Preclinical efficacy of PD-L1/TGF-beta resistant MUC1 CAR T-cells with inducible IL12 against triple-negative breast cancer

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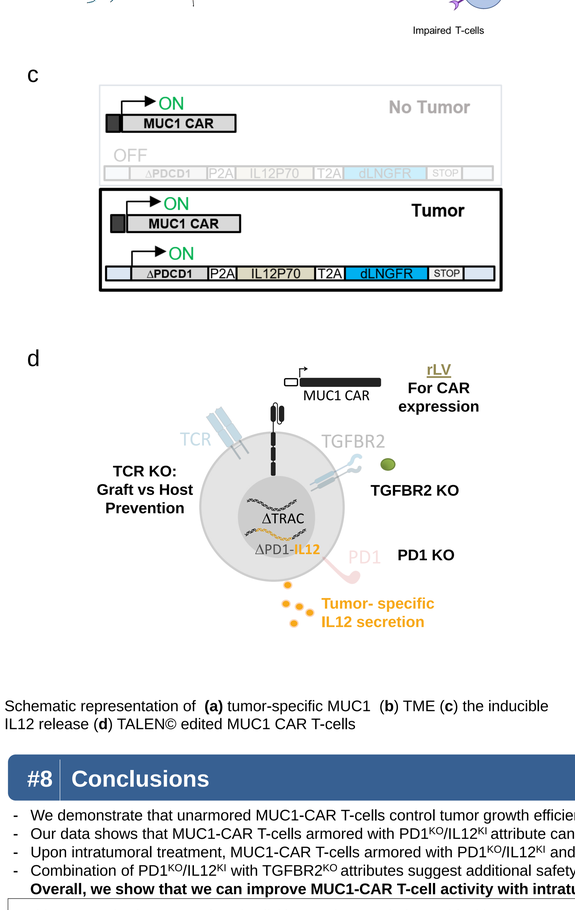
#1 Background

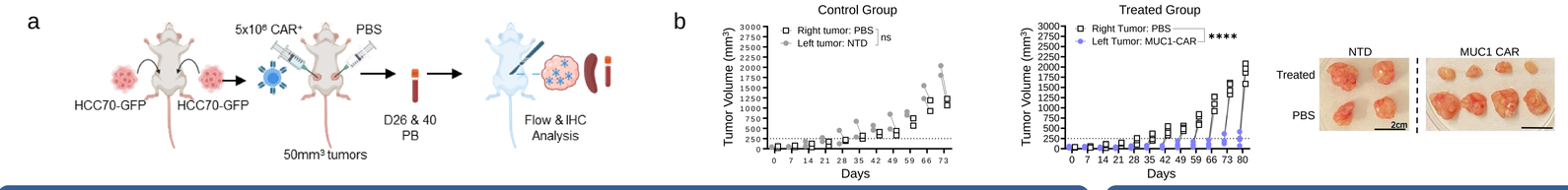
Triple negative breast cancer (TNBC) has limited therapeutic options and the worse prognosis compared to the other subtypes of breast cancer. CAR-T cell therapy could be an invaluable option for TNBC patients. However, the immune-suppressive tumor microenvironment (TME) of solid cancers, such as in TNBC, challenge CAR T-cells to efficiently mount an anti-tumor response. Some of the key mechanisms of immune evasion are mediated by PDL1/PD1 and TGFB1/TGFBR2 interactions resulting in T-cell exhaustion or impaired proliferation. In addition to overcoming the inhibitory effects of the TME, preserving the safety of CAR T-cell therapy while achieving high efficacy of tumor cell killing continues to be under investigation for the treatment of solid tumors. Here, we compare intra-tumoral delivery of TALEN® edited allogeneic MUC1-CAR T-cells to the traditional IV treatment as an alternative route to increase safety and furthermore demonstrate enhanced MUC1-CAR T-cell activity with attributes such as PD1^{KO}, tumor-specific IL12 release and TGFBR2^{KO} that are catered towards the TME.



Schematic representation of **(a)** tumor-specific MUC1 (**b**) TME (**c**) the inducible IL12 release (**d**) TALEN© edited MUC1 CAR T-cells

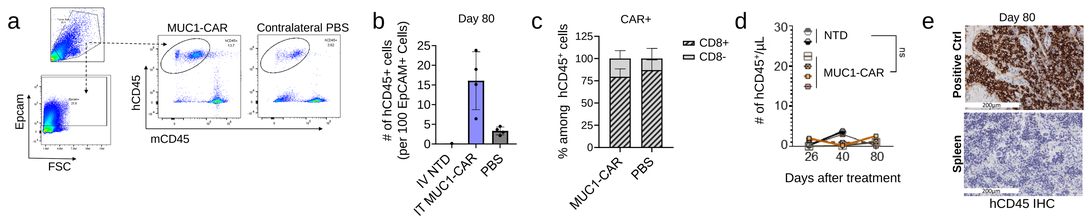
#2 MUC1-CAR T-cells delivered intratumorally show superior tumor control compared to IV treatment.

