CAR Therapy for T-cell Malignancies

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Citation: Cinquina A, Feng J, Zhang H, Ma Y. CAR Therapy for T-Cell Malignances. J Cell Immunol. 2022;4(4):131-133.

Introduction

The introduction of chimeric antigen receptor (CAR) immunotherapy has been revolutionary in the treatment of hematological malignancies [1]. Remarkable success has been achieved in the clinical treatment of B-cell malignancies through the use of CD19 CARs [2-7]. However, translation of CAR therapy for T-cell malignancies has been more difficult for several reasons. As the CAR cells predominantly used are T cells, a CAR T cell manufactured to target malignant T cells using T-cell markers is at risk for fratricide. This would prevent the expansion of the CAR T cell population necessary for proper tumor eradication. Additionally, as both malignant and non-malignant express these T-cell markers, there is concern that a T-cell-directed CAR cell would lead to T-cell deficiency and subsequent opportunistic infections.

T-cell Directed CARs

One potential target for a CAR against T-cell malignancies is CD7, which is often highly elevated in T-cell leukemias and lymphomas. Genetic editing has been utilized to prevent CD7 expression on the CAR T cells to prevent fratricide, resulting in effective tumor lysis *in vitro* and *in vivo* in preclinical studies [8,9]. This genetic editing strategy may be unnecessary, as CD7 CAR T cells have also been shown to downregulate expression of surface CD7 protein to escape CD7-mediated self-killing (unpublished data). CD7 CAR T cells have demonstrated amazing results in a recent clinical study with high remission rates in a trial of twenty patients with relapsed or refractory T-cell acute lymphoblastic leukemia (T-ALL) [10]. Additionally, the associated neutropenia was temporary, and the median

time from infusion to recovery of absolute neutrophil count of 1,000/μL was 60 days [10].

While CD7 is expressed on all T cell populations, CD4 is expressed in a subset of T cells. While fratricide of CD4$^+$ CAR T cells would be expected in a CD4 CAR, memory CD8$^+$ CAR T cells, which are CD4$^-$ are considered the major cell type contributing towards successful engraftment [4]. While it is generally believed CD4$^+$ CAR T cells are necessary for the proper expansion of CD8$^+$ CAR T cells, *in vivo* CD8$^+$ CAR T cells against CD4 have demonstrated potent expansion and anti-tumor activity [11]. In a trial of a patient with Sezary syndrome, CD4 CAR T cells induced remission, and no obvious infections from the CD4$^+$ T cell aplasia was noted [12].

An alternative treatment target is CD5, which is often highly expressed in T-ALL. Similar to CD7 CAR T cells, CD5 CAR T cells have also been shown to downregulate expression of CD5 to escape CD5-mediated fratricide [13,14]. CD5 CAR T cells have shown promising early clinical results, with 4/9 patients achieving an objective response to CD5 CAR T cells with 2/9 patients having prolonged cytopenias at 6 weeks in a dose escalation trial [15]. Similarly, we recently demonstrated that augmented CD5 CAR T cells led to the remission of relapsed T-lymphoblastic lymphoma (T-LBL) with CNS involvement [16].

CD5-IL15/IL15sushi CAR T Cells

To strengthen CD5 CAR T cells, we modified the CAR T cells to secrete an IL-15/IL-15sushi complex. The cytokine IL-15 is posited to have benefits on CAR T functioning by enhancing

expansion, effector functioning, and successful trafficking of effector and memory T cells to locations desired for therapeutic effect. BCMA-directed CAR T cells expanded with IL-15 exhibited improved *in vivo* persistence and efficacy compared with IL-2- or IL-15/IL-7-grown CAR T cells [17]. However, IL-15 itself has a short half-life, which may limit the effectiveness of IL-15-grown CAR T cells after injection in clinical settings. Linking IL-15 with its soluble IL-15 receptor results in a significantly longer half-life and improved *in vivo* survival compared to IL-15 alone [18,19]. Therefore, to potentiate CD5 CAR, we had created a CD5 CAR that secretes a soluble IL-15 protein linked to the IL-15Rα sushi domain, which is the soluble portion of IL-15 receptor (abv. CD5-IL15/IL15sushi CAR).

In our trial, a patient with relapsed T-LBL with CNS involvement received CD5-IL15/IL15sushi CAR T cells after failing to respond to chemotherapy [16]. Prior to therapy, CSF analysis had demonstrated that around 80% of the CSF cells were composed of CD5$^+$ lymphoblasts. After just one week of CD5-IL15/IL15sushi CAR T cells, the lymphoblasts decreased to about 2% and were undetectable after 4 weeks. This correlated with the patient's symptoms, as preexisting left orbital swelling and debilitating headaches were improved within a week after receiving CD5-IL15/IL15sushi CAR T cells.

