CAR-T Cell therapy in T-cell malignancies: limitations and solutions

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Received: 11 May 2022; Revised: 12 July 2022; Accepted: 3 August 2022
Published online: 23 September 2022
DOI 10.15212/HOD-2022-0002

Abstract

CD19-targeted chimeric antigen receptor (CAR)-T cell therapy has shown high potential for treating B-cell hematological malignancies and has been approved by the US FDA. However, CAR-T cell therapy for T-cell hematologic malignancies poses feasibility challenges, including the difficulty of obtaining sufficient healthy cells from patients, CAR-T cell fratricide, and the risk of immunodeficiency. In this review, we discuss bottlenecks and possible solutions in CAR-T cell therapy for T-cell acute lymphoblastic leukemias, as well as future directions in this field.

Keywords: Immunotherapy, Chimeric antigen receptor (CAR)-T cell, T-cell malignancies

1. INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL), a highly aggressive and invasive hematological malignancy, accounts for approximately 25% of adult and 15% of pediatric acute lymphoblastic leukemia (ALL) cases [1, 2]. T-ALL is more likely to relapse than B-cell acute lymphoblastic leukemia (B-ALL) [3]. Treatment of multiple relapsed/refractory (r/r) T-ALL is challenging, and patients have dismal prognoses [4, 5]. The estimated 5-year survival is currently 70–85% in T-ALL but 7% in relapsed T-ALL [6, 7]. Allogenic hematopoietic stem cell transplantation (SCT) is an ideal cure for T-ALL, which is recommended for patients who experience the first relapse and may induce complete remission (CR) [8, 9]. CAR-T cell therapy has been widely used for B-ALL [10-13]. To date, the US FDA has approved five CAR-T cell therapies for hematological malignancies that express the CD19 or BCMA antigen. However, CAR-T cell therapy for non-B hematologic malignancies is more challenging and remains in an early exploration stage [10-14].

Herein, we summarize clinical studies exploring CAR-T cell therapy for T-cell malignancies, and also discuss limitations and potential future research directions in this field.

2. TARGETING THE MAIN ANTIGENS

2.1 CD7

CD7 is a membrane glycoprotein expressed on T lymphocytes and NK cells [15, 16]. Studies have demonstrated that 95% of T-ALL and T-cell lymphomas are CD7 positive [17]. A case report has described autologous CD7 CAR-T cell therapy leading to CR in a high-risk patient. The patient experienced a manageable cytokine release syndrome (CRS). CAR-T cells persisted for approximately 40 days in vivo [18]. In autologous CD7 CAR-T cell therapy, difficulties in isolating and obtaining a sufficient number of healthy T cells without tumor cell contamination may be encountered [19]. CAR-T cell-mediated targeting of the same antigen can cause endogenous T cell depletion or CAR-T cell fratricide. The case study used CAR with inducible caspase 9 to withdraw CAR-T cells if needed. Unexpectedly, the fratricide effect was not observed in the patient [18]. In addition, Dai et al. have used autologous CD7 CAR-T cells to treat a patient with early T cell precursor lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) with the TP53 mutation. The patient achieved TP53 mutation-negativity on day 91 after receiving CD7 CAR-T cells [20]. In another ongoing clinical trial, researchers have treated nine patients: six with r/r T-ALL/LBL and three with r/r ETP-ALL/LBL with
autologous CD7 CAR-T cells. The CR rate at 3 months was 71.4% in the patients who were followed up for 3 months. Of the first six patients, two experienced grade 2 CRS, and the remainder developed grade 1 CRS [21]. In this trial, CD7 CAR-T cells with the CD7 protein expression blocker (PEBL) structure overcame fratricide [22]. In a related meeting abstract, Yang et al. have reported findings in 14 patients with r/r T-ALL who received autologous CD7 CAR-T cells, of whom 13 achieved CR or incomplete count recovery by 28 days post-infusion. Only one patient experienced grade 3 CRS, whereas others experienced grade 1 or 2 CRS. CD7 CAR-T cells persisted in the peripheral blood for a median of 52.5 days at the last evaluation [23].

