Multiplex targeting of immune checkpoints with compact zinc finger repressors to improve anti-tumor activity of T cells

Poster P212

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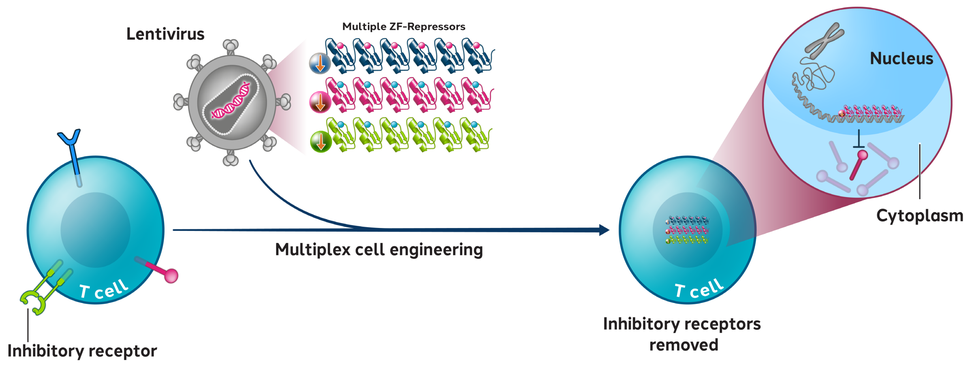
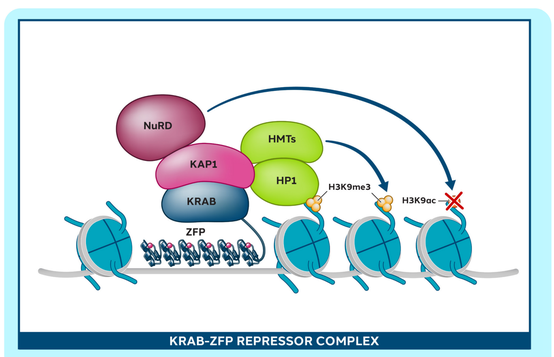
Introduction

The adoptive transfer of gene-modified T cells has recently emerged as a revolutionary new pillar in cancer therapy. Despite its remarkable success, many barriers still limit a more broadened therapeutic efficacy in solid tumors and hematological malignancies. Among these, T cell exhaustion and an immunosuppressive tumor microenvironment (TME) represent critical constraints to maintaining effector functions and persistence and achieving durable clinical potency. To overcome these significant challenges, innovative strategies and approaches are necessary to engineer more potent gene-modified T cells with improved anti-tumor activity.

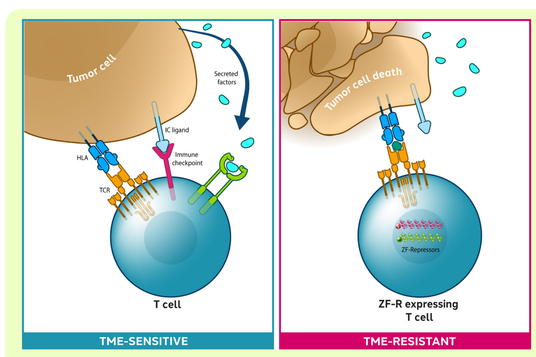
We have developed a platform capable of efficient and durable epigenetic cell engineering using ZF-Repressors (ZF-Rs). These consist of zinc finger proteins (ZFPs) fused to different KRAB repressors, capable of silencing gene expression without the need of DNA double-strand breaks. Their compact size allows multiple ZF-Rs to be combined in a single lentiviral (LV) construct to achieve concomitant multi-gene epigenetic silencing in a single transduction event.



Using this technology, primary human T cells were engineered with a bidirectional LV construct encoding one or two ZF-Rs, either with or without an anti-CD19-CAR, to knock down cell surface proteins described as negative regulators of the anti-tumor activity. These include immune checkpoints, successfully targeted by immunotherapy, and factors playing a key role in the TME and the TCR signaling. Overall, our study proposes an innovative cell engineering approach to generate gene-modified T cells with an improved tumor-eradicating potential.



