Increasing T Cell Versatility with SUPRA CARs

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Chimeric antigen receptor (CAR) T cells offer a promising treatment option for advanced cancers resistant to standard therapy. Here, Cho et al. report a split-CAR design that enables the engineering of multi-feature CAR-T cells, aiming to address current challenges limiting the safety and efficacy of CAR-T cells for cancer treatment.

The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) that target tumor antigens has offered hopes to patients with refractory cancers that otherwise lack effective treatment options. In August 2017, CAR-T cells targeting CD19, a pan-B cell marker found on the majority of malignant B cells, became the first genetically modified cell product to gain FDA approval as cancer therapy. Despite the remarkable clinical efficacy of CAR-T cell therapy for B cell malignancies, a number of obstacles have prevented its immediate application to other cancer types. In particular, challenges such as imperfect tumor-targeting specificity, neurotoxicity, and severe cytokine release syndrome triggered by T cell infusion have raised significant safety concerns (Brudno and Kochenderfer, 2016). Furthermore, antigen loss and inherent tumor heterogeneity provide escape mechanisms for tumor cells faced with conventional, single-input CAR-T cells (Grada et al., 2013; Zah et al., 2016).

To address these challenges, numerous strategies have been developed to functionalize CAR-T cells, including the development of multi-input CARs capable of Boolean-logic signal integration (e.g., OR gate, AND gate, and AND-NOT gate), suicide systems that enable the elimination of engineered T cells, and inducible gene expression systems that can spatially and/or temporally control the availability of CAR signaling (Chang and Chen, 2017). Most engineered solutions reported to date support individual features, but seamless integration of multiple design features will likely be necessary for a fully optimized therapeutic strategy.

In this issue of *Cell*, Cho et al. report an approach to engineer CAR-T cells that enable the generation of "feature-rich" T cells (Cho et al., 2018). Instead of expressing CARs as full-length fusion proteins that include ligand-binding, transmembrane, and signaling domains, the authors devised a two-component, split-CAR system comprising "zipCAR" and "zipFv" fragments (Figure 1). The zipCAR contains intracellular signaling domains connected via a transmembrane segment to an extracellular leucine zipper. The zipFv contains a ligand-binding scFv domain fused to a second leucine zipper. A functional CAR is reconstituted when zipFv proteins are added to engineered T cells that express zipCARs with matching leucine zippers. Conversely, CAR reconstitution can be inhibited by the addition of a competing zipFv that can dimerize with the first zipFv (Figure 1).

This system, termed the split, universal, and programmable (SUPRA) CAR system, enables facile combination of desirable CAR features. For example, OR-gate signal integration can be achieved by adding two zipFv sequences containing the same leucine zipper but different antigen-binding domains. Similarly, "A-and-not-B" signal integration can be achieved by co-administering an antigen-A-specific zipFv that binds the zipCAR plus a competing, antigen-B-specific zipFv that can dimerize with the A-specific zipFv to prevent CAR reconstitution (Figure 1). The split nature of the SUPRA CAR also enables simultaneous calibration of multiple parameters that can influence CAR signaling. Cho et al. demonstrate that adjusting zipCAR expression levels, leucine zipper affinity, and zipFv concentration can all influence the functional output of SUPRA CAR-T cells.

The antigen specificity and binding affinity of the CAR is dictated by the zipFv, which is externally added. Therefore, in principle, one could manufacture a generic zipCAR-T cell product whose antigen specificity can be altered throughout the course of treatment based on patient response. This unique feature could be a powerful tool against highly heterogeneous or genetically unstable cancer types that are resistant to conventional T cell therapy with fixed antigen specificity (Klebanoff et al., 2016; Migliorini et al., 2018), thereby reducing the probability of antigen escape. With an eye toward clinical translation, Cho et al. demonstrated that SUPRA CARs can be engineered using zipper domains derived from human transcription factors and that SUPRA CAR-T cells can control tumor growth in Nod/scid/$\gamma^{-/-}$ (NSG) mice in two different tumor models. Specifically, intraperitoneally (i.p.) injected SUPRA CAR-T cells could induce the regression of i.p. injected SK-BR-3 breast cancer cells almost as effectively as conventional CAR-T cells. Furthermore, intravenously (i.v.) injected SUPRA CAR-T cells could eradicate i.v. injected Jurkat cells as efficiently as conventional CAR-T cells. The authors further demonstrated that *in vivo* interferon (IFN)-γ production levels can be modulated in multiple ways, including adjusting the concentration of zipFv input, changing the binding affinity of the leucine zipper domains of the zipCAR and zipFv, and adding competing zipFvs that prevent the reconstitution of SUPRA CARs. However, it was observed that lowering IFN-γ production by reducing zipFv input or zipper-finding affinity also resulted in decreased tumor killing, and it remains to be seen whether the addition

