Adoptive therapy with T cells genetically modified to express synthetic CD19-specific chimeric antigen receptors (CAR) is successful in inducing remissions in a subset of patients with CD19-positive B-cell malignancies. A similar approach targeting BCMA with CAR T cells has shown promising results in multiple myeloma (1). Genetic instability and immune evasion are hallmarks of cancer and it is perhaps not surprising that targeting a single molecule might lead to the outgrowth of antigen-low or -negative tumor cells. Indeed, relapse with CD19-negative tumor cells is a major mechanism of failure in patients with leukemia and lymphoma receiving CD19 CAR T cells, and targeting more than one B-cell lineage molecule with CAR T cells is under active investigation (2).

In this issue of Blood Cancer Discovery, Fernández de Larrea and colleagues extend this approach to multiple myeloma. They demonstrate in preclinical models of multiple myeloma that targeting BCMA and GPRC5D simultaneously with T cells engineered to express chimeric antigen receptors specific for these antigens may prevent tumor cell escape. Strategies to simultaneous target multiple antigens are needed to fully capitalize on the promise of this therapeutic strategy. In this issue of Blood Cancer Discovery, Fernández de Larrea and colleagues demonstrate in preclinical models of multiple myeloma that targeting BCMA and GPRC5D simultaneously with T cells engineered to express chimeric antigen receptors specific for these antigens may prevent tumor cell escape.

See related article by Fernández de Larrea et al., p. 146 (3).

**Summary:** Adoptive T-cell therapy targeting a single tumor antigen can induce remissions of hematologic cancers but relapses often occur due to the outgrowth of tumor cells with absent or low expression of the antigen. Strategies to simultaneously target multiple antigens are needed to fully capitalize on the promise of this therapeutic strategy. In this issue of Blood Cancer Discovery, Fernández de Larrea and colleagues demonstrate in preclinical models of multiple myeloma that targeting BCMA and GPRC5D simultaneously with T cells engineered to express chimeric antigen receptors specific for these antigens may prevent tumor cell escape.

**Dual Targeting with CAR T Cells to Limit Antigen Escape in Multiple Myeloma**

Sylvain Simon and Stanley R. Riddell

Adoptive therapy with T cells genetically modified to express synthetic CD19-specific chimeric antigen receptors (CAR) is successful in inducing remissions in a subset of patients with CD19-positive B-cell malignancies. A similar approach targeting BCMA with CAR T cells has shown promising results in multiple myeloma (1). Genetic instability and immune evasion are hallmarks of cancer and it is perhaps not surprising that targeting a single molecule might lead to the outgrowth of antigen-low or -negative tumor cells. Indeed, relapse with CD19-negative tumor cells is a major mechanism of failure in patients with leukemia and lymphoma receiving CD19 CAR T cells, and targeting more than one B-cell lineage molecule with CAR T cells is under active investigation (2).

In this issue of Blood Cancer Discovery, Fernández de Larrea and colleagues extend this approach to multiple myeloma and evaluate strategies for simultaneously targeting B-cell maturation antigen (BCMA) and G-protein-coupled receptor class C group 5D (GPRC5D) with CAR T cells (3). Their study demonstrates that it is feasible to simultaneously target GPRC5D and BCMA and provides insight into optimal dual targeting designs, thus broadening the arsenal and potential efficacy of cellular therapies for multiple myeloma.

CARs are most often comprised of the variable region of a mAb (single chain variable fragment, scFv) that is specific for a cell surface tumor antigen and is linked to an intracellular signaling domain containing the CD3ζ chain and either CD28 or 4-1BB costimulatory domains to activate T-cell effector functions. Two CD19-targeted CAR therapies have obtained FDA approval for diffuse large B-cell lymphoma (DLBCL) and B-cell acute lymphoblastic leukemia (B-ALL), paving the way for the development and evaluation of CAR T-cell therapies targeting a vast array of different antigens expressed on other malignancies.

Early-phase clinical trials demonstrated that BCMA (TNF Receptor Superfamily Member 17 or TNFRSF17), which is restricted in its expression to normal and malignant plasma cells, can be targeted effectively with CAR T cells, bispecific T-cell engaging antibodies (BiTES), and antibody conjugates (1). The results of a phase I dose escalation study of a BCMA-specific CAR containing a 4-1BB/CD3ζ signaling domain in patients with refractory multiple myeloma demonstrated frequent but manageable toxicities, antitumor efficacy, and prolongation of progression-free survival (PFS) compared with historical data (1). Although the initial response rates to BCMA directed therapies are impressive, the duration of responses is short in many patients, and disease progression can result from the escape of BCMA-low or -negative tumor cells (4).

