TALEN®-edited SMART CAR T-cells leverage solid tumor microenvironment for specific and effective immunotherapy

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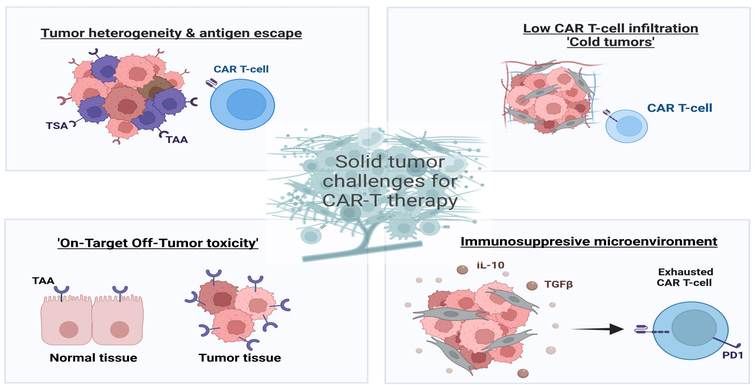
#1 Introduction

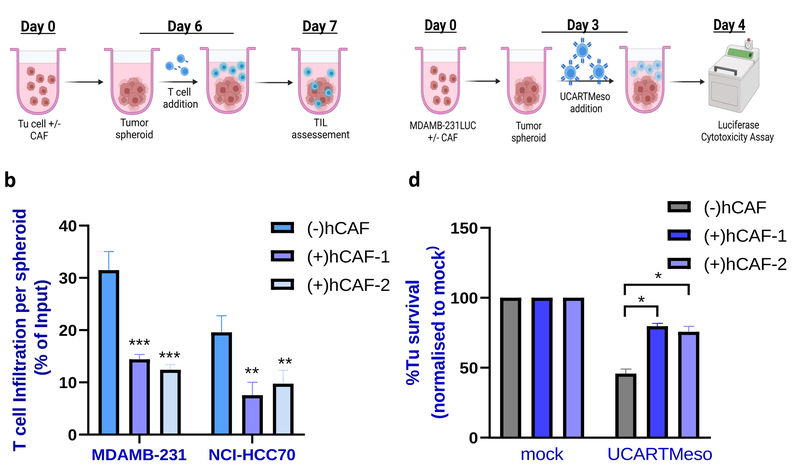
#1.1 Solid tumor challenges for CAR-T therapy

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 severely hampered by several factors, illustrated below.



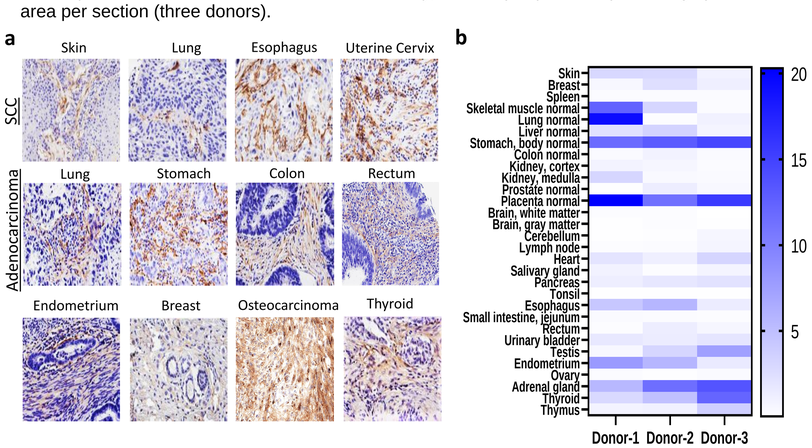
#1.2 Cancer-associated Fibroblasts: modulators of tumor immune evasion

(**a**) Schematic of T cell intra-spheroid infiltration assay. (**b**) Quantitation of T cell infiltration in tumor spheroids +/- with patient TNBC-derived CAFs, as a percentage of input. (**c**) Schematic of UCART-Meso cytotoxicity against MDAMB-231 spheroids +/- patient TNBC-derived CAFs. (**d**) Quantitation of UCARTMeso anti-tumor cytotoxicity elicited in (**c**).



#1.3 Fibroblast Activation Protein (FAP) expressed on CAFs is predominantly localized to the TME

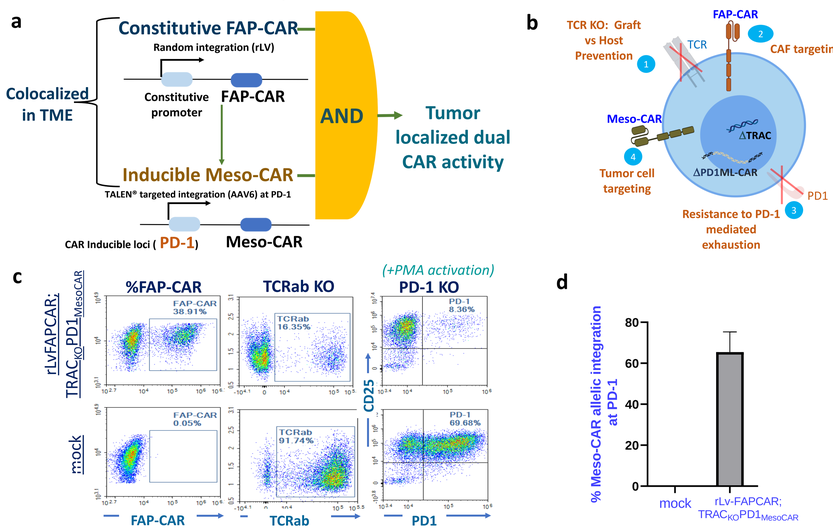
(**a**) Immunohistochemical staining of FAP expression in patient tumor samples (**b**) Quantitation of FAP expression on normal human tissue microarray, heat map represents percentage positive area per section (three donors).



