SYNTHETIC BIOLOGY

CAR-T cells SEAK help from enzymes

Chimeric antigen receptor T cells (CAR-T cells) are often hindered by the concurrent challenges of variable antigen expression patterns and immunosuppressive tumor microenvironments. A new approach enhances CAR-T cells by coexpressing bacterial enzymes that activate prodrugs in high concentrations at the disease site.

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The clinical success of CAR-T cells against B cell malignancies has proven that immune cells engineered to recognize tumor-associated antigens can serve as a powerful new class of living medicines. However, these targeted cell therapies continue to face challenges of variable antigen expression and CAR-T cell dysfunction in the immunosuppressive tumor microenvironment (TME). Now, researchers have reinforced CAR-T cell activity with the help of bacterial enzymes that serve to locally activate chemotherapeutic prodrugs and provide an orthogonal, antigen-independent mode of attack[1].

As a model targeted therapy, even the gold standard CD19 CAR cannot recognize antigen-negative B cells, and antigen loss remains a leading cause of tumor escape and patient relapse[2]. Moreover, frequent occurrences of CAR-T cell exhaustion in the immunosuppressive TME can prevent even the clearance of antigen-positive targets, further adding to rising relapse rates[3]. A straightforward strategy to address these challenges has been to combine cell therapies with existing agents such as chemotherapeutics and checkpoint inhibitors. While such approaches do often produce enhanced therapeutic outcomes, they result in additive toxicities and more severe immune-related adverse events[4].

In this issue of *Nature Chemical Biology*, Gardner et al.[1] develop a novel approach to engineer CAR-T cells as cellular factories that can control and amplify the activity of chemotherapeutics with SEAKER (Synthetic Enzyme-Armed KillER) cells—CAR-T cells genetically equipped with bacterial enzymes that activate systemically administered prodrugs directly at tumor sites (Fig. 1a). The authors introduce a proof-of-concept SEAKER approach using appropriately modified prodrugs of the highly cytotoxic small molecule 5′-*O*-sulfamoyladenosine (AMS), which they used to test the activity of two well-characterized hydrolytic enzymes of bacterial origin: (i) *Pseudomonas* sp. carboxypeptidase G2 (CPG2) and (ii) *Enterobacter cloacae* β-lactamase (β-Lac). They then engineered the SEAKER cells to express the established CD19 CAR (19BBz) downstream of the CPG2 or β-Lac enzymes and assessed the resulting cells for enhanced efficacy in multiple models of human B cell malignancies.

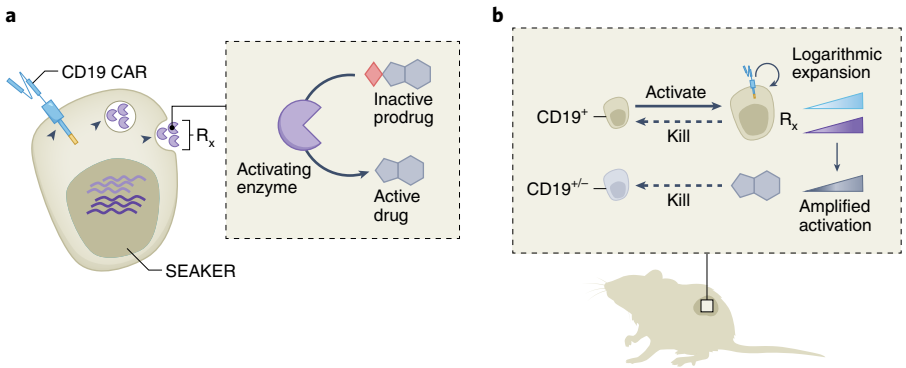

Fig. 1 | 'SEAKER' cells for dual-mode tumor lysis. a, CAR-T cells are genetically engineered to secrete bacterial enzymes that are designed to activate systemically delivered prodrug forms of potent chemotherapeutics and provide the basis of the SEAKER (synthetic enzyme-armed killer) system. **b**, CD19-directed SEAKER cells that encounter CD19-positive cells ($CD19^+$) undergo rapid logarithmic expansion at the disease site, in turn producing high concentrations of the activating enzyme, leading to local amplification of prodrug activation and, ultimately, clearance of heterogenous tumor cells ($CD19^{+/-}$).

Importantly, SEAKER cells expand logarithmically upon engagement with antigen-positive targets, which leads to both the targeted killing of CD19-positive cells and the amplified production of the prodrug-unmasking enzymes that catalytically generate higher concentrations of active AMS—ultimately facilitating dual-mode tumor lysis and clearance of heterogenous tumor cells (Fig. 1b). Indeed, both SEAKER systems were able to clear heterogenous models of leukemia, destroying CD19-positive, and CD19-negative, cells with no detectable systemic toxicity. SEAKER cells were also more effective at lower doses than conventional 19BBz CAR-T cells in an aggressive model of human lymphoma—highlighting the synergy of cytolytic and chemotherapeutic antitumor activity, and the ability of the two therapies to each reduce the dose, and associated toxicities, of the other. Moreover, SEAKER cells displaying several markers of T cell exhaustion continued to produce functional enzymes, sustaining the small-molecule mode of action while T cell effector functions failed. Lastly, the production of bacterial enzymes did not appear to increase the immunogenicity of SEAKER cells that were able to persist long term in syngeneic models.

In the current work, Gardner et al.[1], with their dual-mode approach to tumor lysis, provide an elegant solution to several of the continued challenges facing CAR-T therapies. The novelty of the system resides not only in the combination of the two orthogonal approaches but also in the use of engineered T cells as disease-site factories that sense and respond to their environments. Although other approaches have been developed for targeted drug delivery, such as antibody–drug conjugates (ADC) and antibody-directed enzyme–prodrug therapy (ADEPT)[5], the SEAKER system exploits the proliferative potential of a living medicine and thus enables high levels of drug amplification in situ.

Though the current demonstration aimed to fill the remaining gaps in the treatment of B cell malignancies in particular, the SEAKER approach is inherently modular and well poised to address the additional challenges of solid tumor treatment in the future. Low antigen expression and tumor heterogeneity are major barriers to the successful treatment of solid tumors, and targeted therapies may particularly benefit from being combined with a readily diffusible, orthogonally acting drug. However, the antigen problem is compounded by the lack of tumor-restricted antigens on solid tumors that are not found on healthy tissue, leading to problems of on-target, off-tumor toxicities that SEAKER cells must also navigate.

Next-generation CAR-T cells have been equipped with additional genetic circuitry[6] and protein accessories[7,8] that are designed to address these additional challenges, while other approaches have explored combinations with nanoparticles[9] and oncolytic viruses[10] to deliver CAR targets directly to the tumor. One can imagine that further CAR-T cell modifications and synergy with additional therapeutic platforms could be incorporated to further enhance future generations of the SEAKER approach developed here. As synthetic biology continues to advance concurrently with immunotherapy, there are sure to be further creative solutions to the heterogenous challenges of disease. ❐

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Competing interests

The authors declare no competing financial interests.