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Metabolic Reprogramming Enhances Expansion and Potency of CAR-T cells

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Abstract

Cell therapies, such as Chimeric Antigen Receptor (CAR) T cell therapy, have revolutionized the treatment of hematological malignancies, but their use in solid tumors remains a significant challenge for the field. 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, resulting in mitochondrial dysfunction, exhaustion, and depletion of adoptively transferred cells. Metabolic manipulation of therapeutic cells could overcome these barriers to enable cell therapy success in solid tumors.

To this end, we have employed AVATAR technology, an incubation system that enables precise control of oxygen tension and hyperbaric pressure on cells in culture to improve the manufacture of adoptive T cell therapies. Using a CD19 CAR T model system, we have shown that transduction and expansion of CAR T cells under reduced oxygen and hyperbaric pressure conditions yield greater percentages and total numbers of lentiviral-transduced cells. While cells transduced in a standard CO_2 incubator yielded 10-20% CD19 CAR+ cells, transduction in the AVATAR system with increased pressure yielded 15-40% CD19 CAR+ cells. Similarly, T cell cultures performed under pressurized AVATAR conditions generated ~2X more viable cells after 10 days. Furthermore, these metabolically reprogrammed cells exhibit enhanced potency, with improved anti-tumor cytotoxic activity in vitro. We have extended these studies to measure the ability of AVATAR-expanded CD19 CAR T cells to control the growth of CD19-expressing NALM6-Luc tumor cells in vivo compared to CAR T cells grown in a conventional CO_2 incubator. Mice dosed with the AVATAR-expanded cells exhibited good tumor control and increased persistence of CAR T cells in relevant organs, along with alterations in cell trafficking to the bone marrow and spleen. The transferred cells maintained a less differentiated phenotype, with central memory and effector memory populations, as measured by expression of the markers CD45RA and CD62L, dominating the T cell compartment.

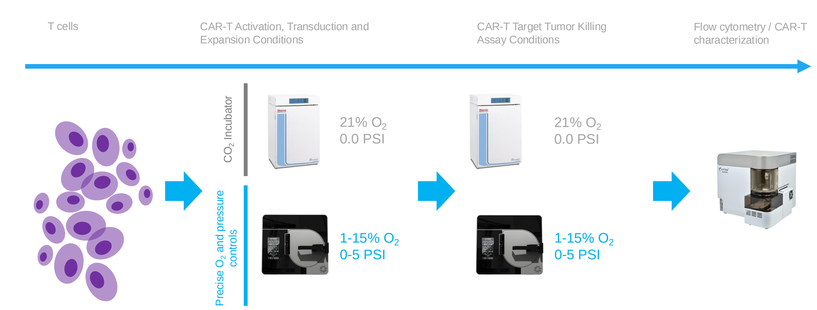
This work showcases the benefits of metabolic reprogramming to improve the yield and functional potency of CAR T cells, which has direct applications to reducing the cost and improving the efficacy of these lifesaving treatments. The AVATAR technology is currently being translated into a closed system bioreactor for use in GMP cell therapy manufacturing.

Methods & Experimental Design

In vitro: Untouched CD3+ T cells from a healthy donor was thawed in the presence of soluble anti-CD3 and cultured under 3 environmental conditions; 1) Normal CO_2 incubator (NI), 2) 15% O_2 + 2 PSI (15_2), and 3) 15% O_2 + 5 PSI (15_5). After 2 days, the cells were transduced with a 2nd generation CD19-CAR tool lentivirus and returned to their respective incubators and expanded for 10 days. The CD19-CAR expression was observed by flow cytometry and cytotoxic activity was tested in a killing assay with Nalm6-mCh-Luc-Puro target cells. These cells were subsequently frozen down to use *in vivo*.

In vivo: Femaile NSG mice were inoculated with 0.5e6 Nalm6-mCh-Luc-Puro cells IV. The following day, CAR-T cells were administered at 2.5e6 and 1.25e6 CD19-CAR+ T cells per animal. BLI tumor measurements and blood samples were taken weekly up to 4 weeks post tumor innoculation. The blood was examined for circulating T cells by ddPCR and flow cytometry using **Labcorp's Custom Expanded Persistence T Memory Panel**.

