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RESEARCH HIGHLIGHT

# Off-the-shelf CAR T cells to treat cancer

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**Chimeric antigen receptor (CAR) T cell therapies approved for cancer treatment are manufactured from a patient's own cells (autologous), entailing challenges such as high costs, inconsistent product quality, and potential treatment failure due to T cell dysfunction. In a study published in *Cell Research*, Hu et al. generated CAR T cells from healthy donor T cells (allogeneic), and these were successful in a clinical trial for the treatment of T cell malignancies**.

CAR T cell therapies, in which T cells are isolated from patients and engineered to kill tumor cells, have shown success for the treatment of various hematological malignancies.[1] However, there are multiple challenges that need to be addressed to make this therapy widely accessible for patients. One of these challenges is the autologous (patient-derived) nature of CAR T cell products, which is associated with a complex manufacturing process, leading to disadvantages such as high costs, potential delays in treatment availability, and inconsistent product quality. The efficacy of bespoke CAR T cells might also be limited by T cell exhaustion, a state of T cell dysfunction that is common in cancer patients.[2]

One solution to these issues could be using "off-the-shelf" (allogeneic) CAR T cells, produced with T cells derived from healthy donors. This would lower production costs, avoid treatment delays, increase product uniformity, and expand access to CAR T cell therapies.[2] Allogeneic CAR T cells would be particularly beneficial for treating the highly aggressive T cell malignancies, because it would avoid potential product contamination with malignant T cells.[3] However, there are barriers to the development of allogeneic CAR T cells, including the potential for life-threatening graft-versus-host disease (GvHD), in which donor cells attack healthy recipient cells, and rejection of infused CAR T cells by host immunity.[2,4] T cell malignancies also remain elusive to treatment due to the potential for self-killing (suicide and fratricide) of CAR T cells, caused by shared expression of target antigens with malignant T cells (Fig. 1a).[3]

In a recent paper in *Cell Research*, Hu et al. generated healthy donor-derived CAR T cells targeting CD7, a transmembrane protein expressed in most T cell malignancies. The authors introduced additional genetic modifications to overcome challenges associated with allogeneic products (Fig. 1b). First, since CD7 is expressed in healthy T cells, CD7 expression was genetically deleted to prevent CAR T cell fratricide.[5] The T cell receptor (TCR) and its associated signaling complex CD3 were also genetically disrupted, to reduce the likelihood of GvHD caused by recognition of recipient antigens by donor TCRs. The authors then addressed the issue of host immune rejection of allogeneic products (allo-rejection). To this end, they genetically ablated human leukocyte antigen (HLA) class II expression in CAR T cells to reduce the risk of rejection by endogenous $CD4^+CD7^-$ cells, which are resistant to CAR T cell killing due to the lack of CD7 expression. To prevent recognition by host natural killer (NK) cells, Hu et al. incorporated an NK cell inhibitory receptor (NKi) generated by fusing the extracellular and transmembrane domains of E-cadherin, a known NK inhibitor, to the intracellular domain of CD28, a T cell co-stimulatory protein. Finally, a modification was incorporated to enhance CAR T cell antitumor efficacy. CD7 plays a role in the production of interleukin-2 (IL-2), a gamma chain (γc) cytokine that is crucial for T cell proliferation and effector function.[6] To compensate for the lack of CD7 expression in CAR T cells, Hu et al. edited the CAR construct by introducing the IL-2 receptor subunit gamma (CD132 or γc) intracellular domain (CAR-g). This led to enhanced IL-2 production, T cell proliferation, and antitumor efficacy. Together, these genetic modifications resulted in potent allogeneic CD7-targeting CAR T cells (RD13-01) that had enhanced expansion and were resistant to fratricide, GvHD and allo-rejection from host $CD4^+$ T cells and NK cells.

To evaluate the clinical application of RD13-01, the authors conducted a phase I clinical trial in patients with relapsed or refractory $CD7^+$ hematological malignancies, including T cell acute lymphoblastic leukemia, T cell lymphoma and acute myeloid leukemia. Although previous studies showed anti-leukemic activity of CD7-targeting CAR T cells,[7–9] there was little evidence about their clinical safety and efficacy prior to this study. Excitingly, RD13-01 treatment had an acceptable safety profile, with no cases of dose limiting toxicity, GvHD or immune effector cell-associated neurotoxicity syndrome. Although some patients developed cytokine release syndrome, this was not severe and could be controlled with standard treatments. There were two patient deaths during this study, one of them resulting from sepsis, and the other one from Epstein-Barr virus (EBV)-associated diffuse large B-cell lymphoma. In this regard, multiple patients presented signs of cytomegalovirus (CMV) and/or EBV reactivation, presumably resulting from chemotherapy-induced immunosuppression and T cell depletion. With regards to efficacy, most patients (82%) achieved an objective response, with multiple patients (64%) achieving complete response (CR) or CR with incomplete hematological recovery. Notably, CRs were observed in a patient with 95% leukemia blasts in the bone marrow and in a patient with extramedullary leukemia. A few patients experienced relapse, with the presence of $CD7^+$ malignant cells in the bone marrow. To investigate this resistance mechanism, Hu et al. studied the phenotype of endogenous T cells following treatment.

