Cancer immunotherapy

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Orthogonal engineering of synthetic T cell states to enhance cancer immunotherapy

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Orthogonal engineering of adoptively transferred CD8$^+$ T cells to co-express two cytokines — an IL-2Rβ/γ-biased IL-2 variant and the proinflammatory alarmin IL-33 — induces an exhaustion-resistant synthetic cell state with potent anti-tumor efficacy in the absence of host pre-conditioning.

Adoptive cell therapy (ACT) involves the transfer of T cells with reactivity to tumor-associated antigens into patients with cancer. ACTs can include T cells with endogenous reactivity to tumor neoantigens, and T cells gene-engineered to express chimeric antigen receptors or T cell antigen receptors that are specific for cancer-associated antigens. Chimeric antigen receptor therapy directed against the B cell co-receptor subunit CD19 is revolutionizing the treatment of certain relapsed and/or refractory B cell malignancies, whereas tumor-infiltrating lymphocyte therapy mediates striking clinical responses in subsets of patients with solid cancers[1,2].

Despite considerable progress, numerous obstacles limit the applicability and efficacy of ACTs, including impaired maintenance and exhaustion of the transferred T cells and the need for host pre-conditioning and/or cytokine support to promote engraftment[2–4]. In a study published in *Nature Immunology*, Corria-Osorio et al.[5] show that orthogonal engineering of CD8$^+$ T cells to co-express two cytokines — an interleukin 2 variant (IL-2v) that preferentially engages the β- and γ-chains of the IL-2 receptor, and the proinflammatory alarmin IL-33 — potently augments responses to adoptive immunotherapy. Acting synergistically, these cytokines program T cells into a previously undescribed synthetic cell state with improved engraftment and resistance to canonical exhaustion, as well as the ability to mediate potent tumor regression in the absence of host pre-conditioning (Fig. 1a).

Despite rapid clinical advances in cell therapy, T cells are naturally hardwired with a set of physiological constraints that can powerfully limit their therapeutic efficacy. At present, clinically effective responses require host pre-conditioning, are limited by poor maintenance and undergo exhaustion, which leads to treatment failure. Although it is tempting to consider these issues as forms of cellular malfunction, they represent fundamental biological properties of T cells critical under normal circumstances for the maintenance of lymphoid homeostasis, immunological tolerance and prevention of excessive immunopathology after infection. For instance, memory T cells require homeostatic cytokines such as IL-15 for their survival, which provides a physiological constraint on the size of the memory pool[6]. Effector T cells require cytokines such as IL-2 for their survival, which usefully constrains the size of effector responses and renders them amenable to suppression by regulatory T cells[7]. Similarly, T cells undergo the physiological process of exhaustion when faced with chronic antigen stimulation, upregulating co-inhibitory receptors to limit immunopathology after infection[8]. In the context of cell therapy, these valuable evolutionary constraints become limitations to therapeutic efficacy and create the need for often toxic and dose-limiting host pre-conditioning regimens and cytokine support. Genetic editing hence opens a window of opportunity for rational gene-engineering approaches to synthetically bypass these physiological constraints. Although numerous transcription factors, cytokines and receptors have been employed in this context[9–11], the use of orthogonal strategies, whereby multiple payloads are combined in a synergistic manner, remains largely unexplored.

In their recent work, Corria-Osario et al.[5] engineered antigen-specific CD8$^+$ T cells to co-express either IL-2v or IL-33 in conjunction with a secreted decoy form of the immunoinhibitory receptor PD-1 (PD-1d) that blocks immunosuppression mediated by the PD-1 ligand PD-L1. The reasoning behind this choice was that IL-2v would maintain the pool of progenitor TCF-1$^+$ T cells[12], while inflammation-promoting IL-33 would induce a sufficiently stimulatory environment to drive effector differentiation and anti-tumor function[13]. Indeed, orthogonal co-expression of both cytokines within the tumor microenvironment was critical for adoptively transferred T cells to mediate increased regression of syngeneic B16 tumors in the absence of host lymphodepletion. This regression was accompanied by substantial expansion of both stem-like TCF-1$^+$ populations and effector TCF-1$^-$ populations within tumors. Although IL-2v was mainly responsible for accumulation of the TCF-1$^+$ population within tumors, the pronounced accumulation of TCF-1$^-$ effector cells was dependent on expression of IL-33 — but only in the context of orthogonal co-expression with IL-2v. Expansion of transferred cells within tumors did not require continuous recruitment of T cells from lymph nodes, as blockade of lymph node egress with the pharmacological bioactive lipid S1P inhibitor FTY720 did not significantly diminish the maintenance of transferred T cells within tumors. The maintenance of orthogonally engineered T cells within tumors was therefore not dependent on TCF-1$^+$ stem-like cells within the tumor-draining lymph node, as was the case in a previously described physiological setting[14].

