

MULTI-ARMORED ALLOGENEIC MUC-1 CAR T-CELLS EFFICIENTLY CONTROL TRIPLE NEGATIVE BREAST CANCER TUMOR GROWTH<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0217>

Piril Erler*, Tomasz Kurcon, Jordan Skinner, Chantel Dixon, Steven Grudman, Ben Mumford, Shipra Das, Alexander Boyne, Alexandre Juillerat, Roman Galetto, Julien Valton, Hana Cho, Laurent Poirot, Beatriz Aranda-Orgilles. *Collectis, New York, NY, USA*

Background CAR T-cell therapy success in solid tumors has been limited due to their complex biology. In solid tumors, lack of tumor-specific antigens, tumor heterogeneity, and immuno-inhibitory nature of tumor microenvironment (TME) demand an optimal therapeutic window where CAR T-cells can be highly efficient while ensuring safety. 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 over-expression of MUC1 (~67%) [1]. 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.

Methods We first 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.

Results Three scFVs we prioritized showed efficient dose-dependent killing of breast cancer cells *in vitro*. Next, we used subcutaneous and orthotopic models to test CAR T-cells armored with IL-12 inducible release *in vivo*. Efficient tumor control and increased CAR-T cell infiltration extended mice survival, and notably antitumor response followed a dose-dependent pattern. Importantly, we introduced multiple edits in CAR-T cells with a high degree of efficacy, and precision using TALEN®. Functionally validating these edits, we demonstrated that TGFBR2-KO circumvents the inhibitory effects of TGFβ1, and IL-12 release follows a CAR T-cell activation pattern restricting it to the tumor site for increased safety.

Conclusions Overall, our data demonstrate that we can efficiently generate allogenic CAR T-cells and equip them through complex engineering to overcome key challenges of solid tumors. We show that MUC-1 CAR T-cells control tumor growth, while infiltrating tumors more efficiently when enhanced with attributes catered towards the TME of TNBC tumors. Altogether, these pre-clinical data suggest that enhanced MUC-1 CAR T-cells could address some of the current challenges in development of CAR-Ts for TNBC patients with unmet medical needs.

REFERENCE

1. Siroy, A., Abdul-Karim, F. W., Miedler, J., Fong, N., Fu, P., Gilmore, H., & Baar, J. MUC1 is expressed at high frequency in early-stage basal-like triple-negative breast cancer. *Human Pathology* 2013; **44**:54.

Ethics Approval All animals in this study were treated humanely and in agreement with IACUC guidelines. IACUC Protocol #:2019-05-10-CEL-02