Commentary

Chimeric antigen receptor T cells get passed by leukemia

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New immunotherapy treatments have led to dramatic responses in patients with chemotherapy-refractory solid tumors, leukemias, and lymphomas. One of these immunotherapies involves the adoptive transfer of autologous T cells gene-engineered to express a chimeric antigen receptor (CAR) that is a hybrid of an antibody and a T cell receptor (TCR) (1). The antibody portion provides new antigen specificity and after binding, the intracellular TCR domains of the CAR induce T cell activation and tumor killing. CAR T cells targeted against CD19, a protein expressed on nearly all normal and cancerous B cells, has mediated impressive responses in patients with relapsed/refractory B cell acute lymphoblastic leukemia (B-ALL). Four groups have reported complete remission (CR) rates up to 90% in pediatric and/or adult patients with B-ALL, while the expected CR rate with chemotherapy alone is <30% (2-5). The number of patients treated with anti-CD19 CAR T cells has increased to beyond 100 and novel forms of relapse that allow tumor cells to avoid killing by CAR T cells have been identified. Although anti-CD19 CAR T cell therapy remains at an early stage of development, an understanding of the mechanisms of treatment escape may identify patients at high-risk for relapse and uncover new avenues to circumvent these relapses.

Gardner *et al*. (6) recently reported details from two relapses out of seven patients with MLL B-ALL treated with anti-CD19 CAR T cell therapy. Both cases were characterized as having ALL to AML lineage switch shortly after CAR T cell therapy indicating the role of phenotype switch in therapy failure. In one case there was retention of the IGH rearrangement after relapse and lineage switch suggesting de-differentiation of a B lymphoid clone. In the other case the IGH rearrangement was not detected in relapsed AML blasts, suggesting a non-committed precursor or selection of a preexisting myeloid clone. The MLL chromosomal translocation involving chromosome 11, band q23 (7) confers a poor prognosis in chemotherapy treated patients and is known to associate with lymphoid to myeloid lineage switch, often shortly after chemotherapy (8). In contrast, out of 62 cases of non-MLL rearranged B-ALL after anti-CD19 CAR T cell therapy none exhibited lineage switch.

The mechanism for lineage conversion of MLL B-ALL after therapy is unclear and possibilities include: early non-committed clonal hematopoietic progenitors able to give rise to either lineage; lymphoid stem cell clone plasticity allowing lineage inter-conversion; or the presence of a bi-lineal population of blasts present at diagnosis, which upon therapeutic selection, leads to emergence of a minor alternate subset (9). These putative mechanisms allow hypotheses to be developed and tested to determine if any may be involved after CD19-targeted CAR T cell treatment (*Figure 1*). This may require more extensive immunophenotypic and genetic characterizations of the leukemia immediately before and after CAR T cell treatment. While these analyses will take time, the report from Gardner *et al*. (6) must give any CAR T cell clinician pause prior to the use of anti-CD19 CAR T cell therapy for patients with a MLL-rearranged B-ALL. The quick rate of relapse suggests that clinical trials should evaluate the utility of allogeneic stem cell transplants immediately after anti-CD19 CAR T cell therapy for MLL B-ALL. Using conventional chemotherapy, such an approach can act as a bridge for relapsed and refractory leukemia patients not expected to achieve remissions with chemotherapy alone (10).

