RESEARCH HIGHLIGHT

A SNIPpet of safety: a Goldilocks approach in CAR-T therapy

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Despite the remarkable efficacy of chimeric antigen receptor (CAR) T-cells in B-cell malignancies, their full potential in solid tumors has not yet been achieved. In a recent publication in *Cell*, Labanieh et al. developed a druggable CAR T-cell construct that allows optimal fine-tuning of CAR T-cells to achieve maximal efficacy and reduce toxicity.

With the advent of CD19 targeting using chimeric antigen receptor (CAR) T-cells, the potential of immunotherapy, in particular adoptive cell therapy, became a reality. CD19-directed CAR T-cells have been transformative–eradicating disease in the most chemotherapy-refractory patients, with potential to induce long-term durable remissions. While inflammatory toxicities related to T-cell expansion can be life-threatening, the iterative recognition of and approach to treatment of cytokine release syndrome (CRS) and sequelae thereof, along with the development of pre-emptive interventions and supportive care measures,[1] facilitated the safe implementation across patient populations. Additionally, the tolerance for on-target, off-tumor targeting enabled implementation of a therapeutic approach where broad antigen targeting was not of substantial concern. Indeed, the loss of normal B-cells, an expected outcome of CD19 targeting, is supported by immunoglobulin replacement and being studied for its use as a surrogate of functional CAR T-cell persistence.[2]

With a desire to improve outcomes in solid tumors, exploring CAR T-cells is a natural transition. However, substantial hurdles have emerged in trying to extend the therapeutic index. Tonic signaling,[3] leading to CAR T-cell exhaustion, and suboptimal CAR T-cell function[4] have emerged as particular challenges in targeting solid tumors. Moreover, in solid tumors where targeted tumoral antigens are often expressed in healthy tissues, albeit at lower densities, on-target off-tumor targeting may not be tolerable.

To address these issues, Labanieh et al.[5] sought to design a druggable system that would allow a fine-tuning of CAR signaling, both ex vivo (during CAR T-cell manufacturing) and in vivo (following infusion). Using grazoprevir (GPV), an FDA-approved drug that blocks the hepatitis C NS3 protease (NS3p), they designed a novel platform named Signal Neutralization by an Inhibitable Protease (SNIP). After generating multiple CAR-NS3p iterations, inclusion of an NS3p cleavage site between the CD8α transmembrane (Tm) domain and the 4-1BB signaling domain of the CAR together with a membrane-bound trans NS3p (Fig. 1, left), abrogated CAR signaling in the basal state (SNIP OFF). Notably though, in the presence of GPV, NS3 protease activity was blocked, resulting in a level of CAR T-cell signaling equivalent to the parental CAR construct (SNIP ON).

Accordingly, the authors assessed whether the efficacy of SNIP CAR T-cells would be augmented if the initial in vitro expansion phase was performed in a SNIP OFF state. To this end, they evaluated GD2-, HER2- and B7H3-CARs, all of which exhibit tonic signaling, and found that the CAR T-cells manufactured in a SNIP OFF state outperformed the respective constitutive CAR T-cells (Fig. 1, middle). Furthermore, phenotypic and transcriptomic analyses revealed reduced exhaustion marker expression on SNIP OFF CAR T-cells as compared to conventional CAR T-cells. These changes had functional sequelae as there was a higher infiltration of SNIP CAR T-cells, as compared to conventional CAR T-cells, in aggressive 143B osteosarcomas. These tumor-infiltrating CAR T-cells displayed unique clusters of $CD4^+$ T-cells enriched in memory markers and $CD8^+$ T-cells exhibiting elevated expression of cytotoxic genes. Thus, abrogation of CAR T-cell tonic signaling during the manufacturing process is a promising approach that may enhance clinical CAR T-cell outcomes in patients with solid tumors.

In addition to improving efficacy, mitigating CAR T cell-associated toxicities is paramount. Incorporation of suicide switches in gene-modified CAR T-cells as a means of selectively eliminating the modified T-cells represents one such strategy. However, this approach results in complete CAR T-cell eradication with no possibility for recovery. In contrast, SNIP CAR T-cells have the potential of being tunable and as such, the authors assessed whether modulation of NS3p-induced cleavage could act as a safety switch in the advent of on-target off-tumor toxicities. Using an ROR1-directed CAR that has been shown to induce lethal on-target, off-tumor toxicity in NSG mice because of cross-reactivity to mouse ROR1,[6] the authors designed a SNIP-ROR1 CAR. While mice treated with constitutive ROR1-CAR T-cells, exhibited rapid toxicities, resulting in weight loss and death within 4 days after treatment, mice receiving SNIP-ROR1 CAR T-cells recovered when GPV treatment was stopped, due to a cessation of CAR T-cell signaling (Fig. 1, right). Accordingly, the withdrawal of GPV could also be used to treat more generalized toxicities, such as severe CRS.

