Abstract #6109

Combined targeting of cancer cells and tumor stroma by engineered dual-specific T cells expressing a TCR and a CAR

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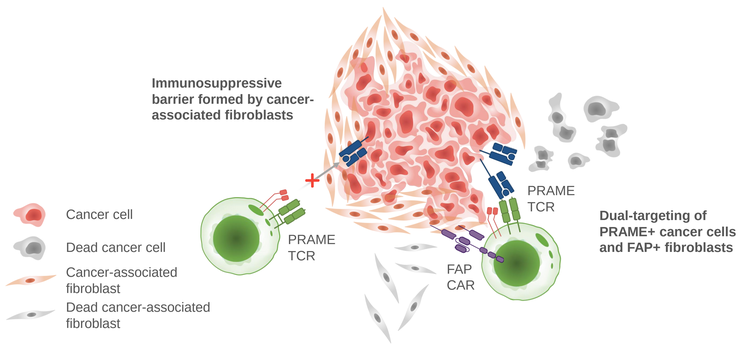
Cancer-associated fibroblasts: a key barrier to solid tumor therapy



Background

Engineered T cells have so far achieved durable responses only in hematopoietic malignancies, melanoma and sarcoma. The immunosuppressive tumor microenvironment (TME), which impairs T-cell infiltration and function is widely recognized as the main barrier to therapeutic efficacy in other solid tumors. Cancer-associated fibroblasts (CAFs) are a key TME component forming a physical and immunosuppressive barrier. CAFs express fibroblast activation protein (FAP), whose elevated expression correlates with poor prognosis. To address these challenges, we developed dual-specific T cells simultaneously targeting cancer cells via a PRAME-specific TCR and CAFs via a FAP-specific CAR.

Methods

We screened ten FAP CARs with different scFvs for compatibility with our proprietary PRAME-specific TCR. T cells expressing selected dual CAR/TCR constructs were tested for cytotoxicity against cancer cell lines expressing either PRAME or FAP. T-cell-mediated killing was assessed by time-lapse live-cell microscopy in 2D cultures and 3D tumor spheroid models.

Engineering of dual FAP/PRAME-specific T cells

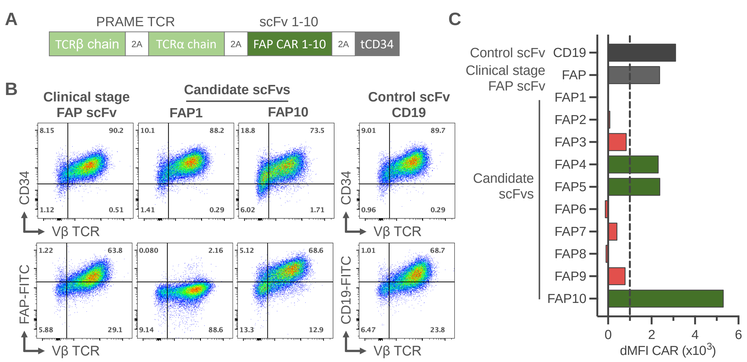


Fig. 1: Screening of CAR constructs containing different FAP scFvs for optimal co-expression alongside a PRAME-specific TCR. T cells were transduced, sorted for CD34 expression on day 8, and analyzed by flow cytometry on day 10. Mock-transduced T cells (Control T cells), and T cells expressing an irrelevant CD19-specific CAR (Control scFv CD19) or a clinical stage FAP-specific scFv derived from Sibrotuzumab (Clinical stage FAP scFv) were used as controls. Cells were gated on PRAME-specific TCR Vβ-chain, and CAR expression was assessed by staining with fluorescently labeled FAP or CD19 protein. (**A**) Retroviral expression cassette. (**B**) Representative flow cytometry plots. (**C**) Background-corrected mean fluorescence intensity (dMFI).

