IN THE SPOTLIGHT

Overcoming Antigen Escape with CAR T-cell Therapy

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Summary: Sotillo and colleagues describe the molecular events associated with apparent loss of target antigen expression following CART-19 therapy. We propose that broader immune activation is required to prevent outgrowth of tumor antigen escape variants following targeted therapies. Cancer Discov, 5(12): 1238–40. ©2015 AACR.

See related article by Sotillo et al., p. 1282 (5).

T-cell function can be specifically redirected to a chosen tumor-associated antigen through expression of a chimeric antigen receptor (CAR). CARs consist of an antigen recognition domain, usually a single-chain variable fragment of an immunoglobulin, linked to T-cell activation (CD3ζ) and costimulation (commonly CD28 or 4-1BB) intracellular signaling domains. Upon antigen recognition, the T cell becomes activated, proliferates, and secretes cytokines, as well as directly lysing the targeted cell. We, and others, have shown that targeting cancer cells with CD19-specific CARs has immense promise in the context of B-cell acute lymphoblastic leukemia (B-ALL; ref. 1).

CD19 is a marker expressed on B cells as well as most B-cell malignancies; therefore, effective CAR T-cell therapy may result in B-cell aplasias. B-cell aplasia is considered to be an acceptable “on-target off-tumor” toxicity that can be managed clinically with intravenous immunoglobulin infusion. In addition, CD19 is proposed to play a role in B-cell malignancy development and progression, indicating that mutation of CD19 may be detrimental to tumor cell viability and proliferation. However, loss of CD19 and outgrowth of CD19- tumor cells have been reported in both pediatric and adult responders following CD19-targeted CAR T-cell therapy. At the 2014 Annual Society of Hematology meeting, The University of Pennsylvania/Children’s Hospital of Pennsylvania reported 10 of 30 pediatric patients relapsed, and 5 of these patients experienced antigen-negative relapse (2). Results from Memorial Sloan Kettering Cancer Center (MSKCC) presented at the 2015 American Society of Clinical Oncology annual meeting demonstrated that 2 of 14 patients experiencing disease relapse had outgrowth of antigen-negative tumor cells (3). The NCI has recently described 2 patients who achieved complete response following CD19-targeted CAR T-cell therapy, but relapsed with CD19-negative disease (4). It is clear from these reports that loss of antigen expression on tumor cells presents a problem that is not specific to any single institute. Antigen loss renders CAR T cells ineffective against B-cell tumors and may have implications for the broader success of CAR T-cell therapies, regardless of the tumor-associated antigen (TAA) targeted.

In this issue, Sotillo and colleagues investigated the molecular events associated with apparent loss of CD19 expression in patients with relapsed disease following CD19-targeted CAR T-cell therapy (5). These studies demonstrated that 1 patient with a CD19 genetic alteration and a second patient without genetic alteration both had increased levels of a CD19 isoform that skipped exon 2 (Δex2). The authors demonstrated that CD19 mRNA can be alternatively spliced, leading to decreased levels of the full-length CD19 isoform and increased levels of the Δex2 isoform. The authors show that the Δex2 isoform of CD19 is more stable than the full-length isoform and can partially rescue functional defects associated with complete loss of expression of CD19 while leading to loss of the cognate CD19 epitope necessary for CART-19 recognition. The authors used cell lines to determine that the splice factor SRSF3 was involved in the inclusion of CD19 exon 2. Subsequently, samples from 2 patients with CD19-negative relapses were shown to have lower amounts of SRSF3 compared with earlier samples. The authors cannot determine whether the mutations identified in these patients select for Δex2 isoform (“permissive” model) or actively redirect the splicing machinery (“instructive” model). It is also plausible that alternative splicing may be epigenetically regulated.

This study, along with evidence of splice variant melanoma cells that are resistant to vemurafenib, is evidence of splice-based adaptations in tumor cells leading to outgrowth of tumor escape variant cells (6). This indicates that future CAR T-cell, monoclonal antibody, or any other targeted therapy may drive the outgrowth of tumor antigen escape variants. Therefore, therapies that recruit a more broadly targeted antitumor immune response will be required if tumor-targeted immunotherapy in general, and CAR T-cell therapy specifically, is going to have universally effective and durable antitumor effects.

