Breaking Barriers in Solid Tumors with SMART allogeneic CAR T-cells

Piril Erler[1], Shipra Das[1], Sonal Dharani[1], Jordan Skinner[1], Sumin Jo[1], Hana Cho[1], Laurent Poirot[2], Beatriz Aranda Orgilles[1]

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

1 Abstract

Background: Despite the remarkable success of CAR T-cell therapies treating blood cancers, effectiveness in solid tumors has encountered additional obstacles impairing the function of these innovative therapies. A main barrier is the hostile tumor microenvironment (TME), which creates an immunosuppressive milieu and restricts T-cell infiltration into the tumor. Moreover, tumor antigen diversity or low expression of CAR-targeted tumor-associated antigens (TAA) in normal tissues can cause antigen-loss or on-target off-tumor toxicity, respectively, leading to relapse and posing serious challenges for therapeutic safety.
To enhance efficacy and specificity of CAR T-cells against solid tumors, it is thus critical to incorporate additional functionalities, which in turn require tight regulation to avoid potential toxicities. Immune pathways provide an attractive variety of tightly regulated genes that can be engineered for this purpose. Here, we show different approaches to demonstrate this concept and improve CAR T-cell activity while preventing potential toxicities.

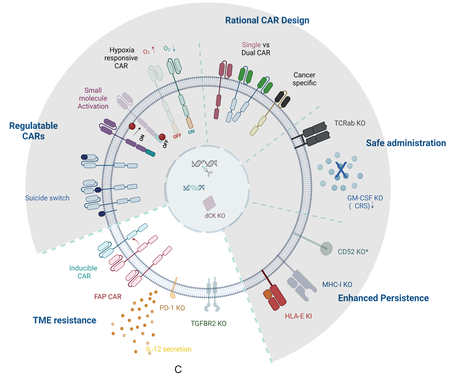
Methods: We use TALEN®-mediated gene editing to generate allogeneic CAR T-cells while repurposing PD-1 function, a key factor of the T-cell activation pathway, for different objectives. We then demonstrate the increased efficacy and reduced toxicities using diverse in vitro and in vivo methodologies.

Results: We first engineered CAR T-cells by integrating IL-12 into PD-1 regulatory elements to confine IL-12 to the TME in a CAR-activation dependent manner. Moreover, we deleted TGFBR2 to overcome TGFB1-mediated resistance in the TME. Using extensive in vitro and in vivo experiments and various routes of CAR T-cell delivery, we demonstrate that multiple engineered cells enhance proliferation and infiltration of CAR T-cells thus reducing tumor burden, enhancing survival and limiting side effects in orthotopic triple-negative breast cancer animal models.

To address the hostile TME and off-tumor toxicity, in our second strategy we designed inducible dual SMART CAR T-cells. We expressed a CAR targeting FAP, a biomarker expressed in cancer associated fibroblasts (CAFs) and linked to tumor immunosuppression. Concomitantly, we engineered CAR T-cells by integrating into PD-1 a CAR targeting Mesothelin, a well-studied TAA. This way, mesothelin CAR expression is limited to the TME when FAP-CAR is engaged. Using *in vitro* and *in vivo* techniques, we show that TME-restricted co-expression of FAP and mesothelin CAR increases anti-tumor cytotoxicity, while minimizing potential "on-target off-tumor" toxicities.

Conclusion: Overall, our data show the potential of repurposing immune pathways to create allogeneic CAR T-cells with increased activity in immunosuppressive microenvironments while minimizing potential safety issues, potentially providing a therapeutic option for patients with solid malignancies.