#2 TALEN® edited Dual Inducible CAR-T cells

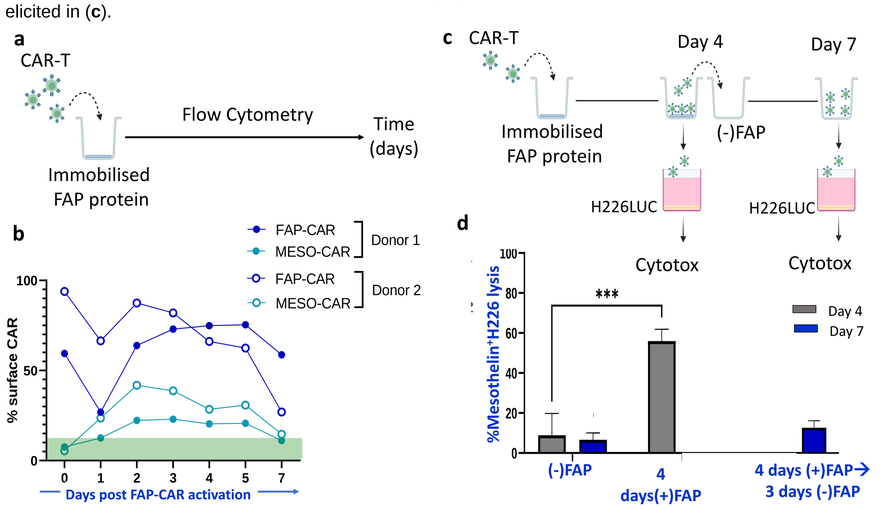
#2.1 Combating 'cold tumors' and 'off-tumor' cytotoxicity with FAPCAR;$TRAC_{KO}PD1_{MesoCAR}$ T cell

(**a**) Dual inducible CAR-T cell strategy for targeting $FAP^{+}Mesothelin^{+}$ tumors. (**b**) TALEN® engineered Allogeneic dual inducible rLv-FAPCAR,$TRAC_{KO}PD1_{MesoCAR}$ T cell. (**c**) Phenotype of rLv-FAPCAR,$TRAC_{KO}PD1_{MesoCAR}$ T cells by flow cytometry. (**d**) Percentage Mesothelin CAR integration at CAR-inducible PD-1 locus, measured by ddPCR.



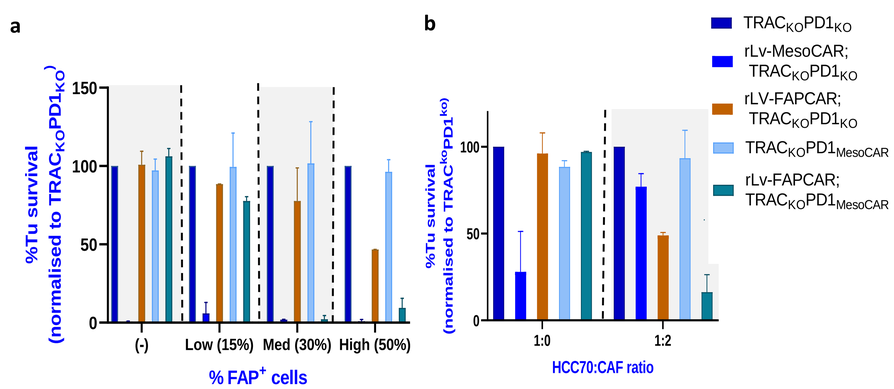
#2.2 MesoCAR expression and activity is stringently regulated by FAPCAR

(**a**) Schematic of FAPCAR activation of rLv-FAPCAR;$TRAC_{KO}PD1_{MesoCAR}$ T cells. (**b**) Flow cytometry analysis of cells from (**a**). (**c**) Schematic for assessing MesoCAR activity against $Mesothelin^{+}FAP^{-}$ H226LUC tumor cells upon FAP-CAR activation and subsequent disengagement. (**d**) Cytotoxicity measurement of MesoCAR elicited in (**c**).



