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Functional potency assay predicts CAR-T effectiveness in tumor microenvironment

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Abstract: Predict CAR T potency in solid tumor microenvironment

BACKGROUND: Chimeric antigen receptor (CAR) T cell therapy holds great promise for the treatment of various cancers, including solid tumors. However, attempts to model the behavior and effectiveness of CAR-T cell therapies for blood cancers and solid tumors have been challenging due to the unique tumor microenvironments in which these cancer cells are found. The tumor microenvironment (TME) is often characterized by hypoxia, increased acidity, and high interstitial fluid pressures, allowing cancer cells to effectively evade immune surveillance. This immunosuppressive TME also contributes to CAR-T cell exhaustion, thereby limiting its antitumor activity and function.

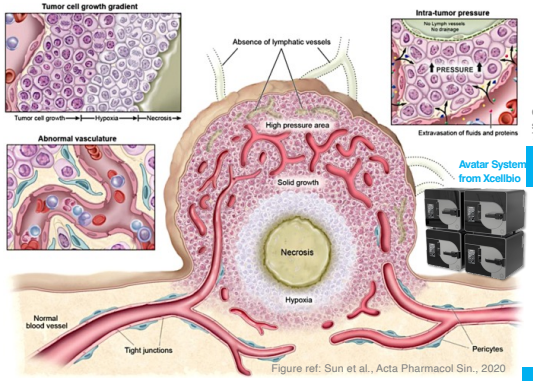
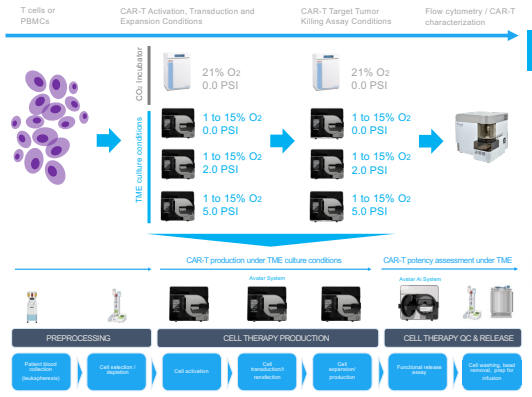


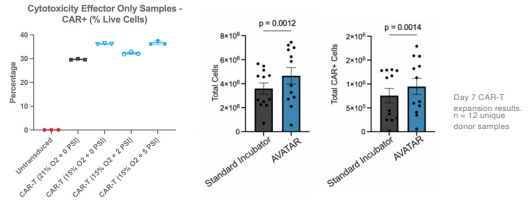
Figure ref: Sun et al., Acta Pharmacol Sin., 2020

METHODS: To address these concerns, we have developed a novel cell-based assay to measure CAR-T cell potency and cytotoxic function in three-dimensional (3D) *in vitro* cell culture system, human acute B cell lymphoblastic leukemia mouse model, and immunosuppressive tumor microenvironments. Utilizing the AVATAR system, we replicated the oxygen and interstitial fluid pressures found in the vasculature, the bone marrow and solid tumor microenvironments. Tumor cytolysis assays were conducted in these environments to measure cell exhaustion as analyzed by flow cytometry and electrical impedance. In addition, serial tumor challenge assays were performed to examine CAR-T potency and effectiveness in TME. RESULTS: Proof-of-concept experiments were performed using anti-receptor tyrosine kinase-like orphan receptor 1 (ROR1) CAR-T cells targeting the ovarian adenocarcinoma cell line, SKOV3. CD19 CAR-T were also used targeting the acute lymphoblastic leukemia cell line, NALM6. Defined ratios of effector T cells to tumor cells was assessed to model CAR-T potency *in vitro* and elevated CD19 CAR-T mediated cytotoxicity was confirmed with the increased ratio of effector T cells to tumor cells. Initial results from these screening experiments show significant decline in ROR1 CAR-T mediated cytotoxicity when performed under TME conditions. However, CD19 CAR-T showed effective cell killing under TME conditions. Interestingly, acclimating and expanding ROR1 CAR-T cells to high pressure and decreased oxygen culture (AVATAR) conditions improved potency levels and warrants further investigation. CONCLUSIONS: In summary, we observe CAR-T cells comprise the tumor cell killing ability in both *in vitro* and *in vivo* animal models. We also describe a physiologically relevant potency assay that incorporates hyperbaric and hypoxic incubation technology to predict the behavior of cell therapies in immunosuppressive tumor microenvironments.