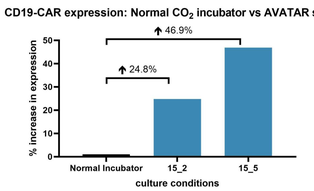
AVATAR and AVATAR Foundry

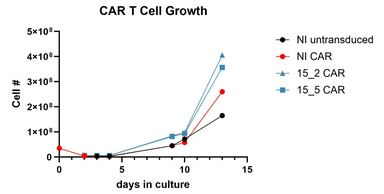


Preclinical discovery: The precisely controlling O_2 levels and pressure within the AVATAR system enables the exploration of optimal environmental conditions for enhancing the potency and yield of cell therapies. Additionally, it facilitates testing under conditions that closely mimic the physiological environment, including the tumor microenvironment (TME).

Environmental conditions identified using the AVATAR system can then be carried out in the fully enclosed, GMP-grade AVATAR Foundry for the production of cell therapies.

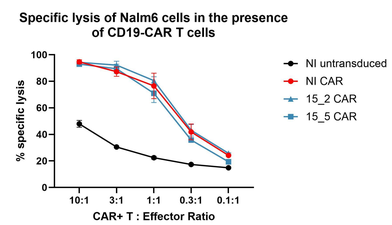
Generating CD19-CAR T cells under pressurized conditions in the AVATAR system results in higher overall yield and CAR expression





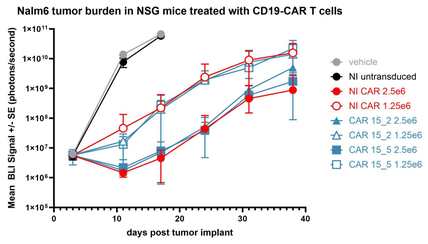
Expression and cell growth of CD19-CAR T cells. Increased CAR+ expression was observed in cells grown under pressure in the AVATAR system compared to a normal incubator. A greater than 2-fold increase in total yield at the end of expansion was enhanced by pressure, which has been consistent and confirmed in other healthy T cell donors.

CD19-CAR T cells grown under pressurized conditions functionally perform as well as cells grown in a normal incubator *in vitro*



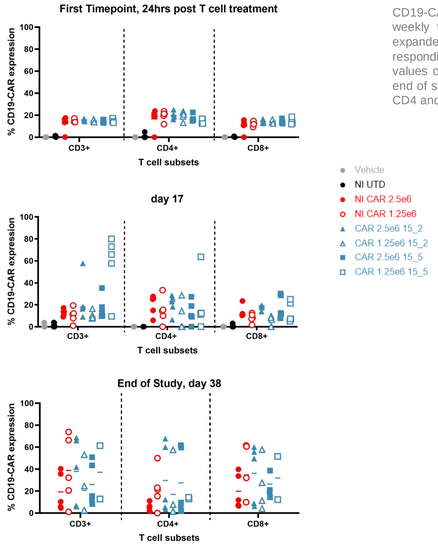
Cytotoxicity assay with Nalm6 target cells. T cells were co-cultured with Nalm6 target cells for 48hrs. Specific lysis was measured by flow cytometry. CD19-CAR specific killing was observed in a dose-dependent manner. T cells grown under pressure in the AVATAR system showed no impairment in their cytotoxic function compared to cells grown in a standard incubator

In vivo Nalm6 challenge of CD19-CAR T cells cultured in the AVATAR system

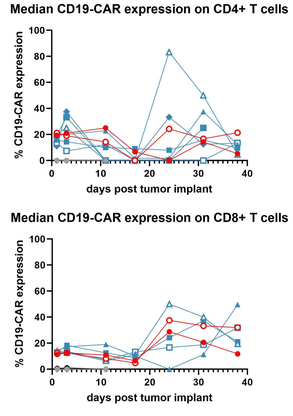


In vivo evaluation of CD19-CAR T cells. BLI measurements were taken weekly post CAR T treatment. CD19-CAR specific tumor growth inhibition of CD19-CAR T cells grown in a pressurized AVATAR system performed as well as cells grown in a standard incubator, showing that pressure has no effect on cytotoxic activity.

