Uncovering the Role of CD4$^+$ CAR T Cells in Cancer Immunotherapy

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Chimeric antigen receptor (CAR) T-cell therapy has transformed clinical care against blood malignancies and is seeing encouraging progress against solid tumors. While scientific advancement has been rapid, our mechanistic understanding of intrinsic features of CAR-engineered T cells is still evolving. CAR products typically consist of CD4$^+$ and CD8$^+$ T-cell subsets at variable ratios, yet a clear understanding of how each subset contributes together and independently to therapeutic response is lacking. CD8$^+$ CAR T cells are well characterized for their perforin-dependent killing effects; however, the role of CD4$^+$ CAR T cells as "helpers" versus "killers" has been variable across models and warrants more in-depth investigation. A recent study by Boulch and colleagues published in *Nature Cancer* demonstrates that CD4$^+$ CAR T cells, alone, can exert potent antitumor activity through a mechanism involving IFNγ. CD4$^+$ CAR T-cell production of IFNγ creates a cytokine field that can act at a distance to kill both antigen-positive and -negative tumor cells that are sensitive to the proapoptotic effects of IFNγ. These new findings reveal important insights for the antitumor effects mediated by CD4$^+$ CAR T cells, which could have significant clinical implications.

Unleashing the full potential of chimeric antigen receptor (CAR) T-cell therapy, for both hematologic and solid cancers, requires mechanistic understanding of the intrinsic features of the CAR T-cell product. In particular, revealing the cytotoxic mechanisms of CD8$^+$ and CD4$^+$ T-cell subsets has the potential to benefit the refinement of CAR design, T-cell manufacturing, and clinical application. "Killer" CD8$^+$ CAR T cells directly eliminate antigen-positive tumor cells through a perforin-dependent mechanism; however, maintenance of their functional potential requires CD4$^+$ T-cell support. "Helper" CD4$^+$ CAR T-cell subsets both promote CD8$^+$ T-cell function, as well as elicit direct antitumor cytotoxicity. However, the mechanisms for CD4-mediated cytolytic function are not clearly delineated.

An elegant study by Boulch and colleagues (1) revealed that IFNγ production is a dominant pathway by which CD4$^+$ CAR T cells can eliminate tumor cells. Using genetically engineered mouse models, the authors generated IFNγ-deficient CAR T cells and demonstrated that the cytotoxic effect of CD4$^+$ CAR T cells was primarily dependent on IFNγ and, to a lesser extent, on perforin-directed killing. IFNγ directly acts on tumor cells to trigger apoptosis through its receptor, IFNγR1, and the sensitivity of tumor cells to IFNγ was associated with the tumor-specific antitumor effects of CD4$^+$ CAR T-cell therapy. Using a mixed IFNγ-sensitivity tumor model, the authors further demonstrated that CD4$^+$ CAR T cells preferentially eliminated tumor cells that are intrinsically sensitive to IFNγ-induced apoptosis. The authors next tested the clinical importance of these findings in a cohort of 63 anti-CD19 CAR T-cell–treated patients with diffuse large B-cell lymphoma. CAR T-cell products displaying high CD4 to CD8 CAR T ratios showed strong correlation between serum IFNγ induction and clinical outcomes, including progression-free survival and overall survival.

These findings by Boulch and colleagues revealed an underappreciated mechanism by which CD4$^+$ CAR T cells mediate cytotoxicity, contributing to our understanding that CD4$^+$ T cells can directly elicit antitumor responses independent of their role as "helpers." Recent studies have illustrated the critical role of CD4$^+$ T-cell activity, especially cytolytic function, in mediating effective cancer immunotherapy (1–4). Oh and colleagues (3) reported that cytotoxic CD4$^+$ T cells are clonally expanded in bladder cancer tissues. The cytotoxic CD4$^+$ signature was correlated with effectiveness of anti-PD-L1 therapy in a cohort of 244 patients with metastatic bladder cancer, suggesting the existence of an intratumoral cytotoxic CD4$^+$ T-cell subset and their responsiveness to immune-stimulatory agents such as checkpoint blockade antibodies. CD4$^+$ T-cell responses are also essential for potent CAR T-cell antitumor function. This study and others (1–4) have highlighted the important role of CD4$^+$ T cells in cancer immunity, particularly their cytolytic function on tumors including antigen-negative tumor variants.

