Enhanced Allogeneic MUC-1 CAR T-cell Response Against Triple Negative Breast Cancer (TNBC) Utilizing Variable Delivery Routes and Molecular Armoring

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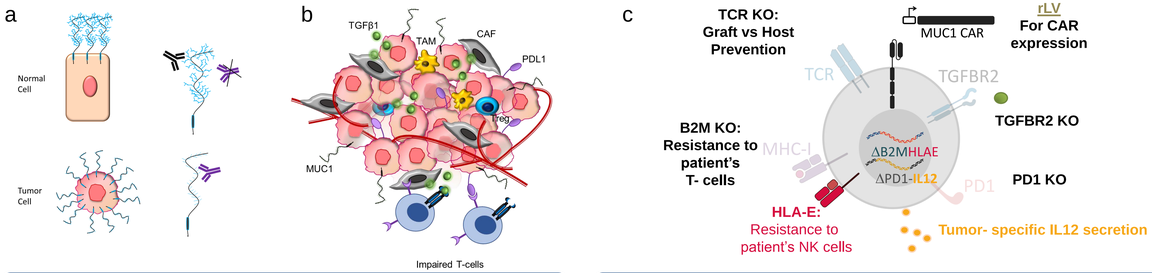
#1 Background

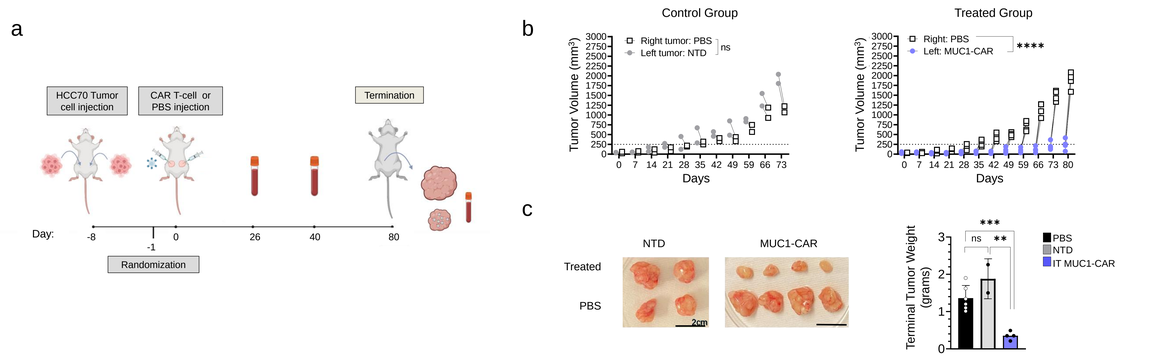
Triple negative breast cancer (TNBC) has limited therapeutic options and the worse prognosis compared to the other subtypes of breast cancer. CAR-T cell therapy could be an invaluable option for TNBC patients. However, the immune-suppressive tumor microenviroment (TME) of solid cancers, such as in TNBC, 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 our TALEN® edited allogeneic MUC1-CAR of the same doses to the traditional IV treatments as an alternative route to increase safety and furthermore demonstrate enhanced CAR-T cell activity with attributes of PD1^{KO}, tumor-specific IL12 release and TGFBR2^{KO} that are catered towards the TME.

(**a**) Schematic representation of tumor-specific MUC1 (**b**) Schematic representation of TME (**c**) Schematic representation of TALEN© edited MUC1-CAR



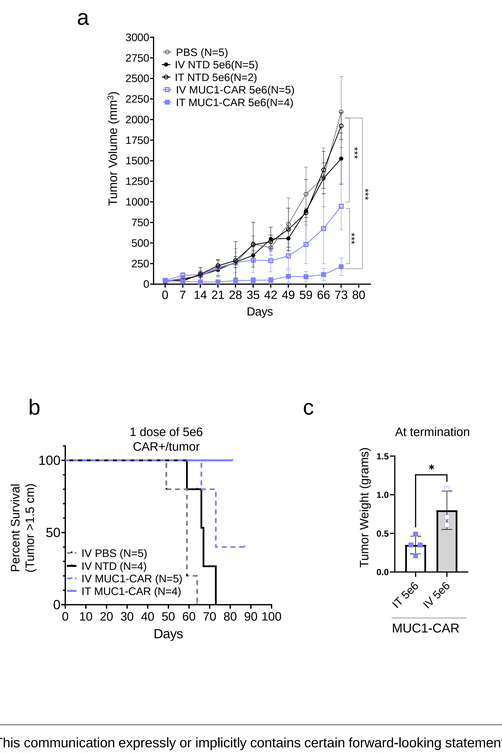
#2 Intra-tumoral delivery of MUC1-CAR efficiently controls tumors.

(**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**) Tumor weight comparison at termination for NTD, MUC1-CAR, and contralateral PBS control tumors (***p*-value < 0.01, ****p*-value < 0.001, *****p*-value < 0.0001)



