the risk of short- and long-term toxicity may outweigh the gain. Specifically concerning are the risk of opportunistic viral infections, such as progressive multifocal leukoencephalopathy, and secondary myelodysplastic syndrome/acute myeloid leukemia. It does remain difficult to attribute the occurrence of these particular risks specifically to lymphodepleting chemotherapy/CAR T-cell therapy or to prior or later treatment lines. Improving our ability to discern patients with a low vs high risk of poor outcome is needed to better select patients for this treatment, which also comes with a great economic burden and is not yet widely available globally due to high costs, complex logistics, and limited manufacturing slots.

Last, there is a negative correlation between long-term remission and POD24 and/or high total metabolic tumor volume, a correlation that also holds for CAR-T cell therapy. Importantly, data from this trial confirm that the use of bendamustine, especially within 6 months prior to apheresis, can negatively affect the starting material and thus CAR T-cell function.

How can we further improve this treatment? Should we administer it as second- or third-line therapy, aim at reducing tumor volume by more aggressive bridging therapy, or find methods of better selection of patients? All of these are valid and open questions.

In conclusion, Neelapu et al show that CD19 CAR T-cell therapy provides a highly effective and relatively safe treatment strategy for patients with R/R FL, but transparent reporting of all long-term side effects and long-term efficacy is important. Several randomized controlled trials are currently ongoing. Our biggest challenge will be to assign CAR T its appropriate position in the treatment algorithm of FL, especially if cure ultimately may not be achieved.

Conflict-of-interest disclosure: S.H.T. reports honoraria from and consulting/advisory role for Incyte, Kite, a Gilead Company, Takeda, Roche, and BMS (all paid to the institution). M.J.K. reports honoraria from and consulting/advisory role for BMS/Celgene, Kite, a Gilead Company, Miltenyi Biotec, Novartis, Adicet Bio, Mustang Bio, and Roche; research funding from Kite, a Gilead Company, and travel support from Kite, a Gilead Company, Miltenyi Biotec, Novartis, and Roche (all paid to the institution). ■

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https://doi.org/10.1182/blood.2023022796

IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Dao et al, page 507

Two to tango: engineered T cells against AML

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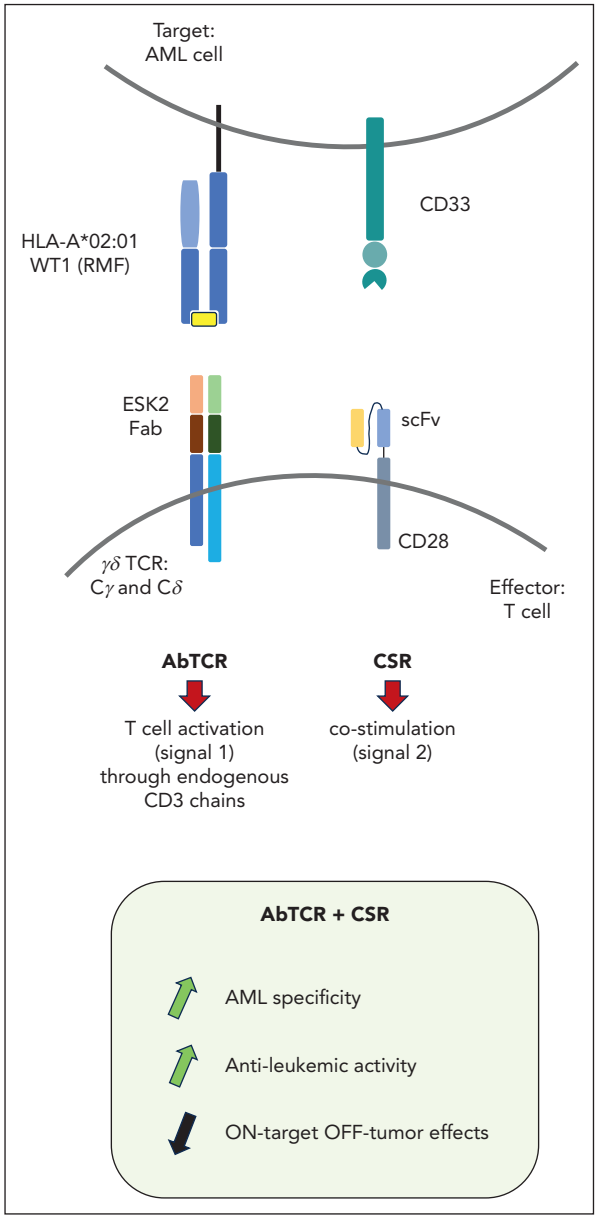
In this issue of *Blood*, Dao et al[1] present a new dual-antigen targeted engineered T-cell platform for safe and efficient T-cell therapy of acute myeloid leukemia (AML).

