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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Rossi et al, page 804

CAR T cells find strength in polyfunction

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In this issue of *Blood*, Rossi et al report that the heterogeneity of personalized chimeric antigen receptor (CAR) T cells has limited the ability to identify product attributes that enhance patient outcomes, but they have showed that by using single-cell analyses, heterogeneity may actually improve clinical outcomes.[1]

CD19-targeted CAR T cells have recently been approved for treating patients with B-cell acute lymphoblastic leukemia (B-ALL) or diffuse large B-cell lymphoma (DLBCL).[2,3] CD19-targeted CAR T cells mediate durable responses in patients, although efficacy could be further improved by increasing the response rate for DLBCL and reducing the relapse rate for B-ALL. This novel therapy is also associated with unexpected adverse effects such as inflammatory and neurologic toxicities related to the en masse activation of CAR T cells.[4] Significant research effort is being directed toward identifying patient characteristics that correlate with CAR T-cell efficacy and/or toxicities. To date, clinical factors that correlate with response or toxicities have included tumor burden, serum cytokine levels, peripheral blood CAR T-cell expansion, and/or persistence.[5-7] However, these correlations are agnostic of the infused CAR T-cell product. Even the dose of CAR T cells approved as a standard of care[2] for B-cell malignancies can vary up to 10-fold, which highlights the challenge of determining the attributes of these heterogeneous cell products that correlate with clinical outcomes. However, Rossi et al used single-cell analyses to identify functional CAR T-cell characteristics that correlate strongly with clinical responses and toxicities.

The novel hypothesis for this study was that transfer of the CAR into a heterogeneous population of cells inclusive of multiple T-cell subsets will support the production of CAR T cells capable of diverse, polyfunctional immune activities. By using technology that allows the detection of 32 different proteins secreted by a single cell that are categorized as effector, stimulatory, chemoattractive, regulatory, or inflammatory, the investigators analyzed aliquots of CAR T-cell products infused into 22 patients with DLBCL or other lymphomas.[8] Surprisingly, polyfunctional CAR T cells, defined as secreting at least 2 of the 32 selected proteins, accounted for only 20% to 25% of the product. Despite this low frequency, the polyfunctionality strength index (PSI), a product of the percentage of polyfunctional cells and the mean fluorescent intensity of the proteins secreted by the cells, correlated with clinical outcomes. Only 2 of the 16 patients with the highest PSI failed to achieve an objective response. Similarly, the PSI correlated with levels of severe (grade $\geq$3) cytokine release syndrome. Although PSI did not correlate with neurologic toxicities, when PSI of CD4 T cells or interleukin-17 was combined with a metric of CAR T-cell peak expansion, there was a much stronger correlation with neurologic toxicities than CAR T-cell peak expansion alone, which suggests the need to evaluate a role for T helper 17 cells in neurologic toxicities.

Rossi et al also determined that the PSI of CD8 CAR T cells did not correlate with clinical responses, whereas the PSI of CD4 CAR T cells did. Furthermore, patients with objective responses had polyfunctional CD4 CAR T cells that secreted inflammatory, regulatory, and effector proteins, whereas CD4 CAR T cells used in nonresponders secreted predominantly effector proteins. Beneficial polyfunctional CD4 T cells may be the scientific rationale for the need to balance the ratio of CD4 and CD8 CAR T cells in infused products, which is being evaluated in clinical trials.[6,7] Although Rossi et al suggest that a minor fraction of the CAR T-cell product is critical for the clinical outcome, they also demonstrate that objective responses do not differ based on the frequencies of effector or memory immune cell subsets. This lack of correlation may be the result of the heterogeneity of the CAR T-cell product or it may suggest that the PSI is a functional definition of CAR T-cell subsets that cannot be distinguished by classical immune phenotypes.

Single-cell polyfunctional analyses provide a measure of product efficacy that can be used to identify patients likely to respond to treatment and those likely to develop toxicities. However, questions remain that will hopefully be addressed when this single-cell technology is applied to CAR T-cell products under clinical evaluation or given as a standard of care. It should be evaluated if PSI is critical for CAR T cells that include other costimulatory domains or target antigens other than CD19 or diseases other than lymphoma. In addition, understanding the relation of PSI and antitumor clinical response will require determining whether PSI is a measure of active antitumor CAR T-cell subsets or simply a measure of product quality. Anecdotal observations suggest that long-lasting CAR T cells in patients are polyfunctional, which argues that PSI may be a measure of clinically active subsets.[9] Furthermore, it should be determined whether the PSI of the CAR T-cell product correlates with the PSI of the apheresis product. If so, there is an opportunity to enhance CAR T-cell products by treating patients with medications that improve

the PSI of their T cells before collection, as suggested in a study of T cells collected from patients with chronic lymphocytic leukemia who were treated with ibrutinib before CAR T-cell production.[10] And importantly, measures of quality or other attributes of commercial CAR T-cell products such as PSI, percent of CAR, CD4:CD8 ratio, and dose should be shared with researchers. Only by pairing product data with clinical outcomes will commercial application of CAR T cells be further optimized and support enhanced safety and efficacy for patients.