Identification of anti-FAP CARs optimally co-expressed in combination with an engineered TCR on T cells

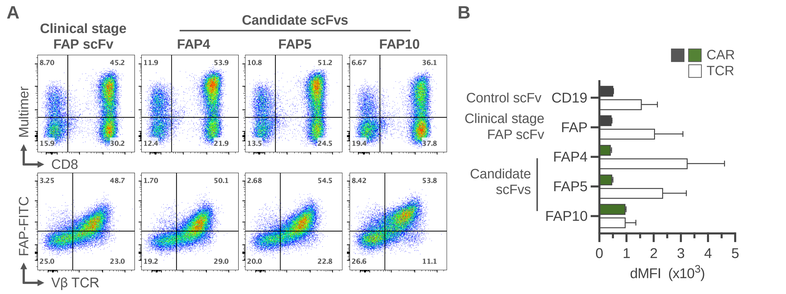


Fig. 2: Antigen-binding capacity of dual FAP/PRAME-specific T cells using HLA multimers and fluorescently labeled protein. Three selected FAP-specific CARs were co-expressed with a PRAME-specific TCR in T cells. Dual specificity of engineered T cells was assessed by staining the TCR with MHC multimer or specific TCR Vβ-chain antibody and the CAR with FAP or CD19 protein. (**A**) Representative flow cytometry plots. (**B**) Background-corrected mean fluorescence intensity (dMFI) values from n = 2 donors ± SEM.

Dual FAP/PRAME-specific T cells mediate concurrent killing of cancer and fibroblast cells

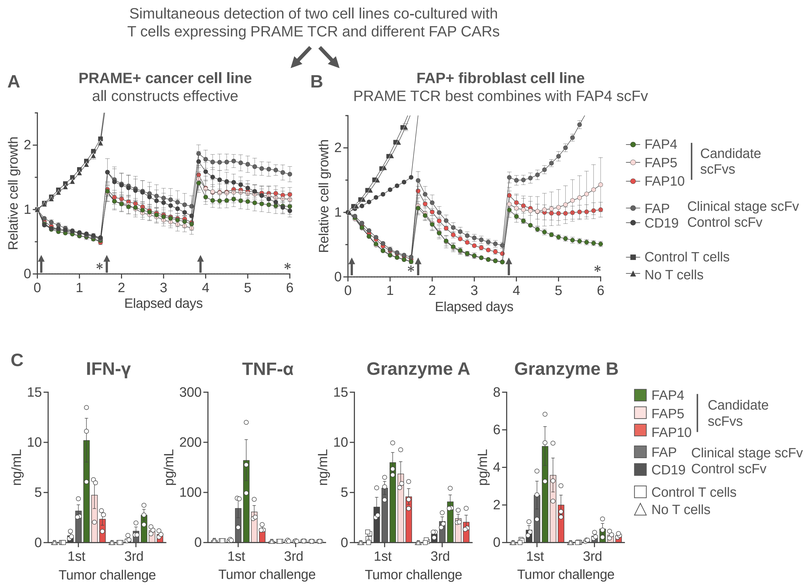


Fig. 3: CAR-mediated cytotoxicity following repeated stimulation with a mix of two target cell lines. To model tumor heterogeneity, a PRAME+ cancer cell line (NCI-H1703) and a FAP+ fibroblast cell line (1BR.3N) were labeled with green and red fluorescent proteins, respectively, and were mixed at an equal ratio. Dual-specific T cells were added to the target cells using a E:T ratio of 1:1. Arrows and asterisks indicate time points at which fresh tumor cells were added, and supernatants were collected for cytokine analysis, respectively. Tumor cell killing was assessed by two-color time-lapse live-cell microscopy. Representative results from one of n = 3 donors showing dual specificity, mediating serial cytotoxicity against FAP+ 1BR.3N cells (**A**) and kill PRAME+ NCI-H1703 cells (**B**) measured simultaneously in the green and red channel, respectively. Error bars are SD from three technical replicates. (**C**) Cytokine secretion was measured using a multiplex cytokine bead array from n = 3 donors ± SEM.

