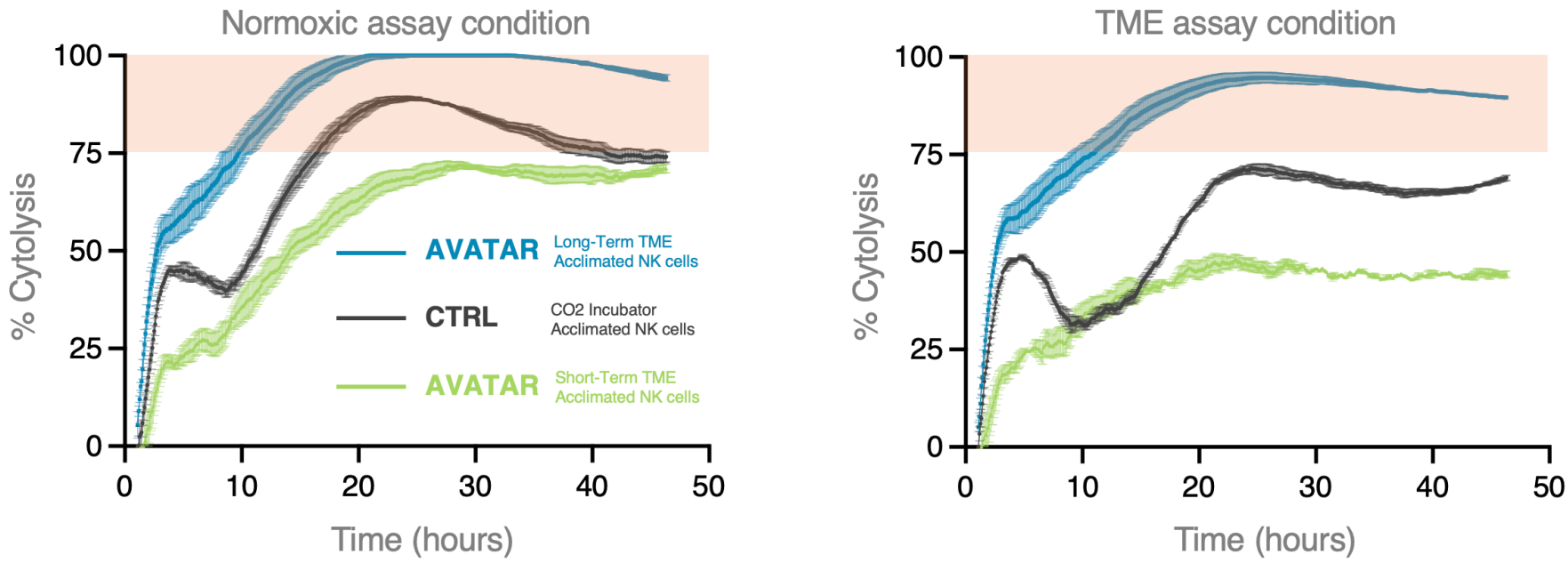
THE SOLID TUMOR MICROENVIRONMENT PROMOTES IMMUNE EXHAUSTION

In the past few years, scientists from around the world have published results about how this technology has driven new insights in their studies. For example, a team in California studied the interaction of T cells and macrophages in a carefully replicated tumor microenvironment, finding that removal of key macrophages from the environment reduces T cell exhaustion and reinvigorates effector potential.[2] They discovered that hypoxic conditions accelerate the processes leading to T cell exhaustion. Other studies have observed similar outcomes involving therapeutic NK cells, where TME culture conditions led to a decrease in tumor killing capabilities.[3]

More recently, scientists at Xcellbio have shown that their smart incubator recapitulates the hallmark features of a solid tumor microenvironment, generating results that match what's seen in the body — including T cell exhaustion and depleted killing abilities that do not occur in standard incubators. The information generated in these studies has revealed important insights for a broad range of cell therapy applications.

