## 280 MULTIPLEX IMMUNOFLUORESCENCE IMAGING OF ARMORED CAR T CELLS IN PANCREATIC DUCTAL ADENOCARCINOMA MOUSE XENOGRAFTS CONFIRMS POTENT ANTI-TUMOR RESPONSE

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**Background** Chimeric antigen receptor (CAR) T-cells are a promising modality for the treatment of solid tumors, however their functionality to date has been hampered by the immunosuppressive tumor microenvironment (TME). Armoring CAR T cells to overcome TME may improve clinical outcomes. We established a subcutaneous BxPC3 xenograft model of pancreatic ductal adenocarcinoma (PDAC), that exhibits hallmark characteristics of human PDAC. In this model, CAR T cells regressed tumors; by contrast, armored CAR T cells designed to resist TME, mediated an apparent tumor volume increase. We utilized MACSima™ Imaging Cycling Staining (MICS) system to elucidate the mechanism of apparent tumor volume increase post armored CAR T cell treatment, and the interactions between tumor, stroma and immune cells.

**Methods** BxPC3 bearing NSG mice were treated with CAR T cells with or without armor, or untransduced T cell control (UTD) at high or low CAR T cell/mouse dose ($2x10^6$ or $1x10^6$) or left untreated. FFPE sections from tumors harvested 7 days post treatment were imaged on MICS by serial immunofluorescence for 29 protein markers and nuclear stain DAPI (ROI of 3mm by 5 mm; ~14.4 $mm^2$). Multiplex image processing and segmentation, unsupervised UMAP clustering, and target quantitation were performed on MACS® iQ view software.

**Results** MICS of BxPC3 xenograft tissues revealed PDAC-characteristic morphology, with distinct tumor cell clusters (Pan-cytokeratin, Cytokeratin 7, MSLN, p53) and mucin vacuoles (CA125), surrounded by dense, activated stroma (Vimentin and αSMA). UMAP dimension reduction for 29 markers yielded distinct tumor and stroma clusters in each treatment group. Following CAR treatment, tumor and stroma were infiltrated by CD4+ and CD8+ human T cells. In the high CAR dose groups, there were 14.7% CD8+ tumor-infiltrating lymphocytes (TILs) in CAR alone, and 51.6% for armored CAR. Armored CAR T cells disrupted the tumor/stroma morphology and mediated a dramatic reduction in tumor cells to 1.8%, as compared to 62% for UTD control and 29.3% for CAR T alone. The armored CD8+ TILs were highly proliferative with high abundance of mitochondria: 28% of armored TILs co-expressed Ki67 and TOM22, versus 2.6% in the CAR T alone group, and 0.6% in UTD.

**Conclusions** Using MICS technology, we therefore delineate the mechanism of action of the armored CAR-T cells in PDAC xenograft model, and rule out tumor progression. Tumor-activated armored CAR T cells manifest high proliferative and metabolic activity, superior tumor penetration and killing.

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