Engineering Potent CAR T-Cell Therapies by Controlling T-Cell Activation Signaling Parameters Using the Stim-R™ Technology, a Programmable Synthetic Cell-Signaling Platform

Abstract No. 252

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Background

- Chimeric antigen receptor (CAR) T-cell therapy has produced profound results in certain hematologic malignancies but has been less successful in the treatment of solid tumors
- Studies suggest that T-cell exhaustion plays a role in limiting the ability of CAR T cells to eradicate solid tumors[1]
- T-cell activation is a formative event that directs cell fate, function, and durability of mature T cells
- During expansion of T cells to generate a CAR T-cell product, parameters related to activation can affect the phenotypic and functional quality of the resulting cells

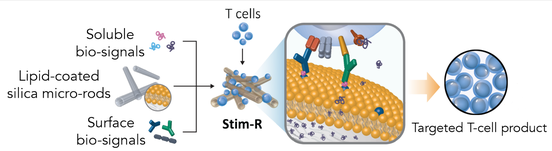
Hypothesis

- Controlled delivery of activation molecules during T-cell production can generate CAR T cells with greater potency

Methods

- To control signaling during T-cell activation, we employed our Stim-R epigenetic reprogramming technology, a synthetic cell mimic that mediates precise signal-molecule presentation (**Figure 1**)

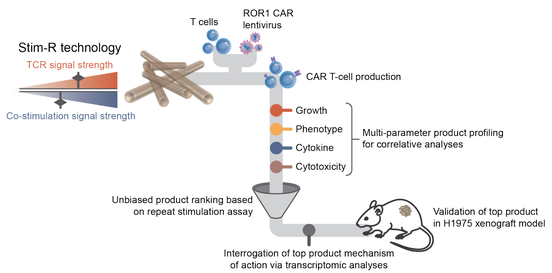
Figure 1: The Stim-R technology is a programmable cell-signaling platform



The Stim-R technology comprises biodegradable lipid-coated silica micro-rods that can present multiple signals in precise densities and stoichiometries. Soluble signals are released in a controlled manner while surface-anchored signals are presented on a synthetic lipid membrane, mimicking physiologic presentation.

- We designed and fabricated Stim-R technology formulations to present T-cell activating signals engaging CD3 and CD28 at different densities and stoichiometries
- Utilizing these formulation variants, we generated arrays of diverse ROR1-targeted CAR T-cell products, which we profiled phenotypically and functionally
- Based on these metrics, we compared Stim-R–generated CAR T cells to CAR T cells generated using a conventional stimulation reagent to identify lead formulations showing superior *in vitro* function (**Figure 2**)
- We also evaluated the *in vivo* efficacy of lead Stim-R–generated CAR T cells

Figure 2: An unbiased screening method using the Stim-R technology to optimize TCR and co-stimulation signal strength to produce potent CAR T-cell products



The Stim-R technology was used to independently tune TCR and co-stimulation signal strength during T-cell activation in order to generate an array of CAR T-cell products for profiling. Stim-R CAR T-cell products were ranked based on performance in an *in vitro* repeated antigen-stimulation assay and the top-performing Stim-R product was benchmarked *in vitro* and *in vivo* against CAR T cells generated using TransAct™, a commercially available T-cell stimulation reagent currently used for clinical CAR T-cell production ("Benchmark").

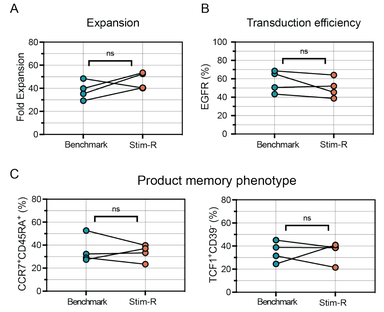
Results

Key Findings

The Stim-R epigenetic reprogramming technology generates potent ROR1-targeted CAR T-cell products with:

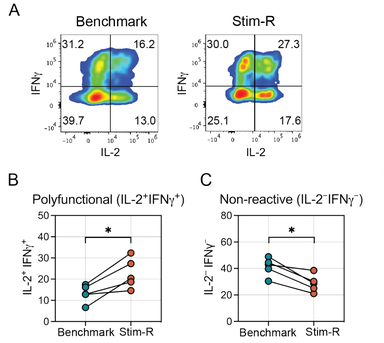
- Increased polyfunctionality
- Enhanced cytotoxicity and proliferation *in vitro*
- Persistence of a unique cell population enriched in both stemness and effector-associated gene signatures following repeated exposure to tumor cell lines *in vitro*
- Higher peak CAR T-cell number and prolonged CAR T-cell persistence *in vivo*
- Improved tumor control *in vivo*

