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Getting (T-cells) ENGaged

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In this issue of *Haematologica*, Vaidya and colleagues report on "Improving the anti-AML activity of CD123-specific Engager T cells by MyD88 and CD40 costimulation"(1). This work focuses on Engager T cells (ENG T cells), an interesting adoptive T cell modality, aimed at combining the benefits of bispecific monoclonal antibodies (mAbs) to engage bystander T cells regardless of their T-cell receptor (TCR)-specificity with the longevity and trafficking capabilities of adoptively transferred T cells.

Bispecific mAbs (BsAbs) engaging both a tumor target and an immune effector cell have emerged as important therapeutic tools. This approach works by bridging T cells and target cells with BsAbs and prompts T-cell activation that is no longer major histocompatibility complex restricted and independent of the specificity of the native TCR. BsAbs can be generated in different formats and may include or lack an IgG backbone with a Fc Domain. Configurations that do not include an Fc linker include BiTEs (bispecific T cell engagers), DARTs (dual affinity retargeting) and Diabodies which are differentiated by the type of linker and configuration of how the single chain variable fragment (scFvs) recognizing the tumor target is linked to the scFv binding the T cell (2). Blinatumomab is an example of a BiTE that has shown tremendous clinical efficacy in engaging T-cells to eliminate CD19+ B-lymphoblastic leukemia (3-5). Similarly, BsAb approaches are being developed for targets such as CD20 in CD20+ Non-Hodgkin Lymphoma and CD33 and CD123 (6) in acute myeloid leukemia (AML).

However, while these molecules can redirect resident T-cells to target tumor targets, they have a short half-life (Blinatumomab is being administered by continuous infusion) and do not self-amplify or promote ongoing T cell engagement. In contrast, adoptive T-cell immunotherapies using antigen-specific T cells such as chimeric antigen-receptor (CAR) T cells mediate cytotoxic effects against tumor cells in target-specific fashion, can persist and mediate tumor control for years. However, although epitope-spreading has been described, they do not activate bystander T cells to mediate antigen-specific tumor-killing in the tumor microenvironment.

ENG T cells are a T-cell platform that secretes bispecific engagers, after having been transduced with a vector encoding for a BsAb consisting of two single chain variable fragments (scFvs), one specific for the tumor target and the other specific for CD3 ϵ (Figure 1A, C). The production and delivery of BsAbs by ENG T cells *in vivo* conceptually allows the continuous local delivery of BsAbs at the tumor site without the need for a continuous infusion. The secreted BsAb engage both untransduced T cells in the microenvironment and the ENG T cells themselves and facilitate antigen-specific tumor killing. Additionally, ENG-T cells can be engineered to provide

co-delivery of costimulatory molecules or cytokines to improve ENG T cell function and overcome immunosuppressive factors in the tumor microenvironment.

Several groups have reported preclinical activity with this approach utilizing different bispecific engagers in models of both hematologic malignancies and solid tumors (7-9). In the current study, the authors focus on CD123-specific ENG T cells, which are genetically modified T cells secreting a bispecific antibody (CD123-ENG) consisting of two scFvs binding CD123 and CD3 ϵ respectively to target AML. Previous studies documented the secretion of the bispecific engager protein (CD123 ENG) by CD123 ENG T-cells and binding of the CD123 ENG protein to both CD123-ENG T cells and nontransduced bystander T cells. CD123 ENG T-cells were able to kill CD123-positive primary AML blasts in an antigen-specific manner, and redirected bystander T cells to induce antigen-specific AML target killing in transwell assays whilst demonstrating activity in AML xenograft models (7). However, a decrease in effector function of ENG T cells upon chronic antigen-stimulation remains a limitation of this and other T-cell based therapies.

To overcome this, Vaidya et al. explore the inclusion of a drug-inducible composite MyD88/CD40 activation receptor to provide costimulation, confer improved expansion capability and antitumor activity, via downstream signaling pathways involving NF- κ B and PI3K/AKT. The inducible MyD88/CD40 switch comprising a Myristoylation-targeting sequence (to increase protein-protein interactions leading to subcellular localization of myristoylated proteins with its signaling partners), MyD88 (lacking its TIR sequence), the CD40 cytoplasmic domain and two tandem FKBP12v36 chemical inducer of dimerization (CID)-binding domains, which dimerize and activate the receptor upon administration of a dimerizing drug (CID), has previously been described in the context of enhancing the function of dendritic cells (10) and CART cells (11, 12) (Figure 1B).