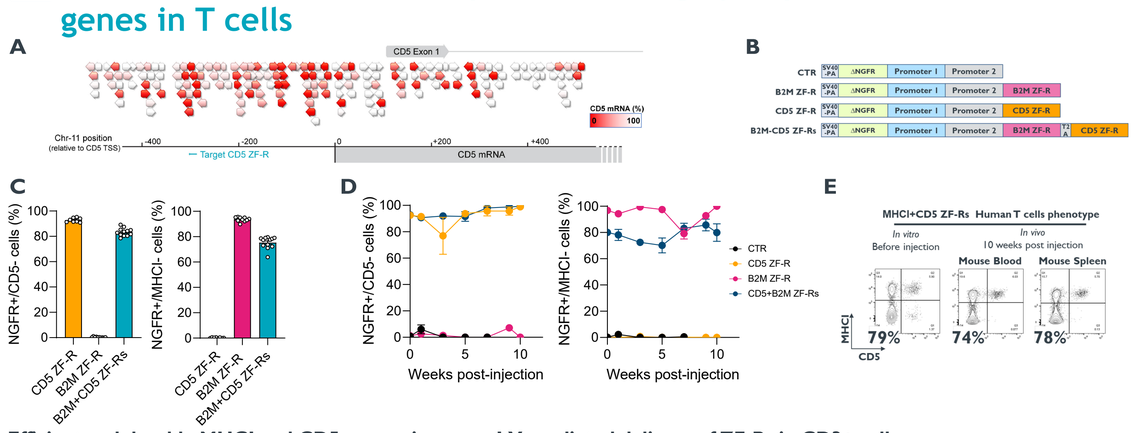
The KRAB-ZFP repressor complex and the induction of heterochromatin formation. Krüppel-associated box domain zinc finger proteins (KRAB-ZFPs) are the largest family of transcriptional regulators in higher vertebrates. They consist of a ZFP that binds to specific DNA target sequences on gene regulatory regions via their zinc fingers, and a KRAB domain required for the recruitment of the scaffold protein KAP1 and its crucial co-factor HP1. These mediate localized compaction of euchromatin to heterochromatin by interacting with the Nucleosome Remodeling Deacetylase (NuRD) complex, which catalyzes the removal of H3K9ac, and histone methyltransferases (HMTs) responsible for the addition of H3K9me3. HP1 interacts with both KAP1 and H3K9me3.



Desensitize human T cell from TME suppressive factors. Cancer cells take advantage of normal cellular homeostatic mechanisms to evade immune surveillance. Immune checkpoint (IC) pathways including PD-1, LAG3, TIM3, CTLA4 and TIGIT are often activated to inhibit anti-tumor immune response. In addition, cancer cells secrete soluble factors, that induce a pro-tumoral state of immune tolerance by deregulating the immune balance towards immuno-suppression. Drugs blocking some of these pathways have demonstrated durable clinical activities in a subset of cancer patients.
Sangamo's strategy aims at desensitizing T cells against these TME inhibitory signals by knocking-down immune checkpoint and other receptors described to dampen T cell response against tumors.

Results

1 Efficient and durable co-repression of highly expressed *B2M* and *CD5* genes in T cells



Efficient and durable MHCI and CD5 repression upon LV-mediated delivery of ZF-Rs in CD3+ cells.

(A) Schematic representation of *CD5* gene mRNA knockdown versus the CD5 ZF-R binding sites (triangles); darker colors indicate stronger repression. Selected CD5 ZF-R is highlighted in blue.

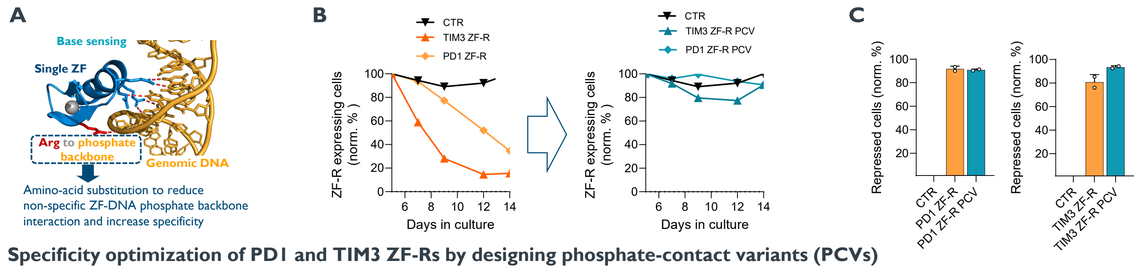
(B) Panel of LV particles delivering up to two ZF-Rs were generated to assess repression efficiency in CD3+ cells.

(C) CD5 (left) and B2M (right) repression efficiency were quantified by measuring the percentage of NGFR+/MHCI- and NGFR+/CD5- CD3+cells by flow cytometry.

(D) Durability of B2M and CD5 repression was assessed by monitoring NGFR+/MHCI- and NGFR+/CD5- CD3+ cells injected in NXG mice for 10 weeks.

(E) FACS plots showing concomitant MHC-I and CD5 repression in transduced T cells before injection (left) and 10 weeks post-injection in blood (middle) and spleen (right).

