217

Multi-armored Allogeneic MUC-1 CAR T-cells Efficiently Control Triple Negative Breast Cancer (TNBC) Tumor Growth

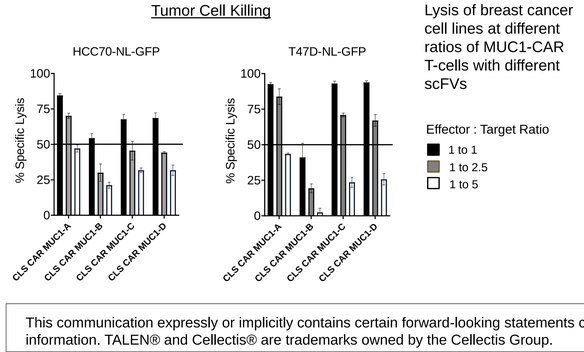
Piril Erler[1], Tomasz Kurcon[1], Jordan Skinner[1], Chantel Dixon[1], Steven Grudman[1], Ben Mumford[1], Sophie Leduc[2], Sandra Rozlan[2], Emilie Dessez[2], Shipra Das[1], Alexander Boyne[1], Alexandre Juillerat[1], Roman Galetto[2], Hana Cho[1], Julien Valton[2], Laurent Poirot[2], Beatriz Aranda-Orgilles[1]

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

#1 Background

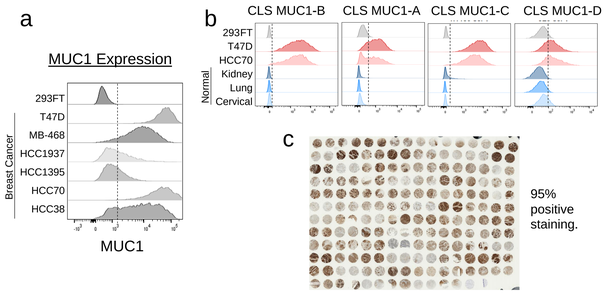
CAR T-cell therapy success in solid tumors has been limited due to lack of tumor-specific antigens, tumor heterogeneity, and immuno-inhibitory nature of tumor microenviroment (TME).To address these challenges, we engineered CAR T-cells that i) use the tumor-specific MUC-1 antigen as a discriminatory target and ii) have enhanced therapeutic properties provided by multiple attributes. We focused on TNBC due to poor prognosis and overexpression of MUC1 (~67%). Here, we describe a universal CAR T-cell therapy for TNBC that can overcome both the host immune rejection and key inhibitory signals from the TME.

(**a**) Schematic representation tumor-specific MUC1 expression (**b**) Schematic representation of TME



#3 Selected scFVs can detect tumor specific MUC1 in breast cancer cell lines and tissue samples.

(**a**) MUC1 expression in breast cancer cell lines (**b**) Binding of MUC1 scFVs to healthy primary cells and breast cancer cell lines (**c**) Representative tissue microarray IHC staining of breast cancer tumors with MUC-1 scFV protein



#4 MUC1 scFVs show dose-dependent killing of breast cancer cells *in vitro*.

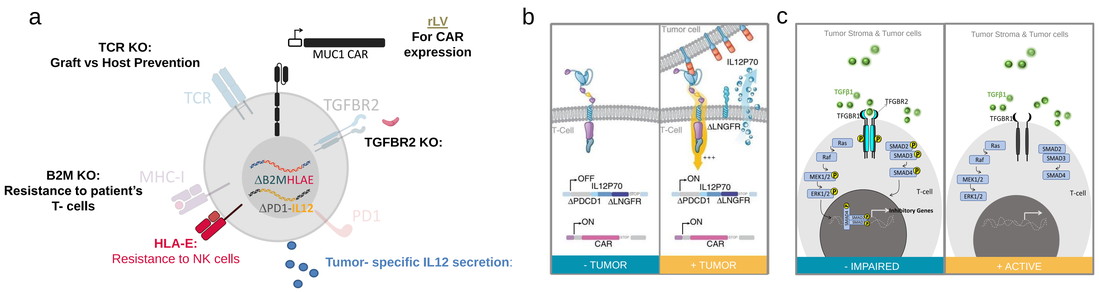
Lysis of breast cancer cell lines at different ratios of MUC1-CAR T-cells with different scFVs