Development and clinical implementation of targeted T-cell therapy for AML has so far been impeded by the phenotypic similarities between malignant and normal myelopoiesis, the paucity of tumor-specific antigens, the complex bone marrow microenvironment, and AML disease heterogeneity. Now, Dao et al redirected T cells against Wilms tumor 1 (WT1) with a novel antibody T-cell receptor (AbTCR) construct and exploited CD33 as input to activate a chimeric costimulatory signaling receptor (CSR) to enhance specificity, safety and efficacy (see figure). From their phage-display library, the authors identified new antibodies (ESK2) recognizing the WT1 RMF (RMFPNAPYL) peptide/HLA-A*02:01 complex with enhanced specificity compared with their previously identified ESK1 antibodies. The fragment antigen binding regions of 2 lead candidates were linked to the constant chains of the γδ T-cell receptor (TCR) and expressed in a viral vector along with a CSR recognizing CD33 through a single-chain variable fragment (scFv) linked to CD28. When human T cells transduced with AbTCR + CSR encountered AML cells expressing WT1 (RMF)/HLA-A*02:01 and CD33, the T cells recognized both target antigens and received T-cell activation signals from the γδ TCR/CD3 complex (signal 1, by AbTCR) and CD28 costimulation (signal 2, by CSR) (see figure). Investigation of engineered T-cell function showed that AbTCR$^+$ T cells specifically recognized and killed WT1 (RMF)/HLA-A*02:01$^+$ AML targets and that both killing and interferon-γ production were increased on delivery of CD28 costimulation through the CSR (see figure). The CSR acted in *cis*, requiring both the WT1 (RMF)/HLA-A*02:01 complex and CD33 expressed on the same target cell. More important, no toxicity against normal



Dual-antigen targeting in AML with chimeric receptors enhances specificity and efficacy of engineered T cells. Schematic of the approach. AbTCR, chimeric receptor using the fragment antigen binding (Fab) region of an ESK2 antibody recognizing the WT1 (RMF [RMFPNAPYL]) peptide/HLA-A*02:01 complex linked to the Cγ and Cδ regions of the γδ T-cell receptor (TCR). CSR, chimeric costimulatory signaling receptor using a single-chain variable fragment (scFv) recognizing CD33 linked to parts of the CD28 costimulatory molecule. Box: overall outcome of the approach engineering T cells with AbTCR + CSR.

peripheral blood mononuclear cells, granulocytes, or cord blood $CD34^+$ cells was detected in vitro. In vivo in mouse xenograft models, strongest antileukemic activity was observed when mice were treated with T cells expressing both AbTCR + CSR. Thus, the proposed AbTCR + CSR combination resulted in increased AML specificity and enhanced antileukemic activity while sparing normal hematopoiesis and reducing on-target off-tumor activity of the engineered T cells (see figure).

Being highly overexpressed in hematologic and solid tumors, with restricted low expression in some adult normal tissues (reproductive organs, kidney podocytes, mesothelial lining, and $CD34^+$ cells), and exerting critical biological functions in tumors, WT1 is an intensively investigated highly prioritized oncofetal tumor-associated antigen. WT1 has several immunogenic epitopes presented in the context of human leukocyte antigen (HLA) class I. The WT1 (RMF)/HLA-A*02:01 epitope targeted in the study by Dao et al is historically the most investigated WT1 epitope in vaccine or TCR-based adoptive cell transfer studies. Safety and therapeutic benefit have been reported in 12 patients with AML infused prophylactically with WT1 TCR-transgenic T cells after allogeneic hematopoietic stem cell transplant.[2] However, the RMF epitope requires the immunoproteasome for natural processing,[3] introducing some uncertainties about cell surface presentation in malignant cells. Some recent analyses are pointing toward a potential immune escape mechanism after WT1 TCR–T-cell therapy, where reduced levels of specific immunoproteasome subunits were reported in 2 resistant patients.[4] Immunoproteasome-independent WT1 epitopes and corresponding class I restricted αβ TCRs have also been identified, opening new interesting therapeutic prospects for targeting WT1.[4-6] Eventually, an AbTCR against a WT1 epitope processed by standard proteasomes could be developed.

