IN THE SPOTLIGHT

Not So FAST: Tumor Cells Resisting Death Drive CAR T-cell Dysfunction

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Summary: In this issue, Singh and colleagues describe a novel tumor-intrinsic mechanism of resistance to chimeric antigen receptor (CAR) T-cell therapy targeting CD19 in B-cell malignancies. They show that reduced expression of death receptor genes in the tumors mediates resistance to killing by CAR T cells, leads to progressive CAR T-cell dysfunction, and is associated with unfavorable clinical outcome in patients.

See related article by Singh et al., p. 552 (7).

Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 is highly effective in refractory B-cell malignancies, inducing response rates in up to 80% to 90% of patients with acute lymphoblastic leukemia (ALL) and various non-Hodgkin lymphoma subtypes (1, 2). However, long-term durability of these responses is observed in only approximately 40% to 50% of these patients, highlighting the need to understand the mechanisms of primary as well as acquired resistance following CAR T-cell therapy. Resistance mechanisms can be categorized into those that are related to the fitness and functional state of the T cells in the apheresis and/or infusion products, and tumor-intrinsic mechanisms of resistance, with multiple levels of cross-talk likely existing between the biology of the tumor cells and the functionality of a patient’s T cells (Fig. 1).

The fitness of the CAR T cells may be affected because of dysfunctional T cells in the leukapheresis product resulting from prior lines of therapy, excessive stimulation or other culture conditions during manufacturing, or insufficient host conditioning preinfusion, or resulting from the effects of the tumor microenvironment on T cells prior to apheresis or after infusion. Indeed, in a cohort of patients with chronic lymphocytic leukemia who were treated with anti-CD19 CAR T-cell therapy, Fraietta and colleagues found that the functional phenotype of the T cells in the leukapheresis product affected the quality of the CAR T-cell product that could be generated, which was in turn associated with clinical efficacy (3). Rossi and colleagues reported an association between the polyfunctionality of the preinfusion CAR T cells and clinical outcome in patients with lymphoma (4). Turtle and colleagues showed that the function of CAR T cells in vivo, as suggested by their expansion and persistence postinfusion, may be influenced by host conditioning prior to infusion (5). The CAR T cells could also become dysfunctional after they traffic to the tumor site due to immunosuppressive mechanisms in the tumor microenvironment mediated by regulatory T cells, myeloid-derived suppressor cells, immunosuppressive cytokines, and/or other immune checkpoints.

Immune escape due to CD19 loss has been reported in a significant proportion in patients with ALL and lymphomas treated with anti-CD19 CAR T-cell therapy (1, 2). Loss of CD19 may be mediated by acquired mutations in CD19 and/or alternative splicing of CD19 with deletion of exon 2 resulting in sequestration of the CD19 protein in the endoplasmic reticulum or deletion of exons 5–6 that encode the transmembrane domain (6). Relapse of the tumor with CD19 loss would suggest that the CAR T-cell product used was of good quality, as it likely eliminated all CD19-expressing tumor cells. In contrast, if the relapsed tumor is CD19 positive, it raises the possibility that the resistance is likely because of impaired T-cell fitness or alternative mechanisms of tumor-intrinsic resistance to CAR T-cell killing.

In this issue of Cancer Discovery, Singh and colleagues describe a novel tumor-intrinsic mechanism that confers resistance to cytotoxicity by anti-CD19 CAR T (CART19) cells and also drives progressive CART19 dysfunction (7). To understand the mechanism of primary resistance to CART19 therapy, they performed an unbiased CRISPR-based genome-wide loss-of-function screen in an ALL cell line, Nalm6, and found that guides targeting genes associated with proapoptotic death receptor signaling pathway including FADD, BID, CASP8, and TNFRSF10B were significantly enriched for CART19 resistance. Conversely, guides targeting antiapoptotic molecules such as CFLAR, TRAF2, and BIRC2 were depleted. They validated these findings by generating CRISPR-edited Nalm6 cells lacking FADD, a critical adaptor molecule for signaling from all proapoptotic death receptors, or BID, a cell death regulator that is activated downstream of FADD and induces release of cytochrome c from mitochondria, leading to apoptosis. Long-term cultures of a mixture of wild-type (WT) and knockout (KO) Nalm6 cells with CART19 resulted in progressive enrichment of KO cells. Additional studies further corroborated the role of death receptor signaling in cytotoxic killing by CART19 cells. Co-culture of WT Nalm6 cells with CART19 in the presence of an inhibitor...
of BIRC2, birinapant, resulted in enhanced killing, whereas KO of FasL or TRAIL in CART19 decreased their cytotoxic function. Similar effects were observed with additional ALL models, a diffuse large B-cell lymphoma model, CAR T cells targeting CD22, and CAR T cells with alternative costimulatory domains. Importantly, the resistance to CART19 therapy was also demonstrated \textit{in vivo} in NOD/SCID/γc−/− mice engrafted with FADD KO or BID KO Nalm6 cells.

