183. Nonviral Generation of Genome-Edited CAR T Cells

(Wisconsin Alumni Research Foundation)

5TH KDDF GLOBAL C&D TECH FAIR

Asset Overview

Product Type	Cell therapy
Disease Area	Oncology
Indication	Cancer
Current Stage	Lead Optimization
Target	Solid tumors
MoA	Non-viral CAR T cells target the TRAC locus to alter basal signaling
Brief Description	• Chimeric antigen receptor (CAR) T cells have demonstrated high clinical response rates against hematological malignancies (e.g., CD19+ cancers) but have shown limited activity in patients with solid tumors. • Recent work showed that precise insertion of a CAR at a defined locus improves treatment outcomes in the context of a CD19 CAR; however, it is unclear if such a strategy could also affect outcomes in solid tumors. Furthermore, CAR manufacturing generally relies on viral vectors for gene delivery, which comprise a complex and resource-intensive part of the manufacturing supply chain. • Anti-GD2 CAR T cells were generated using CRISPR/Cas9 within 9 days using recombinant Cas9 protein and nucleic acids, without any viral vectors. The CAR was specifically targeted to the T cell receptor alpha constant gene (TRAC). T cell products were characterized at the level of the genome, transcriptome, proteome, and secretome using CHANGE-seq, targeted next-generation sequencing, scRNA-seq, spectral cytometry, and ELISA assays, respectively. Functionality was evaluated in vivo in an NSG™ xenograft neuroblastoma model.
Intellectual Property	US20220042048A1
Publication	Production and characterization of virus-free, CRISPR-CAR T cells capable of inducing solid tumor regression. J Immunother Cancer (2022)
Inventors	Krishanu Saah, Christian Matthew Capitini, Katherine Paige Mueller, Nicole Jenine Piscopo, Amritava Das, Matthew Hull Forsberg, Louise Armie Saraspe

Highlights

- Eliminates the need for viral incorporation of the CAR construct into T cells
- May be used to generate CAR T cells active against both hematological malignancies and solid tumors
- Reduces the risk of incorrect insertion and may improve success rates of CAR T cells (through disruption of TRAC and production of more active CAR T cells).

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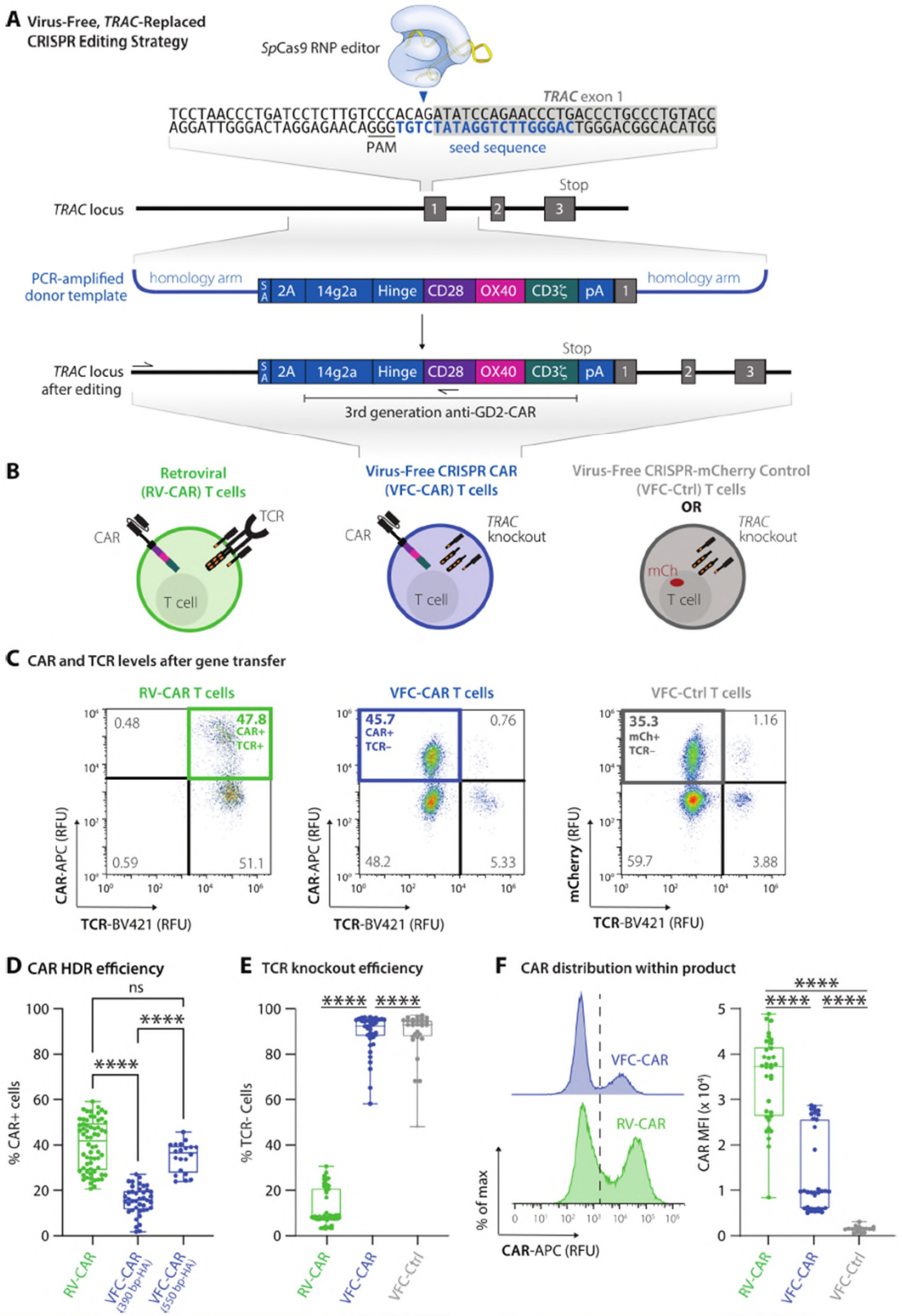
Key Data