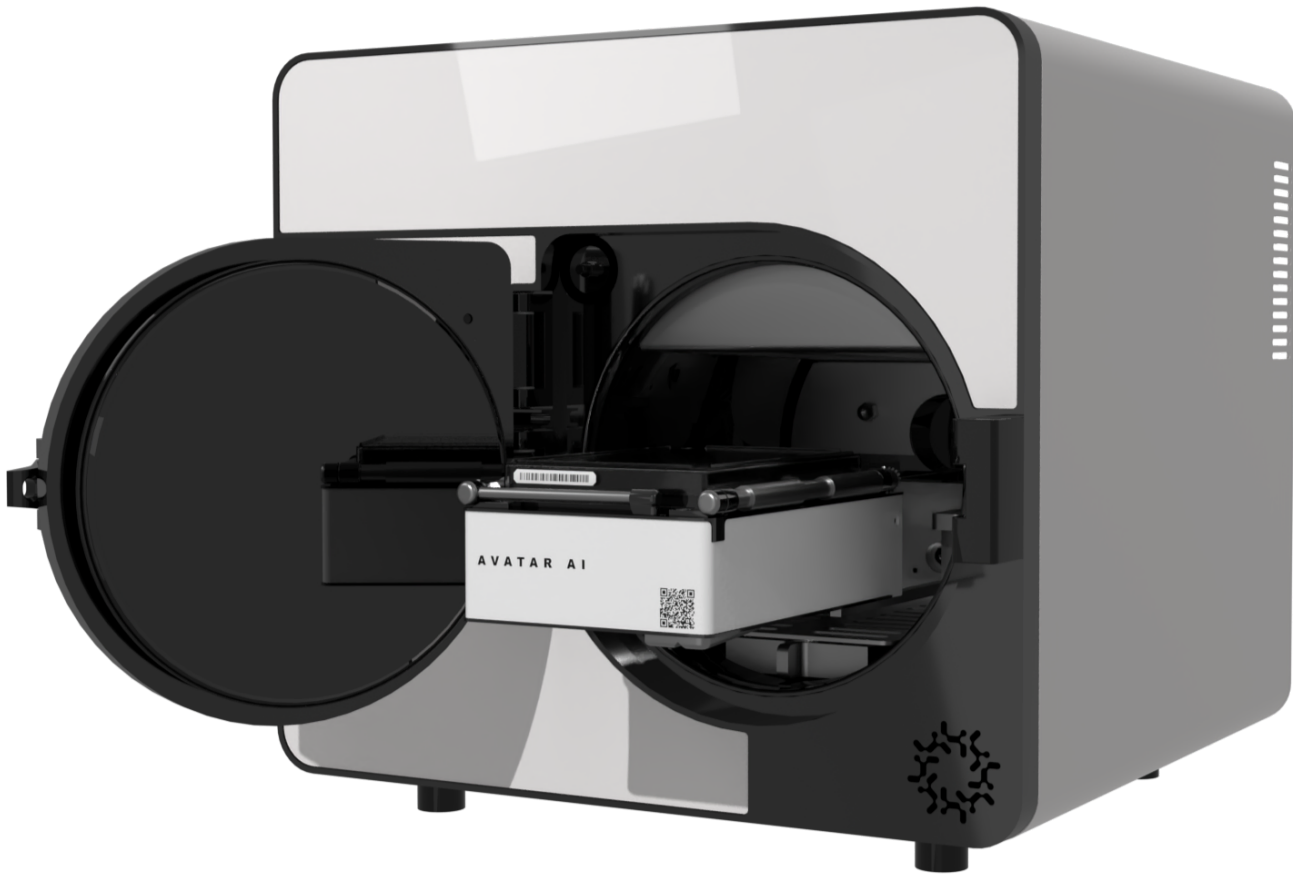




Long-term TME acclimated NK cell line exhibits improved anti-tumorigenic potency under hypoxic and hyperbaric cell killing conditions (TME assay condition)

TRAINING IMMUNE CELLS TO KILL TUMOR CELLS UNDER TME

For example, while NK cells appear to thrive under traditional culture conditions, they often fail to kill effectively under TME culture conditions. By growing NK cells under the same harsh TME conditions they will be expected to face in a patient, their potency levels can be more realistically evaluated. Indeed, scientists have found that it is possible to identify specific populations of NK92 cells that can acclimate to this environment and maintain their energy and killing function; much like tumor cells, they may use alternative energy pathways such as glycolysis to survive. This works best when cell lines are maintained and propagated under TME conditions, allowing for sufficient acclimatization to this harsh environment. After about two months in culture, NK92 cells show significant improvement in potency levels compared to those maintained under conventional culture conditions. This training period may finally allow cell therapy developers to address the challenge of targeting solid tumors.