Combined FAP-CAR and PRAME-TCR mediated cytotoxicity leads to elimination of heterogeneous multicell 3D tumor spheroids

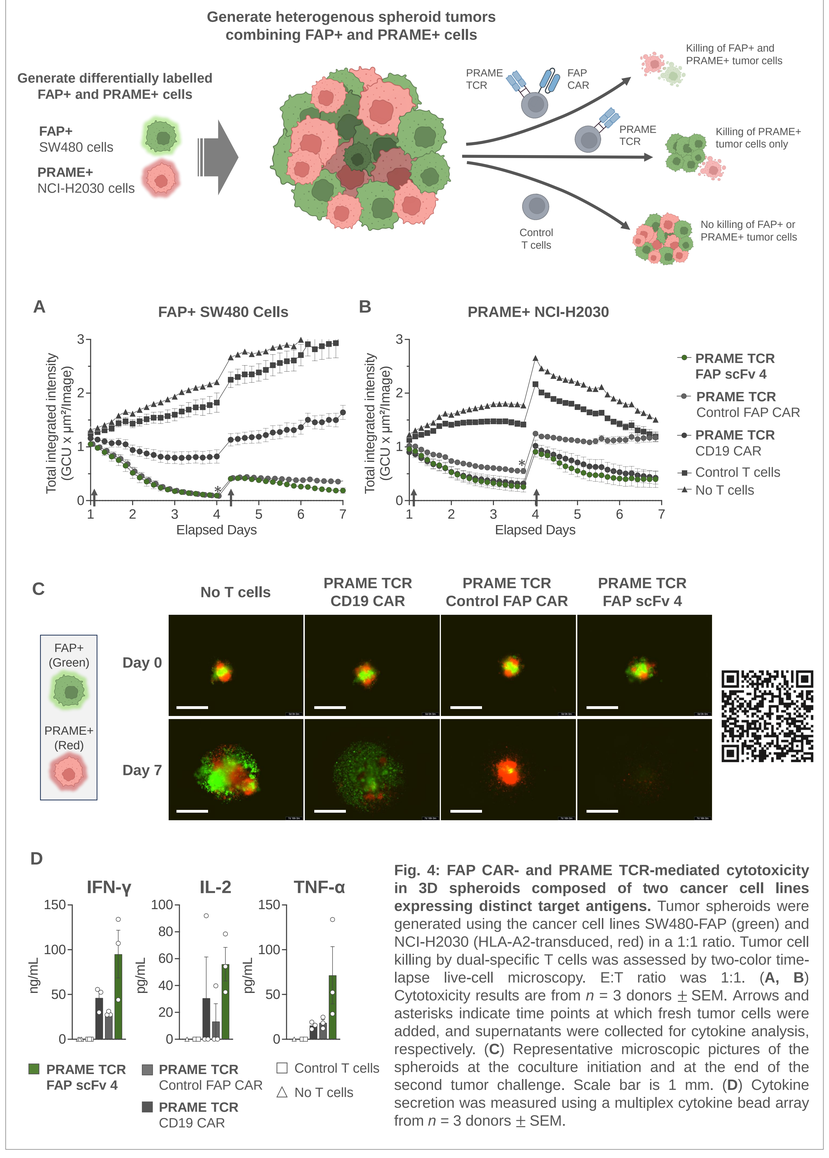


Fig. 4: FAP CAR- and PRAME TCR-mediated cytotoxicity in 3D spheroids composed of two cancer cell lines expressing distinct target antigens. Tumor spheroids were generated using the cancer cell lines SW480-FAP (green) and NCI-H2030 (HLA-A2-transduced, red) in a 1:1 ratio. Tumor cell killing by dual-specific T cells was assessed by two-color time-lapse live-cell microscopy. E:T ratio was 1:1. (**A, B**) Cytotoxicity results are from n = 3 donors ± SEM. Arrows and asterisks indicate time points at which fresh tumor cells were added, and supernatants were collected for cytokine analysis, respectively. (**C**) Representative microscopic pictures of the spheroids at the coculture initiation and at the end of the second tumor challenge. Scale bar is 1 mm. (**D**) Cytokine secretion was measured using a multiplex cytokine bead array from n = 3 donors ± SEM.

Conclusions

- **Synergistic T cell engineering:** Construct design combining selected CAR and TCR in one vector enables one-step engineering of dual-specific T-cells
- **Address tumor heterogeneity:** Sustained killing of heterogeneous tumor spheroids with concurrent killing of cells expressing distinct antigens
- **Broad applicability:** Very high prevalence of PRAME and FAP in high unmet need indications
- **Potential to improve efficacy of T cell therapies:** Simultaneous killing of tumor cells and tumor stroma provides the potential for deep and durable responses in hard-to-treat cancers