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

Sylvain Simon and Stanley R. Riddell

**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*, 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 De Larrea et al.**

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*, 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 clinical translation in multiple myeloma, particularly in combination with BCMA.

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.

**Figure 1.** Dual targeting strategies for eliminating heterogeneous multiple myeloma cells that express BCMA and GPRC5D. Monospecific BCMA CAR T-cell therapy (left) induces robust initial antitumor responses in patients with multiple myeloma, but many patients relapse with BCMA\(^{low/\text{nega}}\) expression. Upfront dual targeting of BCMA and GPRC5D with CAR T cells (right), which can be performed with different strategies can improve the depth and duration of antitumor responses by eliminating antigen loss variants (Created with BioRender.com).
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% BCMA<sup>ko</sup> cells. To determine the origin of eventual tumor outgrowth using imaging, the authors engineered BCMA<sup>ko</sup> and BCMA<sup>wt</sup> tumor cells to express distinct luciferase enzymes that required different substrates. As expected mice treated with the BCMA mono-specific CAR T cells progressed with BCMA<sup>wt</sup> tumors. 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 BCMA<sup>wt</sup> tumors. The three dual targeting CARs with GPRC5D 4-1BB/CD3ζ signaling quickly eradicated both the BCMA<sup>ko</sup> and BCMA<sup>wt</sup> 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 <i>in vivo</i> (9). A similar experiment with persistence of CAR T cells targeting BCMA and CS1 antigens 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 stoichiometry 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 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.

Published first August 3, 2020.

REFERENCES


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

Sylvain Simon and Stanley R. Riddell

Blood Cancer Discov  Published OnlineFirst August 3, 2020.

Updated version  Access the most recent version of this article at:
doi: 10.1158/2643-3230.BCD-20-0122

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://bloodcancerdiscov.aacrjournals.org/content/early/2020/07/30/2643-3230.BCD-20-0122. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.