The density and stability of the target antigen on the surface of the tumor cell is a major factor that affects achieving durable antitumor efficacy with CAR T cells (5). BCMA is subject to cleavage by the ubiquitous γ-secretase complex, which results in variable and heterogeneous expression on tumor cells and poses a barrier to complete tumor elimination with BCMA-targeted therapies (6). Our group recently demonstrated that BCMA expression is markedly increased in multiple myeloma cells in vitro and in vivo by exposure to a small-molecule γ-secretase inhibitor (GSI), and this combination improved antitumor efficacy of BCMA CAR T cells in preclinical models (7). The combination of BCMA CAR T cells with a GSI is now being tested in a clinical trial (NCT03502577) to determine whether this approach is safe and improves the depth and durability of antitumor responses.

Increasing the amount of antigen on tumor cells with a GSI may increase the susceptibility of BCMA-low tumor cells to CAR T cells but would not help eliminate tumor cells with genetic loss or silencing of BCMA expression. Thus, identifying additional targets in multiple myeloma for CAR T cells is a high priority to limit tumor escape from selective immune pressure. To safely accomplish multispecific targeting in MM,
it is necessary to identify molecules that are only expressed on myeloma cells or the plasma cell lineage to avoid recognition of other normal tissues. Smith and colleagues previously identified GPRC5D to be highly expressed on multiple myeloma cells and hair follicles, which are considered an immune privileged compartment. In prior work, they selected a scFv that binds GPRC5D and demonstrated that GPRC5D-specific CAR T cells were effective in eliminating MM xenografts in NSG mice, and safe in nonhuman primates (8). Thus, GPRC5D is an attractive target for clinical translation in multiple myeloma, particularly in combination with BCMA.

One can envision dual targeting of BCMA and GPRC5D in four different ways: (i) the parallel production of two mono-specific CAR T-cell populations that are coadministered or administered sequentially; (ii) genetically modifying T cells to coexpress two distinct CARs by transduction with individual viral vectors encoding each construct; (iii) designing a single “bicistronic” vector that expresses two CARs in the same T cell; and (iv) expressing the two scFv on a single chimeric protein (tandem CAR; Fig. 1). Selecting the optimal approach to translate to patients may depend on the expression of each target antigen, the affinities of each scFv, and structural and signaling features of the individual CARs.

In this study, the authors compared three of these strategies for dual targeting of BCMA and GPRC5D in multiple myeloma. They produced two mono-specific CAR T-cell products in parallel that were pooled together at a 1:1 ratio before infusion, developed bicistronic constructs to coexpress BCMA and GPRC5D-specific CARs in T cells, and designed a tandem dual scFv single-stalk CAR with the ability to bind both BCMA and GPRC5D. In each of the three approaches, the BCMA scFv was constructed with a 4-1BB costimulatory domain because this BCMA 4-1BB/CD3ζ mono-specific CAR is currently being tested in the clinic. The anti-GPRC5D CAR was designed in both CD28/CD3ζ and 4-1BB/CD3ζ formats for single and dual antigen targeting. The authors found that all dual-targeting strategies conferred specific killing of tumor cells expressing both BCMA and GPRC5D antigens and tumor cells expressing only BCMA or GPRC5D. More detailed in vitro characterization of T cells expressing each of the dual or mono-specific CARs including cytokine production, signal transduction, and transcriptional profiling after antigen stimulation might be necessary to reveal important functional differences between these strategies. Phosphoproteomic and RNA-sequencing analysis of mono-specific CARs have uncovered key features associated with different constructs that have provided guidance for designing more effective CAR T cells (9).

The authors then compared each dual targeting approach with BCMA and GPRC5D mono-specific CAR T cells in an in vivo tumor xenograft model of multiple myeloma in which the tumor cells expressed both BCMA and GPRC5D. The infusion of a high dose of mono-specific or dual-specific CAR T cells rapidly cleared tumor cells from all mice. When the mice were rechallenged with tumor cells that lacked BCMA (BCMAko) to mimic relapse after target antigen loss, mice treated with the dual targeting and GPRC5D mono-specific strategies were protected, whereas those treated with mono-specific BCMA CAR T cells failed to control tumor
challenge providing support for the superiority of the BCMA/GPRC5D dual targeting strategy.

