Multiplex silencing of six molecules endows allogeneic anti-CD19 CAR T-cells with properties of enhanced cellular potency and limited rejection

A Roussel-Gervais,[1] M Mansuy,[1] B Ponsard,[1] V Blancheteau,[1] I Lanz I,[1] Y Genolet,[1] B Bourrat,[1] A Turc,[1] O Cherpin,[1] S Ilmjärv,[1] A Šakić,[1] M Alessandrini.[1]
[1] Antion Biosciences SA, Geneva, Switzerland

Introduction

Chimeric antigen receptor (CAR) T-cell therapy is an accepted form of treatment for haematological malignancies. Despite impressive clinical results, several challenges remain, including a lack of efficacy against solid tumour cancers and limited access to these treatments. To overcome this, a next generation of CAR T-cells is needed, so that they can be supplied as an off-the-shelf (allogeneic) solution, capable of durable treatment outcomes. The latter is directly linked to the persistence of the allogeneic CAR T-cells, which relies not only on the potency of these cells, but also on their ability to avoid rejection by the host immune system. We have developed a novel bimodal gene construct for simultaneous CAR expression and microRNA (miRNA) mediated gene silencing (miCAR).[1] Using this construct, our aim was to multiplex engineer anti-CD19 CAR (CAR19) T-cells with silencing of six cell surface receptors, namely the T-cell receptor (TCR), human leukocyte antigen class I (HLA-I), CD52, PD1, TIM3 and TIGIT. Silencing of first three mentioned receptors facilitates the off-the-shelf provision of allogeneic CAR T-cells with the added benefit of being able to employ strategies to limit immune rejection. Functional silencing of the inhibitory receptors (PD1, TIM3 and TIGIT) aims to enhance cellular potency.

Methods

- Primary T-cells were modified in a single vector transduction step, expanded in G-Rex® cell culture plates, and purified by depletion of residual TCR-expressing cells (Fig. 2).
- Characterization included FACS immunophenotyping, cytokine-independent outgrowth assays, functional testing against CD19-expressing tumor cells, and mixed lymphocyte reactions (MLRs).

Results

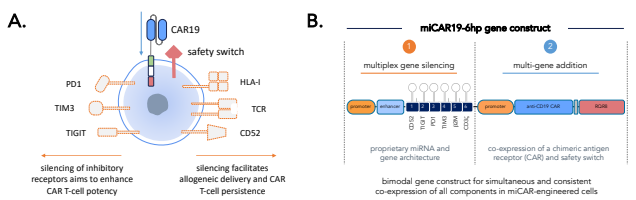

Fig 1. Engineering CAR19 T-cells with silencing of six relevant target genes. (A) Schematic of a next generation CAR T-cell with silencing of six relevant cell surface receptors. (B) Design of a miCAR19 gene construct with six miRNAs for multiplex silencing of the corresponding cell surface receptors; and a multi-gene addition cassette for expression of CAR19 and the RQR8 safety switch.

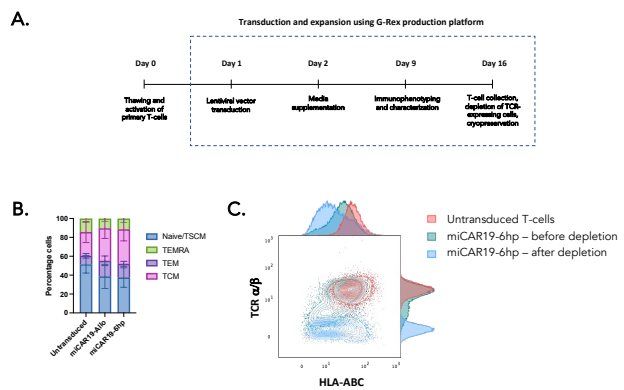

Fig 2. Manufacturing multiplex-engineered miCAR19 T-cells. (A) Schematic representation of the manufacturing protocol. (B) Post manufacturing distribution of T-cell phenotypes based on CD45RA and CD62L expression (miCAR19-Allo cells have only TCR silencing). TCM = Central memory T-cells; TEM = Effector memory T-cells; TEMRA = Effector memory T-cells re-expressing CD45RA; TSCM = Stem cell-like memory T-cells. (B) Contour plot overlay of before and after depletion of TCR-expressing cells to purify miCAR19-6hp T-cells, demonstrating the simultaneous loss of expression of TCR a/b and HLA-ABC.

