Abstract 254

SCAN ME

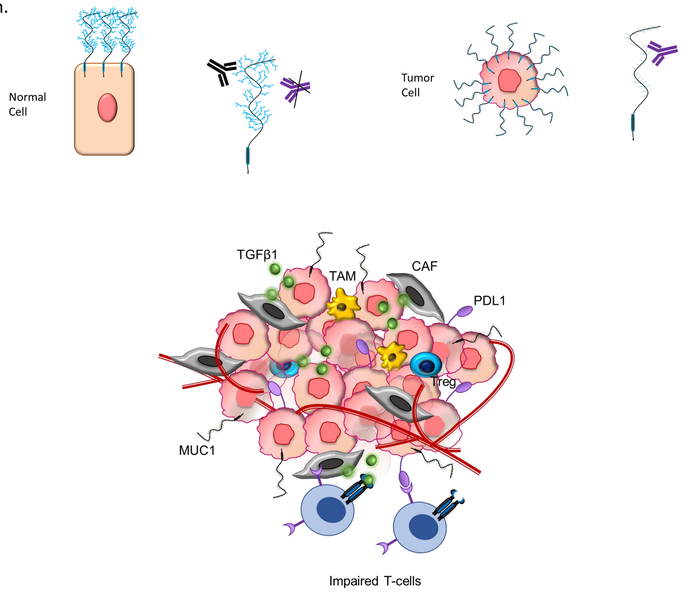
TGFBR2 KO combined with activation-induced IL12 secretion synergize to optimize potency and safety of MUC1-CAR T-cells in preclinical targeting of triple-negative breast cancer

Laurent Poirot[2] , Piril Erler[1], Hana Cho[1], Jordan Skinner[1], Beatriz Aranda-Orgilles[1]

[1] Cellectis Inc, New York, NY; [2] Cellectis SA, Paris, France

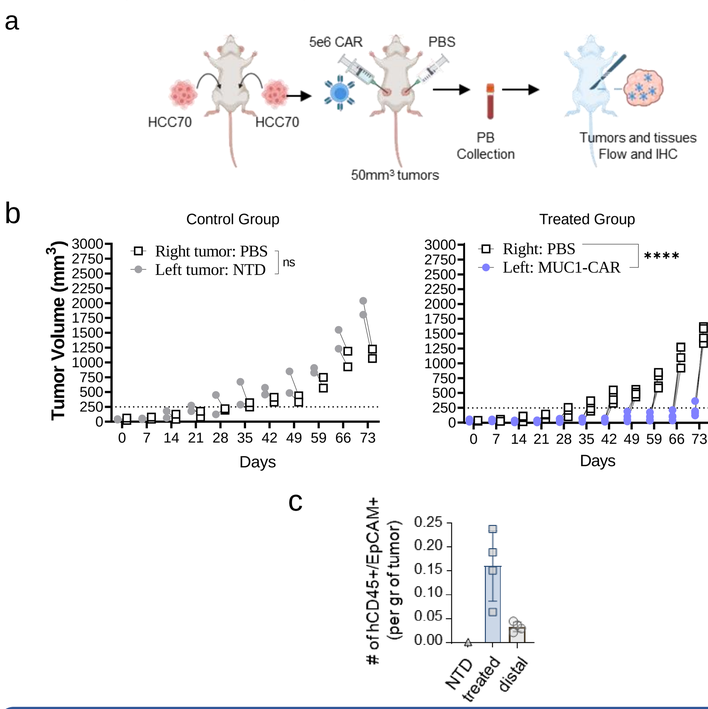
#1 Background

As immune-therapies for solid tumors progress, the tumor microenvironment (TME) presents several hurdles that CAR T-cells need to overcome for efficient tumor control. To limit T-cell effectiveness against the tumor, the TME employs a variety of mechanisms including tumor evasion, secretion of immunosuppressive factors or cellular resistance, among others. Despite being the most aggressive breast cancer subtype with a dismal prognosis, therapeutic options for triple negative breast cancer (TNBC) remain limited to date. Tumor-associated MUC1 antigen is hypoglycosylated in cancer and overexpressed in many TNBC patients, thus providing a useful target for CAR T-cell therapies. TALEN®-based gene editing allows multiplex reprograming of allogeneic CAR T-cells to circumvent TME barriers. Exploring different routes of infusion can also help understanding the best therapeutic approach.



