RESEARCH HIGHLIGHT

Fine-tuning T cell function through engineered orthogonal chimeric cytokine receptors

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In a recent study published in *Nature*, Kalbasi et al. demonstrate that engineering T cells to express a chimeric orthogonal IL-2 receptor, where the intracellular domain (ICD) of the receptor was replaced with the IL-9 receptor ICD, mitigated negative impacts of IL-2 administration and also drove signaling through STAT1, STAT3 and STAT5 to promote anti-tumor function and T cell stemness.

$CD8^+$ T cell-mediated immune responses against antigens are strongly regulated by three signals: (1) direct interaction between antigen and cognate T cell receptor (TCR), (2) costimulatory support through CD27, CD28, 4-1BB and other receptors, and (3) cytokines such as IL-2 driving T cell proliferation and inflammatory signaling.[1] In the context of cellular immunotherapy, a number of studies have examined the impacts of modifying and optimizing features of antigen stimulation and costimulatory signals on T cell products.[2] Recent work has demonstrated that the modification of cytokine signaling in T cells also has profound impacts on anti-tumor activity. While most early cell production processes were reliant on IL-2 to drive T cell expansion, promoting desirable features by utilizing alternative gamma chain (γc) cytokines such as IL-7, IL-15 or IL-21 during T cell culture can increase T cell persistence and function in vivo.[3,4] An additional usage of γc cytokines in adoptive cell therapy (ACT) is the in vivo administration of cytokines such as IL-2 along with T cells.[5] While protocols have utilized IL-2 administration after infusion of chimeric antigen receptor (CAR) or TCR engineered T cells, the predominant role for in vivo administered γc cytokines as a part of ACT has been in the setting of expanded autologous tumor infiltrating lymphocytes (TILs), which require cytokine support to promote T cell engraftment, persistence, and anti-tumor function. Consequently, γc cytokines can play important roles in vitro and in vivo to promote effective anti-tumor responses by T cells.

Despite the clear benefits of incorporating γc cytokines into ACT protocols, a major challenge in their use has been limited ability to control which T cells bind cytokine, and how various subsets of T cells respond to cytokine. Ideally, cytokines would only signal in tumor-reactive T cells, and the downstream signaling driven by cytokine binding would promote favorable features in cells. However, endogenous cytokines are promiscuous T cell binders — any T cell with cytokine receptor expression can bind a cytokine. In ACT this can be problematic, as bystander or suppressive T cell subsets, such as Treg may express higher levels of cytokine receptors, including IL-2R, than tumor-reactive T cells.[6] Consequently, in vivo administration of high-dose IL-2 can promote the preferential expansion and suppressive function of Treg (Fig. 1a), which may also act as cytokine sinks that limit IL-2 availability for tumor-reactive T cells.[7] Additionally, endogenous cytokine signaling can produce unwanted downstream impacts on cell phenotype. IL-2 signaling may promote terminal differentiation, loss of stemness capacity and limited in vivo persistence, resulting in impaired anti-tumor activity.[4,8]

To retain the benefits of γc cytokine signaling in T cells and mitigate drawbacks, emerging strategies use engineered cytokines and receptors to signal in desired cell subsets. These strategies rely on orthogonal IL-2 (oIL-2) and transduction of a modified IL-2R that binds oIL-2 in tumor-specific T cells.[9] While this system necessitates engineering T cells to express the appropriate receptor, it eliminates the need for endogenous IL-2 that can bind to undesired cell types (Fig. 1b). However, this system retains the intracellular signaling portion of the endogenous IL-2 receptor, driving terminal differentiation and limited persistence in tumor-specific T cells. While this may be ameliorated by altering the binding characteristics of IL-2,[10] additional strategies to optimize cytokine signaling may provide further improvements to anti-tumor T cell functionality.

In a study recently published in *Nature*, Kalbasi and colleagues explored further optimizations on oIL-2 systems to generate more potent anti-tumor T cells.[11] The authors replaced the intracellular domain (ICD) of the oIL-2 system with that of other γc cytokine receptors while retaining the oIL-2R extracellular domain (ECD), and profiled the impacts on T cell signaling and function (Fig. 1c). Incorporating the IL-9R ICD, absent in endogenous T cells, resulted in enhanced production of stem cell memory T cells (Tscm), cells associated with favorable anti-tumor features. Binding of oIL-2 to the oIL-9R drove activity in the STAT1, STAT3 and STAT5 signaling pathways, a feature not observed in endogenous or other engineered cytokine signaling pathways. oIL-9R expression in T cells mediated improved anti-tumor function in murine models upon administration of oIL-2, along with increased T cell stemness relative to endogenous or orthogonal IL-2R. The authors observed that transduction of human T cells with oIL-9R and treatment with oIL-2 also improved T cell stemness in vivo and in vitro during chronic antigen exposure. While the authors observed reduced proliferation in oIL-9R cells relative to oIL-2R cells, this did not appear to impact anti-tumor efficacy. However, the authors' observation that the oIL-9R system appeared to increase mesothelin CAR T cell toxicity in murine models emphasizes the need to understand the interplay of synthetic cytokine signaling systems and tumor-targeting receptors in T cells, especially when targets are not uniquely expressed in tumor.

