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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Harnessing TCR/CAR Antagonism to Enhance Immunotherapeutic Precision

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Chimeric Antigen Receptor (CAR) T cell immunotherapy represents a conceptual breakthrough in the treatment of hematological malignancies. However, the rarity of cell surface protein targets specific to cancerous but not vital tissue has hindered its broad application to solid tumor treatment. While new logic-gated CAR designs have shown reduced toxicity against healthy tissues, the generalizability of such approaches across tumors remains unclear. Here, we harness a universal characteristic of endogenous T cell receptors (TCRs), their ability to discriminate between self and non-self ligands through inhibition of response against self (weak) antigens, to develop a broadly applicable method of enhancing immunotherapeutic precision. We hypothesized that this discriminatory mechanism, known as antagonism, would apply across receptors, allowing for a transfer of specificityfrom TCRs onto CARs. We therefore systematically mapped out the responses of CAR T cells to joint TCR and CAR stimulations. We transduced ovalbumin-specific TCR T cells with mouse CD19 CAR to produce T cells expressing both TCR and CAR and evaluated the response of TCR/CAR T cells using *in vivo* and *in vitro* leukemia models (**Figure 1A**). We discovered that strong TCR antigen enhanced CAR T killing of CD19 $^{+}$ leukemia, while weak TCR antigen antagonized CAR T responses as assessed *in vivo* cytotoxicity and *in vitro* multiplexed dynamic profiling (**Figure 1B**). We developed a mathematical model based on cross-receptor inhibitory coupling that accurately predicted the extent of TCR/CAR antagonism across a wide range of immunological settings. This model was validated in a CD19 $^{+}$ B16 mouse melanoma model showing that TCR/CAR antagonism decreased the infiltration of a tumor-reactive T cell cluster (cluster 1), while TCR/CAR agonism enhanced infiltration of this cluster1 (**Figure 1C**). We then applied our quantitative knowledge of TCR/CAR crosstalk to design an Antagonism-Enforced Braking System (AEBS) for CAR T cell therapy. This was assessed in a model system using a CAR targeting the tyrosine-protein kinase erbB-2 (HER2), expressed on a subset of patients with both B-ALL and AML together with a hedgehog acyltransferase (HHAT) specific TCR, which responds strongly to mutated peptides presented on tumor cells and weakly to wild-type peptides presented on healthy tissue. Consistent with our discovery of the TCR/CAR antagonism, TCR signals against healthy cells expressing wild-type HHAT peptide antigen antagonized HER2 CAR T cell responses, minimizing on-target/off-tumor cytotoxicity against healthy cells. Notably though, AEBS-CAR T cells exhibited high anti-tumor cytotoxicity against tumor cells expressing HER2 and mutated HHAT peptides (**Figure 1D**). AEBS CAR T cells sharpen the discriminatory power of synthetic anti-tumor lymphocytes, laying the groundwork for future studies to engineer complex logic into cells with minimal numbers of receptors (**Figure 1E**). Our work highlights a novel mechanism by which TCRs can enforce CAR T cell specificity, with practical implications for the rational design of future anti-leukemia immunotherapies.

Disclosures No relevant conflicts of interest to declare.