2 Enhancing CAR T-cells

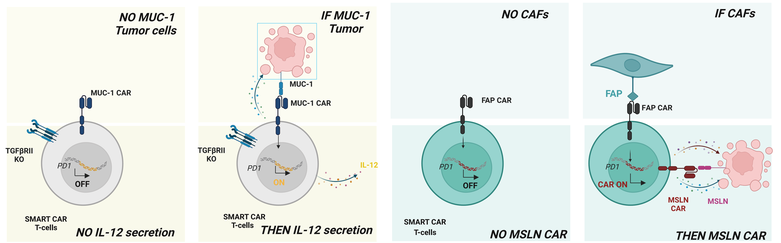


C

3 PD-1: a regulatable locus for armoring

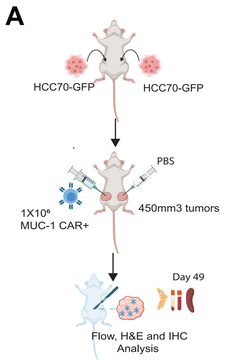
STRATEGY 1 Cytokine Induction

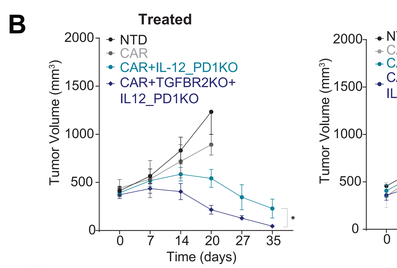
STRATEGY 2 CAR Induction

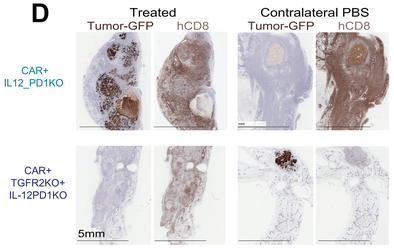


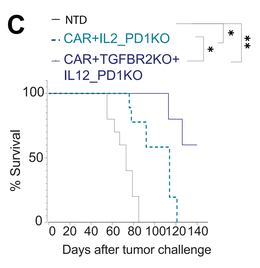
CAFs: Cancer Associated Fibroblasts

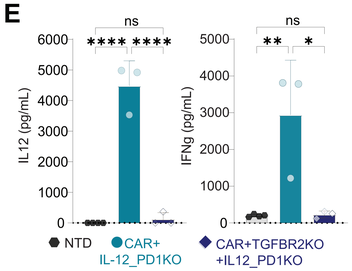
4 PD-1 regulated secretion of IL-12 enhances anti-tumor activity





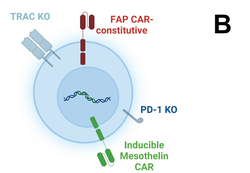


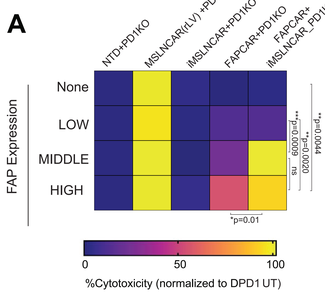


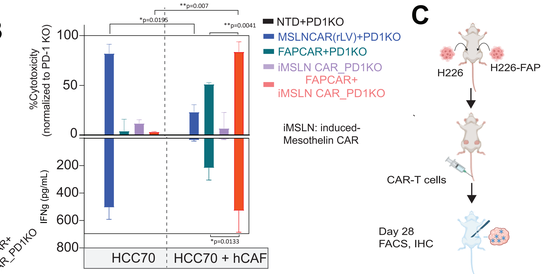


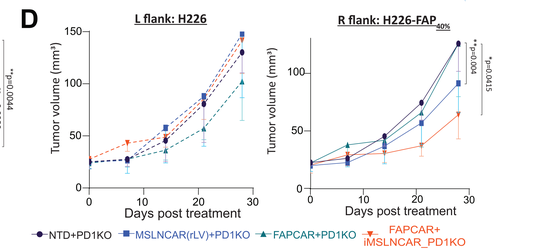
Strategy 1. Induced expression of IL-12 enhances anti-tumor activity and further deletion of TGFBR2 enhances activity while providing safety. A. Experimental overview. **B.**Tumor size over time after injecting 1x10⁶ anti-MUC1 armored CAR T-cells in only one flank. **C**. Kaplan-Meier curve indicating survival. **D**. Analysis of the mammary gland at the end of the treatment showing tumor clearance and reduced immune cell infiltration upon treatment with multi-armored CAR T-cells. **E**. IL-12 and IFNg levels in serum at day 49 after treatment.

5 PD-1 regulated expression of MSLN CAR enhances safety









Strategy 2. Induced expression of Mesothelin CAR via FAP anchoring provides cytotoxic cytoxicity and mitigates on-target off-tumor toxicities. A. Cytotoxicity activity against mesothelin-expressing cells in the presence of different levels of FAP expression. **B.** Cytotoxicity and IFNg release against tumor cells in the presence and absence of hCAFs with spheroid models. **C** *In vivo* experimental overview. **D.** *In vivo* antitumor activity in the presence aand absence of FAP expression. NTD: on-transduced; MSLNCAR: mesothelin CAR; iMSLNCAR: inducible Mesothelin CAR

6 Conclusions

Allogeneic CAR T-cells can be engineered with various functionalities to enhance their effectiveness. By integrating synthetic genes into the PD-1 locus, it is possible to achieve tight regulation of gene expression controlled by external stimuli, such as tumor recognition or interaction with elements of the tumor microenvironment.

Strategy 1 *illustrates the integration of IL-12, a pro-inflammatory cytokine, into the regulatory elements of PD-1, which is secreted upon CAR T-cell recognition of tumor antigens. This approach results in a significant increase in CAR T-cell activity in vivo. The beneficial effects are further augmented by the knockout of TGFBR2, which helps regulate CAR T-cell expansion in peripheral areas and promotes the secretion of IL-12, leading to increased levels of IFNg. These combined mechanisms, facilitated by TALEN® -mediated PD-1 knock-out, enhance CAR T-cell activity while ensuring a higher degree of safety.*

Strategy 2 *involves integrating a tumor antigen CAR, such as Mesothelin, into the PD-1 regulatory elements using TALEN®-mediated specific gene editig. Here, Mesothelin CAR expression is induced upon the interaction of a constitutively expressed FAR CAR with the tumor microenvironment. This method anchors CAR T-cells to the tumor microenvironment, thereby preventing on-target off-tumor activities while preserving and enhancing efficacy.*

These strategies have the potential to be extended to other loci regulated upon CAR T-cell activation and the integration of a diversity of synthetic genes to conquer the challenges of solid tumors and generate more efficient and safer therapeutic alternatives for patients with solid tumors.

SCAN ME

Schematics were done with biorender.com