Figure 3: Stim-R CAR T cells showed production characteristics comparable to CAR T cells generated using TransAct™, a conventional T-cell stimulation reagent ("Benchmark")



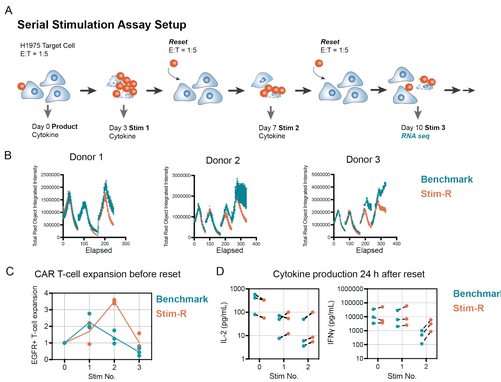
ROR1-targeted CAR T cells produced using the Stim-R technology or Benchmark showed comparable expansion (A), transduction efficiency (B), and frequency of stem-like cells (C). Data in (A–C) represent the same 4 independent donors. Data in (A-C) were analyzed using a Student's *t*-test. ns, not significant.

Figure 4: Stim-R CAR T cells exhibited increased polyfunctionality in response to acute ROR1+ target-cell stimulation



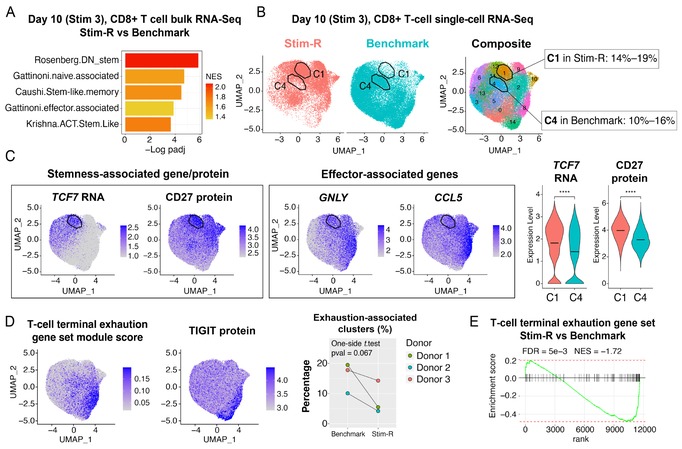
Stim-R CAR T cells and Benchmark CAR T cells were each co-cultured with a ROR1-expressing cell line for 5 hours. Subsequently, the cells were analyzed for IL-2 and IFNγ expression using flow cytometry (A). Stim-R CAR T cells showed higher population of polyfunctional cells, which express both IL-2 and IFNγ (B), and lower population of non-reactive cells, which express neither IL-2 nor IFNγ (C). Data in (B–C) represent the same 4 independent donors. Data in (B–C) were analyzed using a Student's *t*-test; *P<0.05.

Figure 5: Stim-R CAR T cells showed enhanced cytotoxicity, expansion, and cytokine production in response to repeated ROR1+ target-cell stimulation



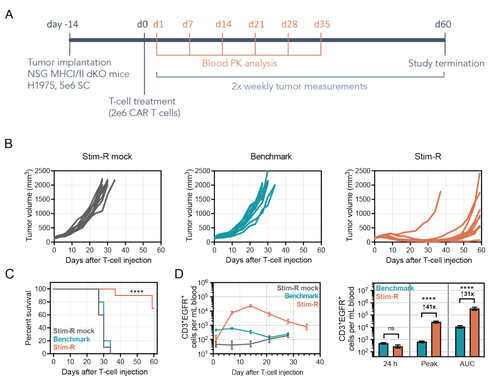
(A) Stim-R and Benchmark CAR T cells (red cells) were exposed to repeated antigen stimulation using the ROR1+ H1975 cell line (blue cells). (B) Stim-R CAR T cells showed superior target clearance compared to Benchmark CAR T cells. Target cell density was measured as total intensity of target cell fluorescence by IncuCyte. (C) Stim-R CAR T cells showed higher proliferation in response to target stimulation. (D) Stim-R CAR T cells showed a trend of higher IL-2 and IFNγ production after repeated stimulation. Data in (B) represent the mean of 2 technical replicates for each donor. Data in (C) represent unique donors (dots) with the mean of the donors superimposed (lines). Data in (D) represent unique donors.