The AVATAR AI System allows users to develop rapid and accurate potency assays for cell therapy products

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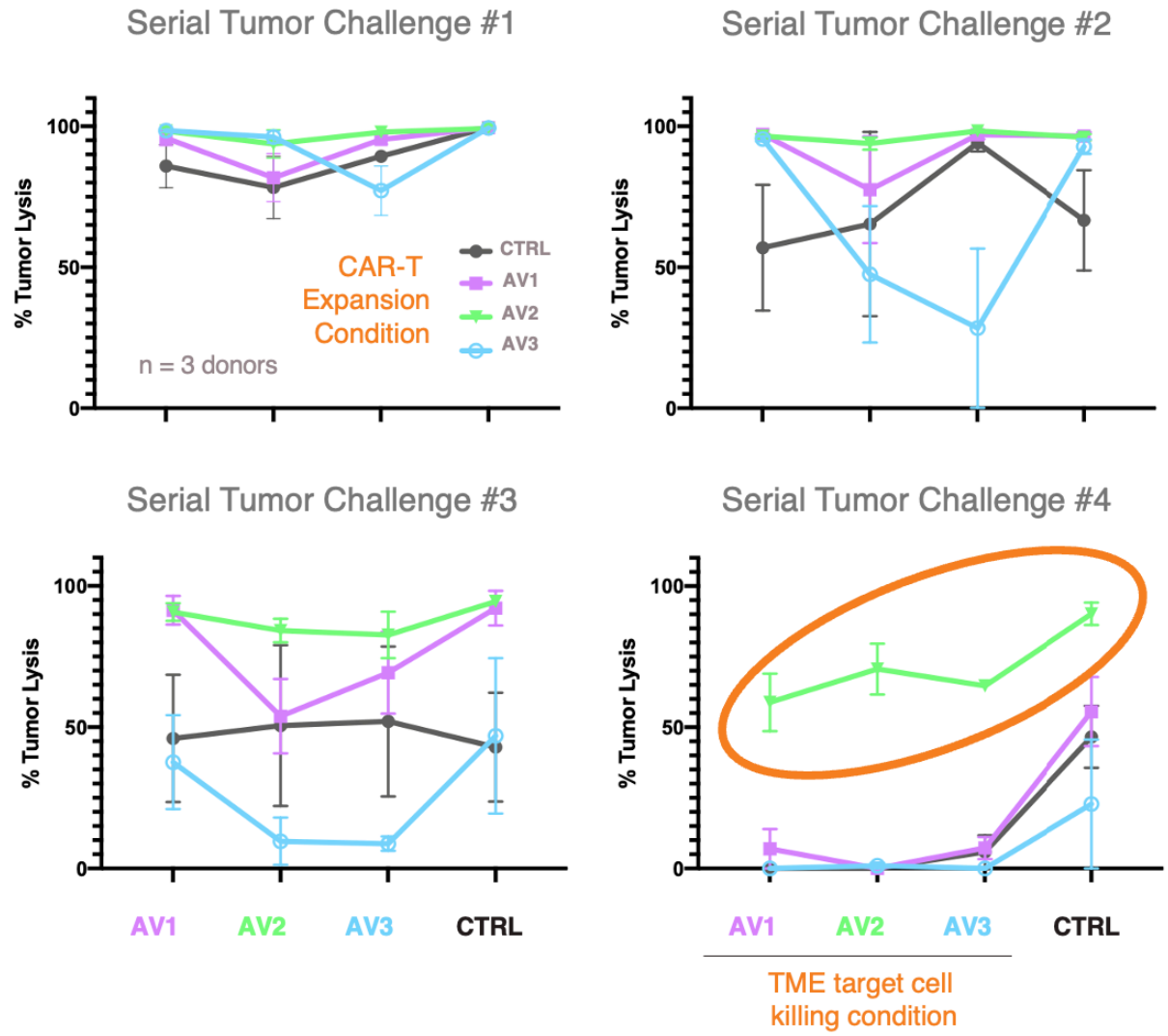