Intriguingly, an in-depth assessment of cellular phenotypes by single-cell RNA sequencing revealed that in the context of co-expression of IL-2v, IL-33 and PD-1d, intratumoral T cells transitioned from naturally occurring to synthetic cellular differentiation states[5]. Transfer of cells that co-expressed IL-2v and PD-1d resulted in cells with gene-expression profiles resembling those of naive or central memory T cells, whereas transfer of cells that co-expressed IL-33 and PD-1d induced the upregulation of genes encoding molecules associated with effector memory differentiation. It was only when IL-2v, IL-33 and PD-1d were co-expressed that T cells acquired a unique gene-expression signature distinct from that of previously described natural T cell states. This synthetic cell state was marked by a TOX$^-$GzmC$^+$ phenotype and

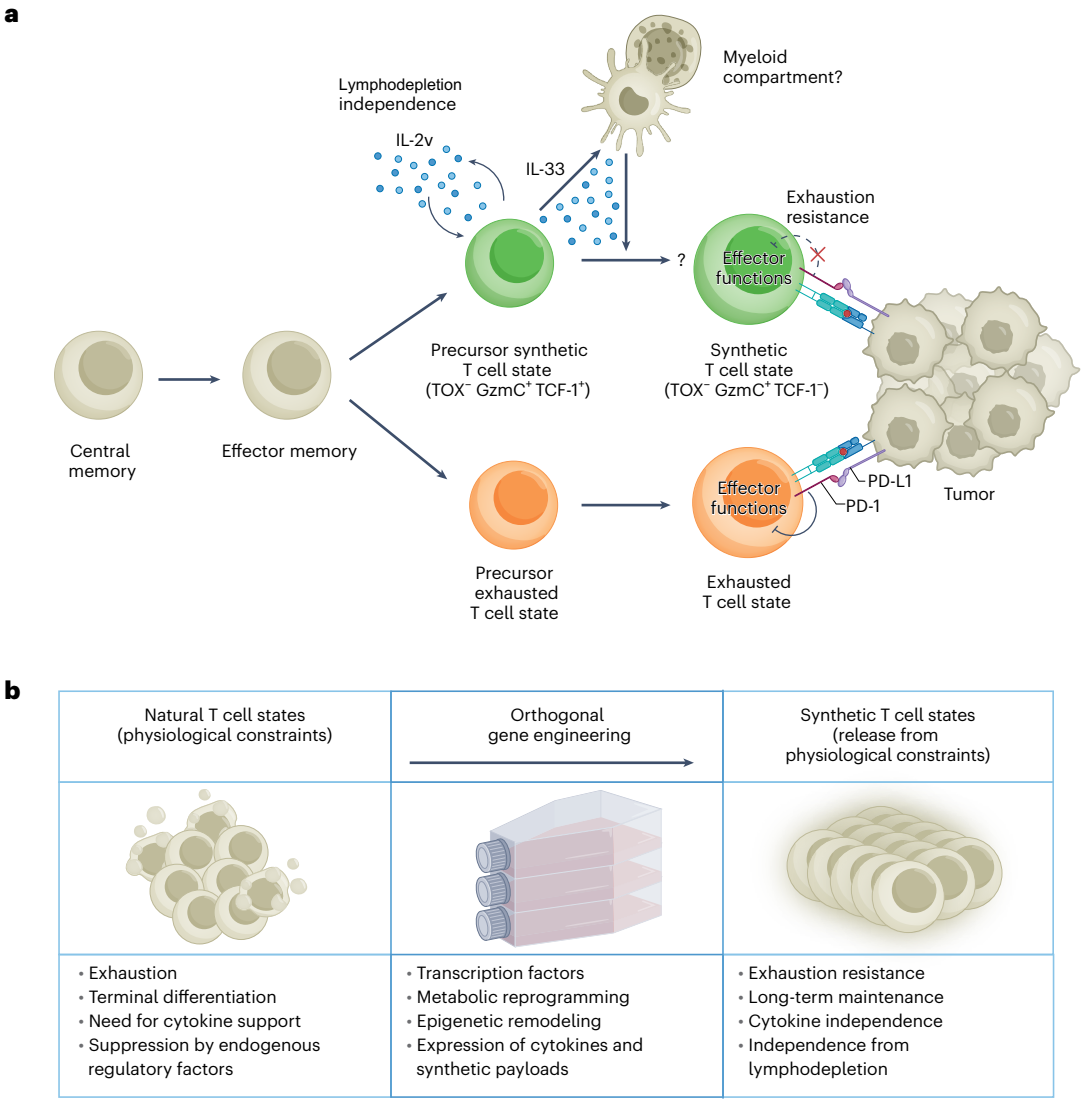

Fig. 1 | Orthogonal engineering of synthetic T cell states to enhance therapy. **a**, Co-expression of IL-2v and IL-33 by orthogonally engineered CD8$^+$ T cells induces their differentiation into an exhaustion-resistant synthetic state that is insensitive to inhibition by PD-L1 signaling. The differentiation of T cells in a TOX$^-$Gzmc$^+$ synthetic state is proposed to occur via a TCF-1$^+$ precursor synthetic state. IL-2v acts in an autocrine and locally paracrine manner to promote the accumulation of TCF-1$^+$ precursor synthetic cells; IL-33 acts on an undefined ST2$^+$ non–T cell subpopulation within tumors to create the conditions required for induction of the TOX$^-$GzmC$^+$ exhaustion-resistant synthetic state. **b**, Orthogonal engineering T cells to improve efficacy and tolerability of adoptive cell therapies. Natural cell states are subject to physiological constraints hardwired into the biology of T cells for the maintenance of lymphoid homeostasis, immunological tolerance and prevention of immunopathology. These constraints become limitations in the context of cell therapy, hindering efficacy and creating the need for often toxic and dose-limiting host pre-conditioning regimens and cytokine support. Orthogonal combinations of various biological factors may be a critical step in overcoming many of the hurdles currently faced by ACTs.

included high expression of genes encoding tumoricidal cytotoxic molecules (for example, granzyme B, granzyme A and perforin) and cytokines (for example, interferon-γ and tumor necrosis factor).

Given those findings, the authors went on to delineate the functional characteristics of orthogonally engineered cells after adoptive transfer[5]. They found orthogonally engineered cells to be resistant to exhaustion despite expressing PD-1, as PD-L1 failed to block stimulation-driven activation, proliferation and induction of cytotoxic molecules when the cells were isolated from tumors and stimulated ex vivo. Consistent with those findings, co-expression of IL-2v and IL-33 with a single vector was sufficient to mediate tumor regression in the lymphoreplete context, and neither additional co-expression of PD-1d nor treatment with blocking antibodies to PD-L1 augmented the anti-tumor response driven by IL-2v- and IL-33-expressing cells. Intriguingly, the authors also found that the observed synthetic phenotype induced was acquired exclusively by adoptively transferred

