CAR T Cells Secreting Synthetic Proteins to Target Solid Tumors

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Introduction

Chimeric antigen receptor (CAR) T cell therapy uses autologous T cells from patients and genetically modifies them to recognize specific surface structures on tumor cells in order to induce tumor cell killing[1]. CAR T cells are very effective in treating hematological malignancies but there still remain obstacles in adopting this treatment option against solid tumors[2,3]. One of these obstacles consists for example of the immunosuppressive tumor microenvironment (TME)[3]. As an attempt to additionally target the TME, CAR T cells have been developed to secrete bispecific T cell engagers (TCEs). Besides CD3 these TCE bind to Fibroblast Activation Protein (FAP) which is expressed on Cancer Associated Fibroblasts (CAFs). Through this binding, the T cells are activated and are instructed to induce killing of the CAFs. These so called CAR^{TEAM} cells showed an increased control of pancreatic tumor growth in different in vitro and in vivo models[4].

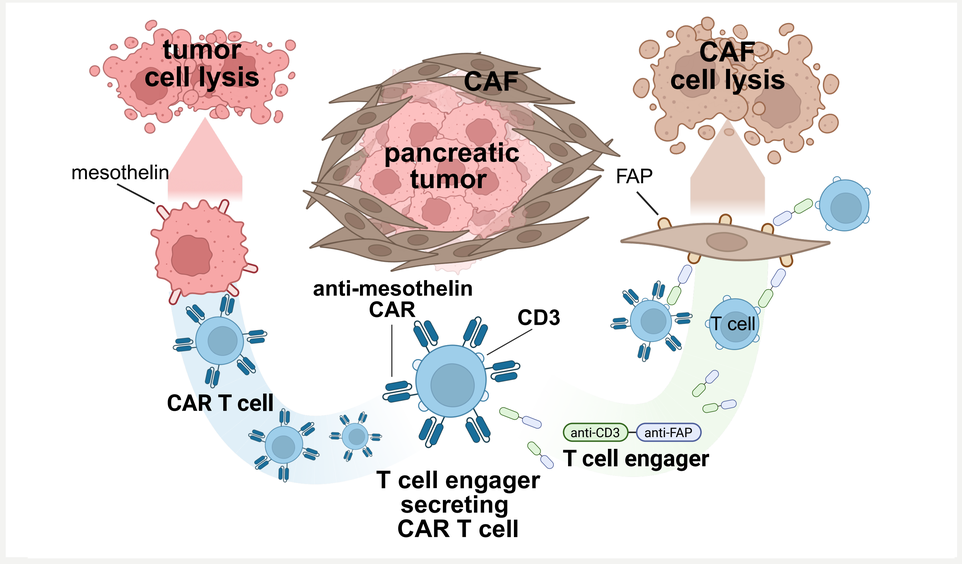


***Figure**: schematic representation of TCE secreting CAR T cells killing tumor cells and CAFs. (created with BioRender)*

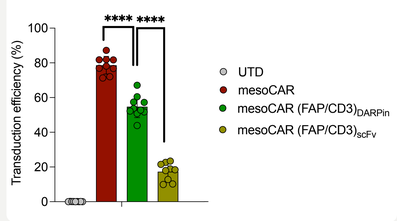
Hypothesis

Based on the CAR^{TEAM} cells, we hypothesized that using smaller sized alternative protein structures, as for example Designed Ankyrin Repeat Proteins (DARPins), might enable the integration of more than one TCE into the CAR T cell. This could help to further improve the performance of CAR T cells in the treatment of solid tumors.

Results

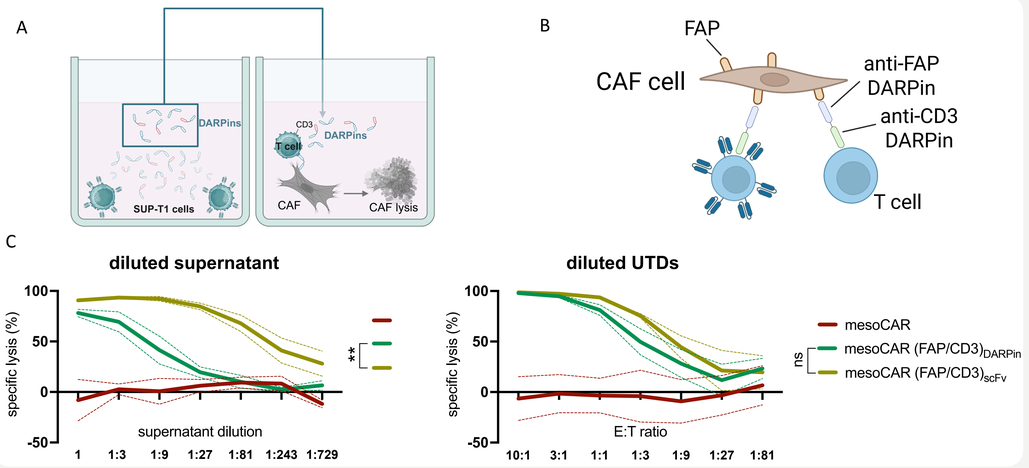
Comparison of DARPin and scFv constructs