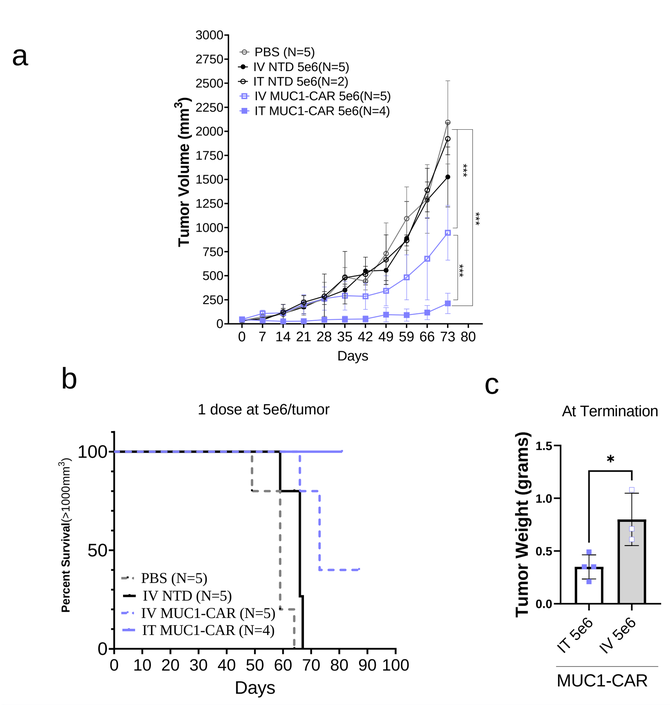
#2 Intra-tumoral delivery of MUC1 CAR-T controls tumor locally

(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 distal PBS-treated control tumors transplanted on the contralateral mammary fat pad) (c) Flow cytometry analysis of the number of hCD45+ cells per EpCAM+ cells per gram of tumor at termination (day 80). (d) Side by side comparison of tumor growth in intravenously (i.v.) vs intratumorally (i.t.) treated mice.



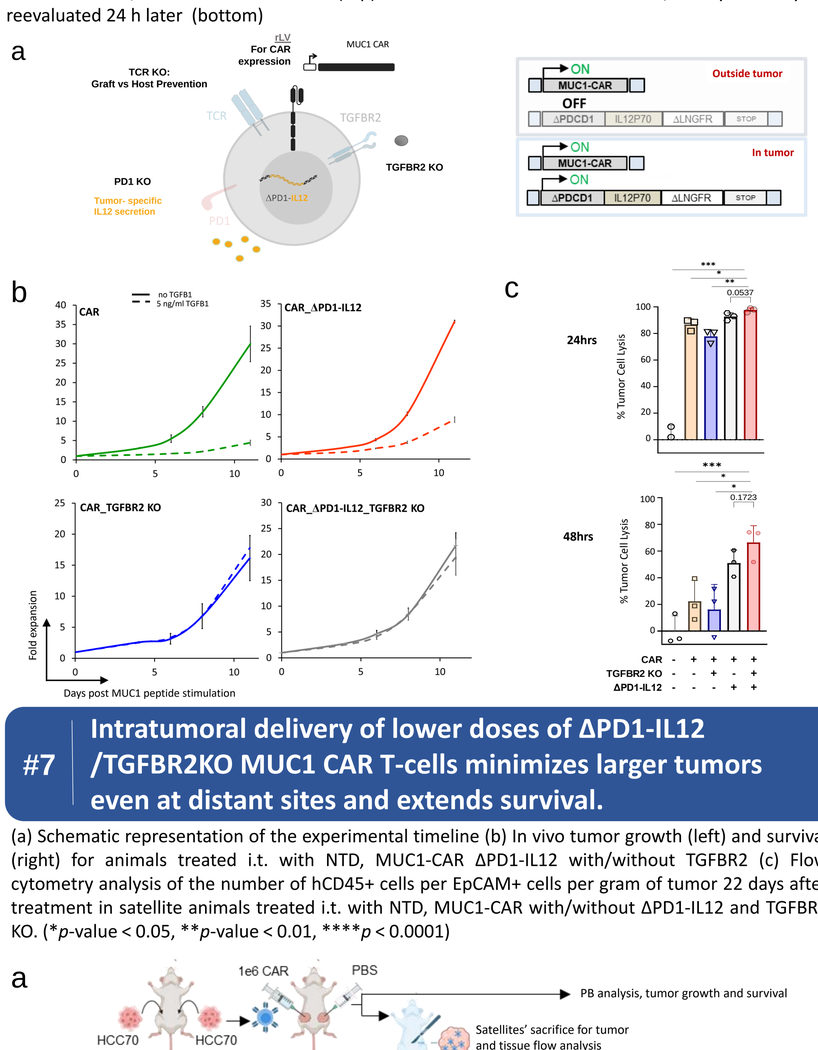
#3 Superior control of tumor growth via intra-tumoral delivery of MUC1-CAR compared to IV delivery.

