In Vivo Pooled Screening Platform for the Discovery of Optimized Chimeric Antigen Receptor (CAR) Design in T cells

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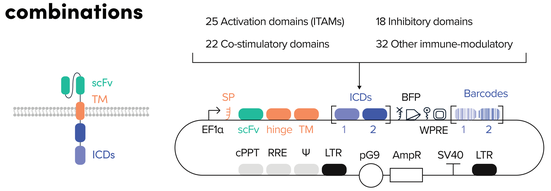
Background

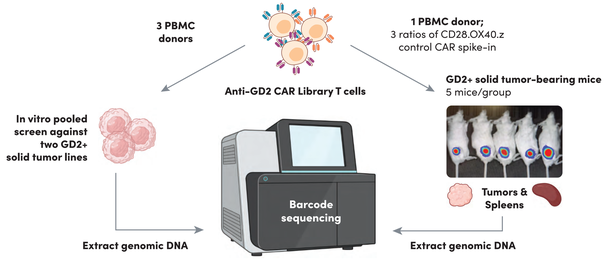
While Chimeric Antigen Receptor expressing T cells (CAR-T cells) have shown high response rates against hematological cancers in the clinic, they have shown limited efficacy against solid tumors due to lack of persistence and immunosuppression in the tumor microenvironment (TME). To address the TME, recent efforts have focused on discovering novel intracellular signaling domains (ICDs) with enhanced anti-tumor efficacy at scale. However, in vitro screens are inherently limited in mimicking the TME and other physiologically relevant aspects of adoptive cell therapy (ACT).

Here, we developed an in vivo pooled library screen approach alongside our existing in vitro platform to identify potent CAR designs in a CHLA-20 neuroblastoma xenograft model. We cloned a DNA-barcoded 10,000-member CAR ICD library into an anti-GD2 CAR backbone. GD2 CAR library-expressing primary T cells were injected into tumor-bearing mice at >100x library coverage, and spleens and tumors were collected 2 weeks post-ACT. Sequencing genomic DNA quantified the abundance of barcodes linked to each CAR ICD combination. We hypothesized that any remaining CAR-T cells at the tumor site and spleen would represent T cells with long-term persistence elicited by antigen-specific CAR ICD signaling. 103 novel CAR constructs were synthesized based on barcode enrichment from both in vitro and in vivo pooled screens, and we validated their anti-tumor function in a serial tumor rechallenge assay.

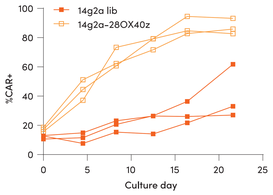
Ginkgo CAR library has 10,000 novel ICD combinations

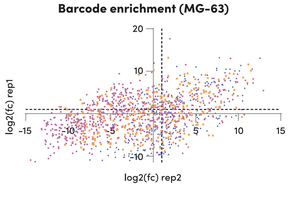


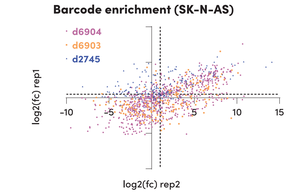
In vitro/In vivo screen schematics



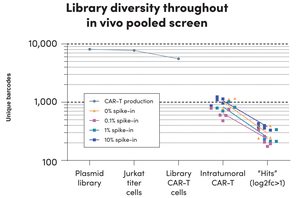
In vitro pooled screen

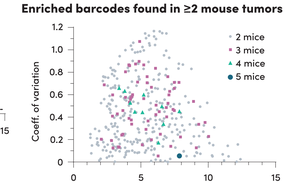




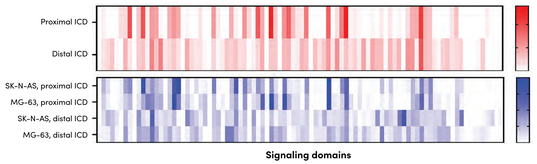


In vivo pooled screen

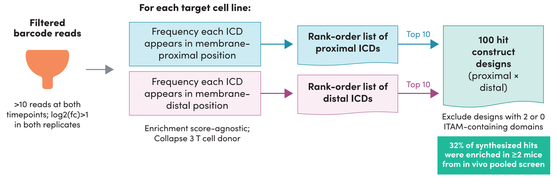




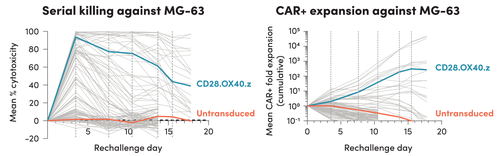
Relative frequency of ICD appearance among enriched barcodes from in vitro and in vivo pooled screens

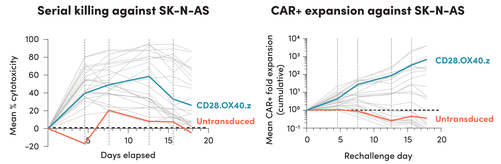


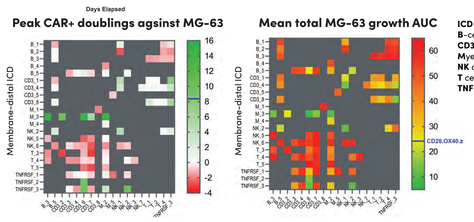
Methodology for selecting in vitro pooled screening hits



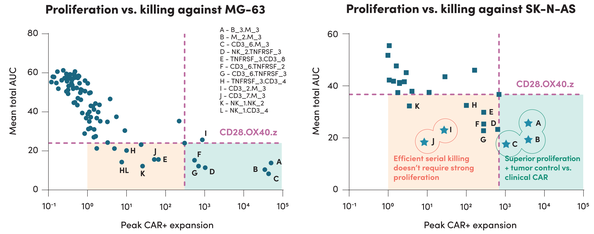
Many novel CARs perform better than clinical anti-GD2 CAR design against two GD2+ tumor cell lines with differing antigen densities

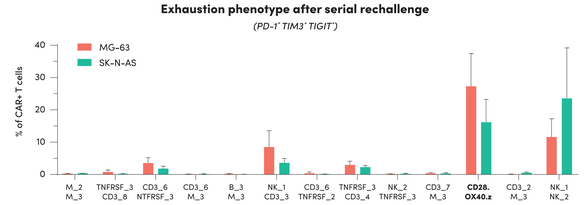






Many novel CARs exhibit better long-term tumor control than clinical CAR, despite less robust proliferation





Conclusions

Here we have demonstrated that both in vitro and in vivo pooled screening can discover novel ICD combinations with superior function against solid tumors compared to current clinical CAR designs. In particular, in vivo pooled screening allowed for high coverage of library diversity, and hundreds of unique enriched barcodes were recovered from digested mouse tumors. Many barcodes were consistently and highly enriched across multiple replicate mice, increasing confidence in the veracity of these hits. To overcome high donor-to-donor variability and mitigate false positives in our in vitro pooled screen, we employed a novel approach wherein hit designs were generated based on frequency of ICD appearance among enriched barcodes.

Small-scale arrayed screening identified numerous novel CAR designs with superior proliferation, cytotoxicity, and phenotype after nearly three weeks of chronic antigen stimulation. ICD identities among the best-performing designs indicate rules for superior CAR architecture; namely, membrane-proximal placement of ITAM-containing domains and inclusion of non-T cell-derived immune signaling motifs. Further development of an in vivo screening platform in humanized mice utilizing multiple PBMC donors could be a promising next step to discovering cell therapy products with strong translational potential.