We and others have also found that CD4$^+$ CAR T cells mediated potent antitumor function in multiple solid tumor models, and in these studies, CD4$^+$ CAR T cells were able to elicit superior antitumor function as compared with CD8$^+$ cells (2, 5). Intriguingly, these studies have blocked the granzyme/perforin pathway, resulting in the reduction of killing potency by CD4$^+$ CAR T cells, suggesting that the granzyme/perforin pathway is important for CD4$^+$ CAR T cytotoxicity. Comparatively, the study by Boulch and colleagues highlighted the importance of IFNγ in the cytolytic function of CD4$^+$ CAR T cells in IFNγ-sensitive tumor models (1). The mechanism of granzyme/perforin versus IFNγ-mediated cytolysis may be tumor dependent and remains to be further evaluated.

CD4$^+$ CAR T cells were also shown to better control large, established solid tumors in the long-term setting, together with the transcriptional signature illustrating the resistance to exhaustion (2, 5, 6). Indeed, a case report has shown long-term CD4$^+$ CAR T-cell persistence (since 2010) in a patient with B-cell chronic lymphocytic leukemia treated with CD19-targeted CAR T cells (6). Therefore, although the intrinsic sensitivity to CD4-mediated cytotoxicity might

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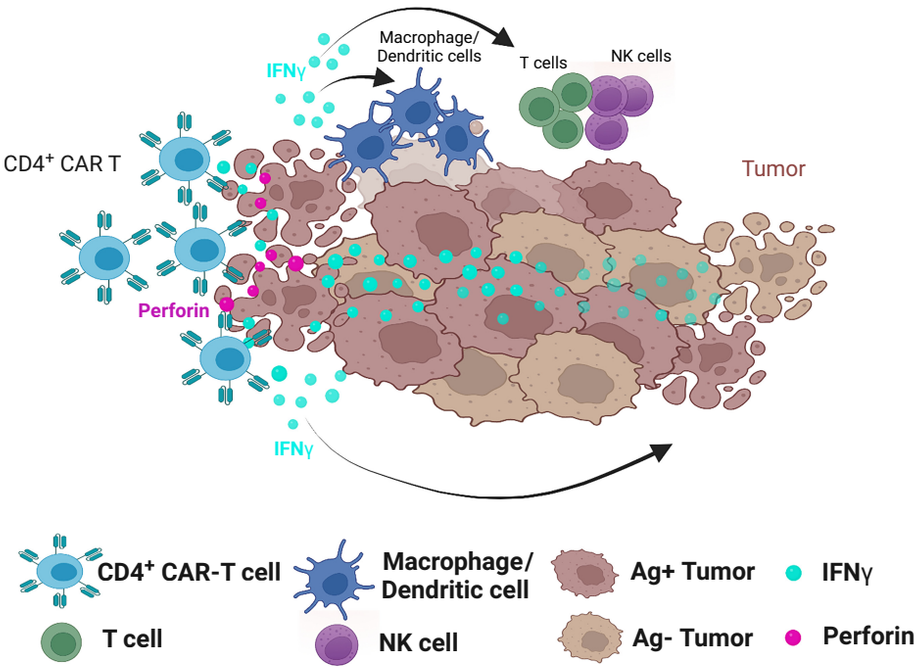

Figure 1.
Multifaceted functions of $CD4^+$ CAR T cells in cancer immunotherapy. Schematic of the multiple roles of $CD4^+$ CAR T cells in antitumor immunity. $CD4^+$ CAR T cells exert direct antitumor activity against antigen-positive tumors through perforin and IFNγ and induce IFNγ-dependent remote cytolytic cell death. In addition, IFNγ produced by $CD4^+$ CAR T cells activate and reprogram the host immune cells. NK, natural killer. (Created with BioRender.com.)

differ across tumors, maintaining the $CD4^+$ subset is critical to manufacture potent CAR T-cell products. One of the major directions of future studies, meanwhile, is the systematic dissection of cytotoxicity versus "helper" function on $CD4^+$ CAR T cells, which will subsequently rationally optimize CD4:CD8 ratio to maximize antitumor potency.