Here the authors report the effect of using an inducible costimulation system in CD123.ENG T cells (Figure 1C) and compare the effects of the inducible MyD88/CD40 molecule (iMC) with inducible MyD88 and inducible CD40 alone. They demonstrate that upon activation of an iMyD88/CD40 switch by a dimerizing CID drug, CD123 ENG T cells maintained their antigen specificity, exhibited superior effector function under conditions of repeat stimulation and mediated enhanced anti-tumor activity in different AML xenograft models. Although the effect of iMC on bystander T cell activation was not specifically evaluated, these data support further development of this promising Engager T cell platform.

REFERENCES:

1. Vaidya A, Doherty E, Wu X, et al. Improving the anti-AML activity of CD123-specific Engager T cells by MyD88 and CD40 costimulation. *Haematologica*. xxx
2. Velasquez MP, Bonifant CL, Gottschalk S. Redirecting T cells to hematological malignancies with bispecific antibodies. *Blood*. 2018;131(1):30-38.
3. Kantarjian H, Stein A, Gokbuget N, et al. Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. *N Engl J Med*. 2017;376(9):836-847.
4. von Stackelberg A, Locatelli F, Zugmaier G, et al. Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. *J Clin Oncol*. 2016;34(36):4381-4389.
5. Foa R, Bassan R, Vitale A, et al. Dasatinib-Blinatumomab for Ph-Positive Acute Lymphoblastic Leukemia in Adults. *N Engl J Med*. 2020;383(17):1613-1623.
6. Al-Hussaini M, Rettig MP, Ritchey JK, et al. Targeting CD123 in acute myeloid leukemia using a T-cell-directed dual-affinity retargeting platform. *Blood*. 2016;127(1):122-131.
7. Bonifant CL, Szoor A, Torres D, et al. CD123-Engager T Cells as a Novel Immunotherapeutic for Acute Myeloid Leukemia. *Mol Ther*. 2016;24(9):1615-1626.
8. Iwahori K, Kakarla S, Velasquez MP, et al. Engager T cells: a new class of antigen-specific T cells that redirect bystander T cells. *Mol Ther*. 2015;23(1):171-178.
9. Liu X, Barrett DM, Jiang S, et al. Improved anti-leukemia activities of adoptively transferred T cells expressing bispecific T-cell engager in mice. *Blood Cancer J*. 2016;6(6):e430.
10. Narayanan P, Lapteva N, Seethammagari M, Levitt JM, Slawin KM, Spencer DM. A composite MyD88/CD40 switch synergistically activates mouse and human dendritic cells for enhanced antitumor efficacy. *J Clin Invest*. 2011;121(4):1524-1534.
10. Mata M, Gerken C, Nguyen P, Krenciute G, Spencer DM, Gottschalk S. Inducible Activation of MyD88 and CD40 in CAR T Cells Results in Controllable and Potent Antitumor Activity in Preclinical Solid Tumor Models. *Cancer Discov*. 2017;7(11):1306-1319.
11. Collinson-Pautz MR, Chang WC, Lu A, et al. Constitutively active MyD88/CD40 costimulation enhances expansion and efficacy of chimeric antigen receptor T cells targeting hematological malignancies. *Leukemia*. 2019;33(9):2195-2207.

LEGENDS:

- A) Transgene encoding for the CD20 transduction marker, CD123ENG BsAb and MyD88/CD40 (iMC) inducible switch
- B) CID activation of the inducible MyD88/CD40 costimulatory switch, containing a myristoylation-targeting sequence, MyD88 (lacking its TIR domain), the cytoplasmic CD40 domain and FKB12v36 CID-binding domains in tandem, leads to dimerization and activation of the iMyD88/CD40 switch activating downstream NF- κ B-transcriptional activity.
- C) Model of CD123 ENG T cell action: Transduced ENG T cells express the CD123 ENG bispecific diabody which binds to CD123 ENG T cells and bystander T-cells directing them to antigen-bearing AML cells. Upon antigen binding CD123 ENG and bystander T cells are activated and mediate anti-tumor activity. CD123 ENG T cells increase CD123 ENG diabody mRNA production. However, without activation or inclusion of a MyD88/CD40 switch, CD123 ENG T cell function may decrease upon chronic antigen stimulation.
- D) To overcome a decrease in effector function upon chronic antigen stimulation, CD123 ENG T cells can be engineered to express the MyD88/CD40 molecule which can be activated by administration of CID as shown in B. Inducible activation of the MyD88/CD40 switch results in superior CD123 ENG T cell antitumor activity in the tumor microenvironment via downstream T-cell signaling pathways including NF- κ B and PI3K/AKT.