Experimental design: Generating CAR-T cells under pressurized TME culture conditions to enhance potency in TME

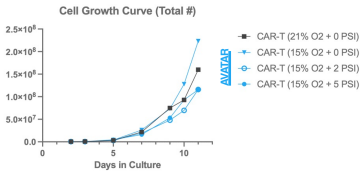


Pressurized TME expansion condition enhances viral transduction, increasing the total number of CD19 CAR-T+ cells



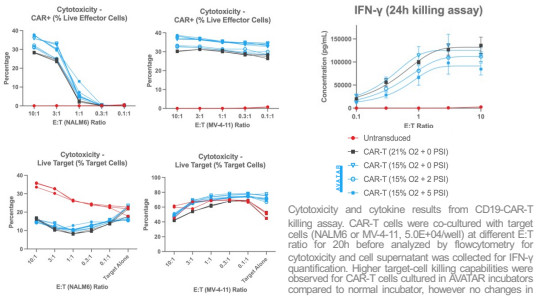
Characteristics of CD19-CAR-T cells. Activated T cells were transfected with CD19-CAR lentivirus and cultured in standard or AVATAR incubators for 7 days before analyzed by flowcytometry. Cells grown in AVATAR system showed a 5-7% increase in transduction efficiency, together with a 20% increase in growth capacity.

TME expansion condition enhances CAR-T expansion rates, increasing the total number of CD19 CAR-T+ cells



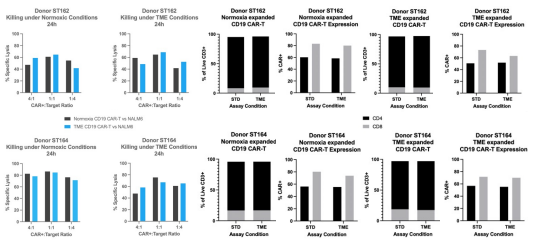
CD19-CAR-T cell growth curves. . Same numbers of CD19-CAR-T cells (5.0 E+05) were cultured under different O_2% and pressure conditions for 11 days and cell numbers were counted everyday with AO/PI method. By end of the culture period, cells in AVATAR incubator with low pressure/ hypoxia condition (CAR-T 15% O_2+ 0 PSI) had the highest growth rate.

Pressurized TME expansion condition enhances CD19-CAR-T survival during target tumor killing assay



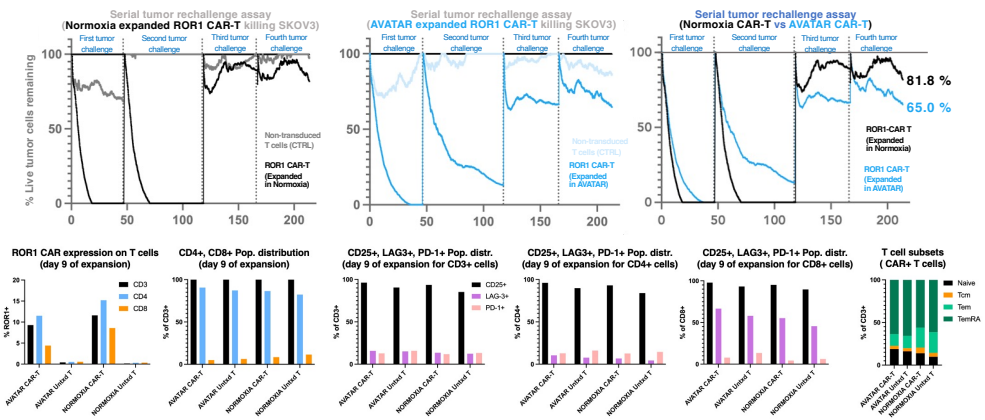
Cytotoxicity and cytokine results from CD19-CAR-T killing assay. CAR-T cells were co-cultured with target cells (NALM6 or MV-4-11, 5.0E+04/well) at different E:T ratio for 20h before analyzed by flowcytometry for cytotoxicity and cell supernatant was collected for IFN-γ quantification. Higher target-cell killing capabilities were observed for CAR-T cells cultured in AVATAR incubators compared to normal incubator, however no changes in IFN-γ secretion were detected in those groups.

