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Modulating Environmental Conditions to Enhance Production of Potent Cell Therapies for the Solid Tumor Microenvironment

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Abstract: Expansion strategies to optimize CAR-T potency in TME

Cell therapy, such as Chimeric Antigen Receptor (CAR)-T cell therapy, has revolutionized the treatment of hematological malignancies, with high response rates that have led to five FDA approvals. In contrast, cell therapy in solid tumors remains a significant challenge for the field with relatively low efficacy and no approved therapies to date. This is due, in part, to immunosuppressive mechanisms in the tumor microenvironment (TME), which include reduced oxygen tension, high interstitial pressure, and an abundance of immunosuppressive proteins. Our work strives to modulate cell culture conditions during T cell activation, transduction, and expansion in order to manufacture the highest number of T cells exhibiting improved fitness in this harsh environment.

The AVATAR technology provides discrete control of oxygen tension and hyperbaric pressure to drive metabolic and mechanoresponsive signaling. We have implemented the AVATAR technology in multiple instruments across the cell therapy development process, from preclinical to GMP, to enable users to develop and manufacture cell therapies that can overcome the suppressive TME. We will showcase data that highlights how the AVATAR technology can enhance the transduction efficiency and expansion rate of CD19 CAR T cells, as compared to culture in a traditional incubator. We will expand these results to showcase enhanced cell expansion and decreased exhaustion of both healthy donor-derived bone marrow infiltrating lymphocytes and multiple myeloma patient-derived tumor infiltrating lymphocytes, as a result of culture in the low oxygen, high pressure AVATAR system.

These studies highlight the integral role that cell culture conditions play in the efficient expansion of therapeutic cell therapies while maintaining phenotypes associated with efficacious and durable anti-tumor effects.

AVATAR Platform Overview: From preclinical discovery to clinical manufacturing

Technologies to develop & manufacture cell therapies that can overcome the suppressive TME

Research & Early Development

AVATAR

Functional potency release assay

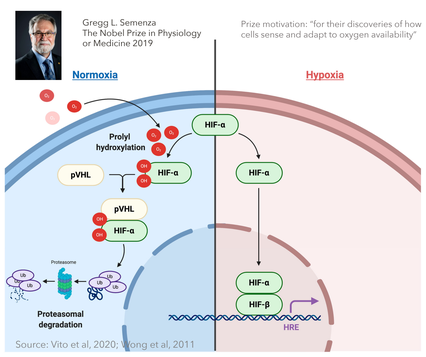
AVATAR ai

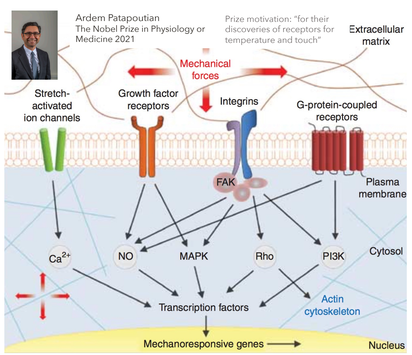
GMP MANUFACTURING

AVATAR FOUNDRY

Modulating oxygen and hyperbaric pressures to metabolically reprogram T cell therapies

AVATAR technology provides control of oxygen tension and hyperbaric pressure regulation to drive metabolic and mechanoresponsive signaling, both subjects of recent Nobel Prizes





