261 SYNERGISTIC ANTITUMOR EFFECTS OF MOUSE MESOTHELIN-DIRECTED CAR (MMESO-CAR) T CELLS AND αCD40 IN A SYNGENEIC MODEL OF PDAC

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Background This study explores the synergistic potential of combining mouse mesothelin-specific chimeric antigen receptor (mmeso-CAR) T cells and a CD40 agonist (αCD40) to enhance CAR T cell and overall immune response against pancreatic ductal adenocarcinoma (PDAC) in syngeneic mouse models.

Methods The subcutaneous syngeneic PDAC model was established using a KPC-derived cell line. Mice were treated with mmeso-CAR T cells, αCD40, or combinations of both. In vivo therapeutic efficacy was evaluated in endpoint and time course models, monitoring tumor volume and histological changes over time, and assessing CAR T cell and immune cell distribution and activation in secondary lymphoid organs (SLOs): tumor draining lymph node (TdLN) and spleen, and the tumor microenvironment (TME). Our methodology encompassed real-time live cell assays, in vivo imaging, multiplex cytokine assays, multi-parametric flow cytometry, histology, RNAscope, digital spatial profiling, and scRNAseq. Additionally, the combination treatment is currently being evaluated in an orthotopic syngeneic model of triple-negative breast cancer (TNBC).

Results Combining mmeso-CAR T cells with αCD40 yielded improved tumor control and long-term survival outcomes. αCD40 treatment induced significant tumor necrosis within 24 hours (39.5 ± 29.1% and 33.8 ± 20.5% of tumor area, with or without mmeso-CAR T cells, respectively). The necrotic effect persisted after seven days when combined with mmeso-CAR T cells (25.9 ± 20.7% versus 2.1 ± 3.3% with αCD40 alone), associated with a greater reduction in tumor weights and PanCK/mesothelin+ tumor areas (figure 1). αCD40 treatment promoted the expansion of mmeso-CAR T cells and modulated their activation in SLOs and within the TME. The combination therapy engaged APCs, host T and NK cells, increasing recruitment and activation in SLOs (figure 2) and in the TME, associated with elevated blood levels of pro-inflammatory cytokines and chemokines. ScRNAseq analysis on immune cells from tumor and TdLN confirmed the involvement of both CAR-dependent and independent antitumor responses.

Conclusions This study unveils the synergistic effect of combining mmeso-CAR T cells and αCD40 in delaying tumor growth in a syngeneic PDAC model. The combination therapy led to rapid and sustained tumor necrosis with increased infiltration and activation of immune cells in the SLOs and the TME. Our comprehensive characterization provided valuable mechanistic insights into the underlying synergistic mechanisms. Ongoing evaluation of the mmeso-CAR T cells/αCD40 therapy in a syngeneic model of TNBC aims to assess the effectiveness in various solid tumors expressing mesothelin. These findings may open potential applications of meso-CAR T cells/αCD40 combination therapy in the clinic.

