

TALEN[®]-edited Inducible Dual CAR T-cells Aimed at Safe and Effective Targeting of Solid Tumors

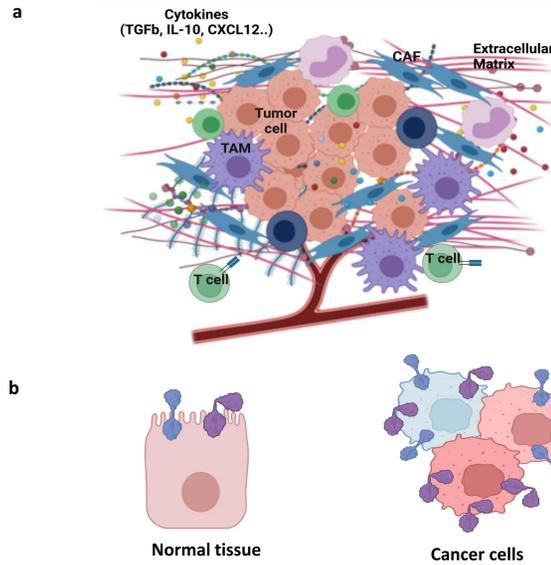
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#1 Introduction: The Solid Tumor Microenvironment

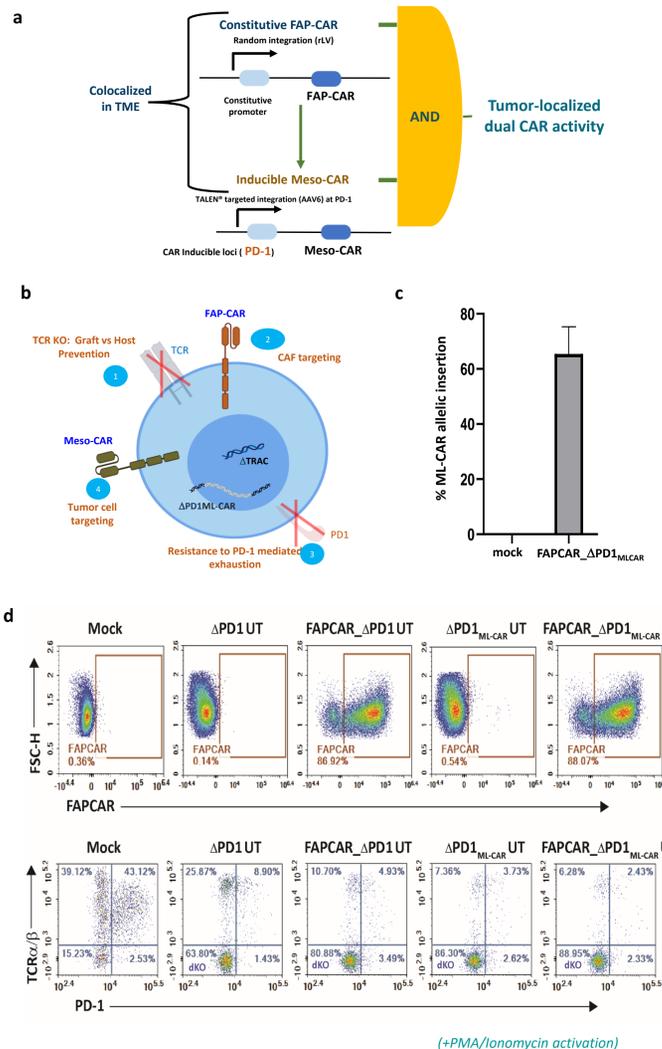
Adoptive cell therapy based on chimeric antigen receptor-engineered T (CAR T) cells has been transformational for selective hematologic malignancies. However, its therapeutic efficacy in solid tumors is severely hampered by several factors. Prominent among these is a complex tumor microenvironment (TME), the components of which subvert immune clearance by **inhibiting intra-tumor T cell infiltration** and establishing an **immunosuppressive milieu**. Furthermore, **tumor antigen heterogeneity** as well as low level expression of CAR-directed tumor-associated antigens (TAA) in normal tissues can result in **antigen-escape** and **“on-target off-tumor”** cytotoxicity respectively, raising significant concerns about therapeutic safety and relapse.