Figure 6: Transcriptomic analysis revealed that Stim-R CAR T cells retained a unique subset of stem-like cells with effector-associated gene signatures and displayed down-regulation of exhaustion-associated gene sets compared to conventional CAR T cells following repeated antigen stimulation



Single-cell RNA-Seq and bulk RNA-Seq were performed on Stim-R and Benchmark CAR T cells collected on day 10 of a repeated antigen stimulation assay (Figure 5A) from 3 donors. (A) Stim-R CD8+ T cells displayed up-regulation of stemness-associated and effector-associated gene sets compared to Benchmark CD8+ T cells in bulk RNA-Seq gene set enrichment analysis. (B) Stim-R and Benchmark CD8+ T cells are separated on UMAP plot generated in single-cell RNA-Seq analysis. Putative stem-like clusters identified in Stim-R (C1) and Benchmark (C4), respectively, are indicated, with proportions shown in boxes. (C) C1 in Stim-R (highlighted) exhibited high expression of stemness-associated gene marker *TCF7* and protein marker CD27, as well as positivity for effector-associated genes *GNLY* and *CCL5* (center panels). C1 in Stim-R showed significantly higher *TCF7* RNA and CD27 protein expression compared to C4 in Benchmark (right panel). (D) Clusters C8 and C14 exhibited enriched module score of TTE gene set[2] and high TIGIT protein expression. The proportion of C8+14 decreased in Stim-R CD8+ T cells compared to Benchmark CD8+ T cells. (E) Stim-R CD8+ T cells displayed down-regulation of TTE gene set[2] compared to Benchmark CD8+ T cells in bulk RNA-Seq gene set enrichment analysis. ****P<1×10^{-4} by Wilcoxon test.

Figure 7: Stim-R CAR T cells exhibited higher peak T-cell numbers in the blood, prolonged persistence, and improved tumor control *in vivo*



(A) *In vivo* study design: Stim-R and Benchmark CAR T cells were evaluated in the H1975 xenograft solid tumor model in mice. Stim-R CAR T cells showed improved tumor control (B) and overall survival (C) compared to mock-transduced Stim-R T cells (Stim-R mock) and Benchmark CAR T cells. Data in (B) represent individual tumor growth curves (n=10 mice per condition). (D) CAR T cells were measured in the blood over time. Despite a comparable number of CAR T cells detected in the blood 24 hours post-injection, Stim-R CAR T cells showed higher peak CAR T-cell numbers, prolonged persistence, and higher total CAR T cells detected over the course of the study (assessed by calculating AUC) compared to Benchmark CAR T cells. Data represent mean ± SE. (n=10 mice per condition). ****P<1×10^{-4} by Mantel–Cox test (C) or by one-way ANOVA, followed by Tukey's HSD post hoc test for each metric (D; only Benchmark and Stim-R CAR T-cell conditions shown); ns, not significant.

Conclusions

- **Optimizing signal presentation during T-cell activation using Stim-R epigenetic reprogramming technology enabled the production of potent ROR1-targeted CAR T cells with improved polyfunctionality, persistence, and anti-tumor activity, attributes associated with improved outcomes with other T-cell therapies**
- **Stim-R CAR T cells retained a stem-like subpopulation with effector-associated gene signatures and showed reduced exhaustion following repeated antigen stimulation**
- **Our results suggest that enhancement of T-cell products with Stim-R technology may improve therapeutic benefit against solid tumors**

Abbreviations

AUC, area under the curve; CAR, chimeric antigen receptor; CCL5, C-C motif chemokine ligand 5; CCR7, C-C motif chemokine receptor 7; CD, cluster of differentiation; CD45RA, CD45 200—245 kDa isoform; E, effector; EGFR, epidermal growth factor receptor; GNLY, granulysin; IFNγ, interferon gamma; IL-2, interleukin 2; MHC, major histocompatibility complex; NES, normalized enrichment score; NSG, NOD scid gamma; PK, pharmacokinetics; RFU, relative fluorescence units; RNA-Seq, RNA-sequencing; ROR1, receptor tyrosine kinase-like orphan receptor 1; SE, standard error of the mean; T, target; TCF1/7, transcription factor 1 protein; TCR, T-cell receptor; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TTE, T-cell terminal exhaustion.

References

1. Fraietta JA, et al. *Nat Med*. 2018;24(5):563-571. 2. Oliveira G, et al. *Nature*. 2021;596(7870):119-125.

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