To mimic the clinical situation where antigen negative variants may be preexisting at the time of treatment, mice were engrafted with a tumor cell mixture containing 5%–10% BCMAko cells. To determine the origin of eventual tumor outgrowth using imaging, the authors engineered BCMAwt and BCMAko tumor cells to express distinct luciferase enzymes that required different substrates. As expected mice treated with the BCMA mono-specific T cells progressed with BCMAko tumor cells. Surprisingly, dual targeting CAR designs in which the GPRC5D scFv had CD28/CD3ζ signaling module also failed to control tumors with all mice showing outgrowth of BCMAko tumor cells. The three dual targeting CARs with GPRC5D 4-1BB/CD3ζ signaling quickly eradicated both the BCMAko and BCMAwt tumors. These data are consistent with findings in other CAR therapy settings where 4-1BB costimulation demonstrated reduced tonic signaling, enhanced noncanonical NFκB signaling and decreased expression of apoptotic factors compared with CD28/CD3ζ CARs, all of which translates into enhanced proliferation and persistence of CAR T cells in vivo (9). A similar experiment with mono-specific GPRC5D CAR T cells alone bearing CD28 or 4-1BB costimulation domains would be required to understand the mechanisms underlying the poor efficacy of the GPRC5D/CD28/CD3ζ design. Nevertheless, these data highlight the need for systematic evaluation of costimulatory domains, including those other than 4-1BB and CD28 to determine whether dual targeting CAR designs might be further improved.

Fernández de Larrea and colleagues next investigated which dual targeting strategy would show better antitumor activity at a suboptimal dose of CAR T cells. Using the BCMA-relapse model, they found that the tandem BCMA/GPRC5D CAR conferred worse survival than the two mono-specific T-cell populations pooled together or the bicistronic approach. The design of tandem CAR constructs can be challenging, and in the absence of structural guidance may require the empiric comparison of different scFvs, linkers, and spacer sequences to achieve optimal function. Indeed, minor modifications within the amino acid sequence or the hinge’s length have been shown to have a major impact on the expression and functions of tandem CARs. Notably, a recent study demonstrated the importance of structural studies and systematic construct optimization to generate highly functional tandem CARs targeting BCMA and CS1 antigens in multiple myeloma mouse models (10). CS1 (also known as SLAMF7 or CD3319) is highly expressed by most myeloma cells, but absent from most nonhematologic tissues and represents another relevant target for combination therapies with BCMA antigen for multiple myeloma treatment. Therefore, it remains to be determined whether alternative designs of tandem BCMA/GPRC5D CARs might improve efficacy. The authors also performed therapy at a suboptimal T-cell dose in mice engrafted with multiple myeloma cells expressing both antigens. In the absence of BCMAko cells, the bicistronic approach showed moderately superior efficacy when compared with the mono-specific CAR products pooled together. The tandem CAR strategy performed equivalently to the bicistronic CAR suggesting that, in the BCMA-relapse model, the anti-BCMA moiety of the construct might have been the defective one. The strength of cell–cell interaction assessed by exposure of CAR T cells cocultured with aAPCs to increasing acoustic force ramp was also increased with bispecific T cells compared with mono-specific ones as a putative explanation for the improved survival. Comparison of synapse organization, strength, and composition between the bicistronic and tandem CAR would also be of interest for understanding CAR T-cell–tumor cell interactions.

The collective data from the experiments by Fernández de Larrea and colleagues supports the superiority of the bicistronic approach to dual targeting of BCMA and GPRC5D in multiple myeloma. Although confirmatory studies in larger cohorts of mice and different myeloma models would be ideal, the bicistronic approach possesses inherent advantages that makes it an attractive strategy for initial clinical testing. A single vector that encodes for the two CARs is an easier and less costly manufacturing process than producing two mono-specific products in parallel or transducing a single cell with two different constructs where gene transfer efficiencies can be variable. The stochiometry of surface expression of the two CARs is also likely to be more homogeneous than the cotransduction of two constructs, and the design of bicistronic constructs is less challenging than tandem CARs. The work by Fernández de Larrea and colleagues provides the first evidence that dual targeting of BCMA and GPRC5D will be feasible and this approach has considerable promise for improving progression-free survival after CAR T-cell therapy in multiple myeloma. This approach would not address resistance mechanisms distinct from antigen loss, and clinical trials should be accompanied by detailed analysis of CAR T-cell persistence, migration and function, and of persisting tumor cells to identify other potential barriers to durable efficacy.

Disclosure of Potential Conflicts of Interest

S.R. Riddell reports grants and personal fees from Lyell Immunopharma and personal fees from Juno Therapeutics/a BMS company outside the submitted work; in addition, S.R. Riddell has a patent for “Combination Therapies for Treatment of BCMA-related cancers and autoimmune disorders” pending and licensed to Juno Therapeutics, a BMS company. No potential conflicts of interest were disclosed by the other author.

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