Safety of CD5-IL15/IL15sushi CAR T cells

CAR-related toxicities largely revolve around cytokine-release syndrome (CRS) and neurotoxicity due to widespread immune activation resulting in elevated levels of inflammatory cytokines. Proper dose escalation studies are essential to determine the optimum concentration of CAR cells to promote antitumor efficacy while minimizing their toxicity. While CD5-IL15/IL15sushi CAR T cell was only associated with a mild, Grade I CRS [16], various "safety switches" may be utilized to quickly eliminate CAR cells. While the risk of toxicity appears to be limited, incorporated "safety switches" have been developed as a means to quickly reverse the effects of CAR cells. We have previously demonstrated that low-doses of CAMPATH (alemtuzumab), which binds to CD52 and induces cell death, has resulted in rapid and efficient depletion of CAR T cells *in vivo* [14,20]. Alternatively, CD5-IL15/IL15sushi CAR includes two rituximab (RTX)-binding epitopes in the hinge region. RTX has been routinely used in the treatment of lymphomas, and administration of RTX has previously led to the lysis of other CAR T cells containing RTX-binding epitopes [21].

The risk of CRS might be more substantial in CAR cells which secrete cytokines, such as CD5-IL15/IL15sushi CAR T cells. Additionally, while IL-15 may promote the antitumor efficacy of CAR T cells, excessive levels of IL-15 have also been associated with uncontrolled lymphocytic expansion [22]. However, serum levels of IL-15 in the patient treated with CD5-IL15/IL15sushi CAR T cells remained only minimally elevated (<60 pg/mL) throughout the post-infusion period [16]. Similarly, a phase I and II trial of NK cells transduced with both CD19 CAR and IL-15 demonstrated no increase in IL-15 levels over baseline [23]. These clinical trials suggest that CAR T cells may be useful as a vehicle to deliver IL-15, requiring lower concentrations to bring therapeutic utility without the toxicities associated with higher concentrations associated with IL-15 injections.

Use of T-cell-directed CAR cells is expected to result in T-cell aplasia, similar to the B-cell aplasia observed in CD19 CAR T cells, which can increase the risk of opportunistic infections. In a clinical trial of CD7 CAR T cells, 20% (4/20) of the patients had viral reactivations that resolved with antiviral therapy, and 1 patient with a pre-enrollment history of fungal infection died of pulmonary hemorrhage associated with fungal pneumonia 5.5 months post-therapy [10]. In early reports of phase I trials of CD4 CAR and CD5-IL15/IL15sushi CAR, therapy was not associated with any obvious infections during the treatment period [12,16]. Importantly, CD5$^+$ T cells returned to normal levels nine days post-CD5-IL15/IL15sushi CAR T cell infusion, and such rapid recovery may substantially mitigate the risk of infection following CAR therapy.

Future Direction

Initial clinical trials have shown that T-cell-directed CAR therapy may be a promising therapeutic approach for patients with relapsed or refractory T-cell malignancy, where current treatment options are limited. By inducing remission, patients curative bone marrow transplantation may be offered to more patients who would have previously been ineligible due to persistent disease. While early clinical data has demonstrated CAR efficacy and limited adverse effects due to CRS and T-cell aplasia, larger clinical studies of CD4 CAR (NCT04162340, NCT03829540), CD5 CAR (NCT04594135, NCT05032599, NCT03081910), and CD7 CAR (NCT04689659, NCT05212584, NCT04934774) are currently underway that will further assess the feasibility of T-cell-directed CAR therapy.

While single-targeting CD19 CAR T cells have shown impressive outcomes in treatment of B-ALL, complete clinical response has often been limited by the emergence of CD19-negative tumors through antigen escape [24]. If T-ALL relapses or is refractory following CAR therapy, subsequent treatment with a CAR directed against a different target antigen may be effective to eliminate any tumor cells that may have escaped initial CAR immunotherapy due to antigen escape. Alternatively, dual-targeting CAR therapies have been developed that simultaneously target two separate antigens, with promising results in early clinical trials of relapsed or refractory B-ALL [25]. As CD5 and CD7 are both highly expressed in most T-ALL leukemias, a dual-targeting CD5-CD7 CAR may produce more sustained remission than treatment aimed at only one antigen.

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