CAR induced expression of synthetically engineered FAP-IL2v immunocytokine boosts persistent anti-tumor activity of TALEN®-edited allogeneic CAR T-cells without associated IL-2 toxicity.

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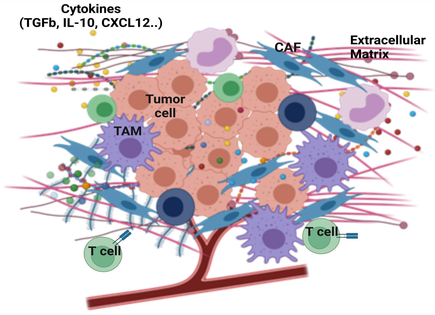
#1 Introduction

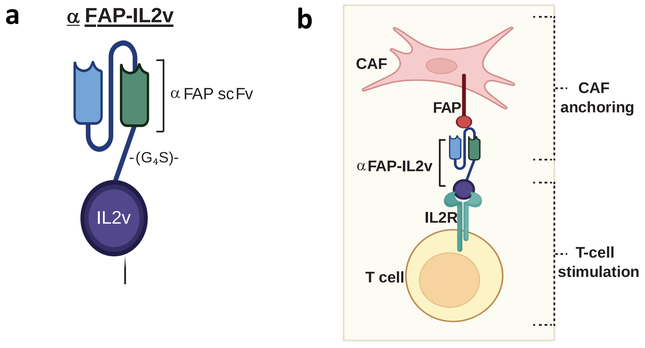
1.1 Solid tumor challenge for CAR-T therapy

Adoptive cell therapy based on chimeric antigen receptor-engineered T (CAR-T) cells has been transformational for selective heme malignancies. However, its therapeutic efficacy in solid tumors is significantly hampered by an immune suppressive, T-cell exclusionary microenvironment. Armored CAR T-cells expressing stimulatory cytokines can address this challenge by boosting intra-tumor CAR T-cell expansion and activity. Potential systemic toxicity of these cytokines however necessitates the development of strategies to localize activity of these cytokines to the tumor microenvironment.



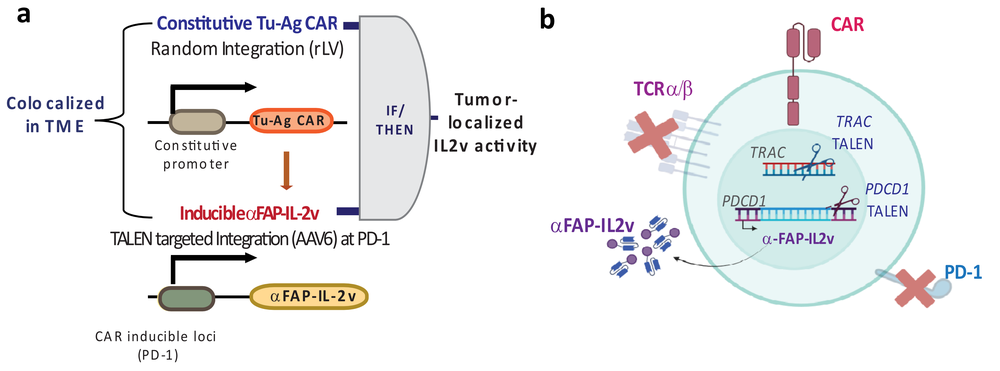
#2 TALEN®-edited immunocytokine-armored CAR-T cells

2.1 Single chain α-FAP Immuno-cytokine



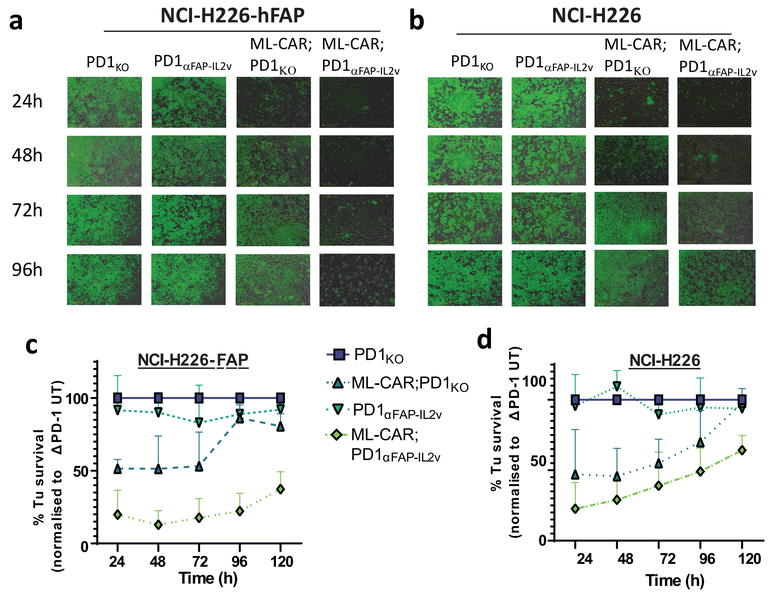
Developing single chain immunocytokine to localize IL-2 activity to TME. (**a**) Single chain immunocytokine fusion protein with N-terminal anti-FAP scFv and C-terminal IL-2 variant (IL-2v) cytokine, linked through a G_4S linker. (**b**) Schematic of αFAP-IL-2v immmunocytokine tethering to FAP^+ cancer-associated fibroblasts (CAFs) and activation of T cells through the IL2R.