The direct antitumor activity of $CD4^+$ CAR T cells has been well documented, and Boulch and colleagues now demonstrate an indirect antitumor mechanism that is IFNγ dependent. Proinflammatory cytokines secreted by CAR T cells such as IFNγ have a multifunctional role in the setting of immunotherapy. The antitumor effects of IFNγ can be on the tumor or by activating and reprogramming immune cells in the tumor microenvironment. The effects of IFNγ on tumor cells can be through induction of antigen presentation machinery, adhesion molecules, cell-cycle arrest, cell death or paradoxically upregulation of inhibitory pathways (i.e., immune checkpoints). Indeed, several studies have shown different mechanisms of IFNγ-mediated changes on tumors, leading to immune recognition and increased sensitivity to CAR T-cell killing (1, 7–9). In line with the importance of tumor-intrinsic features as determinants of response, Boulch and colleagues also supported the idea that the intrinsic sensitivity of tumor cells to the proapoptotic effects of IFNγ is a major factor for response to $CD4^+$ CAR T-cell treatment, which was observed in both hematologic and solid tumors (1).

IFNγ can also indirectly induce antitumor responses by activating and engaging host immune cells. Our team and others have shown that CAR T cells can activate host immune cells such as myeloid and endogenous T cells primarily through secretion of IFNγ in the tumor bed (7, 9). The activation of intratumoral myeloid cells results in induction of endogenous T-cell responses coupled with feed-forward propagation of CAR T-cell responses (7). This is consistent with a recent study by Kruse and colleagues that described the mechanism by which a small number of effector $CD4^+$ T cells eradicated tumors by recruitment and induction of IFN-activated myeloid cells. Combination of the $CD4^+$ T cells and tumoricidal myeloid cells induced remote cell death, which indirectly eliminated IFN unresponsive and MHC-deficient tumors (4). Using intravital imaging, Boulch and colleagues also reported IFNγ-dependent tumor elimination by $CD4^+$ CAR T cells that occurred at a distance from the CAR T-cell location, in line with studies highlighting the spatiotemporal spreading of IFNγ in the tumor microenvironment (10). IFNγ diffusion in the tumor has provided a new perspective on the global effects of IFNγ on both tumors and immune cells, independent of direct cell-cell contact. IFN-dependent antitumor function of $CD4^+$ T cells can act through direct induction of tumor death, as well as indirect induction of myeloid activation and endogenous immune responses. In CAR T-cell therapy, IFNγ-induced apoptosis of remote tumor cells and/or myeloid cell activation is especially important for elimination of antigen loss variants, therefore enabling the eradication of IFN unresponsive and heterogeneous tumors, which also represent a broad range of cancers such as gliomas (**Fig. 1**).

Delineating the mechanisms that augment CAR T-cell–mediated antitumor immunity is critical for the development of an effective cancer immunotherapy. Studies have shown that in addition to direct antitumor activity, CAR T cells have immune-stimulatory effects that result in activation of host innate immune subsets and subsequent engagement of adaptive immunity (7, 9). IFNγ signaling, both in CAR T cells and host lymphocytes, tightly controls adaptive and innate immunity. Recent reports have provided strong evidence for the cytolytic role of $CD4^+$ population (2, 4). The study by Boulch and colleagues (1) highlights the antitumor effect of $CD4^+$ CAR T cells primarily through IFNγ and how $CD4^+$ CAR T-cell therapy may be more powerful when treating IFNγ-sensitive tumors. Furthermore, for IFNγ-resistant

tumors, the spatial temporal activity and diffusion of IFNγ in the tumor microenvironment could have broader impact through an IFNγ-dependent stimulation and recruitment of host immune cells. These studies collectively highlight critical roles of CD4$^+$ T cells and IFNγ signaling in the context of CAR T-cell therapy and other immunotherapies.

Authors' Disclosures

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