Getting (T cells) ENGaged

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In this issue of *Haematologica*, Vaidya and colleagues report on "Improving the anti-acute myeloid leukemia activity of CD123-specific engager T cells by MyD88 and CD40 co-stimulation".[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 (BsAb) to engage bystander T cells, regardless of their T-cell receptor (TCR)-specificity, with the longevity and trafficking capabilities of adoptively transferred T cells.

BsAb 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 bispecific monoclonal antibodies and prompts T-cell activation that is no longer major histocompatibility complex-restricted and independent of the specificity of the native TCR. BsAb 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 bispecific T-cell engagers (BiTE), dual affinity retargeting (DART) and Diabodies which are differentiated by the type of linker and configuration of how the single chain variable fragment (scFv) 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 in acute myeloid leukemia (AML).[6]

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 a target-specific fashion, and 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 scFv, one specific for the tumor target and the other specific for CD3ε (Figure 1A, C). The production and delivery of BsAb by ENG T cells *in vivo* conceptually allows the continuous local delivery of BsAb 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 co-stimulatory 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 scFv binding CD123 and CD3ε 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 non-transduced bystander T cells. CD123 ENG T cells were able to kill CD123$^+$ primary AML blasts in an antigen-specific manner, and redirected bystander T cells to induce antigen-specific AML target killing in transwell assays while 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 co-stimulation, and confer improved expansion capability and antitumor activity, via downstream signaling pathways involving NF-κB 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 their 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 (i.e., a CID), has previously been described in the context of enhancing the function of dendritic cells[10] and CAR T cells[11,12] (Figure 1B).

Here the authors report the effect of using an inducible co-stimulation system in CD123 ENG T cells (Figure 1C) and compare the effects of the inducible MyD88/CD40 molecule with inducible MyD88 and inducible CD40 alone. They demonstrate that upon activation of an inducible

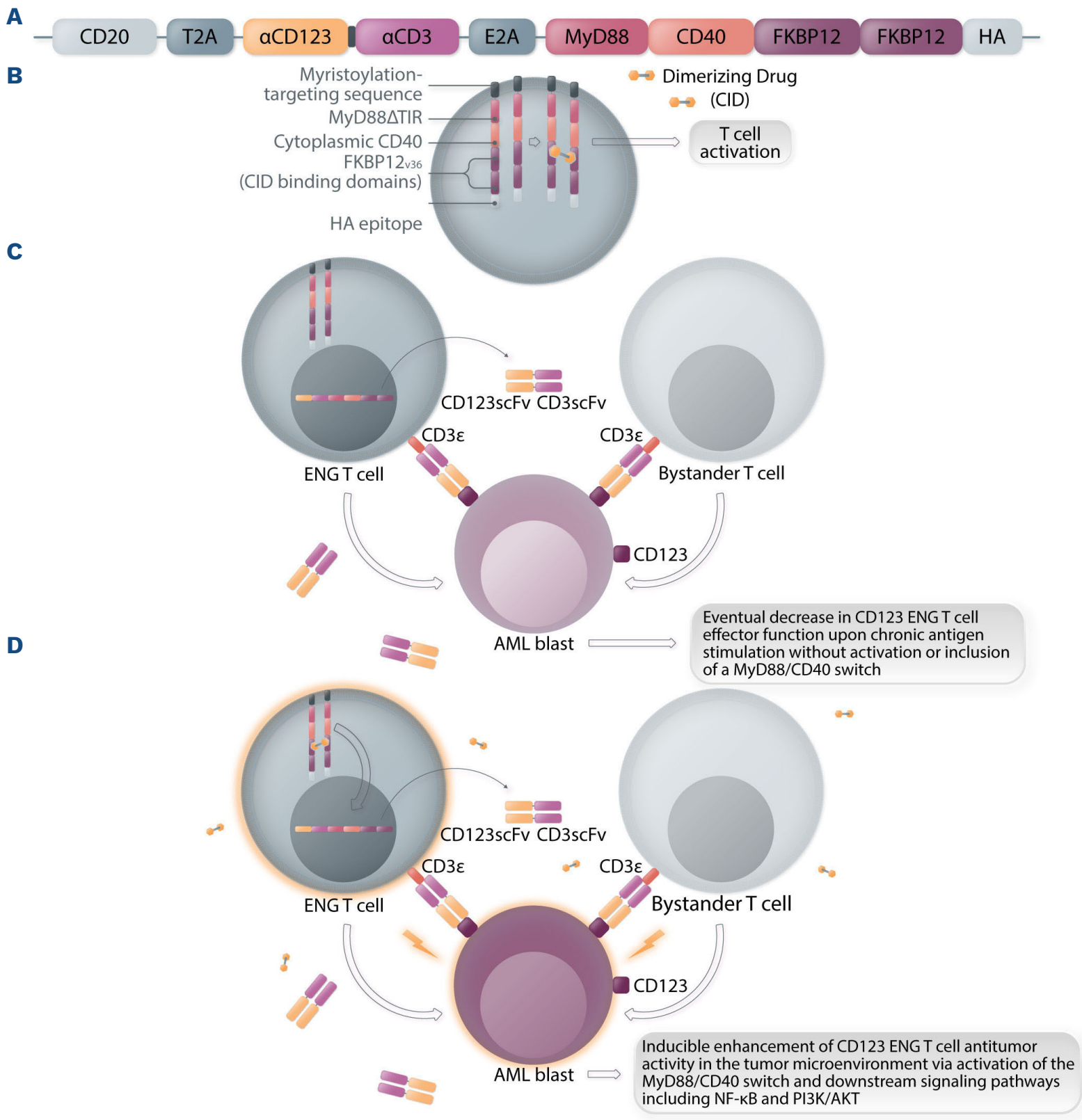

Figure 1. Features of the inducible co-stimulation system in CD123 engager T cells. (A) Transgene encoding for the CD20 transduction marker, CD123 engager (CD123 ENG) T cells bispecific monoclonal antibody and MyD88/CD40 inducible switch. (B) Chemical inducer of dimerization (CID) activation of the inducible MyD88/CD40 co-stimulatory 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 inducible MyD88/CD40 switch activating downstream NF-κB-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 antitumor 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 a 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-κB and PI3K/AKT.

MyD88/CD40 switch by a CID, CD123 ENG T cells maintained their antigen specificity, exhibited superior effector function under conditions of repeated stimulation and mediated enhanced anti-tumor activity in different AML xenograft models. Although the effect of the inducible MyD88/CD40 molecule on bystander T-cell activation was not specifically evaluated, these data support further development of this promising engager T-cell platform.

Disclosures

No conflicts of interest to disclose.

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