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CLINICAL TRIALS AND OBSERVATIONS

Comment on Lu et al, page 321

CD7 CAR: sword and shield

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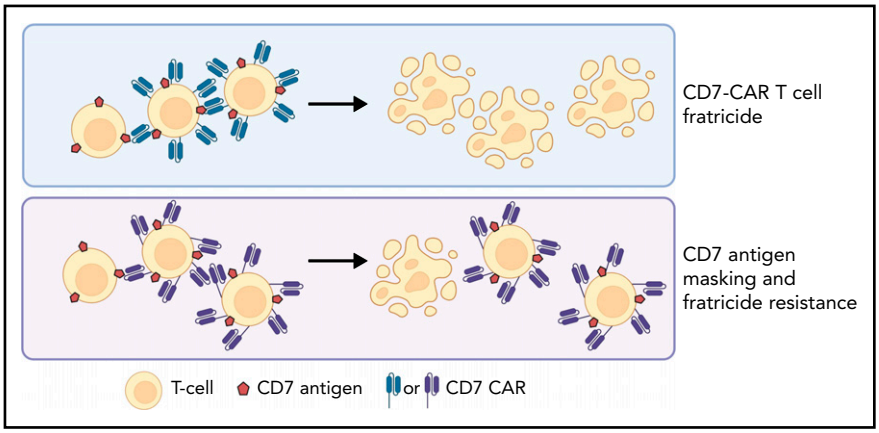
In this issue of *Blood*, Lu et al[1] describe a novel approach for generating autologous CD7-specific chimeric antigen receptor (CAR) T cells from peripheral blood T cells that can naturally overcome CD7-directed fratricide; these T cells could be useful for treating patients with T-lineage malignancies. Expression of a CD7 CAR in T cells promotes sequestration of the available CD7 antigen, resulting in an expansion of naturally selected fratricide-resistant CD7 CAR T cells. The authors report that these CD7 CAR T cells were well tolerated in 20 patients with relapsed or refractory T-cell acute lymphoblastic leukemia (T-ALL) or T-cell acute lymphoblastic lymphoma (T-LBL) and induced complete remission (CR) in most of them.

Developing CAR T-cell therapies for T-lineage leukemia and lymphoma has been complicated by the lack of differential antigen expression between cancerous and healthy T cells. CD7, a transmembrane glycoprotein member of the immunoglobulin superfamily, has been widely evaluated as an antigen target, given its high expression on malignant T cells.[2] However, its expression on normal T cells induced strong fratricide in CD7 CAR T cells, thus requiring additional genetic modifications to prevent their self-elimination. These approaches include disrupting *CD7* gene expression using CRISPR or base editing[3-5] or retaining CD7 protein intracellularly via a protein expression blocker.[6,7]

In their article, Lu et al explained how they generated CD7 CAR T cells by using lentiviral transduction of bulk T cells followed by a selection of CAR T cells that retain CD7 expression but resist self-targeting (NS7CAR). The presence of both the CD7 CAR and the CD7 protein in these T cells indicated that fratricide resistance was achieved by CAR-mediated CD7 epitope masking or by intracellular sequestration of the CD7 protein (see figure). Substantial T-cell fratricide that was observed after CAR transduction suggests that expanded NS7CAR T cells had a suitable ratio of the CD7 and CAR expression levels required to provide effective shielding. The expanded NS7CAR T cells had a predominantly $CD8^+$ memory phenotype and demonstrated specific antitumor activity in preclinical models of human T-ALL.



Expression of CD7-specific CARs on T cells promotes fulminant fratricide of $CD7^+$ T-cell subsets. Lu et al generated a CD7 CAR that sequesters the CD7 antigen away from external recognition, which leads to an expansion of fratricide-resistant CD7 CAR T cells. In a phase 1 clinical study, these naturally selected CD7 CAR T cells produced CRs in patients with refractory or relapsed T-cell malignancies.

On the basis of these results, the authors developed a first-in-human phase 1 clinical study and established a current good manufacturing practice (cGMP) protocol in which NS7CAR T cells were generated from patients with treatment-refractory T-ALL or T-LBL or from their hematopoietic stem cell donors. For autologous products, circulating malignant blasts were removed by positive selection of $CD3^+$ or $CD4^+/CD8^+$ normal T cells. Patients were infused by using 1 of 3 different dose levels determined by the medical team on the basis of the patient's clinical condition. Twenty patients ranging in age from 3 to 47 years received the therapy, with 15 of 20 receiving a dose of 1×10^6 NS7CAR T cells per kilogram after fludarabine-cyclophosphamide lymphodepletion. Of note, 2 patients with a history of previous hematopoietic stem cell transplantation received NS7CAR T cells generated from donor-derived T cells because of lymphopenia. Nineteen patients developed cytokine release syndrome (CRS), 18 had mild symptoms with 1 patient developing grade 3 CRS, and only 2 patients developed neurotoxicity (grade 1). Other toxicities included neutropenia, lymphopenia (n = 20), thrombocytopenia (n = 15), and transient elevation of liver function tests

(grade 4). There was only 1 case of cytomegalovirus reactivation.