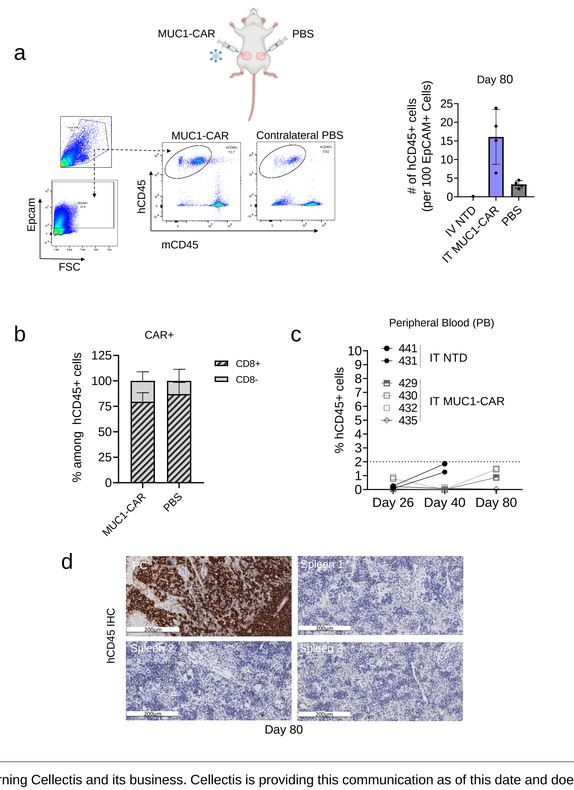
#3 Superior control of tumor growth via intra-tumoral delivery of MUC1-CAR compared to IV delivery.

(a) In vivo tumor growth curve for MUC1-CAR, NTD and PBS treated tumors with intra-tumoral (IT) or intra-venous (IV) delivery (**b**) Survival curve showing exten
(b) ded survival for animals treated with MUC1-CAR with IT delivery (**c**) Tumor weight comparison for MUC1-CAR treated tumors using IT or IV delivery (**p*-value < 0.05, ****p*-value < 0.001)



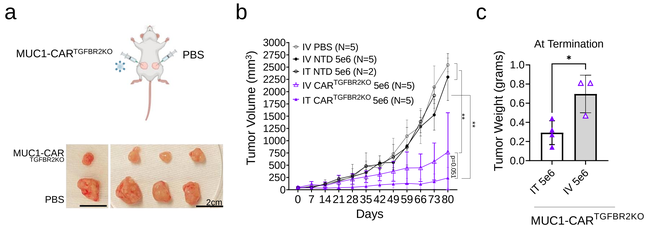
#4 MUC1-CAR migration to the secondary antigen site.

(**a**) 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 (**b**) Phenotype of T cells detected in the MUC1-CAR IT treated tumors and contralateral PBS tumors (**c**) Quantification of MUC1-CAR and NTD control in circulation overtime (**d**) IHC analysis of spleens showing no MUC1-CARs at sites without the antigen



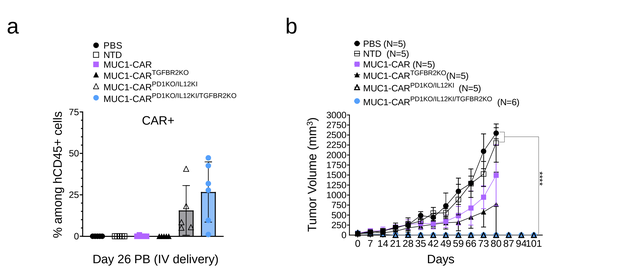
#5 TGFBR2^{KO} MUC1-CAR effectively controls tumors, and tumor control is enhanced with intra-tumoral delivery.

(**a**) Tumor images from intra-tumoral treatment with PBS or MUC1-CAR^{TGFBR2KO} (**b**) *In vivo* tumor growth curve for animals treated with MUC1-CAR with TGFBR2^{KO} attribute, NTD or PBS via IT or IV delivery (**c**) Tumor weight comparison at termination for MUC1-CAR^{TGFBR2KO} treated tumors using IT or IV delivery (**p*-value < 0.05, ***p*-value < 0.01)



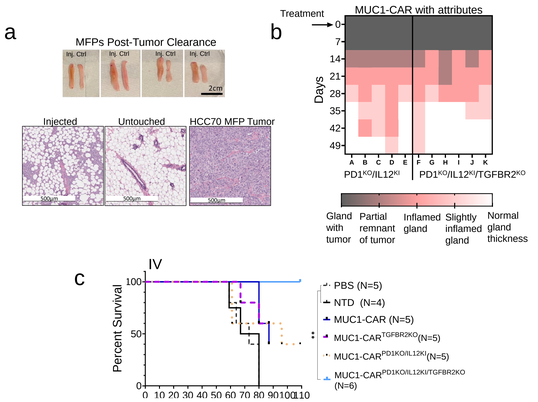
#6 With PD1^{KO}/IL12^{KI} and TGFBR2^{KO} attributes CAR T-cells expand in circulation and clear tumors within weeks without relapse.

(**a**) MUC1-CAR expansion with different attributes in the peripheral blood (b) *In vivo* tumor growth 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 < 0.0001)



#7 MUC1-CAR with PD1^{KO}/IL12^{KI} TGFBR2^{KO} recovers normal glands faster and extends survival.

(**a**) Dissection images and H&E analysis of mammary fat pads (MFP) collected post-treatment with MUC1-CAR with PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes: paired visual of HCC70 tumor cell injected MFP and contralateral untouched MFP (**b**) Heatmap representation of tumor clearance overtime for tumors treated with MUC1-CAR with PD1^{KO}/IL12^{KI} or PD1^{KO}/IL12^{KI}/TGFBR2^{KO} attributes (**c**) Percent survival of mice treated IV with PBS, NTD, MUC1-CAR and MUC1-CAR with various attributes (***p*-value < 0.01)



#8 Conclusions

- We demonstrate that MUC-1 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 with several attributes catered towards the key challenges of immune suppressive TME can clear tumors within weeks without relapse resulting in extension of survival. Additionally, when we combine TGFBR2^{KO} attribute with PD1^{KO}/IL12^{KI} the recovery of the MFP is faster.
- Overall, we show that we can improve CAR T-cell activity with alternative routes of delivery and/or through armoring of the CAR with several attributes.

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