$CD8^+$ T cells, and was not acquired by endogenous tumor-infiltrating lymphocytes that were bystanders to the therapy[5]. This result was surprising, as both IL-2v and IL-33 are secreted payloads and are expected to exert paracrine activity. However, a potential role for highly localized secretion cannot be excluded, especially given that systemic administration of IL-2v and IL33 recombinant proteins in the context of cell therapy failed to fully recapitulate the therapeutic efficacy of orthogonal overexpression of IL-2v and IL-33 by transferred cells and resulted in higher levels of toxicity.

Although the study by Corria-Osario et al.[5] provides exciting proof of concept that orthogonal engineering of adoptively transferred T cells can bring about improvements to cell therapy, the study leaves a number of open questions for future work. In particular, the full nature of the observed exhaustion resistance and loss of sensitivity of orthogonally engineered cells to PD-L1-mediated inhibition has yet to be determined, especially as the proximal inhibitory intracellular signal-transduction processes triggered by PD-1 are expected to be intact. It is also unclear which specific non–T cell subpopulation within tumors receives the IL-33-driven signal and what the subpopulation then produces to drive the observed synergistic effect with IL-2v. Finally, it will be important to clarify whether IL-2v is required for the observed effect, or whether wild-type IL-2 would suffice.

Collectively, these data show that orthogonal engineering of adoptively transferred T cells to co-express IL-2v and IL-33 enables programming of a synthetic T cell state that features high levels of polyfunctionality and exhaustion resistance, associated with potent tumor regression in the absence of prior lymphodepleting pre-conditioning, with important clinical implications[5]. The findings also provide proof of principle for the broader development of orthogonal gene-engineering approaches to overcome the naturally hardwired limitations of T cells and induce synthetic cell states with enhanced efficacy (Fig. 1b).

The potential for seemingly unrelated orthogonal genetic payloads to synergistically augment cell therapy responses leaves open a critical outstanding question: how can the right combinations of genetic payloads for optimal orthogonal cell therapy be best identified? Is enough known about T cell biology within tumors for rational design of orthogonal approaches, or would a higher-throughput combinatorial strategy be useful in arriving at optimal orthogonal configurations?

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Competing interests

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