Molecular Therapy
Oncology
Commentary

Minimally modified off-the-shelf allogeneic CAR T cells

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https://doi.org/10.1016/j.omton.2024.200851

T cells modified with synthetic chimeric antigen receptors (CARs) have been enormously successful in treating hematological malignancies, and multiple products are now approved for use in patients with relapsed/refractory leukemia, lymphoma, and myeloma. However, on-demand manufacturing of an autologous engineered T cell product creates considerable financial, logistical, and availability challenges to the widespread implementation of the therapy. To complicate matters, patients with aggressive disease may require bridging therapy while the T cell product undergoes manufacturing and release testing, and the quality and function of the CAR T cells may be variable between patients following multiple courses of therapy. For these reasons, off-the-shelf strategies using alternative allogeneic cell sources for *ex vivo* engineering including healthy donor T cells, pluripotent stem cell-derived T cells[1], and natural killer (NK) cells[2], and direct *in vivo* CAR T cell engineering[3] are being explored preclinically and clinically.

Using therapeutic allogeneic αβ T cells creates two challenges: (1) the native T cell receptor (TCR) can cause graft versus host disease (GVHD), and conversely, (2) alloreactive host T cells and NK cells can quickly reject the cells. To address the former, components of the TCR complex such as the TCR α constant (*TRAC*) gene have been disrupted by clustered regularly interspaced short palindromic repeats (CRISPR) or transcription activator-like effector nucleases (TALENs), or the locus repurposed to express the CAR.[4] To address the latter problem of the mismatched human leukocyte antigen (HLA), deletion of the β2 microglobulin (*B2M*) and class II-transactivator (*CIITA*) genes creates stealth T cells that fail to express HLA class I and II respectively.[5] Since HLA-negative cells can be targeted by NK cells, overexpression of the minimally polymorphic HLA-E molecules that engage the NKG2A inhibitory receptor on NK cells can compensate for this problem.[6] An alternative strategy involves targeting alloreactive host T cells by either deleting *CD52* in the therapeutic T cells and concurrently treating with the anti-CD52 antibody alemtuzumab[7] or by expressing an alloimmune defense receptor in therapeutic T cells that recognizes the activation marker 4-1BB.[8] These approaches can deplete host T cells globally or with some selectively (Figure 1 left).

In a recent issue of *Molecular Therapy Oncology*, Quach et al. have astutely leveraged the biology of CD30 (also known as *TNFRSF8*), combined with the expansion of TCR antigen-specific T cells, to bypass the multiplexed genetic modifications required to prevent rejection and GVHD associated with allogeneic T cell therapies.[9] Using Epstein-Barr virus (EBV)-specific T cells (EBVSTs) expanded *in vitro* with peptides representing latent and lytic genes (*EBNA1, LMP1, LMP2*, and *BZLF1*) simultaneously reduces the risk of GVHD and enables targeting of EBV-positive cells through the native TCR. CD30 is expressed not only on Reed-Sternberg cells in Hodgkin lymphoma and anaplastic large cell lymphomas but also subsets of activated T cells, B cells, and NK cells,[10] and they demonstrated multi-specificity toward EBV-positive and CD30-positive targets through the TCR and the CAR. Instead of genetically engineering hypoimmunogenic T cells, they exploited the expression of CD30 on activated T and NK cells. In mixed lymphocyte reactions (MLRs), they demonstrated that CD30 CAR EBVSTs inhibited the expansion of alloreactive T cells and thus resisted their killing. Given that CD30 is also upregulated on CAR T cells upon antigen recognition, it was surprising that the transduced cells did not undergo fratricide. However, the authors showed that the CAR binds to CD30 in *cis*, masking the epitope from surrounding CAR T cells (Figure 1 right).

The effectiveness of the approach will be borne out in an ongoing phase 1 clinical trial where CD30 CAR EBVSTs are manufactured from healthy EBV-seropositive donors (NCT04288726). The study has already reported early positive outcomes, including a tolerable safety profile, and, of seven evaluable patients, two complete responses and three partial responses.[11] The efficacy of the CD30 virus-specific T cell strategy will likely be further improved by combination with vaccines[12] or following viral reactivation.[13] Of note, the CD30 CAR EBVSTs were detected in the peripheral blood of patients, but there was no evidence of T cell expansion.[11] Although *in vitro* MLR cultures suggest that alloreactive T cells are eliminated, the lack of expansion of the CAR T cells in patients suggests that further insight is required into the levels and kinetics of CD30 expression *in vivo* across subsets of activated T cells and NK cells that contribute to rejection.