Use of donor-derived CAR-T cells rather than autologous CAR-T cells can circumvent the challenges of obtaining adequate healthy T cells from patients and tumor cell contamination. Donor-derived CAR-T cells are not affected by patient disease status, but may cause graft-versus-host disease (GVHD) and rejection [24]. In our center, we have treated 20 patients with r/r T-ALL with CD7 CAR-T cells derived either from new donors or from prior transplantation donors [25]. When patients received new donor-derived CAR-T cell therapy, they underwent SCT derived from the same donors to alleviate long-term hematologic toxicity [25]. However, this strategy is limited to patients who have received prior SCT or those who are eligible for transplantation and have matched donors. A total of 90% of patients achieved CR, whereas only 10% of patients developed grade 3 or higher CRS. CD7 CAR-T cells proliferated effectively and persisted in vivo for more than 3 months [25].

The CAR construct also incorporated a PEBL sequence causing retrograde transport of CD7 protein to the endoplasmic reticulum (ER). Consequently, the antigen was entrapped in the ER/Golgi, thus blocking its normal expression and minimizing fratricide [25, 26]. Previously, other researchers advocated for a similar technique for decreasing CD7 expression and preventing fratricide [27]. CD7 CAR-T cells can still target endogenous CD7-positive T and NK cells, thus increasing infection risk. A total of 25% of patients developed viral activation, and one patient with a fungal infection died of fungal pneumonia. In vitro analysis showed that the CD7-negative T cells reacted to fungi and viruses, thus indicating that they might have had some immunoprotective activities [25]. A total of 60% of patients had grade 1 or grade 2 GVHD, but all adverse effects were managed with ruxolitinib and/or methylprednisolone [25]. The above evidence indicates that donor-derived CD7 CAR-T cell therapy is highly efficient, but care should be taken to manage the related adverse effects, including infections and GVHD.

Universal CAR-T (UCAR-T), as an “off-the-shelf” product, is under intensive investigation. Gene-editing systems such as TALEN and CRISPR/Cas9 have been used to delete endogenous TCR and MHC genes to prevent GVHD and rejection [28, 29]. UCAR-T cells may offer the benefit of preventing tumor cell contamination in T-cell malignancies. UCAR-T cells are not affected by patient disease status, thus allowing patients to receive standard and timely treatment. In 2018, Cooper et al. used CRISPR-Cas9 to eliminate TCR alpha chain and CD7 expression on CD7 CAR-T cells. Consequently, the CD7 CAR-T cells not only demonstrated efficient tumoroidal activity against T-ALL primary cell lines without GVHD but also increased proliferation efficiency in vitro [30]. However, to date, UCAR-T cell amplification and persistence in vivo and the possible safety issues associated with gene-editing remain limitations of UCAR-T cell therapy. In 2020, Li et al. used CD7 UCAR-T cells to treat two patients with T-ALL, both of whom achieved CR. One patient has been in remission for more than 1 year after infusion of CAR-T cells [31]. Because T and NK cells express CD7, CD7 CAR-T cells target patients’ alloreactive T and NK cells, thereby preventing rejection [32]. However, UCAR-T cells may have less ability to persist in vivo than autologous and donor-derived CAR-T cells [25, 31].

From the above-mentioned CAR-T cell targeting of CD7, the persistence of autologous and donor-derived CAR-T cells appears to be much higher than that of UCAR-T cells. The efficacy of autologous CAR-T cells has been confirmed, although tumor cell contamination remains a challenge. Donor-derived CAR-T cell therapy has achieved convincingly high efficacy, but it involves donors, thus posing obstacles under some conditions (such as a lack of suitable donors) [33]. The efficacy of UCAR T cell therapy, despite its ability to avoid tumor cell contamination, fratricide, and GVHD, requires confirmation through more clinical trials. Nonetheless, endogenous T-cell depletion is a common problem awaiting resolution.

2.2 CD5
CD5 is a glycoprotein with an extracellular domain that spans the cell membrane. CD5 is expressed on thymocytes, T lymphocytes, and B-1a cells [34, 35]. In 2015, Mamonkin et al. revealed that CD5 CAR-T cells exhibit partial and temporary fratricide, and mediate antitumor activity in vitro [36]. Unlike CD7 CAR-T cells, CD5 CAR-T cells can proliferate without knockdown of CD5 gene expression. In 2018, Mamonkin et al. found that CD5 CAR-T cells with the 4-1BB co-stimulatory domain, instead of CD28, can enhance antitumor activity but may enhance CAR-T cell fratricide [37]. However, stringent CD5 knockdown may favor CAR-T cell proliferation [38].