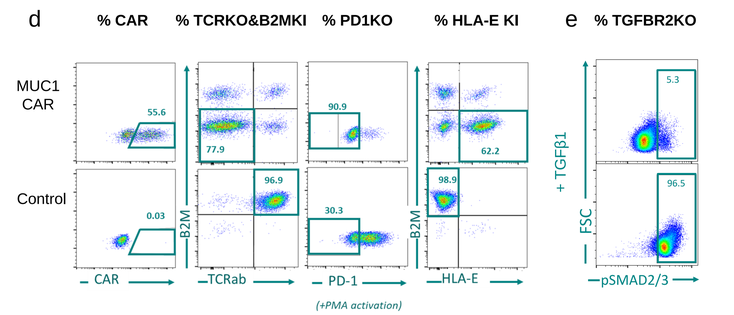
Effector : Target Ratio
- 1 to 1
- 1 to 2.5
- 1 to 5

#2 Methods : Complex Engineering

We screened several tumor-specific scFVs for MUC-1 CARs, assessing their binding and safety profiles. Then, we generated allogenic CAR T-cells by leveraging our TALEN® technology. TCR-alpha and B2M were knocked-out to prevent host-versus-graft disease, and to evade host T-cell attack. HLA-E was knocked-in at the B2M-KO site to provide resistance to host NK cell rejection. To increase activity and to overcome inhibitory signals from the TME, we introduced a PD-1 knock-out, a tumor-specific IL-12 release, and TGFBR2 knock-out. We tested these CAR T-cells *in vitro* using target specificity and cytotoxic assays, and *in vivo* by assessing tumor growth, survival, and tumor infiltration.

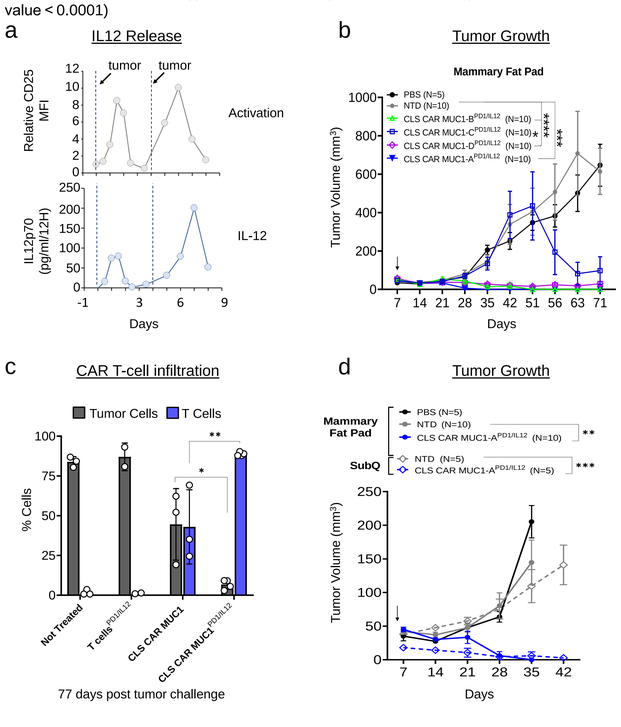
(**a**) Schematic representation of Armored MUC1-CAR (**b**) Schematic representation of inducible IL12 release under PDCD1 promoter (**c**) Schematic representation of TGFBR2 KO Pathway (**d, e**) Phenotyping of the complex engineered CAR- T cells via FACS analysis