Because T cells may mediate killing of target cells by activating the extrinsic apoptotic pathway via the surface death receptors or by activating the intrinsic apoptotic pathway through secretion of cytotoxic molecules such as granzymes and perforin, the resistance observed with only disruption of the death receptors was unexpected. To explain this finding, the authors reasoned that impairing the extrinsic apoptotic pathway likely led to CAR T-cell dysfunction. Consistent with this notion, they found that long-term culture of CART19 with BID KO Nalm6 cells caused reduced proliferation, cytokine secretion, and production of perforin and granzymes. Similar effects were observed with continuous exposure of CART19 to WT Nalm6 cells, suggesting that the induction of CAR T-cell dysfunction may have been mediated by prolonged antigen exposure analogous to classic T-cell exhaustion rather than impaired death receptor signaling. Transcriptional profiling of CART19 cells exposed long-term to BID KO Nalm6 cells was also consistent with a dysfunctional T-cell signature compared with CART19 cells exposed to WT Nalm6. Moreover, evaluation by ATAC-seq revealed an epigenetic signature indicative of exhaustion, although not completely identical to changes observed with classic T-cell exhaustion. Integrative analysis of transcriptomic and epigenomic data showed upregulation of immunosuppressive transcription factors (TOX2, IRF8, and PRDM1) along with increased promoter accessibility of these loci. Taken together, these results suggested that disruption of the death receptor signaling pathway leads to decreased tumor killing and persistent antigen exposure that in turn results in global transcriptomic and epigenetic reprogramming of CAR T cells, adversely affecting their function (Fig. 1).

To determine whether these observations are relevant clinically, the authors then assessed the baseline tumor samples from patients with ALL treated on two independent clinical...
trials with tisagenlecleucel, an anti-CD19 CAR T-cell product with 4-1BB and CD3ζ signaling domains (1). They found that a lower death receptor gene signature score representative of the extrinsic apoptotic pathway, but not a gene signature representative of the intrinsic apoptotic pathway, was associated with primary resistance with persistent CD19-expressing tumors following tisagenlecleucel. In contrast, a higher death receptor gene signature score was associated with durable remission and better overall survival in these patients. Moreover, consistent with preclinical studies, patients with a lower death receptor gene signature score had decreased expansion and persistence of CAR T cells in vivo. Finally, single-cell transcriptomic analysis of CAR T cells obtained from patients 10 days after infusion showed a higher level of exhaustion-associated genes in a non-responder compared with a patient achieving durable response.

Collectively, this study has important implications for both basic and translational research in the rapidly growing field of cellular therapy. From a basic biology perspective, it suggests that, like T cells with native T-cell receptors, CAR T cells may mediate cytolysis by activating the intrinsic or extrinsic apoptosis pathway and that they can acquire exhaustion-like features with chronic antigen exposure. From a translational standpoint, it sheds light on a novel mechanism of resistance to CAR T cells that is antigen-independent and mediated via the death receptor pathway. Functional read-outs of death receptor signaling could therefore potentially serve as a diagnostic biomarker, as well as to select patients for future combination therapies of CAR T cells with SMAC mimetics and/or inhibitors of IAP family proteins to enhance the susceptibility of tumors with low expression of death receptor genes. However, the study also raises several outstanding questions. It is important to elucidate what drives impaired death receptor function in ALL. Is this something that exists clonally due to genetic alterations, or is it due to epigenetically controlled stochastic variation that is selected for under pressure? How do these results relate to other diseases where CAR T-cell therapy is promising, such as lymphomas and multiple myeloma, or responses to other cellular therapies such as natural killer cell therapy? What are the mechanisms driving progressive CAR T-cell dysfunction during chronic antigen exposure? And can the resistance to death receptor-mediated killing be overcome by preventing exhaustion of CAR T cells through overexpression of JUN (8) or by enhancing their function and persistence by deletion of REGNASE1 (9) or PTPN2 (10)? The preclinical models described in this study provide a platform to address these questions, and investigating this mechanism further could lead to development of novel strategies to improve the efficacy of CAR T-cell therapy.

Disclosure of Potential Conflicts of Interest

M.R. Green is a consultant at VeraStem Oncology and has ownership interest in KDAc Therapeutics. S.S. Neelapu is a consultant at Kite/Gilead, Celgene, Calibra, Legend Biotech, Novartis, Unum Therapeutics, Pfizer, Merck, Precision Biosciences, Cell Medica, Incyte, and Allogene; reports receiving commercial research grants from Kite/Gilead, Cellectis, Poseida, Merck, Acerta, Karus, Bristol-Myers Squibb, Unum Therapeutics, Allogene, and Precision Biosciences, and has patents related to cell therapy. No other potential conflicts of interest were disclosed.

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