Hill et al. have performed a clinical trial in which four patients with T-ALL and five patients with T-cell non-Hodgkin lymphoma received autologous CD5 CAR-T cell therapy. Three of nine patients achieved CR, one of whom had T-ALL. Three of nine patients experienced grade 1 or 2 CRS [39]. Interestingly, fratricide was not a major problem, because CAR-T cells expanded in the patients from 0.7 to 6 months, according to polymerase chain reaction (PCR) detection, and normal CD3-positive T cells were not completely depleted. This result might have been because CD28-costimulation and CD5 down-regulation
on T cell surfaces cause CAR-T cells to experience only transient fratricide. In 2021, the same team updated their data and reported that four of nine patients with T-cell lymphoma achieved responses. CR was observed in two patients (22.2%): one with angioimmunoblastic TCL and one with peripheral T-cell lymphoma. Grade 1 CRS and grade 2 CRS were observed in three patients and one patient, respectively. No other neurotoxicity events were observed in this clinical trial [40]. Despite these encouraging findings, more trials are needed to verify the safety and efficacy of CD5 CAR-T cell therapy.

In 2020, Feng et al. treated a patient with T-LBL with donor-derived CD5 CAR-T cells [41]. The researchers produced CD5 CAR-T cells that secreted IL-15 protein to potentiate CD5 CAR-T cell function [42]. Blasts in the cerebrospinal fluid decreased from approximately 80% to approximately 2% 1 week after CD5 CAR-T cell infusion and were undetectable by the fourth week. GVHD was not observed in this patient. The patient subsequently underwent SCT [41].

Table 1 summarizes the above-mentioned preliminary results from several clinical trials of CD5 or CD7 CAR-T cell therapy.

3. TARGETING OTHER ANTIGENS

CD3 is a pan-T cell antigen, and cytoplasmic CD3 is considered an indicator of T-cell lineage [43]. CD3 is not an ideal antigen target for CAR-T cell therapy, because of fratricide. Researchers have used TALEN to knock out the endogenous TCRαβ/CD3 before modifying CD3 CARs. CD3 CAR-T cells have been found to kill primary T cells with high specificity and potency [44]. CD1a is expressed on cortical T-ALL cells, but not on normal T cells or CD34-positive progenitor hematopoietic cells [45-48]. These characteristics make this antigen suitable for cortical T-ALL. CD1a CAR-T cells have shown robust anti-tumor activity in preclinical investigations, but more clinical trials are needed [48].

T-cell-derived hematologic malignancies may come from CD4+ T cells [49]. CAR-T cells targeting CD4 may spare endogenous CD8 T cells, thus avoiding complete T cell immunodeficiency after infusion in patients. Preclinical assays have demonstrated that CD4 CAR-T cells can efficiently eliminate CD4-positive leukemic cells in co-culture assays [50]. However, we did not see any evidence of CD4 CAR-T cell therapy in clinical trials.

Most T cells express the TCR chain, which is encoded by the T cell receptor beta constant 1 (TRBC1) or TRBC2 gene [51]. TCR is expressed in more than 95% of peripheral T cell lymphoma (PTCL) and 30% of T-ALL cases [52, 53]. TRBC1 CAR-T cell therapy may decrease fratricide to some extent by sparing TRBC2 T cells [54]. CAR-T cell therapy is currently being developed for PTCL. In clinical trials, CD30 CAR-T cell therapy for Hodgkin lymphoma has been demonstrated to be effective. CD30 is also present on a subset of PTCL, including anaplastic large cell lymphoma, and may serve as a promising target [55, 56]. In one clinical trial, two patients with anaplastic large cell lymphoma received CD30 CAR-T cell therapy, but the efficacy was limited [57].

4. LIMITATIONS OF CAR-T THERAPY FOR T-ALL/T-LBL

4.1 Difficulty in obtaining autologous healthy T cells

Normal and malignant T cells usually have some overlap in phenotypes. Therefore, obtaining healthy T cells without tumor cell contamination from patients who have tumor cells in the peripheral blood or considerable lymphopenia after intensive therapy may be difficult [19]. The incorporation of tumor cells may cause the emergence of treatment-resistant cells, through a mechanism of antigen masking, as previously reported in CD19 CAR-T cell therapy [19]. As described earlier, generating allogeneic CAR-T cells from transplantation donors or healthy third-party donors may be a viable option. Furthermore, UCAR-T cells also serve as good sources.