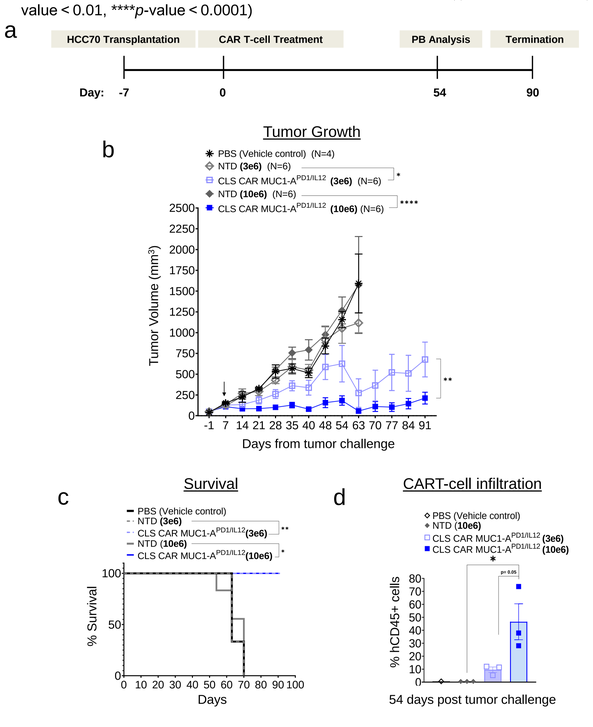
#5 Tumor-specific IL-12 release and PD1KO equipped MUC1-CARs show TNBC tumor control and T-cell infiltration.

(**a**) Secretion of IL-12 in synchronization with T-cell activation measured by CD25 expression (**b**) *In vivo* tumor growth for PBS, NTD and 4 MUC1-CARs with the PD1KO/IL12KI attribute subcutaneous model (**c**) Superior infiltration of HCC70 tumors by MUC1-CAR T-cells enhanced with PD1KO/IL12KI attribute (**d**) MUC1-CAR activity with PD1KO/IL12KI attribute in the (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, ****p-value < 0.0001)



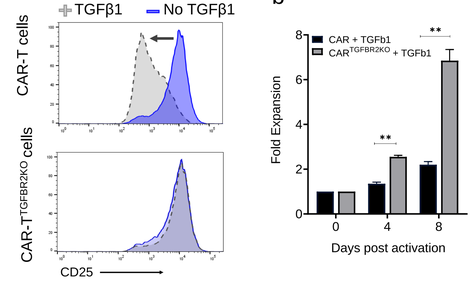
#6 Armored MUC1-CARs control tumors in a dose dependent manner and extend survival.

(a) Schematic representation of timeline of *in vivo* experiment (Similar for each experiment) (**b**) *In vivo* tumor growth for animals treated with PBS, NTD or different doses of different MUC1-CAR T-cells (**c**) Survival curve showing extended survival of animals treated with MUC-1 CAR with attributes (**d**) Dose-dependent HCC70 tumor infiltration of MUC1-CAR with PD1KO/IL12KI attribute (*p-value < 0.05, **p-value < 0.01, ****p-value < 0.0001)



#7 TGFBR2KO Attribute restores activity of CAR-T cells in a TGFβ1 rich environment.

(**a**) Histogram of impaired CD25 expression following CAR-T cell activation in the presence of TGFB1 and its restoration with TGFBR2KO attribute (**b**) Superior expansion of MUC1-CAR with TGFBR2KO attribute in the presence of TGFβ1 (**p-value < 0.01)



#8 Conclusions

- Overall, we demonstrate that MUC-1 CAR T-cells control tumor growth, while infiltrating tumors more efficiently and extending survival when enhanced with attributes catered towards the TME of TNBC tumors.
- Our data shows that MUC1-CAR T cells can be efficiently edited through our complex engineering, and we can generate allogeneic CAR T-cells to overcome key challenges of immune suppressive TME.