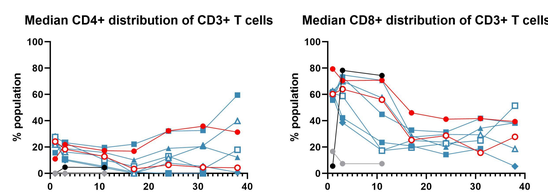
Phenotypic analysis of circulating CD3/4/8 populations: CD19-CAR expression and subsets



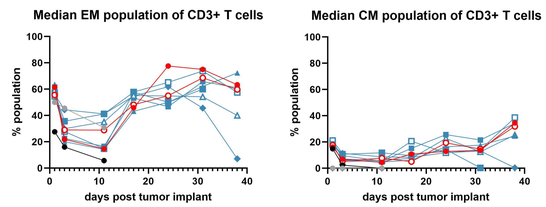
CD19-CAR expression in circulating T cells. Blood was taken weekly through the duration of the study. CD19-CAR+ T cells expanded over time, indicating that the transduced cells were responding to the Nalm6 tumors. Figures on the left are median values of individual mice within a cohort at the start, middle, and end of study. Figures below show the median CAR+ expression of CD4 and CD8 subsets over time.

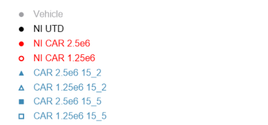


Phenotypic analysis of circulating CD3/4/8 T cells: Population Distribution and Memory Subsets



CD4 and CD8 distribution. Blood samples from each timepoint were analyzed for T cell populations. The figures on the left show the distribution of CD4+ and CD8+ T cell within the total CD3+ gate. The CD4 population increased overtime, while the CD8+ population decreased. The drop in the number of CD8+ T cells present correlated with the increasing tumor burden.

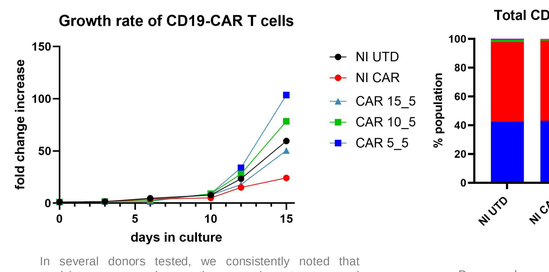




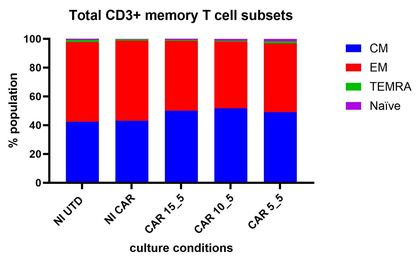
Effector memory (EM) and central memory (CM) distribution. Both EM and CM populations were also assessed with in the CD3/4/8 subsets. EM and CM were defined as CD3/4/8+CD45RA-CCR7- and CD45RA-CCR7+ respectively. Overall, the cells remained within the EM and CM populations and did not terminally differentiate or appear exhausted via LAG3+ expression. LAG3+ expression was barely detectable in any of the major subsets.

Next steps: Further exploring the effects of O_2 levels and pressure on transduced T cells *in vitro* and future *in vivo* experiments

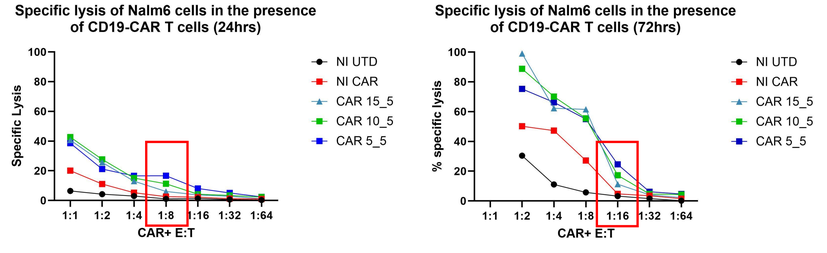
On-going *in vitro* investigations. More studies are underway to understand the effects of oxygen and pressure on transduction efficiency, expansion, phenotype and potency on T cells grown in the AVATAR.



In several donors tested, we consistently noted that applying pressure enhances the expansion rate compared to a conventional incubator. Reducing O_2 on top of pressure further potentiates the growth of these T cells.



Decreased oxygen levels in addition to pressure have no significant effect on T cell phenotype.



CD19-CAR T cells grown in 5_5 AVATAR conditions kill better at low E:T ratios than the other conditions. These T cells performed well in an acute (24hr) killing assay and demonstrated good serial killing at 72hrs.

Conclusions

- Introducing pressure in the T cell manufacturing process enhances the overall yield.
- Phenotype and function of CD19-CAR T cells grown in the AVATAR have no significant effect on phenotype and cytotoxic function *in vitro* or *in vivo*.
- Experiments are currently being conducted to further validate these discoveries. The next step is to determine the correlation between *in vitro* and *in vivo* efficacy. Additionally, we are investigating solid tumor models using a similar approach.

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