VFC-CAR T cells are efficiently generated in one step by replacing the T cell receptor with the CAR



To be continued

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(A) Schematic showing the CAR genetic construct and virus-free strategy to insert the CAR into the first exon (gray box) of the human *TRAC* gene. No viral components are necessary, and the CRISPR-Cas9 ribonucleoprotein is delivered transiently via electroporation. The seed sequence of the gRNA is in blue and the protospacer adjacent motif (PAM) for SpCas9 is underlined. 14g2a: single chain variable fragment clone targeting GD2; 2A: self-cleaving peptide, pA: rabbit ß-globin polyA terminator. Arrows indicate positions of primers for in-out PCR assay shown in figure 2. (B) Schematic of T cell products used in this study with receptors and expressed transgenes. VFC-CAR T cell product generated by electroporation. RV-CAR, donor-matched CAR T cell product generated by retroviral transduction with the same third generation anti-GD2 CAR shown in A; VFC-Ctrl, donor-matched control T cell product manufactured as in A but with an mCherry fluorescent protein instead of a CAR. (C) Representative density flow cytometry plots for transgene and TCR surface protein levels on the manufactured cell products. Y-axis shows CAR or mCherry levels and x-axis shows TCR levels on day 7 post-isolation (day 5 post-electroporation for VFC-CAR and VFC-Ctrl, and day 4 post-transfection for control RV-CAR). Thick colored boxes delineate cell populations selected for downstream analysis. (D) Boxplots show the percentage of CAR positive cells from gene editing for VFC-CAR cells and from retroviral transduction for RV-CAR cells in each sample. The first VFC-CAR product featured homology arms (HA) of 383 (left) and 391 (right) bp, respectively. The homology arms on the second VFC-CAR product were extended to 588 (left) and 499 (right) bp, respectively. (E) Boxplots show the percentage of TCR negative cells from gene editing in VFC-CAR cells and in RV-CAR cells. RV-CAR TCR negativity likely results from endogenous repression of the TCR. (F) Mean fluorescence intensity (MFI) values for the CAR expression levels with associated histograms. Boxplots show the percentage of CAR positive cells in each sample.
****p≤0.0001. CAR, chimeric antigen receptor; ns, not significant; SA, splice acceptor.