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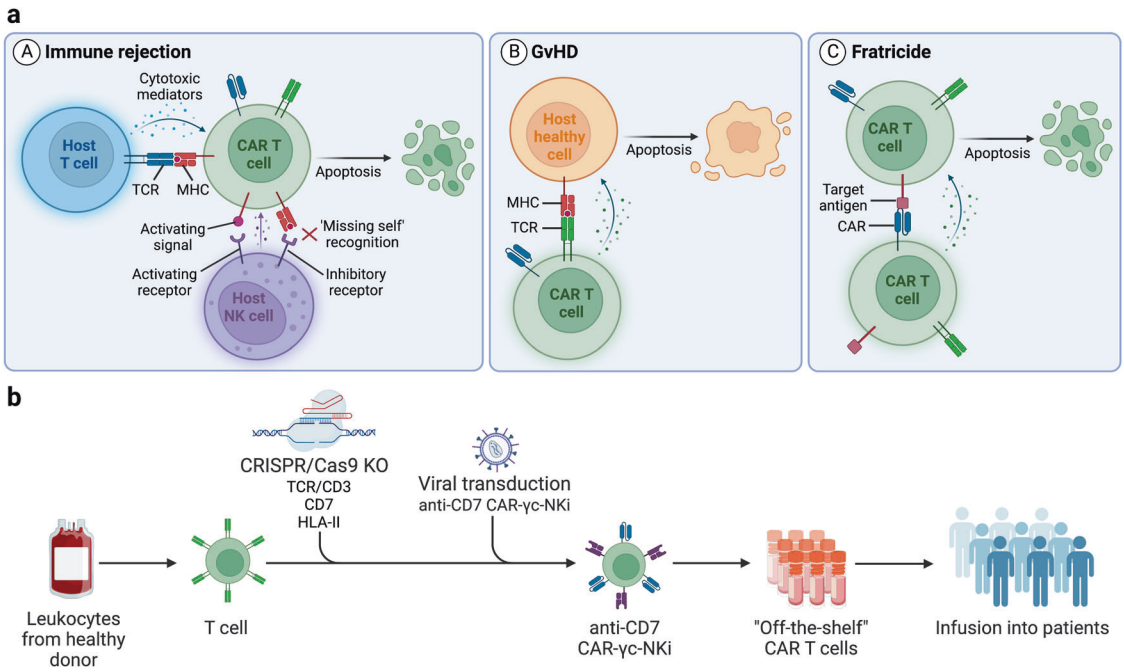


**Fig. 1 Off-the-shelf CAR T cells for the treatment of T cell malignancies. a** Main challenges for the development of allogeneic CAR T cells for the treatment of T cell malignancies. (A) Immune rejection, (B) GvHD, (C) fratricide or suicide. **b** Illustration of RD13-01 manufacturing process. Figure created with BioRender.com.

Interestingly, a previously minor population of $CD8^{+}CD7^{-}$ T cells was greatly expanded after CAR T cell infusion. Data from alloreactivity assays suggested that this endogenous population contributed to CAR T cell allo-rejection and limited persistence in some patients, leading to antigen-positive tumor relapse. This raises an important point of consideration for the development of CAR T cell therapies against T cell malignancies, as negligible antigen-negative T cell subsets ($CD7^{-}$ in this case) might expand after treatment, inducing rejection of the allogeneic CAR T cells and treatment failure.

In summary, this exciting paper by Hu et al. reports the first completed Phase I clinical trial for CD7-targeting allogeneic CAR T cells armed with multiple genetic modifications to potentiate antitumor activity and to resist fratricide, GvHD and immune rejection. This study illustrates the potential of donor-derived CAR T cell therapies for the treatment of T cell malignancies. Further investigations are warranted to optimize antitumor efficacy and persistence to move closer towards the goal of universally accessible off-the-shelf CAR T cell therapies.

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## ADDITIONAL INFORMATION

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