One strategy to overcome the outgrowth of antigen loss tumor cells is to equip T cells with two CARs targeted to different TAs. Targeting two antigens would mean that escape of CAR T-cell-mediated destruction would require simultaneous mutations in two genes. Preclinical studies

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have demonstrated that this approach resulted in increased antitumor function compared with a T cell expressing a single CAR (7). Importantly, expression of two CARs in one T-cell population was demonstrated to offset tumor antigen escape. This approach may be a promising strategy for the future CAR T-cell therapy. However, identifying two TAAs on one tumor that can be both safely and effectively targeted with CAR T cells may prove difficult for some malignancies. Recruitment, restimulation, or rescue of an existing endogenous antitumor immune response may allow for a more broadly targeted antitumor response involving many different effector cell types. Indeed, the endogenous immune system is capable of epitope spreading, which is the generation of an immune response to epitopes distinct from the initial immune-targeted epitope. Immune effector cells associated with tumor cells are often suppressed by the inhibitory immune microenvironment. Inhibitory factors may include ligands for receptors that dampen immune responses (e.g., PD-L1 and/or PD-L2). One approach to modulate the tumor microenvironment is through the addition of checkpoint inhibitors. Immune checkpoint inhibitors prevent ligation of inhibitory receptors (e.g., PD-1 and/or CTLA-4) expressed on T cells, natural killer cells, and other immune effector cells of the immune system. Checkpoint blockade therapy, specifically using monoclonal antibodies that bind and block CTLA-4 or PD-1 T-cell–inhibitory receptors or block the ligands (PD-L1) from interacting, has shown promise in clinical trials. A recent study investigated the molecular determinants of responders and nonresponders to PD-1 antibody and CTLA-4 antibody therapy in patients with non–small cell lung cancer and metastatic melanoma, respectively. Clinical benefit was associated with mutational load and neoantigens in the patient tumor cells (8). It is hypothesized that mutations in tumor cells may generate neoantigens that are presented in
In order to optimize immunotherapy of cancer, the mechanisms whereby tumor cells mediate immune escape need to be understood. Thus, targeting other tumor-associated antigens (TAAs) in both the hematologic and solid tumor settings, this report highlights the need to understand the unresponsiveness of these tumor-specific T cells. Indeed, combining CAR T-cell therapy with checkpoint blockade has shown promise in preclinical studies and will be tested in an upcoming clinical trial at Baylor College of Medicine in Texas (NCT00586391). However, this may not be sufficient to prevent outgrowth of tumors that are not recognized by the CAR or endogenous T cells.

Other tactics to improve antitumor efficacy and recruit endogenous immune cells include the development of “armored” CAR T cells that are further modified to secrete immune stimulatory cytokines (e.g., IL12) or express co-stimulatory molecules (e.g., CD40L or 4-IBBL). Further modifying CAR T cells to secrete IL12, a proinflammatory cytokine, resulted in increased antitumor efficacy, as we and others have previously shown (9). Not only does IL12 act in an autocrine fashion and increase the antitumor function of the CAR T cell itself, it is also found to modulate the tumor microenvironment, rendering T cells resistant to suppression from regulatory T cells and myeloid-derived suppressor cells. This approach will be evaluated in the context of solid tumors in patients with ovarian cancer in a trial at MSKCC (NCT02498912). In another strategy, CAR T cells modified to constitutively express CD40L were shown to mature and activate dendritic cells, as well as modulate the tumor phenotype, leading to increased tumor cell immunogenicity (10). Expression of other co-stimulatory ligands, specifically 4-IBBL and CD80, on CAR T cells has also been demonstrated to enhance CAR T-cell function (11). We hypothesize that these immune-stimulatory effects combined will increase the antitumor effect mediated by CAR T cells, as well as recruit an effective endogenous tumor response to act against the tumor and enhance functional activity of adoptively transferred T cells in glioblastoma. Mol Ther 2013;21:2087–101.

In this issue, Sotillo and colleagues demonstrate unique tumor-specific T cells resistant to suppression from regulatory T cells and myeloid-derived suppressor cells. This report highlights the need to understand the mechanisms whereby tumor cells mediate immune escape in order to optimize immunotherapy of cancer.

Disclosure of Potential Conflicts of Interest
R.J. Brentjens is a scientific co-founder of, reports receiving a commercial research grant from, has ownership interest (including patents) in, and is a consultant/advisory board member for JUNO Therapeutics. No potential conflicts of interest were disclosed by the other author.

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