4.2 CAR-T fratricide

The developed targeted antigens in CAR-T cell therapy to treat T-ALL, such as CD7 and CD5, are expressed on healthy T cells and CAR-T cells [58, 59]. CAR-T cell fratricide results, thus decreasing CAR-T cell amplification [30, 32]. As described earlier, researchers have used the PEBL system, composed of a target-targeting scFv associated with a retention domain, which entraps antigen in the ER/Golgi and hinders expression [27]. In addition, UCAR-T cells are resistant to fratricide after deletion of the antigen via a gene-editing system [30].

4.3 Immunodeficiency

T-cell aplasia and severe immunodeficiency occur when CAR-T cells deplete endogenous normal T cells. Exogenous immunoglobulin replacement therapy can be used to treat B-cell aplasia caused by CAR-T cell persistence in patients with B-ALL [60, 61]. In contrast, T-cell aplasia may be more serious or even life-threatening and have no effective treatment [62]. T-cell aplasia may be prevented through several suggested methods. Targeting an antigen that is absent on normal T cells or is expressed on only a small percentage of normal T cells may leave at least some of the normal T cells intact [63]. Using CAR-T cells with a regulated lifespan or activity whose anti-tumor effects are limited can be advantageous for preventing the onset of T-cell aplasia [64]. In addition, bridging to SCT after CAR-T cell therapy may be an additional choice that can be made to decrease the risk of CAR-T-associated T-cell aplasia [65, 66]. The ultimate strategy to circumvent this problem may involve deletion of the target antigen gene from hematopoietic stem cells to differentiate T cells lacking target antigen expression. In a preclinical study by Kim et al., stem cells with CD7 deletion have been transplanted into recipient mice before CD7 UCAR-T cell therapy. Stem cells can successfully differentiate into CD7-negative T cells and CD7-negative NK cells in vivo without dysfunction, and these
## Table 1 | Outcomes and characteristics of clinical trials of CAR-T cell therapy for T-ALL.

<table>
<thead>
<tr>
<th>Study</th>
<th>Autologous CD7 CAR-T</th>
<th>Autologous CD7 CAR-T</th>
<th>Autologous CD7 CAR-T</th>
<th>Autologous CD7 CAR-T</th>
<th>Donor-derived CD7 CAR-T</th>
<th>CD7 UCAR-T</th>
<th>Autologous CD5 CAR-T</th>
<th>Donor-derived CD5 CAR-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>18</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>25</td>
<td>20</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>No. of patients</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>14</td>
<td>20</td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Dose</td>
<td>2×10⁶/kg</td>
<td>5×10⁶/kg</td>
<td>1–2×10⁶/kg</td>
<td>0.5×10⁶/kg to 2×10⁶/kg</td>
<td>0.5–1×10⁶/kg</td>
<td>6.44×10⁶/kg, 1.1×10⁷/kg</td>
<td>1×10⁷/m² to 1×10⁸/m²</td>
<td>2×10⁶/kg</td>
</tr>
<tr>
<td>Disease phenotype</td>
<td>T-ALL</td>
<td>ETP-ALL/LBL</td>
<td>6 T-ALL/LBL and 3 ETP-ALL/LBL</td>
<td>T-ALL</td>
<td>T-ALL</td>
<td>T-ALL</td>
<td>2 AITL, 6 TCL, and 1 T-ALL</td>
<td>T-LBL</td>
</tr>
<tr>
<td>Efficacy</td>
<td>CR</td>
<td>CR</td>
<td>CR: 88.9% NR: 11.1%</td>
<td>CR: 92.9% NR: 7.1%</td>
<td>CR: 90% PR: 5% NR: 5%</td>
<td>CR: 100%</td>
<td>CR: 22.2%</td>
<td>CR</td>
</tr>
<tr>
<td>CAR-T fratricide</td>
<td>No</td>
<td>Decreased fratricide with ER retention of CD7</td>
<td>Decreased fratricide with ER retention of CD7</td>
<td>NA</td>
<td>Decreased fratricide with ER retention of CD7</td>
<td>Decreased fratricide with knockout of CD7</td>
<td>Minimal and transient*</td>
<td>NA</td>
</tr>
<tr>
<td>CAR-T persistence in PB (by FCM)</td>
<td>Approximately 1.3 mo</td>
<td>Approximately 1.2 mo</td>
<td>NA</td>
<td>Approximately 1.7 mo</td>
<td>3 mo, &gt;6 mo (by PCR)</td>
<td>&lt;1 mo</td>
<td>3 wk to 9 mo (by PCR)</td>
<td>NA</td>
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<tr>
<td>CRS grade ≥1; ≥3</td>
<td>100%; 0</td>
<td>100%; 0 (in case 1–6)</td>
<td>100%; 7.1%</td>
<td>100%; 10%</td>
<td>100%; 100%</td>
<td>NA; 0</td>
<td>100%; 0</td>
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<tr>
<td>ICANS grade ≥1; ≥3</td>
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<td>NA; NA</td>
<td>7.1%; 0</td>
<td>15%; 0</td>
<td>NA; NA</td>
<td>NA; NA</td>
<td>NA; NA</td>
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<tr>
<td>Incidence of GVHD</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>High (60%)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Risk of T cell deficiency</td>
<td>NA</td>
<td>Depletion of CD7+ T cells (CD7- T cells expanded)</td>
<td>Severe and transient T cell deficiency</td>
<td>NA</td>
<td>Depletion of CD7+ cells (CD7- T cells expanded)</td>
<td>Depletion of CD7+ cells</td>
<td>Decreased, but not complete depletion of CD3+ T cells</td>
<td>Mild and transient T cell deficiency</td>
</tr>
</tbody>
</table>