2 ZF-R specificity optimization through phosphate-contact variants design



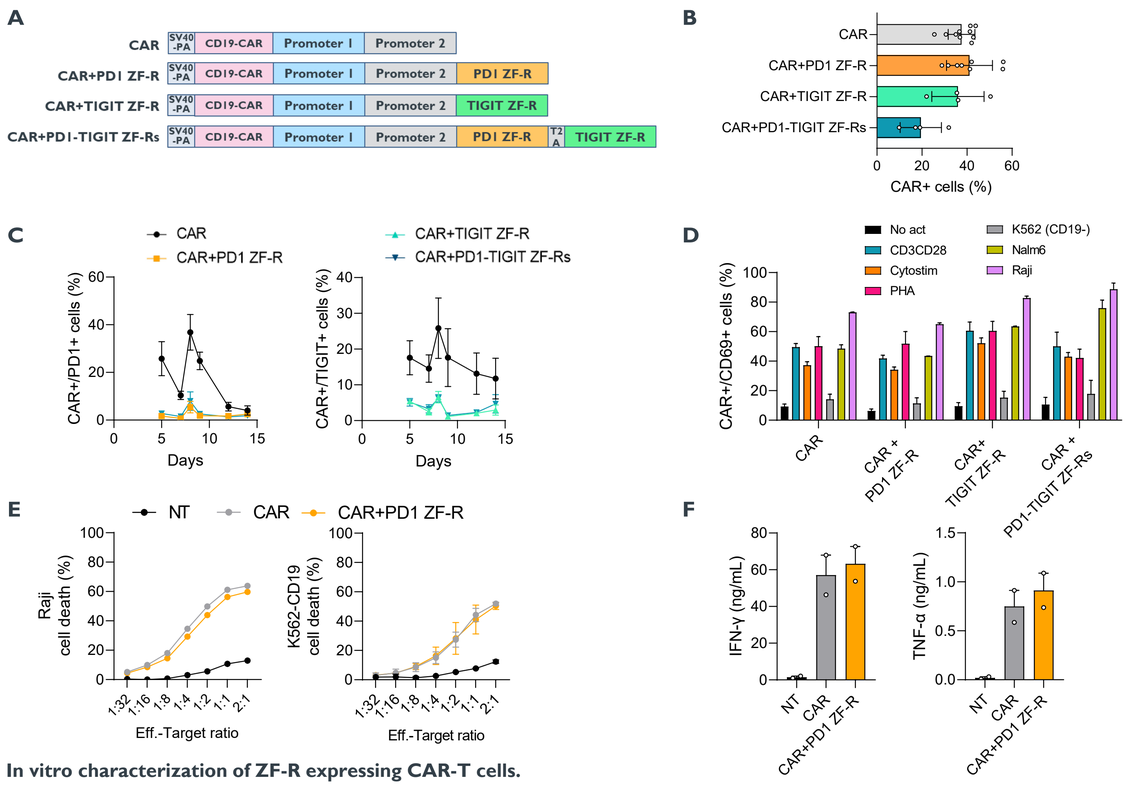
Specificity optimization of PD1 and TIM3 ZF-Rs by designing phosphate-contact variants (PCVs)

(A) Schematic of the interaction between zinc finger domain and genomic DNA[1,2].

(B) The percentage of PD1- and TIM3-ZF-R-expressing T cells decreases rapidly over time using non-optimized ZF-Rs (left). In contrast, the percentage of PD1- and TIM3-ZF-R PCV-expressing T cells is stable following introduction of PCV mutations reducing non-specific interactions of ZF-Rs with the DNA phosphate contact backbone (right).

(C) PD1 (left) and TIM3 (right) repression efficiency were quantified by measuring the percentage of PD1+ and TIM3+ transduced cells by flow cytometry.

3 Generation of fully functional ZF-R-expressing CAR-T cells



In vitro characterization of ZF-R expressing CAR-T cells.

(A) Panel of LV constructs delivering CD19-CAR and up to two ZF-Rs targeting PD1 and TIGIT.

(B) Transduction efficiency of the indicated LVs was quantified as the percentage of CAR+ cells.