The concept of splitting the T-cell activation and costimulation signals to enhance target specificity and engineered T-cell function has been explored in the field for over a decade,[7] but has recently gained momentum in various combinations with conventional chimeric antigen receptors (CARs) or native or transgenic αβ TCRs.[8,9] The possibility of simultaneously targeting both intracellular and cell surface antigens in a cooperative manner, with αβ TCRs or with AbTCRs, significantly broadens the number of potentially targetable antigens and allows the exploitation of their different signaling sensitivity thresholds. A novel dual-antigen targeting strategy for AML was recently presented, targeting 2 cell surface receptors with scFvs, combining a CAR with a chimeric costimulatory receptor.[10] Target antigens were chosen on the basis of differential antigen density between normal and malignant hematopoiesis identified on a set of samples from patients with AML and healthy donors.[10] This T-cell engineering strategy allowed tuning of engineered T-cell sensitivity by targeting adhesion G protein-coupled receptor E2 (ADGRE2) and C-type lectin domain family 12 member A (CLEC12A), and improved antileukemic activity with reduced toxicity to normal hematopoiesis, compared with single-antigen targeting. The study by Dao et al combined WT1 with CD33 targeting, WT1 by itself already having an excellent safety and specificity

profile. When combined with a CSR exploiting the highly expressed pan-myeloid antigen CD33 for the delivery of costimulation, enhanced leukemia specificity and antileukemic activity were demonstrated. Although CD33 antigen densities were characterized recently,[10] we have no information available regarding the densities of WT1 (RMF)/HLA-A*02:01 on AML and normal hematopoietic stem and progenitors. It would be interesting to evaluate whether the combination of AbTCR + CSR shifts the activation threshold of the AbTCR toward recognition of lower-density target cells.

In conclusion, Dao et al present a compelling preclinical proof of concept for a novel approach of dual-antigen targeting for AML with engineered T cells conferring enhanced specificity, safety, and antileukemic activity. Clinical translation is eagerly awaited.

Conflict-of-interest disclosure: C.A. receives licensing fees and royalties from Immatics (through previous institution, Baylor College of Medicine); participated in advisory boards for Kite/Gilead, Janssen, and Celgene/Bristol Myers Squibb; received sponsored travel from Gilead; and has several patents and pending patent applications in the field of engineered T-cell therapies. ■

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https://doi.org/10.1182/blood.2023023004

LYMPHOID NEOPLASIA

Comment on Heger et al, page 522

Predicting outcomes in CNS lymphoma with ctDNA

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In this issue of *Blood*, Heger and colleagues describe a novel tool for predicting clinical outcome in central nervous system (CNS) lymphomas that is based on clinical features, radiographic response to induction, and circulating tumor DNA (ctDNA) detection.[1]

Lymphomatous involvement of the CNS is a much-feared clinical scenario with an uncertain prognosis.[2] Whether it represents a primary CNS lymphoma (PCNSL) or occurs in the context of a systemic aggressive lymphoma at initial diagnosis or relapse, outcomes are relatively poor. Even today, clinical features such as age and performance status remain the basis for risk stratification.[3,4] High-dose methotrexate-containing regimens that culminate in myeloablative chemotherapy and autologous stem cell transplant are associated with the best outcomes in young fit patients and may be curative. Older patients or those with comorbidities are often ineligible for intensive strategies and are consolidated with radiotherapy, with increased risk of cognitive impairment. Response assessments rely on magnetic resonance imaging (MRI), which may not discriminate between residual scar and persistent disease. Consequently, new tools that can reliably distinguish risk subgroups and define tumor response are needed to inform treatment intensity and avoid unnecessary toxicity in these often frail patients.

In systemic diffuse large B-cell lymphoma, ctDNA is a promising indicator of residual disease that is under intensive investigation.[5] In CNS lymphomas, the very low levels of ctDNA present in plasma and difficulties in obtaining primary tissue and serial cerebrospinal fluid (CSF) specimens for study have challenged investigators working to apply a similar strategy to patients with lymphoma limited to the CNS.[6] Both single-gene polymerase chain reaction assays for the detection of *MYD88*^{L265P}, the most frequent mutation in PCNSL, and next-generation sequencing (NGS)-based approaches have been applied to CSF and plasma with variable results. In a recent study by Mutter and colleagues, an ultrasensitive high-throughput sequencing approach detected ctDNA in 61 of 78 plasma specimens and 24 of 24 CSF samples collected from patients with PCNSL or isolated secondary CNSL.[7] Notably, detection of plasma ctDNA at diagnosis, during treatment, or at completion of therapy, representing residual disease, was associated with poor outcomes.

Heger et al here report on development of a robust prognostic model based on an ultrasensitive ctDNA sequencing approach in samples from 67 patients with CNSL (58 with PCNSL; 9 with