(**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 extended survival for animals treated with MUC1-CAR with IT delivery (**c**) Tumor weight comparison at termination for MUC1-CAR treated tumors using IT or IV delivery (*$p < 0.05$, ***$p < 0.001$)



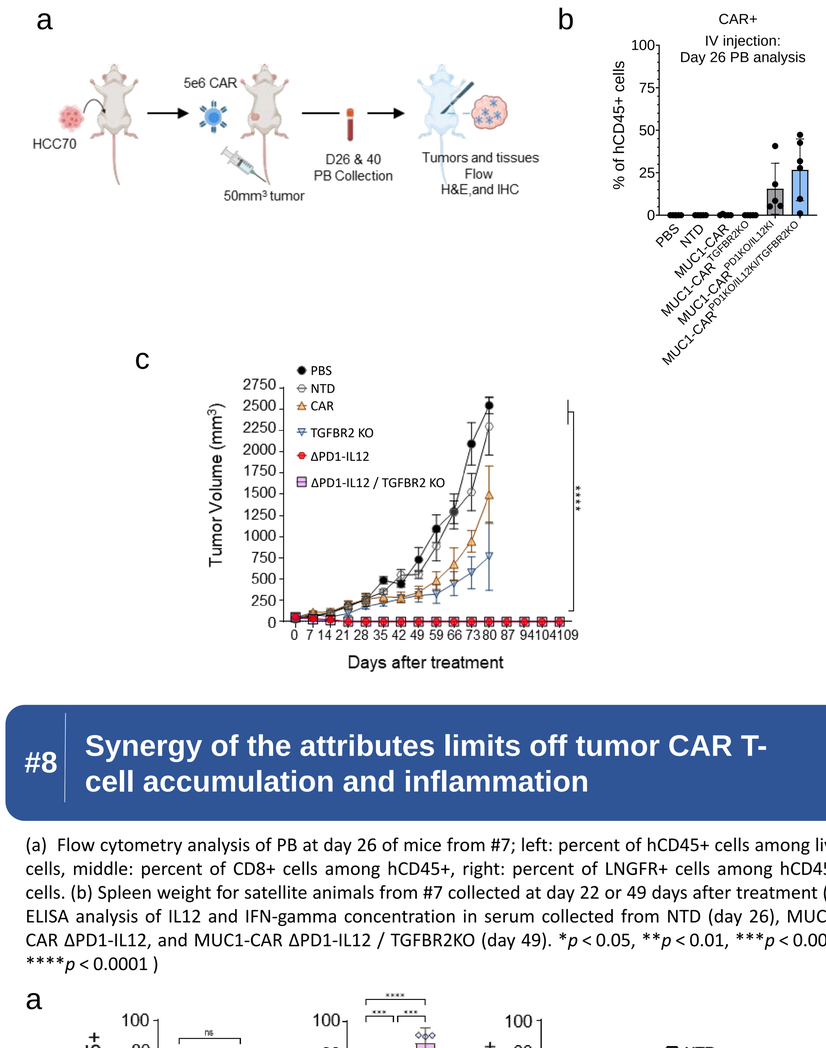
#4 Increased in vitro potency of CAR T-cells using TALEN®-mediated gene editing

(a) Schematic representation of the multiplex engineered cell (left) and of the activation-induced PD1-targeted IL12 cassette (b) In vitro proliferation assay using MUC1 peptide in the presence or absence of TGFB1 for the different combination of TGFBR2 KO and ΔPD1-IL12 attributes (c) Cellular cytotoxicity assay (7:1 E:T ratio) against HCC70 cells in presence of 5 ng/ml TGFB1. % cytoxicity was evaluated after 24 h of coculture of HCC70 cells and MUC1 CAR T-cells (7:1 effector:target ratio) with or without TGFBR2 KO and/or ΔPD1-IL12 attributes (top) then fresh HCC70 cells were added, and cytotoxicity was reevaluated 24 h later (bottom)