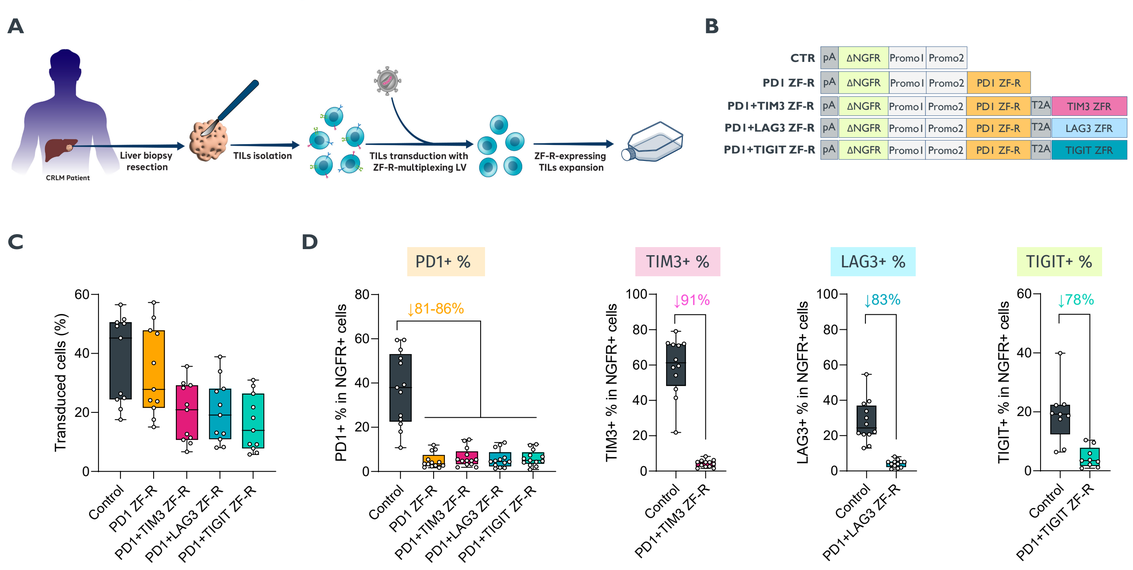
(C) Durability of PD1 and TIGIT repression was assessed in vitro by monitoring CAR+/PD1+ (left) and CAR+/TIGIT+ cells (right) from day 5 to day 14. Of note, cells were re-activated at day 7 with anti-CD3/CD28 beads.

(D) CAR-T cell activation was determined by measuring the percentage of CAR+/CD69+ cells following overnight incubation with the listed activating agents.

(E) Killing activity of CAR-T cells was evaluated by measuring the cell death of Raji (left) and K562-CD19 (right) at different effector (Eff.)-target ratio upon overnight incubation.

(F) IFN-γ and TNF-α were measured following overnight incubation of CAR-T cells with PDL1-expressing Nalm6 cells.

4 Efficient repression of immune checkpoints expression in ZF-R-expressing metastatic TILs



Generation of TILs expressing ZF-Rs targeting immune checkpoints.

(A) Schematic representation of the workflow leading to the generation of ZF-R-expressing TILs. Briefly, TILs were isolated from biopsies resected from colorectal liver metastatic (CRLM) patients following mincing, enzymatic and mechanic dissociation and ficoll separation. Next, isolated TILs were transduced and expanded in vitro for at least 2 weeks.

(B) Panel of LV constructs delivering dNGFR and up to two ZF-Rs targeting PD1, LAG3, and TIGIT.

(C) Transduction efficiency of the indicated LVs was quantified as the percentage of NGFR+ cells.

(D) PD1, TIM3, LAG3 and TIGIT expression were quantified in transduced TILs (NGFR+) by flow cytometry, respectively.

Conclusion

- A bidirectional LV architecture was designed to deliver multiple ZF-Rs in T cells with a single LV particle.
- As a proof of concept, ZF-R-mediated co-repression of constitutively and highly expressed *B2M* and *CD5* genes resulted in efficient and long-term MHC-I and CD5 protein expression knockdown *in vivo*.
- Introduction of PCV mutations to abrogate some interactions between ZF-R and the DNA phosphate backbone strongly improved the specificity of ZF-Rs without compromising on-target repression efficiency.
- PD1 and TIGIT ZF-Rs did not affect in vitro functionality of gene-modified CAR-T cells, warranting further evaluation of their anti-tumor activity in animal models.
- TILs from CRLM patients were efficiently engineered with ZF-Rs targeting immune checkpoints.
- Multiplexed, epigenetic cell engineering using ZF-R-expressing LVs offers an innovative approach to the development of highly customized T-cell therapies with improved therapeutic potential.

References

1. Miller, J. C. et al. Enhancing gene editing specificity by attenuating DNA cleavage kinetics. Nat. Biotechnol. 37, 945–952 (2019)
2. Paschon, D.E. et al. Diversifying the structure of zinc finger nucleases for high-precision genome editing. Nat. Commun. 10, 1133 (2019)

Acknowledgments

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Disclosures

DM, MD, YZ, ST, CD, CJ, GS, IM, AM, JF, AR, MdlR and DF are Sangamo Therapeutics employees. Sangamo Therapeutics owns patents covering the technology described in this poster. PS, MF and IKN received funding from Sangamo Therapeutics for the experiments.

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