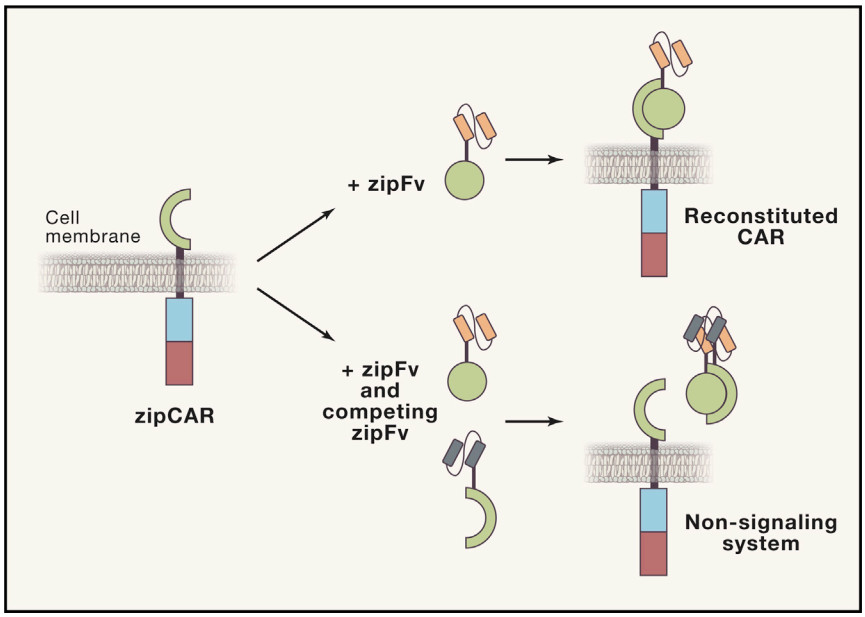

Figure 1. Schematic of the SUPRA CAR System
T cells engineered to express a zipCAR remains signal incompetent until an antigen-binding zipFv containing a matching leucine zipper is added externally to reconstitute a full-length CAR. The system can be modulated by the addition of a competing zipFv species to reduce or prevent CAR reconstitution. Additional control nodes for the system include zipCAR expression level, the concentration of zipFv input, ligand-binding affinity of the zipFvs, and affinity of the leucine zipper components for each other.

of competing zipFv would be able to induce a sufficiently rapid reduction of cytokine levels to effectively address toxicities such as cytokine release syndrome (Brudno and Kochenderfer, 2016; Lee et al., 2014). Nevertheless, the fact that one could easily modulate the performance of CAR-T cells by adjusting the quality and quantity of the zipFv input highlights a unique advantage of the SUPRA CAR system.

The flexibility of the SUPRA CAR system does come at the cost of additional moving parts in the system. As described in this work, *in vivo* application of SUPRA CAR-T cells requires repeated injection of high-dose zipFv proteins, which must be manufactured separately from the zipCAR-expressing T cells. A full characterization of potential toxicity, as well as the practical implementation of this system, will no doubt be the focus of continuing research. The SUPRA CAR represents the latest iteration of CAR design that enables multiple features to be engineered into one CAR-T cell system. As adoptive T cell therapy broadens its reach toward a wider array of malignancies, engineering solutions that support adjustable yet robust control over cellular function will play a critical role in the development of next-generation cancer therapeutics.

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