*Probably because of CD5 down-regulation in CAR-T cells.

**Abbreviations:** AITL, angioimmunoblastic TCL; CAR, chimeric antigen receptor; CR, complete remission; CNS, central nervous system; CRS, cytokine release symptom; ETP-ALL, early T cell precursor lymphoblastic leukemia/lymphoma; FCM, flow cytometry; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; ICANS, immune effector cell–associated neurotoxicity syndrome; mo, months; NA, not available; NR, not remission; PB, peripheral blood; PCR, polymerase chain reaction; PR, partial remission; T-ALL, T-cell acute lymphoblastic leukemia; TCL, T-cell lymphoma; T-LBL, T-cell lymphoblastic lymphoma; wk, weeks.
CD7-negative cells can tolerated well to CD7 UCAR-T cells [67]. Transplantation of gene-edited stem cells may be applied to other targets, such as CD5. However, gene-editing of stem cells is in the immature stage poses safety concerns and may require further optimization before widespread use in clinics.

These bottlenecks and strategies in CAR-T cell therapy for T-ALL/T-LBL are illustrated in Figure 1.

5. CONCLUSION

Currently, chemotherapy and SCT are recommended for ALL therapy but are limited by the problem of relapse [3]. CAR-T cell therapy has been found to improve outcomes in patients with r/r B-ALL, but the difficulties in obtaining sufficient healthy T cells from patients, CAR-T cell fratricide, and the risk of immunodeficiency limit its clinical applications in T-ALL.

Preliminary outcomes have been obtained for CD7, the most common target of CAR-T cell therapy for T-ALL. However, the same antigens shared by malignant T cells, CAR-T cells, and healthy T cells can cause tumor cell contamination, fratricide, and immunodeficiency. As previously described, allogeneic CAR-T cell therapy has demonstrated several advantages over autologous CAR-T cell therapy in overcoming the problem of tumor cell contamination in manufacturing CAR-T cells [25, 31]. The problem of fratricide can be solved by decreasing the expression of the target antigen on CAR-T cells with PEBL or a gene-editing system [27, 30]. Furthermore, major efforts should be focused on finding solutions to prevent immunodeficiency. Screening for novel and specific antigens restricted to malignant cells, equipping CAR-T cells with safety switches, and post-CAR SCT may be beneficial in controlling immunodeficiency [63-66]. In addition, transplanting stem cells with deletion of target antigen genes before CAR-T cell infusion may be a promising strategy to prevent immunodeficiency [67]. All these strategies will require in-depth evaluations to validate their safety and efficacy in preclinical and clinical trials.

ACKNOWLEDGEMENTS

The figures were drawn with BioRender (https://app.biorender.com/). We regret not including all related studies in this review. This work was supported by funding from CAMS Innovation Fund for Medical Sciences (CIFMS,2021-I2M-1-017).

DISCLOSURE OF INTEREST

We declare no competing interests in relation to this work.
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