Characterization of miCAR19 T-cells

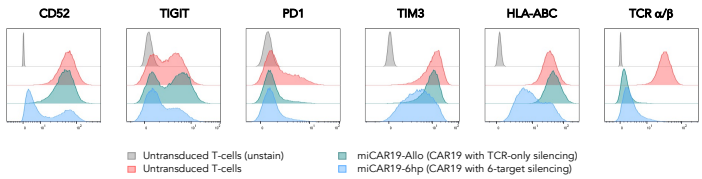

Fig 3. Multiplex gene silencing in miCAR19-6hp T-cells. Representative histograms of each of the relevant cell surface receptors demonstrate efficient silencing of all targets in miCAR19-6hp T-cells when compared to untransduced and miCAR19-Allo T-cells (CAR19 with TCR-only silencing).

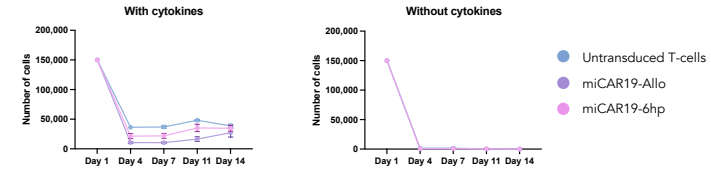

Fig 4. No cytokine-independent outgrowth of multiplex-engineered miCAR19 T-cells. Engineered T-cells were cultured either with or without IL-7 and IL-15 over a period of 14 days. While cell survival was observed in all cell populations in the presence of cytokines, no outgrowth was reported for cells in their absence.

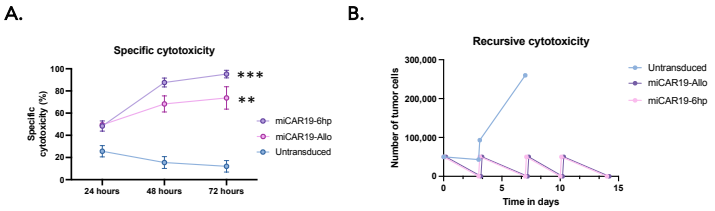

Fig 6. miCAR19-6hp T-cells are functionally active against CD19-expressing tumor cells. (A) In short-term cytotoxicity assays, miCAR19-6hp T-cells efficiently lysed CD19-expressing tumor cells, performing better than miCAR19-Allo T-cells. (B) In recursive killing assays at an E:T ratio of 3:1, both miCAR19 T-cells efficiently lysed target cells over four rounds of JeKo-1 tumor cell exposure, while untransduced T-cells were unable to control tumor growth.

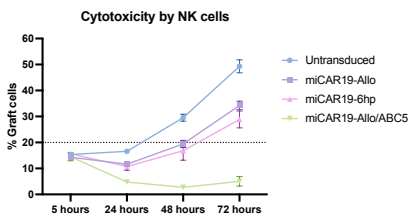

Fig 5. miCAR19-6hp avoid NK cells induced rejection. In MLRs, host NK cells (effector cells, E) were co-cultured with graft miCAR19 T-cells (target cells, T) at an E:T ratio of 5:1. Untransduced graft T-cells with full expression of HLA-I were not prone to NK cell rejection as expected, while miCAR19 T-cells with 95% silencing of HLA-I (miCAR19-Allo/ABC5) were the most sensitive to NK cell mediated cytotoxicity. Notably, graft miCAR19-6hp T-cells with 70% silencing of HLA-I and miCAR19-Allo T-cells with full expression of HLA-I were also largely protected from NK cell mediated cytotoxicity. Dotted line represents initial seeding ratio of T-cells to NK cells.

Summary and Conclusions

- Using a single gene construct, and from a single gene-engineering step, we successfully created next generation CAR T-cells with efficient silencing of six relevant cell surface receptors.
- Multiplex engineered miCAR19 T-cells maintained a favorable immunophenotypic profile, showed improved cellular potency against tumor cells, and were hypoimmunogenic when exposed to host NK cells.
- These data provide proof-of-principle for use of Antion's miCAR platform to multiplex engineer off-the-shelf CAR T-cell therapies with enhanced cellular persistence.

References: [1]Šakić et al., *Multiplex cell engineering of next generation CAR T-cells with functional silencing of six target molecules. Molecular Therapy, 31, 4S1, 2023.* Golinelli et al., Multiplex genetic engineering using microRNA (miRNA)-mediated gene silencing in mesothelin-directed CAR (M5CAR) T cells. Cytotherapy, 25, S7–S283, 2023.