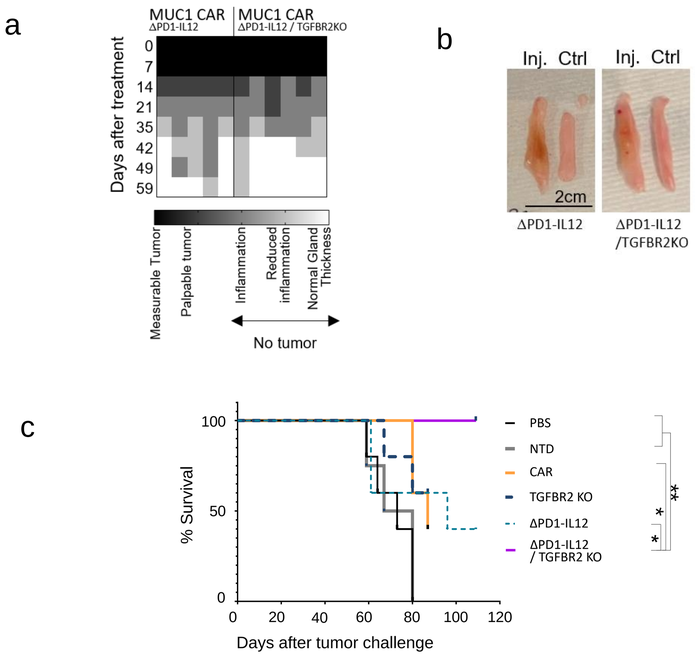
#5 i.v. infused ΔPD1-IL12 MUC1 CAR T-cells expand and clear tumors without relapse.

(a) Schematic representation of the experimental timeline (b) Flow cytometry of MUC1-CAR expansion with different attributes in the peripheral blood. (c) In vivo tumor growth for animals treated i.v. with PBS, NTD, MUC1-CAR with/without ΔPD1-IL12 and/or TGFBR2 KO (****$p < 0.0001$)



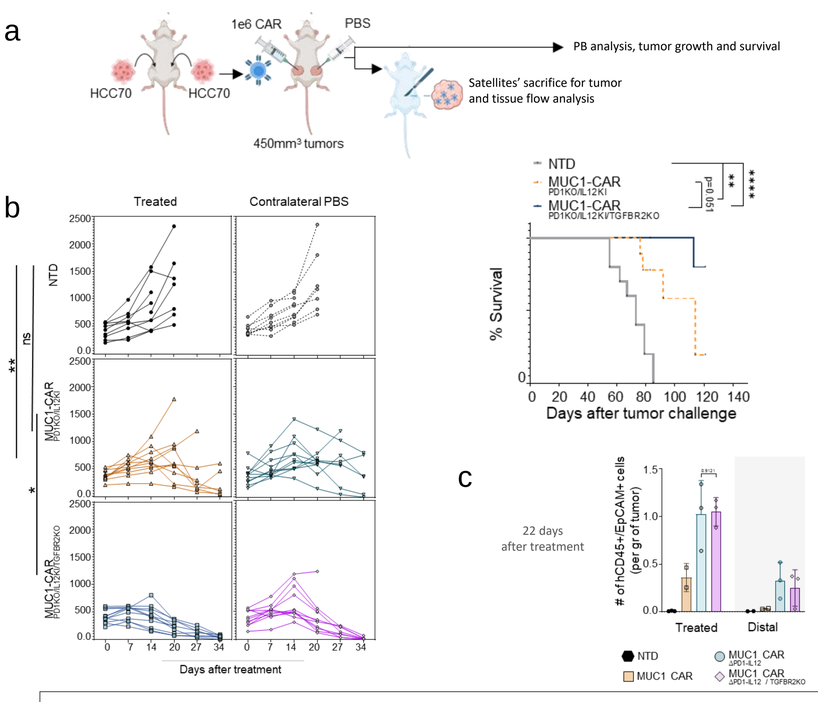
#6 MUC1 CAR T-cells with ΔPD1-IL12 and TGFBR2KO recover normal glands faster and extend survival.

(a) Heatmap representation of tumor clearance overtime for tumors treated with MUC1-CAR (b) Paired visual of HCC70 cell-injected mammary fat pad (MFP) and contralateral untouched MFP in mice treated with ΔPD1-IL12 MUC1 CAR T-cells with or without TGFBR2 KO after tumor clearance. (c) In vivo survival curve for animals treated i.v. with PBS, NTD, MUC1-CAR with/without ΔPD1-IL12 and/or TGFBR2 KO (*$p < 0.05$, **$p < 0.01$)