Methods & Experimental Design

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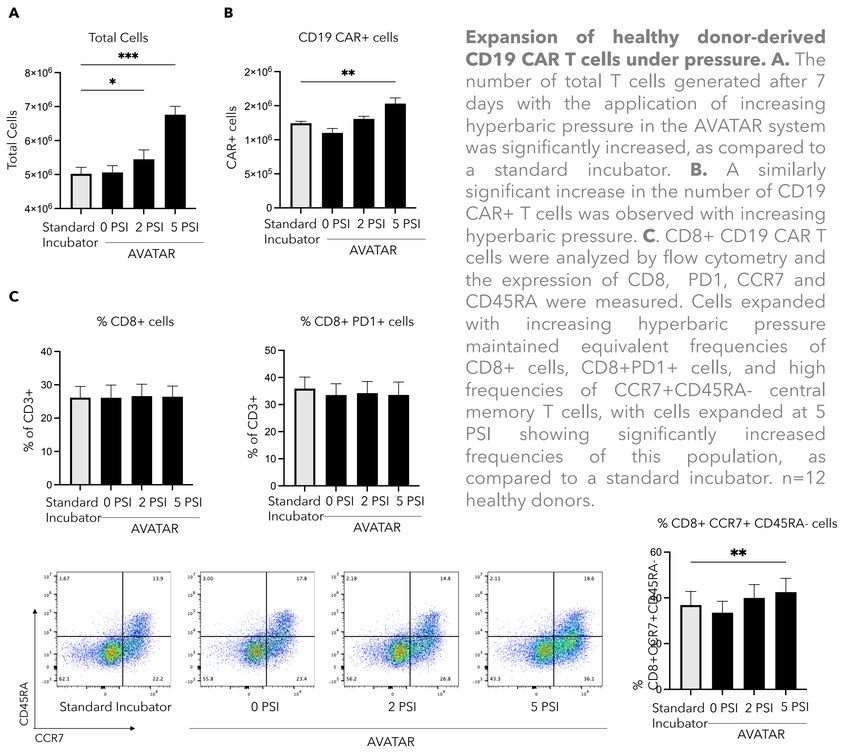
CD19 CAR T cell: CD3+ T cells were isolated from peripheral blood and activated in well plates in the presence of α-CD3/CD28 antibody-coated beads in under 4 experimental conditions: 1) Standard CO_2 incubator, 2) AVATAR at 0 PSI, 3) AVATAR at 2 PSI, or 4) AVATAR at 5 PSI. After 2 days, the cells were transduced with a 2nd generation CD19 CAR lentivirus and returned to their respective incubators and expanded for a further 5 days. The CD19 CAR lentivirus and phenotypic marker expression was measured by flow cytometry. Cytotoxicity assays were performed by incubating defined ratios of CD19 CAR+ T cells with NALM6-mCherry-Luciferase-Puro target cells for 24 or 72 hours and assessing specific lysis of the mCherry+ target cells by flow cytometry.

Marrow Infiltrating Lymphocytes: Bone marrow cells were activated in the the presence of α-CD3/CD28 antibody-coated beads and cultured either in a chamber filled with hypoxic gas within a standard CO_2 incubator or within the AVATAR at 1% O_2 and 5 PSI. Cells were expanded for 10 days and phenotypic marker expression was measured by flow cytometry.

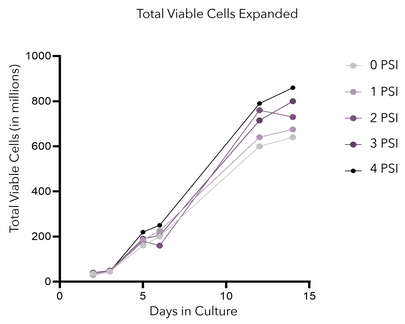
Hyperbaric culture conditions enhances transduction and expansion of CAR T cells, while maintaining Tcm phenotype

Healthy Donors



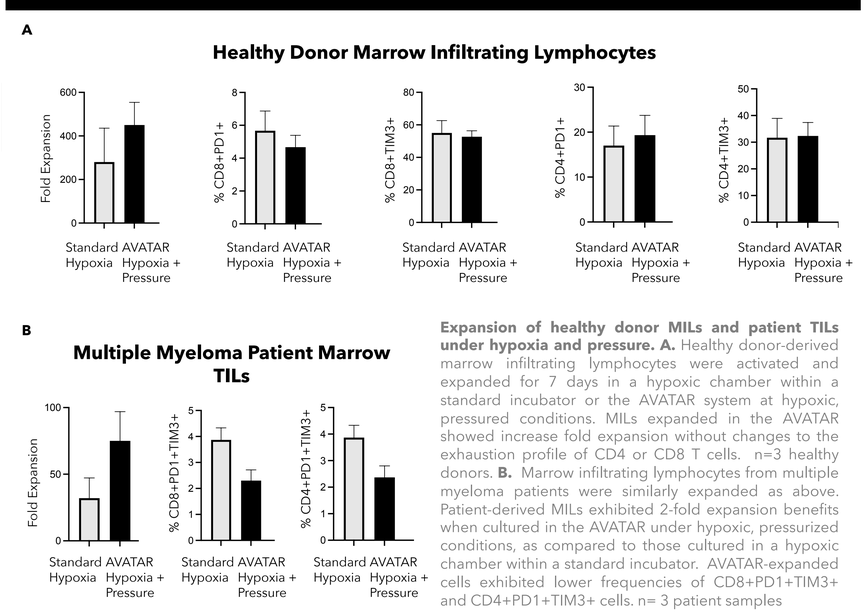
Expansion of healthy donor-derived CD19 CAR T cells under pressure. A. The number of total T cells generated after 7 days with the application of increasing hyperbaric pressure in the AVATAR system was significantly increased, as compared to a standard incubator. **B.** A similarly significant increase in the number of CD19 CAR+ T cells was observed with increasing hyperbaric pressure. **C.** CD8+ CD19 CAR T cells were analyzed by flow cytometry and the expression of CD8, PD1, CCR7 and CD45RA were measured. Cells expanded with increasing hyperbaric pressure maintained equivalent frequencies of CD8+ cells, CD8+PD1+ cells, and high frequencies of CCR7+CD45RA- central memory T cells, with cells expanded at 5 PSI showing significantly increased frequencies of this population, as compared to a standard incubator. n=12 healthy donors.