(**a**) Schematic representation of the experimental timeline (**b**) *In vivo* tumor growth of MUC1-CAR or NTD (Non-transduced T-cells) treated tumors and their paired PBS treated control tumors transplanted on the contralateral mammary fat pad (**c**) *In vivo* tumor growth curve for MUC1-CAR, NTD and PBS treated tumors with intra-tumoral (IT) or intra-venous (IV) delivery (***p-value < 0.001, ****p-value < 0.0001)



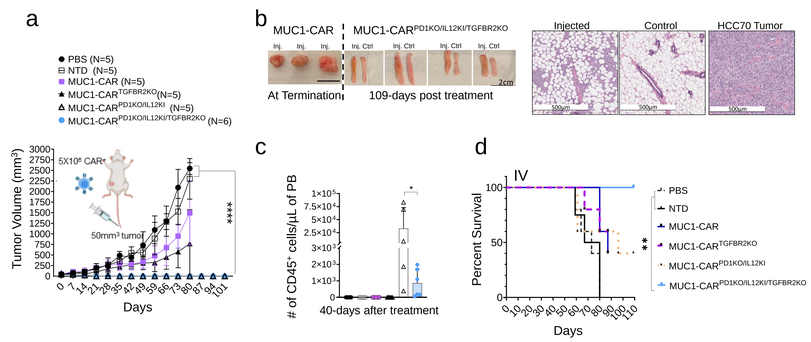
#3 Intratumoral treatment with MUC1-CAR T-cells can still recognize antigen at distant tumor sites while maintaining low number of cells in circulation.

(**a-b**) Flow cytometry analysis of tumors showing MUC1-CAR both in the MUC1-CAR IT treated tumors and the contralateral PBS injected tumors expressing the antigen (**c**) Flow cytometry analysis of CD8+ cells among hCD45+ cells in the MUC1-CAR IT treated tumors and contralateral PBS tumors (**d**) Quantification of MUC1-CAR and NTD control in circulation overtime (**e**) IHC analysis of spleens showing no MUC1-CARs at sites without the antigen



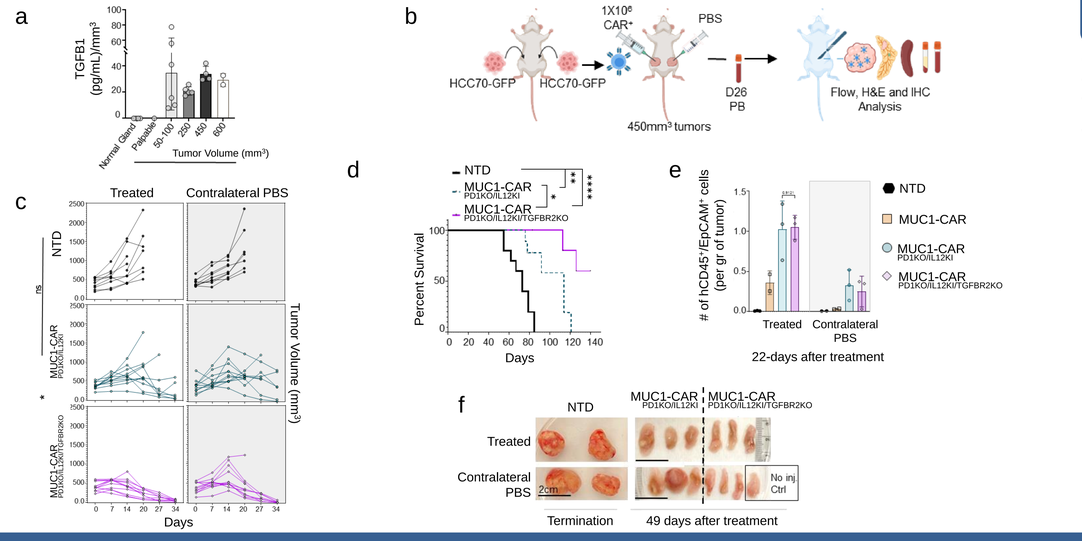
#4 With PD1^{KO}/IL12^{KI} and TGFBR2^{KO} attributes (IV) MUC1-CAR T-cells clear tumors within weeks without relapse and extend survival.