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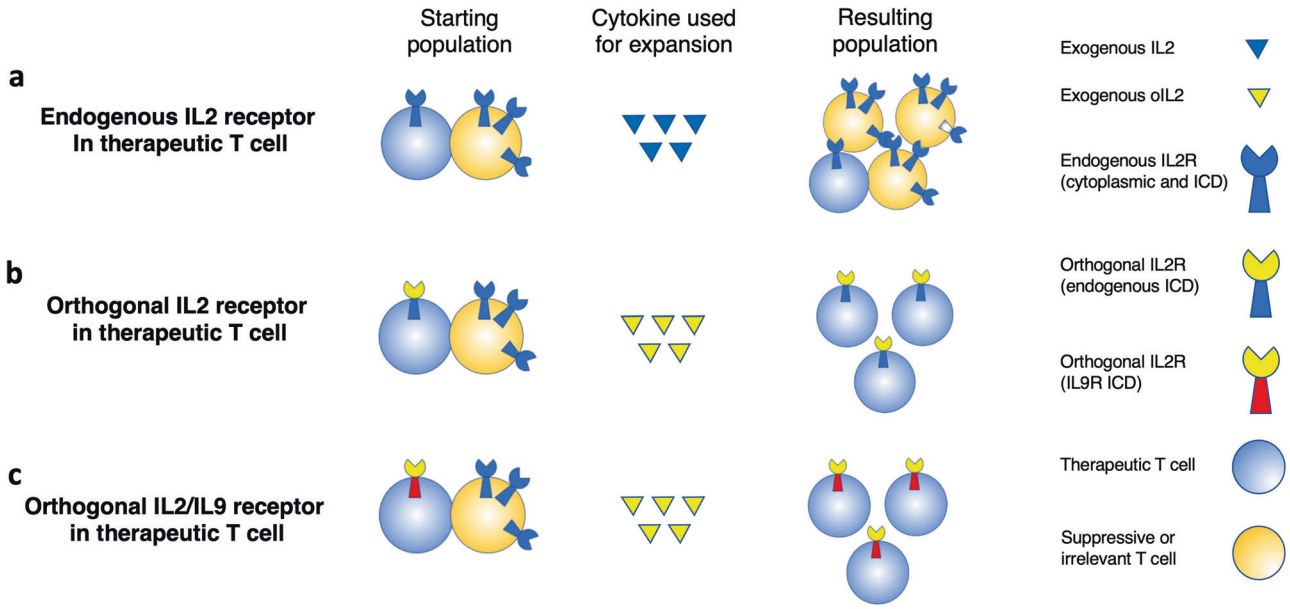

Fig. 1 Orthogonal IL-2 systems mediate specific expansion of therapeutic T cells. a Expansion of mixed populations of therapeutic and suppressive/irrelevant T cells with endogenous IL-2 may lead to preferential expansion of clinically irrelevant T cells. **b** Engineering therapeutic T cells with an orthogonal IL-2R can allow for preferential expansion of these cells through administration of orthogonal IL-2. **c** Engineering therapeutic T cells with a chimeric orthogonal IL-2R expressing ICD of the IL-9R can allow for preferential expansion of these cells and engage alternative signaling cascades to promote desired cellular phenotypes.

An interesting notion raised by Kalbasi et al. is the prospect of utilizing the same signaling molecule to drive differentiated downstream cascades in a single T cell infusion product. If technical and manufacturing challenges could be overcome, it may be possible to separately engineer fractions of infused T cells to express various chimeric cytokine receptors, such that the administration of oIL-2 in vivo would drive effector signaling in some cells, while promoting stemness and durable effector function in other cells. Further work to explore the functional consequences of expressing the numerous possible chimeric cytokine receptors may yield a diverse array of potential T cell phenotypes. While emerging work has demonstrated that the presence of stemlike cells in T cell infusion products is associated with clinical responses,[12] the optimal blend of effector and stemlike cells remains to be determined. While stemlike cells may help to promote persistent immune responses against cancer, more differentiated effector T cells, with high cytolytic ability, may provide an important initial anti-tumor response. Further, heterogenous tumor biology may provide support for tailoring cytokine signaling to match the functional needs of histology-specific clinical applications.

In summary, the authors present an exciting finding that the fusion of orthogonal cytokine signaling systems with chimeric cytokine receptors can create tunable signaling cascades that increase anti-tumor activity. Further explorations of the tolerance for non-γc cytokine receptor ICDs, along with exploring the potential of fusing oIL-2R with the ICDs of non-cytokine signaling proteins, may yield additional novel T cell signaling cascades. The oIL-9R system, along with other novel systems, may allow for controlling T cell features for treating a variety of human diseases.

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COMPETING INTERESTS

R.J.K. and N.P.R. receive compensation and own equity in Lyell Immunopharma.

ADDITIONAL INFORMATION

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