Patient Sample at clinical scale



Manufacturing patient-derived CD19 CAR T cells at clinical scale. Patient sample were used to manufacture CD19 CAR T cells at scale in the AVATAR system at a commercial cell therapy manufacture site. Increasing numbers of viable cells were generated as increasing hyperbaric pressure was applied to the cells.

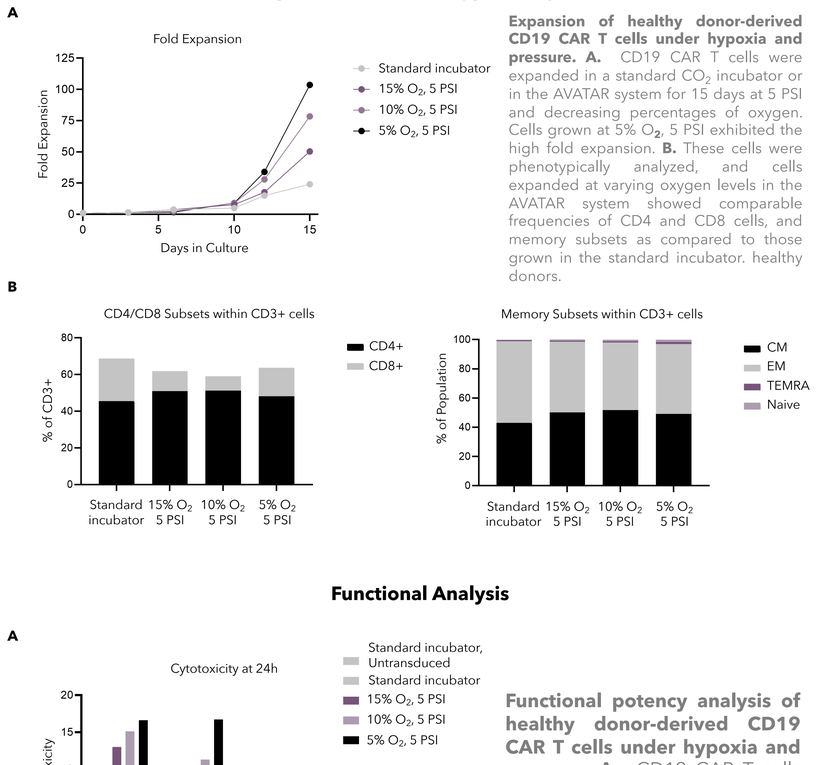
Expanding patient-derived TILs under hypoxic/pressurized in the AVATAR system yields higher cell numbers and reduced exhaustion



Expansion of healthy donor MILs and patient TILs under hypoxia and pressure. A. Healthy donor-derived marrow infiltrating lymphocytes were activated and expanded for 7 days in a hypoxic chamber within a standard incubator or the AVATAR system at hypoxic, pressured conditions. MILs expanded in the AVATAR showed increase fold expansion without changes to the exhaustion profile of CD4 or CD8 T cells. n=3 healthy donors. **B.** Marrow infiltrating lymphocytes from multiple myeloma patients were similarly expanded as above. Patient-derived MILs exhibited 2-fold expansion benefits when cultured in the AVATAR under hypoxic, pressurized conditions, as compared to those cultured in a hypoxic chamber within a standard incubator. AVATAR-expanded cells exhibited lower frequencies of CD8+PD1+TIM3+ and CD4+PD1+TIM3+ cells. n= 3 patient samples