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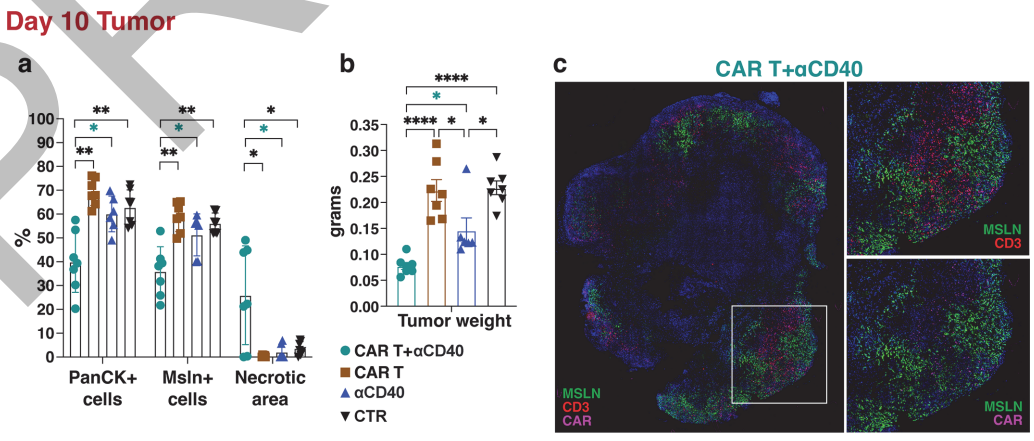
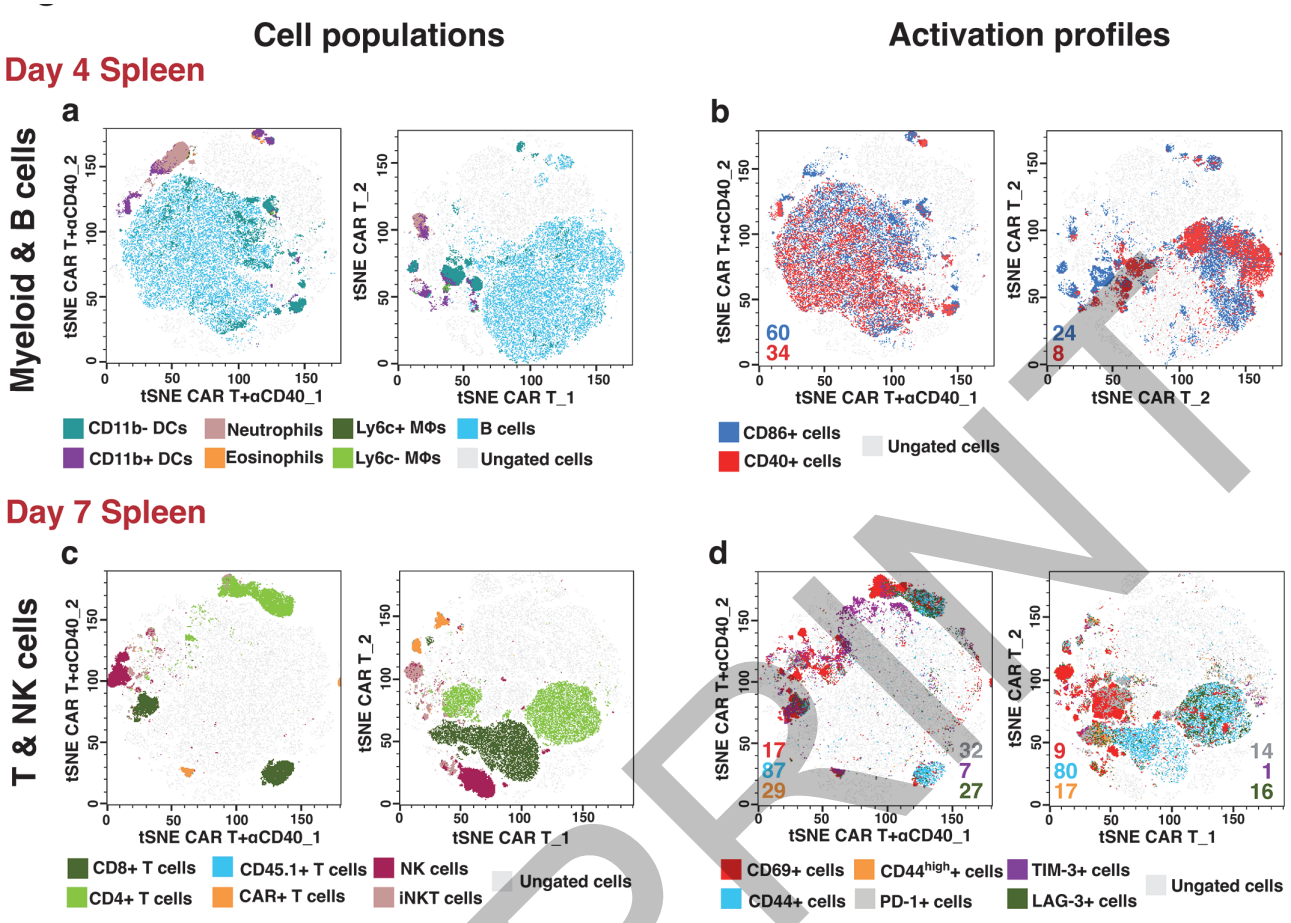
Ethics Approval The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) approved animal experiments (protocol n°804226). Animal procedures were performed in the animal facility at the University of Pennsylvania in accordance with Federal and Institutional IACUC requirements.



Abstract 261 Figure 1 Enhanced tumor control of the mmeso-CAR T cells/αCD40 combination treatment over both αCD40 and mmeso-CAR T cells as monotherapies. (A) Percentage of PanCK+, mesothelin (Msln)+ tumor cells, tumor necrosis, (B) as well as tumor weights, at ten days from mmeso-CAR T cell injection. (C) Using a 3-plex RNAscope™ assay, tumor-infiltrating mmeso-CAR T cells (pink), host T cells (red), and mesothelin (green) were quantified. Representative images of a mmeso-CAR T cell/αCD40-treated tumor at day ten revealed necrosis in the core region, while CAR T cells were observed in close proximity to viable tumor areas along the edges. These regions were enriched with host T cells, and minimal expression of mesothelin RNA was detected compared to adjacent mesothelin+ viable tumor areas.*p<0.05;**p<0.005, p<0.0001 by unpaired t-test.



Abstract 261 Figure 2 Distribution and activation of immune cells in the spleen following mmeso-CAR T cells/αCD40 combination therapy. (A-D) tSNE plots show myeloid (A-B) and lymphoid (C-D) clusters in the spleen at four and seven days after mmeso-CAR T cell injection, corresponding to one and four days after αCD40 treatment, respectively, assessed by flow cytometry (pooled data from n=6/7 samples for each treatment group). Distinct distributions between mmeso-CAR T cells/αCD40 and mmeso-CAR T cells alone treatments reflected variations in activation profiles based on expression of markers CD40, CD86, MHCII and CD80 for myeloid and CD69, CD44, PD-1, LAG-3, and TIM-3 for lymphoid panels. Numbers are overall expression percentage of the respective marker. Differential tSNE plots were also observed at later time points and in the tumor-draining lymph node (not shown).

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