When a CAR T cell expresses high levels of the target antigen, it can be subject to fratricide by other CAR-expressing T cells, for example as described with CD7.[14] What physical properties of a CAR allow for good *cis* binding and protection from fratricide? The location of the scFv epitope and the length and source of the hinge domain may determine whether the CAR molecule has enough flexibility to bind in the *cis* conformation. It would be interesting to test whether other CD30 CARs have similar functionality or whether it is specific to the construct used by Quach et al. Protection from fratricide may also be antigen dependent. Binding of the CAR to a surface-expressed antigen in *cis* could potentially activate signaling through both the CAR itself and the antigenic receptor. In this case, CD30 ligation could promote signal transduction through MAPK and

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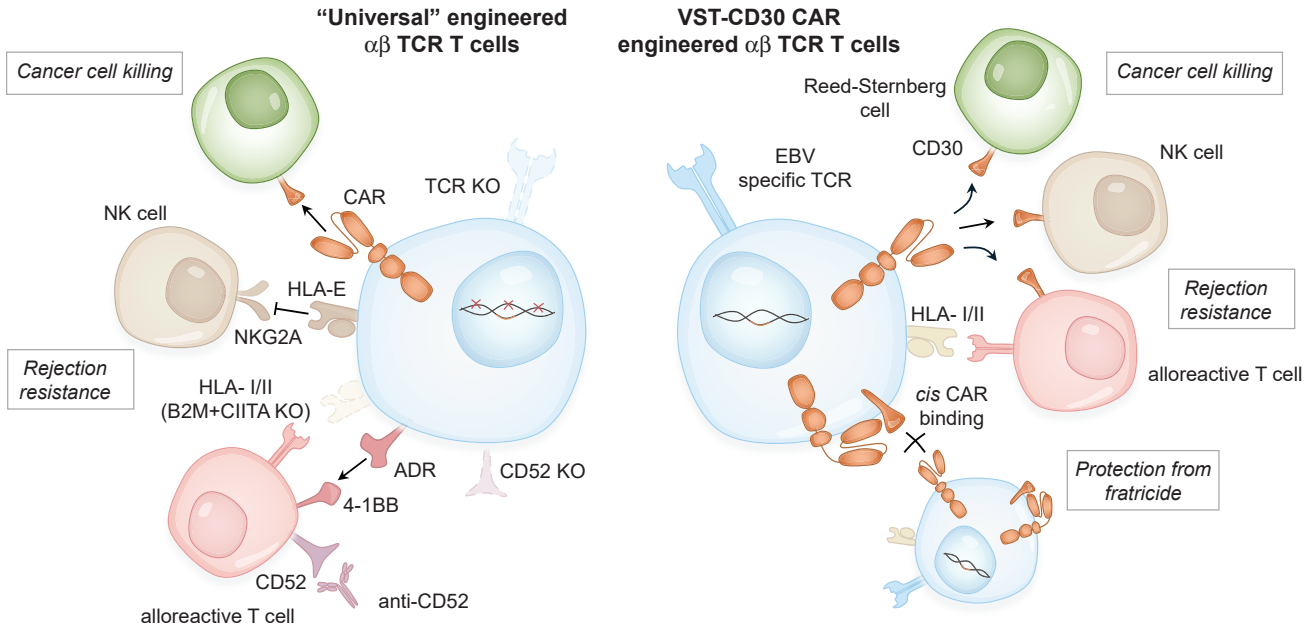

Figure 1. Engineering strategies to avoid GVHD and prevent rejection for allogeneic sources of T cells

(Left) Polyclonal allogeneic αβ T cells derived from healthy donor T cells or from pluripotent stem cells are transduced with a CAR and further edited to disrupt the native TCR and HLA molecules (through beta 2 microglobulin [*B2M*] and class II-transactivator [*CIITA*]). To avoid rejection, *CD52* can be knocked out (KO), and HLA-E and alloimmune defense receptors (ADRs) can be co-expressed. Host T cells are depleted by treatment with an anti-CD52 antibody. (Right) Allogeneic αβ T cells derived from healthy donor T cells are expanded for EBV reactivity and transduced with a CD30 CAR. The CD30 CAR can target both malignant cells and activated NK and T cells that contribute to rejection. The CD30 CAR binds in *cis* to protect from fratricide.

NFKB, which could help the cell survive and proliferate.[10] Delineation of these properties may enable the design of other *cis*-protective CAR molecules.

ACKNOWLEDGMENTS

P.L. is supported by the BC Cancer Foundation and the Fondation Léon Fredericq. L.E. is supported by the Canadian Institute for Health Research, BC Cancer and the BC Cancer Foundation, Michael Smith Health Research BC, and the Terry Fox Research Institute.

DECLARATION OF INTERESTS

All authors declared no potential conflicts of interest.

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