(**a-b**) *In vivo* tumor growth, (**b**) tissue analysis post-tumor clearance (c) flow cytometry analysis of hCD45+ cells in PB and (**d**) percent survival for animals treated intravenously with PBS, NTD, MUC1-CAR, and MUC1-CAR with TGFBR2^{KO}, or PD1^{KO}/IL12^{KI} or PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes. (**p-value < 0.01, ****p-value < 0.0001)



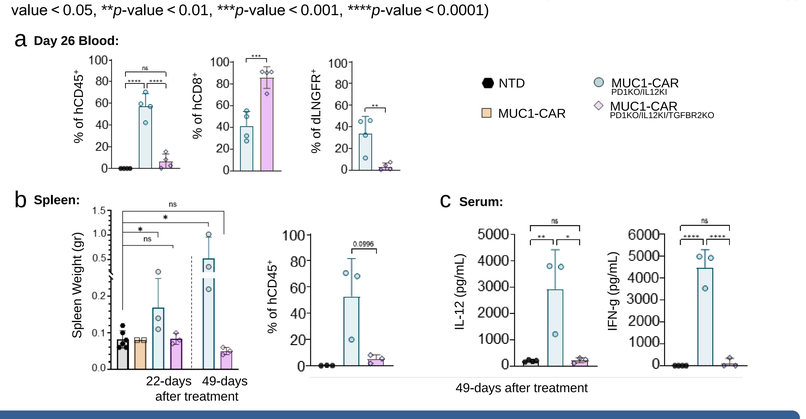
#5 PD1^{KO}/IL12^{KI} and TGFBR2^{KO} armored MUC1-CAR T cells can clear TGFB1 rich tumors at the local and distant sites and extend survival following intratumoral delivery.

(**a**) ELISA analysis of TGFB1 concentration at different sizes of tumor progression. (**b**) Schematic representation of the experimental timeline (**c**) *In vivo* tumor growth and (**d**) percent survival for animals treated intratumorally with NTD, or MUC1-CAR with PD1^{KO}/IL12^{KI} or PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes. (**e**) Flow cytometry analysis of number of hCD45+ and EpCAM+ cells in the local and contralateral tumors. (**f**) Dissection images after treatment with NTD or MUC1-CAR with PD1^{KO}/IL12^{KI} or PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes. (*p-value < 0.05, **p-value < 0.01, ****p-value < 0.0001)



#6 Synergy of the attributes limit off tumor presence of MUC1 CAR T-cells and IL12.

(**a**) Flow cytometry analysis of hCD45+, hCD45+CD8+ and hCD45+LNGFR+ cells in PB of animals treated with NTD, an MUC1-CAR with PD1^{KO}/IL12^{KI} or PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes. (**b**) Spleen weight and flow cytometry analysis of hCD45+ cells in spleens of animals treated with NTD, MUC1-CAR with PD1^{KO}/IL12^{KI} or PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes. (**c**) ELISA analysis of IL-12 and IFN-gamma concentration in serum. (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, ****p-value < 0.0001)



#8 Conclusions

- We demonstrate that unarmored MUC1-CAR T-cells control tumor growth efficiently when administered intra-tumorally, suggesting that we can use lower doses for optimal activity while still being able to recognize the distant antigen-positive tumor sites with only a few CARs detectable in the circulation.
- Our data shows that MUC1-CAR T-cells armored with PD1^{KO}/IL12^{KI} attribute can clear tumors within weeks without relapse and when the TGFBR2^{KO} attribute is added similar tumor clearance is possible with lower number of cells.
- Upon intratumoral treatment, MUC1-CAR T-cells armored with PD1^{KO}/IL12^{KI} and TGFBR2^{KO} attributes show complete tumor reduction in a high TGFB1 and high tumor burden model, at both local and distant tumor sites (potentially recapitulating control of metastatic sites).
- Combination of PD1^{KO}/IL12^{KI} with TGFBR2^{KO} attributes suggest additional safety benefits by resulting in a decrease in MUC1-CAR T-cell and IL12 accumulation outside the tumor, thereby, limiting the risks of off-tumor toxicity as well as potential IL12 related adverse events.

Overall, we show that we can improve MUC1-CAR T-cell activity with intratumoral delivery and/or through armoring of the MUC1-CAR T-cells with several attributes.