(a) Pictorial representation of the solid tumor microenvironment. (b) Schematic representation of heterogeneous antigen expression in normal and cancer cells.



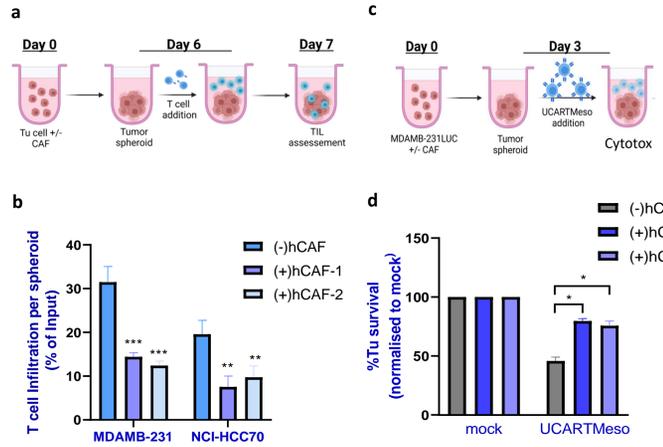
#4 Combating “cold tumors” and “on-target, off-tumor” cytotoxicity with TALEN[®] edited Dual Inducible CAR T-cells

(a) Schematic of engineering dual inducible CAR T-cells targeting FAP⁺Mesothelin⁺ tumors. (b) Pictorial representation of allogeneic dual inducible FAPCAR_ΔPD1_{MLCAR} TRAC knockout T cells, henceforth referred to as Universal T or UT cells. (c) Graphical depicting percentage Mesothelin CAR integration at CAR-inducible PD-1 locus, measured by ddPCR. (d) Phenotype of TALEN[®] engineered dual inducible FAPCAR_ΔPD1_{MLCAR} UT cells by flow cytometry.



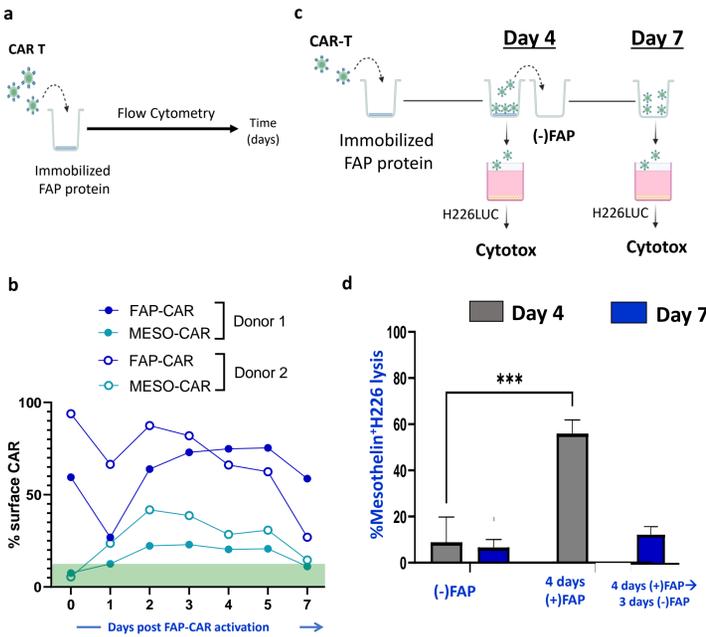
#2 Cancer-associated Fibroblasts inhibit T cell infiltration and CAR T-cell cytotoxicity against solid tumors

(a) Schematic of T cell intra-spheroid infiltration assay. (b) Quantitation of T cell infiltration in tumor spheroids alone or mixed with patient TNBC-derived CAFs, as a percentage of input. (c) Schematic of Mesothelin CAR; TRAC_{KO} T cells cytotoxicity assay against MDAMB-231 spheroids alone or mixed with patient TNBC-derived CAFs. (d) Quantitation of Mesothelin CAR; TRAC_{KO} T cell anti-tumor cytotoxicity assay elicited in (c).