TME-acclimated CAR-T cells exhibits improved anti-tumorigenic potency in specific AVATAR TME settings. ROR1-CAR T cells expanded with AV2 setting in the Avatar System show persistent lysis of Jeko1 tumor cells (Y-axis) compared to standard CO2 incubator (CTRL) in this serial tumor challenge assay. This screening assay was designed to evaluate CAR-T potency and exhaustion under tumor microenvironment (TME) cell killing conditions (X-axis). Results indicate that CAR T cells expanded under AV2 settings show improved tumor killing under TME cell killing conditions, whereas conventionally expanded CAR T cells (CTRL) exhibit significant drop in tumor killing capacity during the fourth tumor challenge under TME cell killing conditions

ASSAYS TO MEASURE CELL EXHAUSTION IN TME

Another important application in cell therapy comes from exhaustion studies, which can be carried out using a serial tumor challenge assay. This kind of study aims to determine that therapeutic candidates are truly potent and resistant to exhaustion by measuring their ability kill tumor cells through repeated challenges against fresh tumor cells. In work performed with Xcellbio, scientists repeatedly challenged CAR T cells with tumor cells, allowing for brief recovery periods in between challenges. Each cell population was cultured under slightly different oxygen and pressure conditions and monitored for signs of exhaustion. In addition, the team ran cell killing assays in the same conditions under which the cells were grown, which are designed to approximate the real biological conditions of the TME. Their results show that CAR T cells expanded under TME-like conditions had superior tumor-killing function compared to cells grown in conventional culture conditions. This work confirms the hypothesis that it is possible to improve potency under immunosuppressive conditions by acclimating cells to these conditions in vitro. It also demonstrates that CAR T studies — particularly cell killing assays — based on cells grown in traditional incubators will not offer a realistic view of how those cells will perform *in vivo*.

Indeed, while evaluations of cell therapy candidates in traditional incubators often make it look as though immune cells will be potent killers when in reality, they are ineffective. What's wrong? One theory is that cells are grown in unrealistic conditions, and when they are reintroduced to the patient, they cannot function well in the new environment. But in the technology developed at Xcellbio, cells can be conditioned for the exact environment they will encounter. Under these harsher culturing conditions, cells may not divide as much, but they become far more potent and persistent killers.