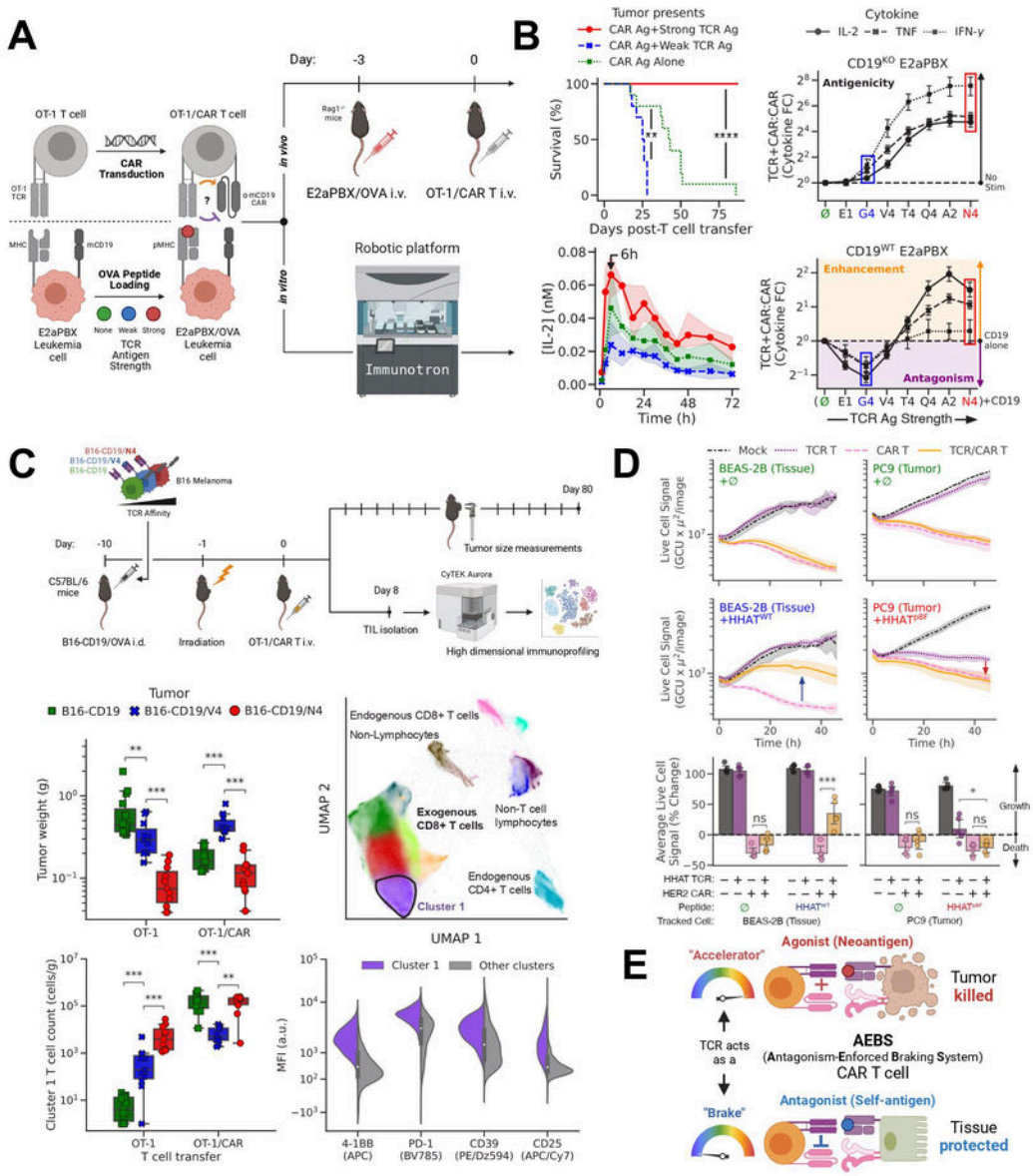

Figure 1. TCR signals of different strengths can antagonize or enhance CAR activity. (A) Experimental scheme to evaluate TCR/CAR crosstalk. Naive OT-1 T cells were transduced with a murine CD19 CAR and then stimulated with CD19⁺ E2aPBX leukemia cells expressing either no additional antigen or one of several ovalbumin peptide variants with differing antigenicities. **(B)** Survival curves for mice bearing E2aPBX/OVA leukemia cells treated with OT-1/CAR T cells (n = 10 mice per group) (**p < 0.01, ****p < 0.0001). A representative example of *IMMUNOtron*-acquired cytokine dynamics quantifying the divergent effects of TCR on CAR activation, as a function of the TCR antigen strength (n = 3 biological replicates). TCR/CAR crosstalk for dual OT-1/CAR T cells stimulated with $CD19^{KO}$ (top) or $CD19^{WT}$ (bottom) E2aPBX leukemia cells. Y-axis represents the fold change (FC) of responses for a combination of CAR and TCR ligands compared to CAR ligand alone; an *FC* above 1 indicates that the strength of TCR signal enhances the overall response of the CAR, while an *FC* below 1 indicates antagonism of the CAR response. The degree of antagonism was evaluated by examining fold changes in the production of the cytokines IL-2, TNF and IFN-γ (n = 6 biological replicates). **(C)** Experimental scheme to evaluate TCR/CAR crosstalk in OT-1/CAR T cells in response to dual antigen B16-CD19/OVA melanoma cells. UMAP plot of 3,514,537 tumor-infiltrating leukocytes colored by cluster membership (16 clusters, far left). Tumor weights at Day 8 across all samples (left) anti-correlated with the frequency of Leukocyte Cluster 1 (right) and its phenotype (far right) revealed a tumor-reactive population of $CD8^+$ T cells (n = 10-15 mice per group). **(D)** Cell growth curves (top) and average growth over time (bottom) for GFP-tagged target cells, monitored as green calibrated units (GCU), show lower cytotoxicity of AEBS-CAR T cells against healthy tissue compared to conventional CAR T cells (upward arrow) and increased cytotoxicity against tumors compared to TCR T cells (downward arrow, n = 5 biological replicates). **(E)** General design for AEBS-CAR T cells resulting in enhanced targeting of tumors and reduced toxicity against healthy tissues.

Figure 1

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