SNIP-CAR system may also promote CAR T-cell cytotoxicity against cells expressing high levels of the targeted antigen while sparing cells expressing low densities of the targeted antigen. Labanieh et al. further demonstrated that reduced dosing of GPV into $ROR1^+$ tumor-bearing NSG mice treated with SNIP-ROR1 CAR T-cells resulted in tumor regression without the rapid toxicity due to on-target killing of healthy $ROR1^+$ mouse tissues.

It has become increasingly evident that a multimodal approach to the development of CAR T-cells in solid tumors will be needed to optimize the therapeutic potential while balancing toxicities. In the development of this novel SNIP CAR, the authors further personalize this therapeutic approach using a Goldilocks-style "just-right" strategy, with significant clinical implications for the

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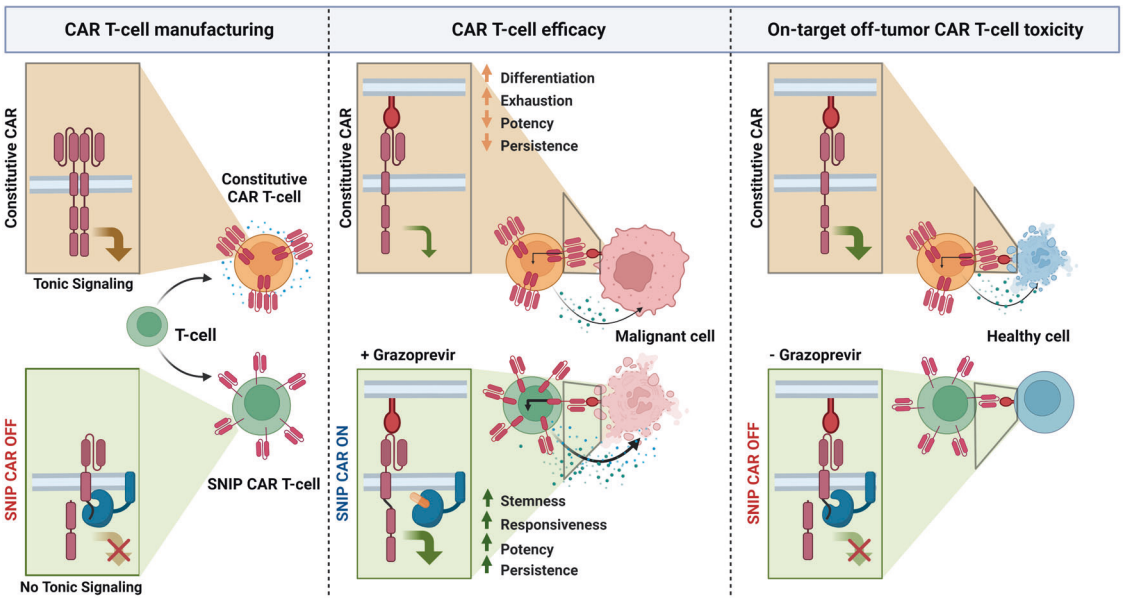

Fig. 1 SNIP CARs as a druggable platform to enhance CAR T-cell efficacy and safety against solid tumors. Left: Many constitutively expressed CARs exhibit tonic signaling in the absence of ligand, resulting in a pre-infused product that is enriched in exhausted CAR T-cells (top). In the SNIP CAR system, the trans expression of a hepatitis C protease together with inclusion of its cleavage site upstream of the CAR intracellular signaling domain abrogates tonic signaling (SNIP OFF), thus conferring a less exhausted T-cell phenotype. Middle: Upon encounter with malignant cells expressing the target antigen, constitutive CAR T-cells are less responsive because of their exhausted state. However, optimal signaling in non-exhausted SNIP CAR T-cells can be induced by inhibiting the protease with the FDA-approved grazoprevir (GPV) drug (SNIP ON), resulting in increased anti-tumor cytotoxicity. Right: While constitutive CAR T-cells can induce severe toxicities, secondary to CRS or targeting of healthy cells, the activity of SNIP CAR T-cells can be inhibited by pausing GPV administration, resulting in a reversal of toxicities. Figure made with permission from BioRender.com.

prevention of on-target off-tumor toxicities, treatment of generalized inflammatory toxicities, and potential improvement and persistence of CAR T-cell functionality.

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ADDITIONAL INFORMATION

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