Lineage-switch is just one mechanism of immune escape

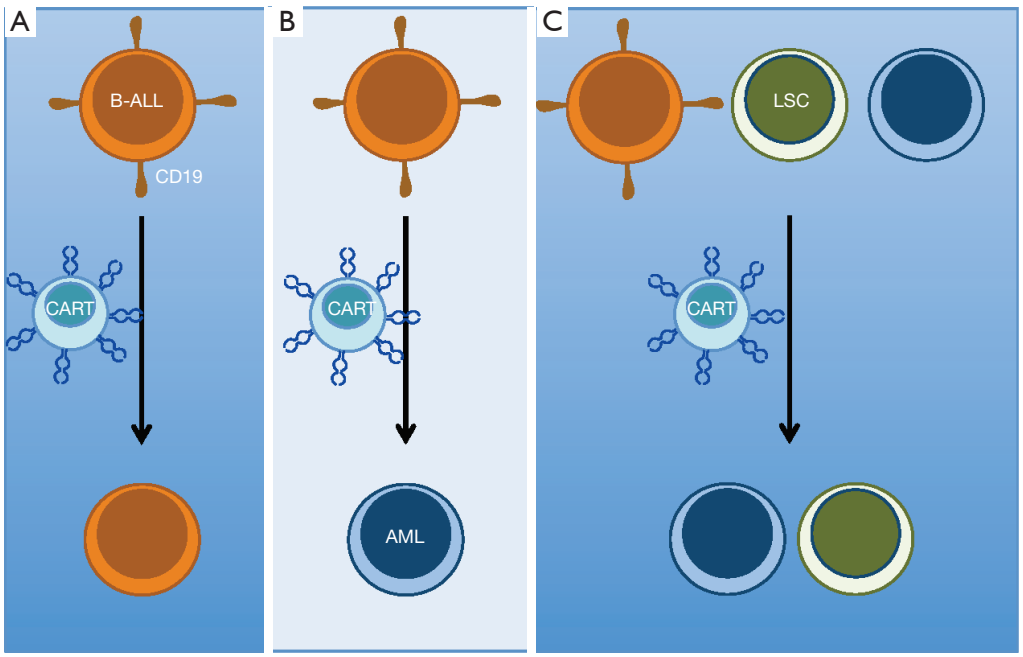

Figure 1 Mechanisms of immune-mediated escape that lead to relapse after adoptive therapy with CD19-targeted chimeric antigen receptor (CAR) T cell therapy for B-ALL. (A) B-ALL tumor cells lose the CD19 epitope recognized by CAR T cells; (B) B-ALL tumor cells undergo de-differentiation or reprogramming to AML; (C) clonal heterogeneity which may include leukemic stem cell (LSC) or a clonal progenitor, leads to incomplete targeting of the leukemia population allowing the AML clone to expand and predominate after treatment. ALL, acute lymphoblastic leukemia; B-ALL, B cell ALL.

after CD19-targeted CAR T cell therapy. Relapses after blinatumomab, a CD19/CD3 bi-specific T cell engager recently FDA approved for B-ALL, harbor CD19-negative clones in 50% of cases (11). This loss of epitope (i.e., antigen) is now being detected after treatment with CD19-targeted CAR T cells as well (3). Recent work published by Sotillo *et al.* (12) elucidated one origination for epitope/antigen loss via an alternative splice mechanism. They demonstrated that in several cases relinquishment of the CAR targeted CD19 surface epitope was due to the emergence of variant CD19 epitope. This N-terminally truncated variant is encoded by mRNA having missense mutations within, or complete loss of, exon 2. The resulting leukemic clones no longer express the CD19 CAR T cells cognate epitope, yet the alternate CD19 isoform at least partially rescues defects in cell signaling and proliferation associated with complete loss of CD19. These CD19 variants are associated with a decrease in SRSF3, a splicing factor they elegantly demonstrated is essential for splicing exon 2 of CD19. This mechanism of resistance to targeted therapy was similarly demonstrated with aberrantly spliced BRAF (V600E) mediating resistance to vemurafenib (13). In the future new therapies should be developed to combat this mechanism of CD19 epitope loss. One option could include CARs targeting CD19 extracellular domains other than exon 2.

These recent reports identify novel forms of ALL resistance to CD19-targeted CAR T cell therapy. Considering recent successes with CD19-targeted therapies for B-ALL, diffuse large B cell lymphoma, and mantle cell lymphoma (14,15) it is likely this therapy will be approved for disease control in patients with B cell malignancies. Therefore, we foresee a significant need to anticipate and potentially prevent relapses in these patients to preserve overall survival. To avoid immune escape by antigen-downregulation or epitope loss: bi-specific targeting with two CARs against distinct epitopes on the same antigen or even different antigens altogether could be employed. In the case of myeloid-switch relapses the potential interventions will depend on the mechanism of relapse. If there are *de novo* heterogenous myeloid and lymphoid blasts at the time of treatment then targeting both with separate CD19-specific and myeloid-specific CARs would be indicated. In contrast, if the mechanism of relapse is de-differentiation then serial treatments first with a CD19-specific CAR followed by a myeloid-specific CAR would be indicated. Considering that trials testing such theories may not be available for some time, we strongly suggest that for the near-term any patient with a MLL-rearranged B-ALL should be considered for allogeneic hematopoietic stem

cell transplant after treatment with anti-CD19 CAR T cells even if in CR. The report by Gardner *et al.* (6) revealed that utilizing anti-CD19 CARs to treat MLL B-ALL may not get the patient all the way to a durable CR. This report also allows us to speculate on new ways to make this treatment more successful for MLL B-ALL patients: taking CARs on a bridge to allogeneic transplant or selecting a new ride on CARs targeting myeloid blasts toward prolonged remissions.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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