#2.3 FAPCAR;$TRAC_{KO}PD1_{MesoCAR}$ T cells efficiently kill $FAP^{+}Mesothelin^{+}$ tumors spheroids with minimal 'off-tumor' cytotoxicity

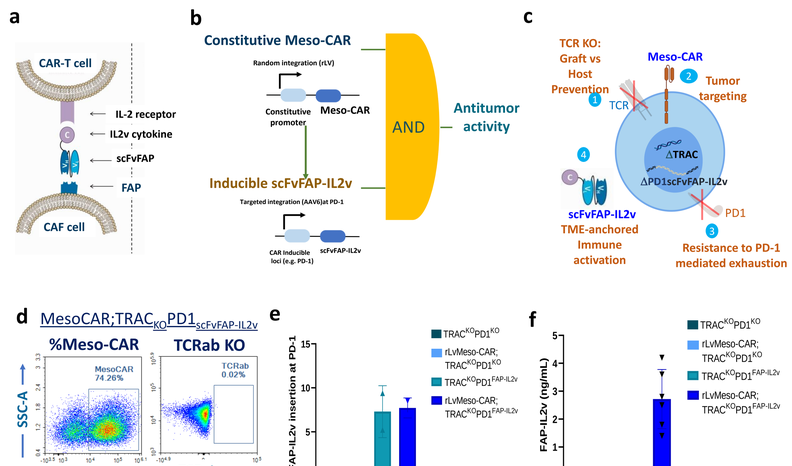
(**a**) Graphical representation of CAR-T cytotoxicity against H226-FAP 3-D spheroids. (**d**) Graph depicting CAR-T cytotoxicity against HCC70 +/- CAF spheroids.



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

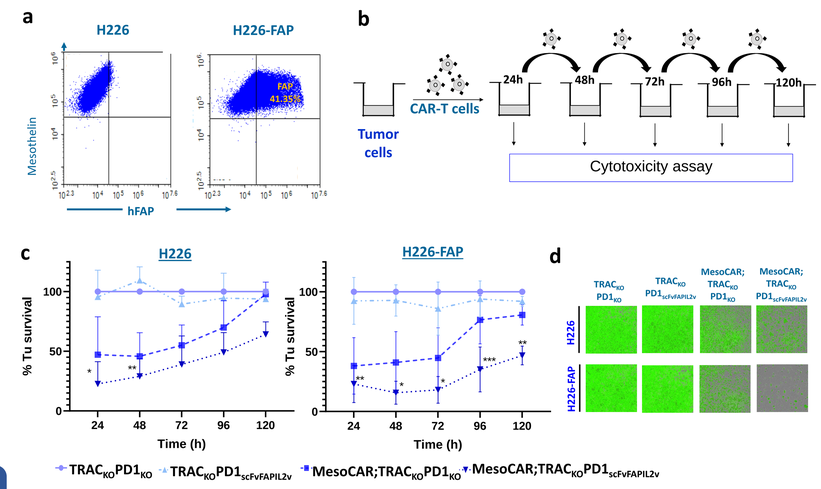
#3.1 Combating immunosuppression and 'off-tumor' cytotoxicity with rLv-MesoCAR;$TRAC_{KO}PD1_{scFvFAP-IL2v}$

(**a**) Schematic of scFvFAP-IL2v immmunocytokine tethering to CAFs and activation of T cells. (**b**) Strategy for engineering and function of immunocytokine armored CAR-T cells. (**c**) TALEN® engineered allogenic armored rLv-MesoCAR;$TRAC_{KO}PD1_{scFvFAP-IL2v}$ T cell. (**d**) Phenotype of rLv-MesoCAR;$TRAC_{KO}PD1_{scFvFAP-IL2v}$ T cells by flow cytometry. (**e**) Percentage scFvFAP-IL2v integration at CAR-inducible PD-1 locus, measured by ddPCR. (**f**) scFvFAP-IL2v protein in MesoCAR;$TRAC_{KO}PD1_{scFvFAP-IL2v}$ T cell supernatant post MesoCAR activation, detected by ELISA.



#3.2 scFvFAP-IL2v boosts antitumor cytotoxicity of UCARTMeso

(**a**) Flow cytometry plots of human FAP transduced H226 mesothelioma cell line. (**b**) Schematic for assessing serial killing activity of MesoCAR;$TRAC_{KO}PD1_{scFvFAP-IL2v}$ T cells against cell lines in (**a**). (**c**) Results of serial killing assay outlined in (**b**). (**d**) Incucyte images of target tumor cells at 72h time point of serial killing assay.



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. **Dual Inducible CAR-T cells** combine the **advantage of targeting heterogenous tumors with the safety of avoiding 'off-tumor' cytotoxicity,** thus expanding potential CAR-T target antigen portfolio.
3. **CAF targeting** by **FAPCAR;$TRAC_{KO}PD1_{MesoCAR}$ dual inducible T cells** make immune evasive solid tumors **susceptible to MesoCAR activity, without off-tumor cytotoxicity** at $FAP^{-}Mesothelin^{+}$ sites
4. **TALEN® and AAV6-mediated** knock-in of immunocytokine **scFvFAP-IL2v** at CAR-inducible loci **boosts CAR cytotoxicity** by generating a tumor site specific immune reactive milieu.
5. Inducible scFvFAP-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.