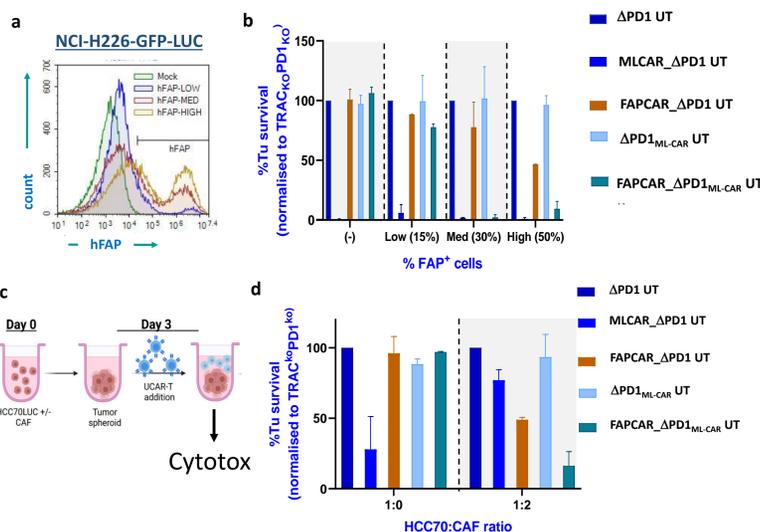
#5 MesoCAR expression and activity is stringently regulated by FAPCAR engagement

(a) Schematic of Dual Inducible FAPCAR_ΔPD1_{MLCAR} UT cell activation with FAP protein. (b) Flow cytometry analysis of cells for FAP-CAR and Meso-CAR expression. (c) Schematic for assessing MesoCAR activity against Mesothelin⁺FAP⁺ H226LUC tumor cells upon FAP-CAR activation and subsequent disengagement. (d) Quantitation of cytotoxicity of MesoCAR elicited in (c).



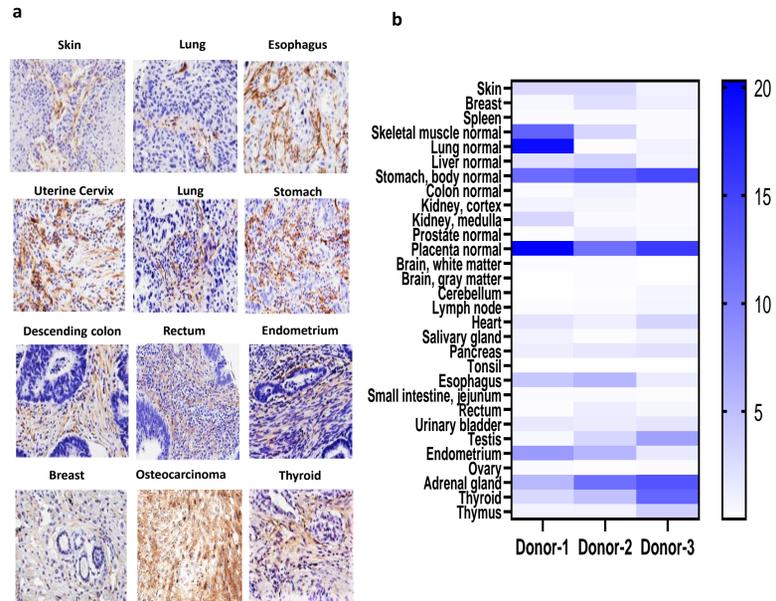
#6 FAPCAR_ΔPD1_{MLCAR} T-cells display superior dual CAR killing against FAP⁺Mesothelin⁺ tumor spheroids with minimal ‘on-target off-tumor’ cytotoxicity

(a) Mesothelioma cell line NCI-H226-LUC transduced to express human FAP protein at different cellular abundance. (b) Quantitation of CAR T-cell cytotoxicity against 3-D spheroids formed by tumor cells from (a). (c) Schematic of CAR T-cell cytotoxicity assay against 3-D spheroids of TNBC cell line HCC70LUC alone or mixed with TNBC-derived CAFs. (d) Graph depicting results of CAR-T cytotoxicity assay outlined in (c).