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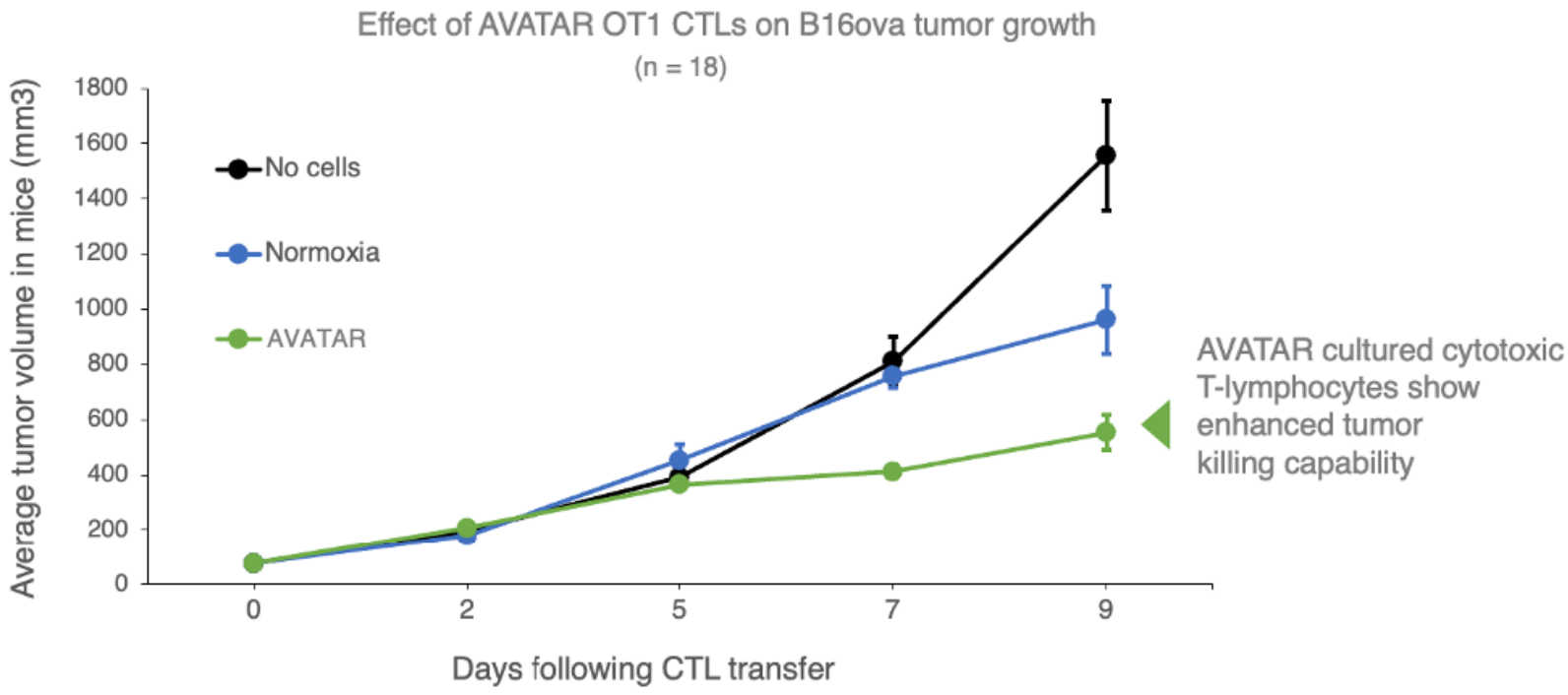

Optimized culture conditions can enhance CAR T potency and reduce cell exhaustion. Mouse studies demonstrate that cytotoxic T lymophocytes (CTLs) cultured under conditions replicating the tumor microenvironment (AVATAR) exhibit enhanced tumor killing capacity compared to conventionally grown T cells (Normoxia)

TECHNOLOGIES FROM XCELLBIO CAN ENHANCE CELL THERAPY POTENCY IN TUMORS

Xcellbio's new incubator system is based on core technology from the AVATAR platform. The original AVATAR incubator, released in 2018, has been validated by peer-reviewed studies from labs around the world. The instrument is much smaller than traditional incubators, but its stackable design allows for convenient scale-up to accommodate larger capacity needs. It also uses far less gas and provides a more streamlined workflow, reaching gas and temperature set-points faster. This diminutive bioreactor has been adopted by leading cell therapy developers, providing a reproducible and robust approach to test cell growth under many different conditions to see which deliver the best results.

Additional technology development paved the way for a second-generation system called the AVATAR AI platform. This new version pairs the foundational tumor microenvironment control technology with real-time, label-free measurement of tumor cell viability and kinetics. By modeling the potency of an immune cell therapy in an immunosuppressive TME environment, researchers now have a solution for identifying the best candidates for progressing into clinical trials. Designed to measure cell therapy potency levels, AVATAR AI's XcellSoft software reduces steps via automated data processing. It supports rapid functional release assays to gauge the quality and efficacy of cell therapies as they are developed and manufactured, enabling users to select candidates based on their performance under hostile TME conditions.