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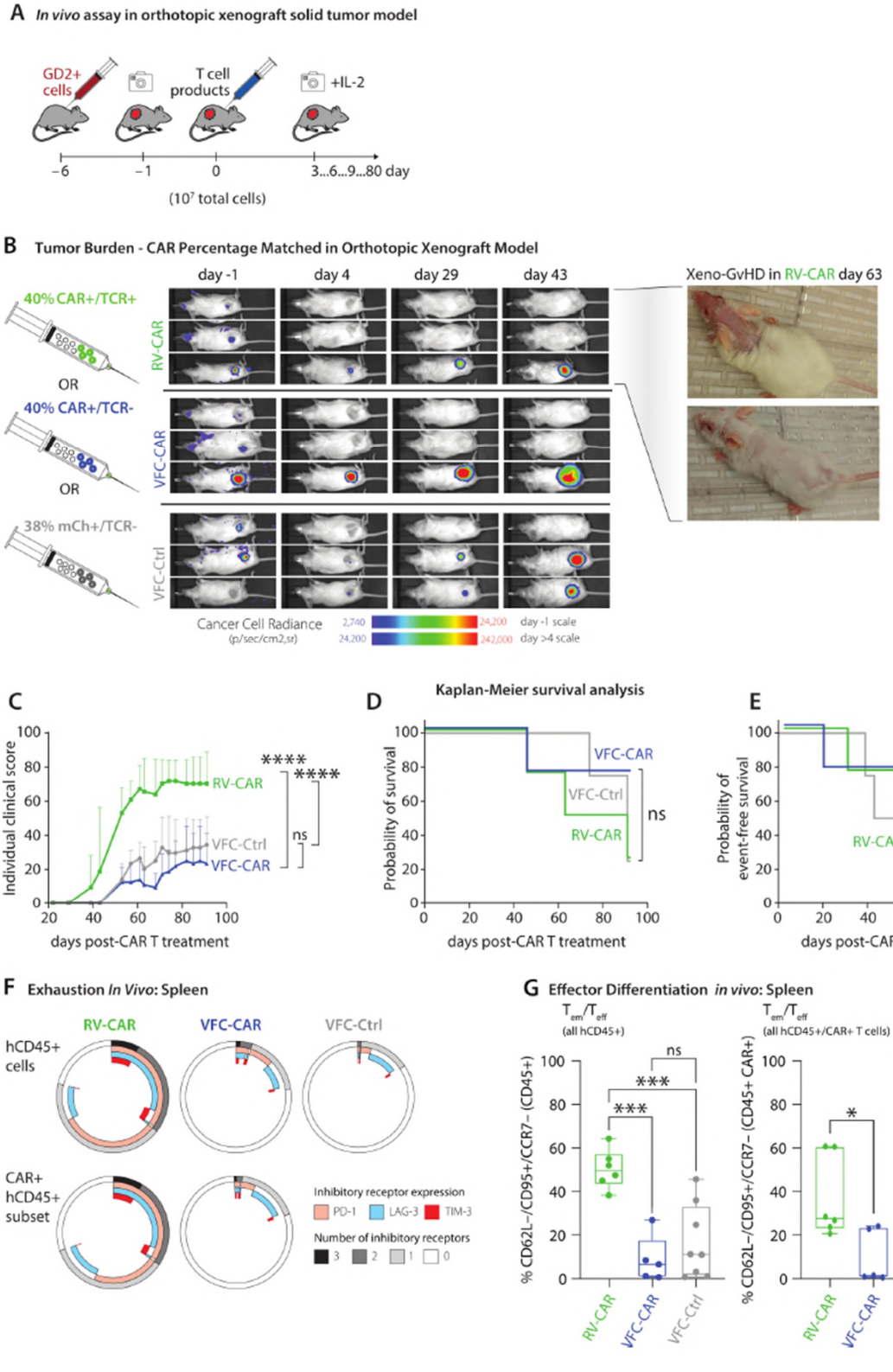
Virus-free CAR T cells exhibit in vivo activity against GD2+ solid tumors with high event-free survival and low exhaustion.



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(A, left) representative IVIS images of NSG™ mice with CHLA20 tumors that were treated with either 10 million VFC-CAR, RV-CAR, or VFC-Ctrl T cells. VFC-CAR and RV-CAR products were 40% CAR-positive for a total dose of 4 million CAR+ cells per mouse. VFC-Ctrl products were 38% mCherry-positive for a total dose of 3.8 million transgene+ cells per mouse.
GD2+ solid tumors were established in the side flank of each mouse as detected by IVIS imaging at day −1. At day 0, three different CAR T products as shown below were infused into the tail vein. (A, right) pictures of RV-CAR T-treated mice showing xeno-GvHD symptoms from the intact TCR function within the RV-CAR T cells. none of the mice infused with VFC products displayed signs of xeno-GvHD. (C) Individual adverse clinical score of each mouse treated. Higher score indicates more adverse symptoms observed in the mice, such as elevated weight loss, hunched posture, ruffled fur, scaly or flaky skin, and decreased activity. (D) Kaplan-Meier curve for total probability of survival. VFC-CAR (blue) N=4; RV-CAR (green) N=4; VFC-Ctrl (gray) N=4. (E) Kaplan-Meier curve for probability of event-free survival, defined as the absence of a palpable tumor or development of an individual clinical score of 4 or above (E) Donut plots show expression of exhaustion-associated markers detected within T cells collected from mouse spleens. RV-CAR, N=6; VFC-CAR, N=7, VFC-Ctrl, N=6. (F) T cell differentiation immunophenotypes detected within mouse spleens. RV-CAR T cells showed significantly higher proportions of more differentiated effector memory (Tem) and terminal effector (teff) T cells relative to VFC T cells. CAR, chimeric antigen receptor; IVIS, *in vivo* imaging system; RV, γ-retroviral; VFC, virus-free CRISPR.