Pressurized TME expansion condition maintains CD19 CAR-T potency during target tumor killing assay

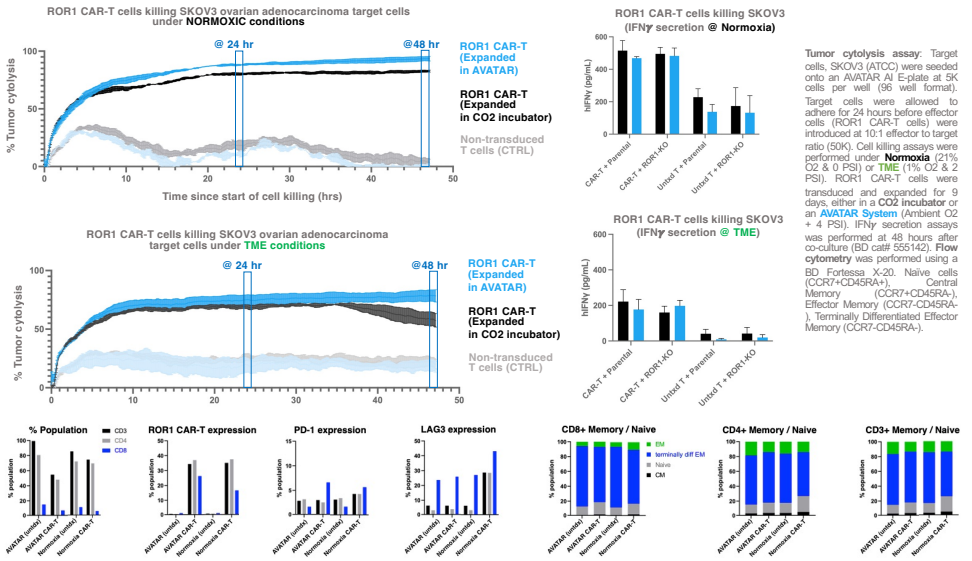


CD19 CAR-T specific killing NALM6 cells in coculture by flow cytometry. CD19 CAR-T cells were expanded in either Normoxic (21% O2 + 0 PSI) or TME (AVATAR: 1% O2 + 2PSI) culture conditions. NALM6 killing by CD19 CAR-T cells were performed in either Normoxic or TME conditions. ST164 donor derived CD19 CAR-T cells showed a 10% increase in cytotoxicity compared ST162 donor derived CD19 CAR-T cells. Donor derived CAR-Ts exhibited a significant shift in CD4/8 populations, skewing towards CD4 populations. Interestingly, higher E:T ratios did not lead to significantly more target killing.

Pressurized TME expansion condition enhances ROR1 CAR-T potency during serial tumor challenge assay



Pressurized TME expansion condition enhances ROR1 CAR-T potency under immunosuppressive TME screening conditions



Tumor cytolysis assay: Target cells, SKOV3 (ATCC) were seeded onto an AVATAR AI E-plate at 5K cells per well (96 well format). Target cells were allowed to adhere for 24 hours before effector cells (ROR1 CAR-T cells) were introduced at 10:1 effector to target ratio (50K). Cell killing assays were performed under **Normoxia** (21% O2 & 0 PSI) or **TME** (1% O2 & 2 PSI). ROR1 CAR-T cells were transduced and expanded for 9 days, either in a **CO2 incubator** or an **AVATAR System** (Ambient O2 + 4 PSI). IFNγ secretion assays was performed at 48 hours after co-culture (BD cat# 555142). **Flow cytometry** was performed using a BD Fortessa X-20. Naïve cells (CCR7+CD45RA+), Central Memory (CCR7+CD45RA-), Effector Memory (CCR7-CD45RA-), Terminally Differentiated Effector Memory (CCR7-CD45RA-).

Conclusions: The immunosuppressive tumor microenvironment may be overcome by manufacturing cell therapy products under physiological culture conditions and warrants further investigation

Normoxia expanded CAR-T potency performance

Target Killing Environment	Tumor Cytolysis % @ 24 hr	Tumor Cytolysis % @ 48 hr
Normoxia	80.2	82.7
TME	69.1	57.7

AVATAR expanded CAR-T potency performance

Target Killing Environment	Tumor Cytolysis % @ 24 hr	Tumor Cytolysis % @ 48 hr
Normoxia	88.1 (+7.9%)	93.5 (+10.8%)
TME	75.2 (+6.1%)	78.5 (+20.8%)

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