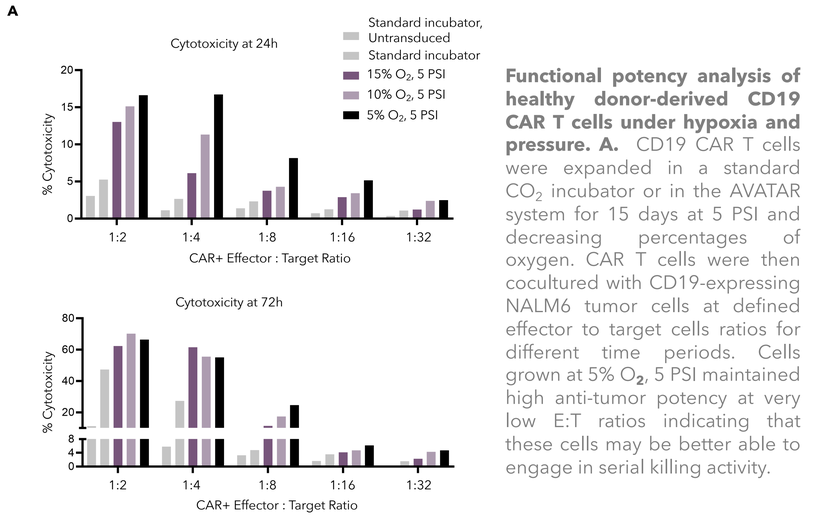
Pressurized and Hypoxic growth conditions promote increased potency of CAR T cells

Expansion and Phenotypic Analysis



Expansion of healthy donor-derived CD19 CAR T cells under hypoxia and pressure. A. CD19 CAR T cells were expanded in a standard CO_2 incubator or in the AVATAR system for 15 days at 5 PSI and decreasing percentages of oxygen. Cells grown at 5% O_2, 5 PSI exhibited the high fold expansion. **B.** These cells were phenotypically analyzed, and cells expanded at varying oxygen levels in the AVATAR system showed comparable frequencies of CD4 and CD8 cells, and memory subsets as compared to those grown in the standard incubator. healthy donors.

Functional Analysis



Functional potency analysis of healthy donor-derived CD19 CAR T cells under hypoxia and pressure. A. CD19 CAR T cells were expanded in a standard CO_2 incubator or in the AVATAR system for 15 days at 5 PSI and decreasing percentages of oxygen. CAR T cells were then cocultured with CD19-expressing NALM6 tumor cells at defined effector to target cells ratios for different time periods. Cells grown at 5% O_2, 5 PSI maintained high anti-tumor potency at very low E:T ratios indicating that these cells may be better able to engage in serial killing activity.

Conclusions

- Introducing hyperbaric pressure in the T cell manufacturing process using the AVATAR system enhances the overall yield of total T cells and CD19 CAR+ T cells in healthy donors, while maintaining similar frequencies of CD8+ cells, CD8+ PD1+ cells, and significantly increases the frequencies of CD8+ CCR7+ CD45RA- central memory T cells at higher pressure conditions.
- This effect is recapitulated using a patient sample at clinical scale at a commercial cell therapy site, where the application of increased hyperbaric pressure is associated with increased expansion of CD19 CAR T cells.
- Hyperbaric pressure, in combination with hypoxia, in the AVATAR system leads to increased expansion of healthy donor-derived marrow infiltrating lymphocytes, as compared to an existing hypoxic culture method. There were no changes observed in the frequency of CD4 and CD8 T cells with exhausted phenotypes.
- This effect was extended to multiple myeloma patient-derived tumor infiltrating T cells, in which hypoxic, pressurized culture in the AVATAR system yielded higher expansion of these cells. This was associated with a small reduction in the frequency of exhausted CD4 and CD8 T cells.
- CD19 CAR T cells grown in the pressurized conditions with a further titration of oxygen levels yielded further increases in cell expansion rates that were not accompanied with changes to CD4/CD8 ratios, the frequencies of CD3+ PD1+ cells, or the composition of memory subsets within the T cell compartment.
- Functional analysis of CD19 CAR T cells expanded in pressurized conditions with titrated oxygen levels revealed that cells expanded at lower oxygen levels were able to maintain their anti-tumor potency even at very low effector: target ratios, indicating that these cells may be better able to engage in serial killing activity.