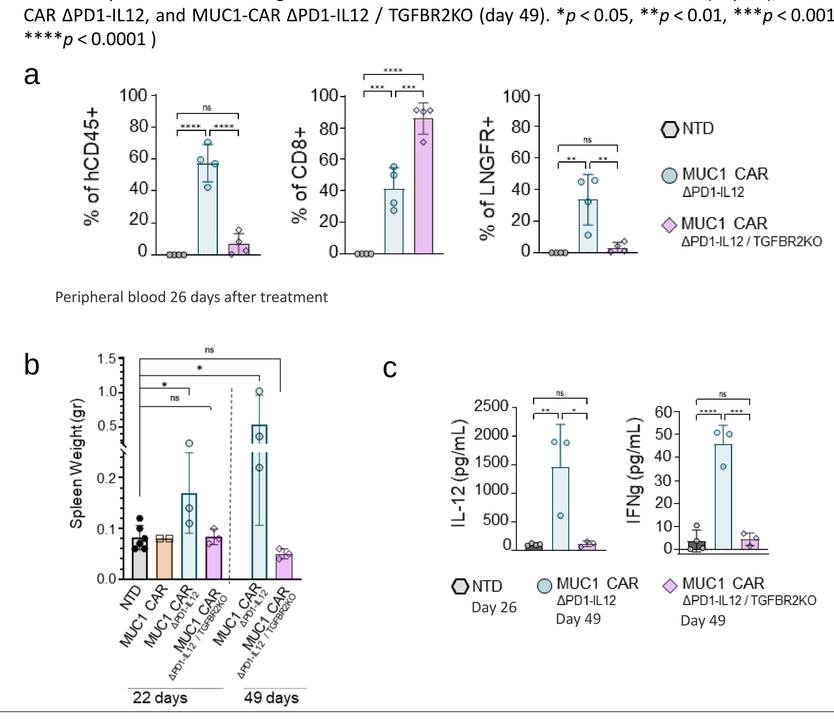
#7 Intratumoral delivery of lower doses of ΔPD1-IL12 /TGFBR2KO MUC1 CAR T-cells minimizes larger tumors even at distant sites and extends survival.

(a) Schematic representation of the experimental timeline (b) In vivo tumor growth (left) and survival (right) for animals treated i.t. with NTD, MUC1-CAR ΔPD1-IL12 with/without TGFBR2 (c) Flow cytometry analysis of the number of hCD45+ cells per EpCAM+ cells per gram of tumor 22 days after treatment in satellite animals treated i.t. with NTD, MUC1-CAR with/without ΔPD1-IL12 and TGFBR2 KO. (*p-value < 0.05, **p-value < 0.01, ****$p < 0.0001$)



#8 Synergy of the attributes limits off tumor CAR T-cell accumulation and inflammation

(a) Flow cytometry analysis of PB at day 26 of mice from #7; left: percent of hCD45+ cells among live cells, middle: percent of CD8+ cells among hCD45+, right: percent of LNGFR+ cells among hCD45+ cells. (b) Spleen weight for satellite animals from #7 collected at day 22 or 49 days after treatment (c) ELISA analysis of IL12 and IFN-gamma concentration in serum collected from NTD (day 26), MUC1-CAR ΔPD1-IL12, and MUC1-CAR ΔPD1-IL12 / TGFBR2KO (day 49). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)



#9 Summary and Conclusions

- Unarmored MUC1-CAR T-cells only showed antitumor activity when infused intratumorally and activity was limited to site of infusion.
- TALEN®-mediated TGFBR2 KO confers resistance to the immunosuppressive effect of TGFB1 and IL12 knock-in at PD1 locus (ΔPD1-IL12) aims at local activation-induced IL12 secretion to create a proinflammatory tumor microenvironment.
- The ΔPD1-IL12 attribute enabled antitumor control by intravenously infused MUC1 CAR T-cells.
- Upon intratumoral infusion, CAR T-cells programmed with ΔPD1-IL12 attribute show complete tumor control at 5 times lower doses than their CAR-only counterpart, despite a tumor burden at least 8 times greater.
- CAR T-cells engineered with ΔPD1-IL12 infused intratumorally not only demonstrated tumor control locally but also against a distal lesion, potentially recapitulating control of metastatic sites.
- We demonstrated that the IL12, PD1 and TGFBR2 pathways interact. When combining the ΔPD1-IL12 with TGFBR2 KO, CAR T-cells preserved their activity while mitigating potential accumulation outside the tumor and, thereby, limiting the risks of off-tumor toxicity.

Altogether, our pre-clinical data highlight the capability of multi-armored allogeneic CAR T-cells to preserve their activity despite the immunosuppressive microenvironment, while mitigating potential safety concerns and offering a potential therapeutic option for patients with TNBC.