Conflict-of-interest disclosure: The author receives consultant fees from Novartis, Servier, and Celyad, as well as research funding from Celgene. ■

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DOI 10.1182/blood-2018-06-858712

LYMPHOID NEOPLASIA

Comment on Cader et al, page 825

The run-down immunologic neighborhood in Hodgkin lymphoma

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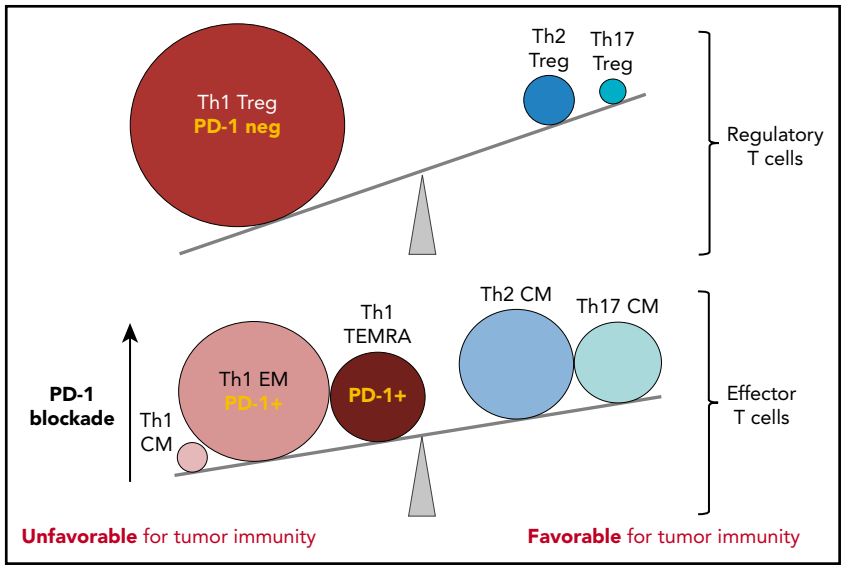
In this issue of *Blood*, Cader et al dissect the T-cell subsets within the tumor microenvironment of Hodgkin lymphoma, revealing an overabundance of $CD4^+$ regulatory T cell (Treg) and exhausted T helper 1 (Th1) effector cells within the Reed-Sternberg cell's dysfunctional immunologic milieu.[1]

The discovery that classical Hodgkin lymphoma (cHL) is extraordinarily responsive to treatment with PD-1 blocking antibodies has begged the question of the effector mechanism(s) behind this singular intervention.[2,3] The unique tumor microenvironment of cHL comprises rare malignant Hodgkin Reed-Sternberg (HRS) cells within an extensive inflammatory and immune cell infiltrate, in which the malignant cells evade immune destruction by multiple mechanisms.[4] HRS cells almost invariably exhibit increased copy number alterations in the 9p24.1 locus that encodes the programmed death 1 (PD-1) receptor ligands PD-L1 and PD-L2, which



T-cell subsets within the Hodgkin lymphoma tumor microenvironment tip the balance away from tumor immunity. In comparison to reactive lymph nodes and tonsils, T cells infiltrating cHL tumors have marked expansions in T-cell subsets that can suppress tumor-specific killing (Treg cells, PD-1 low/negative; >10-fold expanded) and EM $CD4^+$ T cells with PD-1 intermediate/high expression (Th1 EM, Th1 TEMRA; >fivefold expanded), rendering them sensitive to the suppressive effects of the high levels of PD-L1 expressed by HRS cells. The effectiveness of PD-1 blockade in cHL may rely on the release of these effector $CD4^+$ T-cell populations from PD-L1-mediated suppression, achieving tumor control presumably via class II MHC-mediated cytotoxicity. CM, central memory. The figure has been adapted from Figure 7C in the article by Cader et al that begins on page 825.