1) Transduction efficiencies



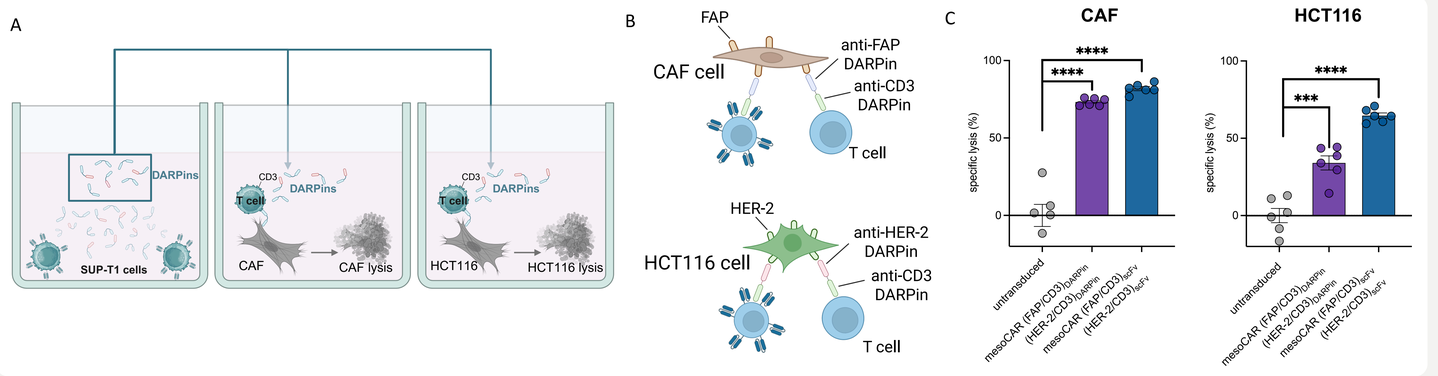
***1)** transduction efficiencies of the different constructs determined by flow cytometric analysis of mCherry positive cells.*
***2) A.** schematic illustration of experimental setup.*
***B.** schematic illustration of CAF cells.*
***C.** cytotoxicity of CAR T supernatant in combination with UTDs against CAF cells assessed by a Luciferase based cytotoxicity assay.*

2) Killing capacity of DARPins/scFvs in the supernatant of CAR T cells



CAR T cells secreting two TCEs

3) SUP-T1 cells secreting two TCEs



***3) A.** schematic illustration of experimental setup. **B.** schematic illustration of CAF and HCT116 cells. **C.** cytotoxicity of transduced SUP-T1 supernatant in combination with UTDs against CAF and HCT116 cells assessed by a Luciferase based cytotoxicity assay.*

Conclusions

- Higher transduction efficiencies with DARPins compared to scFvs.
- Comparable killing capacity of DARPins and scFvs, with scFvs being superior to DARPins when diluted, suggesting that DARPins are most effective at high concentrations, such as the secretion site.
- Transduced SUP-T1s can secrete two functional DARPins/scFvs

References:
1. Baker, D.J., Arany, Z., Baur, J.A. *et al.* CAR T therapy beyond cancer: the evolution of a living drug. *Nature* **619**, 707–715 (2023).
2. Larson, R. C. & Maus, M. V. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer* **21**, 145–161 (2021).
3. Hou, A. J., Chen, L. C. & Chen, Y. Y. Navigating CAR-T cells through the solid-tumour microenvironment. *Nat Rev Drug Discov* **20**, 531–550 (2021).
4. Wehrli, M. *et al.* Mesothelin CAR T-cells secreting anti-FAP/anti-CD3 molecules efficiently target pancreatic adenocarcinoma and its stroma. *Clin Cancer Res* (2024).

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