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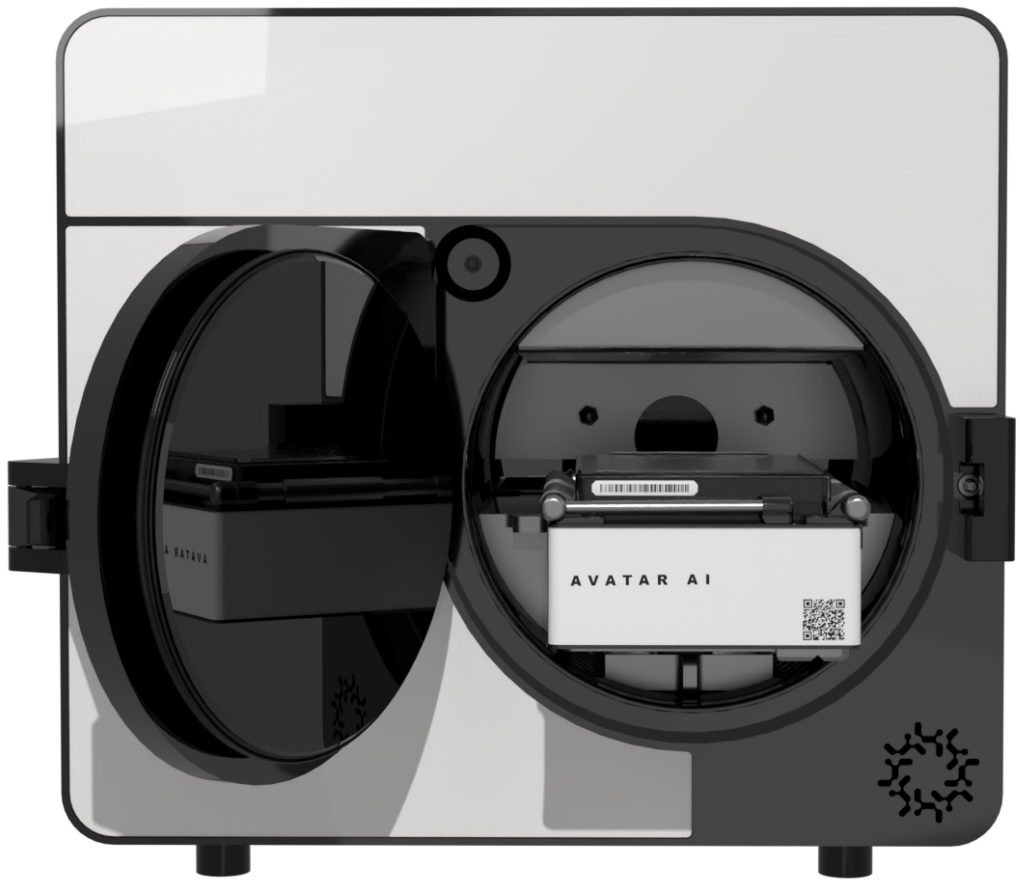
DEVELOP CELL THERAPIES THAT WORK EFFECTIVELY IN TUMOR MICROENVIRONMENT

Looking ahead, the next iteration of the AVATAR-based incubator technology will be the KALI Cell Foundry, a cell manufacturing platform designed to support the development of therapeutic products with improved potency and persistence as well as reduced cell exhaustion by conditioning cell populations to tumor microenvironments as they grow. To date, manufacturing this new class of therapies has been fraught with challenges — largely because established manufacturing protocols at biotech companies have no precedent for the development of one-off treatments that are bespoke for each patient. When released, this system will allow developers of adoptive cell therapies to focus their manufacturing efforts on quality rather than quantity, with enormous potential to make a difference in treatment efficacy. A carefully controlled incubator with sophisticated AI capabilities could allow for optimization of each cell therapy for the specific tumor microenvironment of each patient, enabling scalability and compliance with GMP requirements.

All of these AVATAR-enabled systems are based on the core principle that the immunotherapy field requires smarter, more customizable approaches to cell incubation to improve the persistence, potency, and quality of cell therapies. Collectively, these platforms represent the full spectrum of immunotherapy development: preclinical research can be done in the original AVATAR system; the fidelity and potency of potential treatments can be tested in the AVATAR AI system; and, finally, cell therapies can be manufactured for patient use in the KALI Cell Foundry. By facilitating a more faithful representation of native biology, AVATAR technology can grow cells that more closely mirror *in vivo* biology — potentially revolutionizing scientists' ability to make meaningful discoveries from cultured cells and to manufacture cell-based therapies with improved patient outcomes.

The AVATAR AI can be used to pre-condition target cells and/or effector cells in multiple TME conditions. This allows users to recapitulate the TME and perform cell killing assays under immunosuppressive conditions to screen and select the best performing cell therapy candidates



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