At the 28-day evaluation, 19 patients achieved measurable residual disease–negative CR or incomplete CR (CRi), and 5 of 9 patients who had extramedullary disease also achieved a response. Most patients subsequently proceeded to transplantation, with no relapses documented after a median follow-up of 142.5 days. Notably, the activity of NS7CAR T cells induced rapid ablation of circulating $CD7^+$ T cells and natural killer (NK) cells, which were promptly replenished by $CD7^-$ subsets, thus avoiding prolonged T-cell and NK-cell aplasia. Lu et al concluded that NS7CAR T cells are well tolerated and are effective against $CD7^+$ T-cell malignancies.

The study by Lu et al adds to the mounting evidence that CD7 is a clinically effective CAR target for T-cell malignancies.[7,8] NS7CAR T cells show excellent preliminary results, comparable to the results of the previously published phase 1 trial that used donor-derived cells modified to induce intracellular retention of CD7.[7] Limitations of the Lu et al study include the limited number of patients and the fact that the trial design did not allow for maximum tolerated dose determination, and most patients received a dose of 1×10^6 T cells per kilogram. More data are needed to gain better insights regarding the safety of this procedure.

Generation of naturally fratricide-resistant CD7 CAR T cells has an impact on streamlining the manufacturing process and on the cost-effectiveness of therapy. However, factors affecting the selection of NS7CAR T cells ex vivo and regulating their antileukemic potency warrant additional investigation. In a recent report, Cooper et al[4] demonstrated that lentiviral expression of a similar second-generation CAR (endowed with a 41BB costimulation domain and a binder identical to the one used by Lu et al [clone TH69]) induced fulminant T-cell fratricide, resulting in the development of CD7/T-cell receptor double-edited allogeneic CD7 CAR T cells. These double-edited CAR T cells are currently being evaluated in a phase 1 clinical trial (NCT04984356). In addition, a clinical trial of unedited T cells that express a different CD7 CAR construct has recently been opened at Baylor College of Medicine (NCT03690011). These clinical studies should help establish whether CD7 is a prime target in T-cell malignancies and should contribute to the identification of effective methods of fratricide evasion in CD7 CAR T cells. Most importantly, these studies should provide much-needed targeted immunotherapies for patients with refractory or relapsed T-cell malignancies who otherwise have few effective treatment options.

Conflict-of-interest disclosure: M.P.V. is a member of the Rally! Foundation Medical Advisory Board. The remaining author declares no competing financial interests. ■

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Tracy et al, page 335

Uncovering $CD4^+$ T-cell exhaustion in B-ALL

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In this issue of *Blood*, Tracy et al[1] describe a key subset of exhausted $CD4^+$ T cells in Philadelphia chromosome positive (Ph^+) B-cell acute lymphoblastic leukemia (B-ALL) that modulates antileukemic effects. The authors use a nonirradiated immunocompetent murine model of Ph^+ B-ALL and primary patient samples to dissect the CD4 T-cell immune response and to provide a rationale for combination therapies of tyrosine kinase inhibitors that target the BCR-ABL oncoprotein and programmed death-ligand1 (PD-L1) blockade to reverse the exhausted state of $CD4^+$ T cells and enhance the clearance of leukemia.

T-cell activation is orchestrated by intrinsic positive and negative T-cell regulators, with inhibitory molecules setting thresholds to avoid uncontrolled immune activation. However, within tumors, continuous antigen exposure in the setting of an immunosuppressive microenvironment shifts the T-cell activation toward an inhibitory exhausted state that results in loss of antitumor function. Although $CD8^+$ T-cell exhaustion in chronic infections and cancers has been explored in detail,[2] the characteristics and pathological impact of $CD4^+$ T-cell exhaustion in cancer, and specifically in hematologic malignancies, is now becoming apparent. The expanded cytotoxic role of $CD4^+$ T cells predicts a pattern of T-cell exhaustion comparable to that of the $CD8^+$ exhausted T-cell phenotype. Indeed, recent discovery of $CD4^+$ T cells with exhaustion properties in murine melanoma models and the association of phenotypically exhausted $CD4^+$ T cells