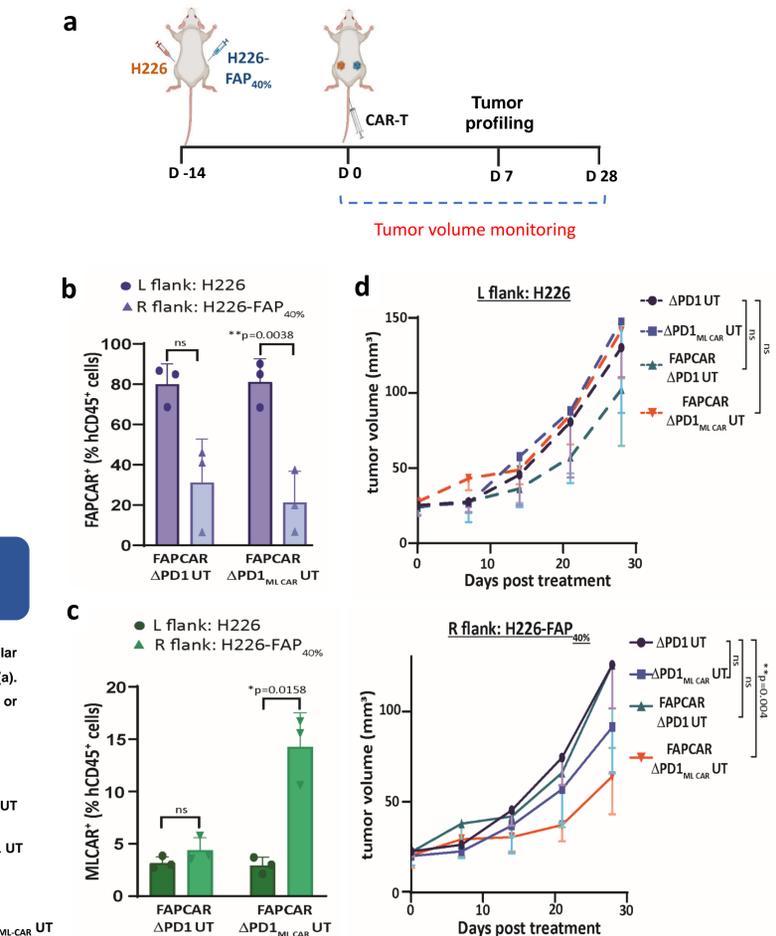
#3 Fibroblast Activation Protein (FAP) expressed on CAFs is predominantly localized to the TME

(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).



#7 FAPCAR_ΔPD1_{MLCAR} UT-cells effectively control FAP⁺ tumors with no bystander toxicity

(a) Schematic of *in vivo* mouse study to assess specificity of FAPCAR-dependent MesoCAR expression and activity. (b,c) Cellular profile of H226 or H226-FAP tumors treated as in (a), as determined by flow cytometry. (d) tumor volumes at endpoint.



Conclusions

1. Dual Inducible FAPCAR_ΔPD1_{MLCAR} Universal T-cells display higher cytotoxicity against FAP⁺Mesothelin⁺ tumors than either of the single CAR-T cells alone.
2. CAR-targeting of CAFs increases cytotoxicity of tumor cell-targeting CAR in physiologically relevant models of solid tumors.
3. Dual inducible FAPCAR_ΔPD1_{MLCAR} UT cells are unable to kill Mesothelin⁺ tumors with lower than physiological FAP⁺ cellular abundance, exhibiting limited ‘on-target off-tumor’ toxicity.
4. TALEN[®] engineered Dual Inducible CAR-T strategy of constitutive TSA-CAR inducing expression of TAA-CAR can increase solid tumor targeting efficacy while limiting off-tumor toxicities.