2.2 Combating immunosuppression and systemic toxicity with rLv-CAR;$TRAC_{KO}PD1_{\alpha FAP\text{-}IL2v}$ T-cells



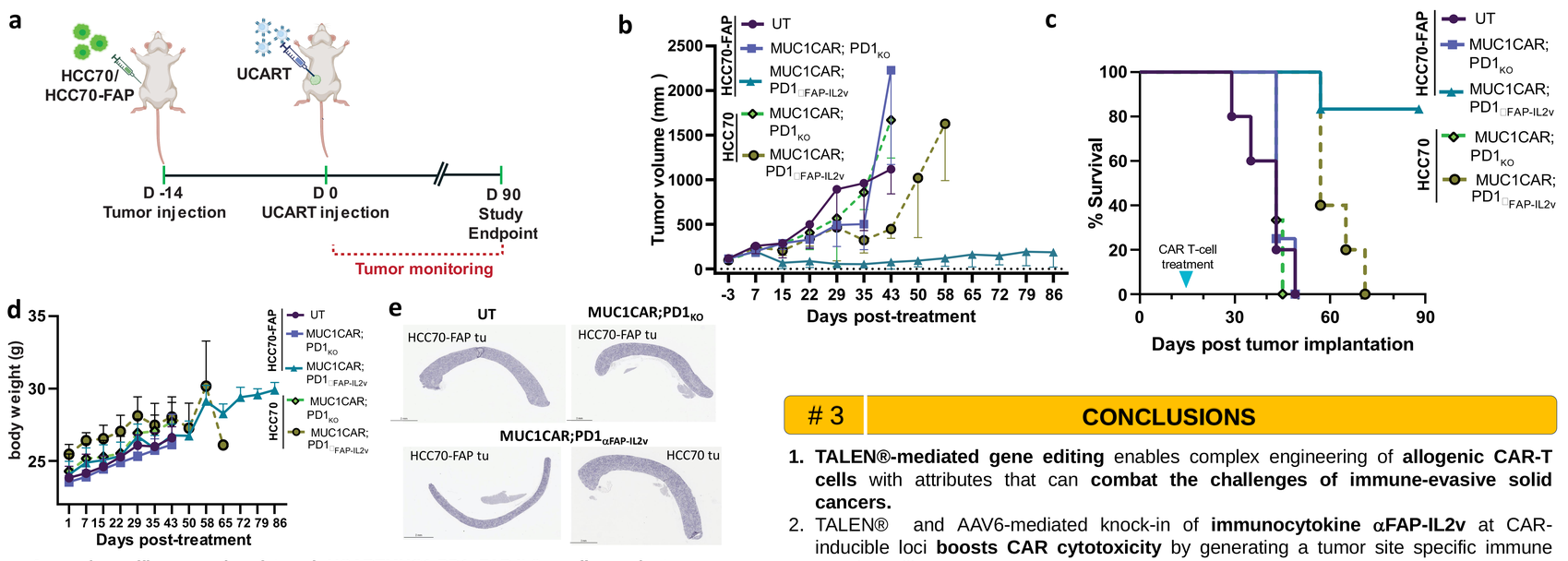
Armored Universal CAR T-cells inducibly expressing αFAP-IL2v immunocytokine. (**a**) Strategy for engineering IF/THEN gated Universal CAR T-cells expressing tumor antigen-targeting CAR and an αFAP-IL-2v immmunocytokine encoding transgene inserted at the CAR-inducible *PDCD1* locus. (b) Graphical representation of TALEN®-engineered, allogenic rLv-CAR;$TRAC_{KO}$ T cells armored with *PDCD1*-inserted αFAP-IL-2v immmunocytokine transgene.

2.3 FAP-anchored αFAP-IL2v immunocytokine boosts enhanced and persistent anti-tumor cytotoxicity of UCAR-ML T-cells



Serial killing assay of MLCAR;$TRAC_{KO}PD1_{\alpha FAP\text{-}IL2v}$ T cells against NCI-H226 human mesothelioma cell line with or without human FAP overexpression. (**a**), (**b**). Representative Incucyte fluorescence images of target tumor cells every 24 hours of serial killing assay, performed at a CAR T-cell to target tumor cell ratio of 5:1. (**c**), (**d**) Percentage of surviving NCI-H226-Luc target cells with and without hFAP expression respectively, as determined by luminescence of residual target cells measured every 24 hours of serial killing assay.

2.4 αFAP-IL2v immunocytokine armored UCARTMUC1 cells safely and effectively target orthotopic mammary FAP^+ tumors *in vivo*



Assessing efficacy and safety of UCARTMUC1;PD1aFAP-IL2v cells against mouse orthotopic mammary tumors. (**a**) Schematic of UCAR T-cell treatment of orthotopic TNBC tumors implanted in NSG mice. (**b**). Graph representing growth kinetics of orthotopic TNBC tumors in mice treated as indicated over time. (**c**) Kaplan–Meier curve for survival analysis of orthotopic TNBC tumor-implanted NSG mice treated as indicated (**d**) Graph representing body weight of tumor-bearing mice as indicated. (**e**) Representative images of immunohistochemical staining of human $CD8^+$ T cells in mice spleen treated as indicated.

3 CONCLUSIONS

1. **TALEN®-mediated gene editing** enables complex engineering of **allogenic CAR-T cells** with attributes that can **combat the challenges of immune-evasive solid cancers.**
2. TALEN® and AAV6-mediated knock-in of **immunocytokine αFAP-IL2v** at CAR-inducible loci **boosts CAR cytotoxicity** by generating a tumor site specific immune reactive milieu.
3. **Inducible αFAP-IL2v immunocytokine armored CAR-T cells** are a safer alternative to cytokine therapy or cytokine armored CAR-T cells due to (a) **Tumor-site specific** production of the cytokine and (b) **TME-retention of the cytokine** thus limiting systemic toxicities.