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REVIEW ARTICLE

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Abstract

Autologous chimeric antigen receptor (CAR) T cell therapy has been extensively studied over the past decades. Currently, autologous CAR T products are FDA-approved to treat B cell acute lymphoblastic leukemia (B-ALL), large B cell, mantle cell, and follicular lymphomas, and multiple myeloma. However, this therapy has drawbacks including higher cost, production lead time, logistical complexity, and higher risk of manufacturing failure. Alternatively, allogeneic CAR T cell therapy, currently under clinical trial, has inherent disadvantages, including cell rejection, graft versus host disease, and undetermined safety and efficacy profiles. Different strategies, including modifying HLA and T cell receptor expression using different effector cells, are under investigation to circumvent these issues. Early allogeneic CAR T therapy results for B-ALL and B-NHL have been promising. Large sample clinical trials are ongoing. Here, we discuss the pros and cons of allo-CAR T for hematologic malignancies and review the latest data on this scalable approach.

1. Introduction

Immunotherapy has been in the spotlight in cancer treatment for decades; chimeric antigen receptor (CAR) T cell therapy is the most promising strategy [1]. Six CAR T products, Kymriah (tisagenlecleucel), Yescarta (axicabtagene ciloleucel), Breyanzi (lisocabtagene maraleucel), Tecartus (brexucabtagene autoleucel), Abecma (Idecabtagene vicleucel), and Carvykti (ciltacabtagene autoleucel), have yet been approved by the FDA for treatment of B cell acute lymphoblastic leukemia (B-ALL), B-NHL and multiple myeloma (MM) [2–6].

All approved CAR T products are engineered using autologous T cells. Although autologous CAR T (auto-CAR T) cell therapy is associated with the absence of allogeneic rejection and thus, a longer persistence, it also has well-known disadvantages, such as higher cost, difficulty to obtain adequate T cells in some circumstances, not being readily accessible, a complex logistical process, risk of failure of manufacturing, and a long lead time to produce individual cells [7].

Allogeneic CAR T (allo-CAR T) cell therapy is an alternative approach that aims to produce "off-the-shelf' ready-to-use CAR T cell products that can circumvent the obstacles of auto-CAR T therapy. It uses third-party donor-derived T cells; it is readily available to eligible patients and potentially has a lower cost than auto-CAR T. In this review, we will discuss the design of allo-CAR T, its pros and cons, current clinical experiences, and future directions.

2. CAR T cell design

There are four major components in CAR T cell structure: the antigen-binding domain, hinge, transmembrane domain, and intracellular signaling domain.

The antigen-binding domain is the extracellular portion of a CAR T cell. It is a single-chain variable fragment (scFv) derived from a monoclonal antibody with variable heavy and light chains. In Kymirah and Yescarta, it is derived from murine anti-human CD19 antibody and targets CD19-expressing cells [8]. The scFv is a critical component of CAR T cell design, given that it determines the specificity and affinity of CAR T cells to the target antigen [9].

The hinge and transmembrane domains connect the antigen binding domain to the intracellular

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signaling domain. Different hinge designs can affect antigen binding, CAR T cell signaling, and CAR T cell cytokine production. The transmembrane domain, commonly derived from CD3ζ, CD28, CD4, or CD8α, influences the stability of CAR T cells [10].

The intracellular signaling domain contains one activation domain and one or more co-stimulatory domains. The activation domain is usually a CD3ζ from a T cell receptor, and the co-stimulatory domain is from the CD28 tumor necrosis factor receptor family (including 4-1BB, OX40, and CD27) [11]. The co-stimulatory domain plays an important role in CAR T cell activation, survival, and efficacy. Various domains have unique properties, and their selection is based on the target disease. For example, the CD28 domain functions through the phosphatidylinositol 3-kinase (PI3K)-ArK pathway and results in high interleukin (IL)-2 production [12–14]. CAR T cells with a CD28 domain have rapid T cell activation, proliferation, and cytolysis, but they are short lived; they are usually undetectable after 3 months. Hence, they are ideal for transient use, with rapid elimination of a tumor, such as a bridge therapy to allogeneic hematopoietic stem cell transplantation (allo-HCT). Conversely, 4-1BB, which signals through TRAF proteins, induces slow T cell effector response, promotes CAR T cell differentiation into central memory T cells, and increases mitochondrial biogenesis and oxidative metabolism. 4-1BB-based CAR T cells can persist longer in patients compared to CD28-based CAR T cells [15].

Currently, there are five generations of the CAR T design. The first generation has a CD3ζ domain that activates ZAP70/Syk tyrosine kinase and, subsequently, the downstream signaling cascade. The second generation has a co-stimulatory domain, either CD28 or 4-1BB. The third generation CAR T includes two co-stimulatory domains, resulting in more cytotoxicity to tumor cells. The fourth generation CAR T has an additional nuclear factor of activated T cell (NFAT) gene for IL-12 production to activate the innate immune system. Lastly, the fifth generation CAR T has a JAK-STAT domain to stimulate CAR T cell proliferation [16].

CAR T therapy is associated with several unique adverse events, including but not limited to cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

Researchers have been investigating the use of safety genes as "off switches" in CAR T cell design. This includes CD20, which is targeted by rituximab, and epidermal growth factor receptor (EGFR), which is targeted by cetuximab [17,18].

3. Pros and cons of allo-CAR T

3.1. Pros

Compared to auto-CAR T, Allogeneic CAR T derived from a third party could be an important resource for treatment of hematologic malignancies (HM), given its immediate availability, lower manufacturing complexity, and economy to scale.

3.1.1. Logistical complexity

Auto-CART is a personalized therapy that requires the collection of a patient's T lymphocytes, which can be challenging because lymphopenia is not uncommon in patients with HM due to prior treatments or involvement of the diseased marrow. Patients in whom the absolute lymphocyte count was below the threshold were excluded from the pivotal clinical trials [3,6,19,20]. Allo-CAR T bypasses the initial individual patient's lymphocyte collection step since the CAR T cells are engineered from healthy donors or umbilical cord cells. Thus, Allo-CAR T cells can be readily available to scale without the complex individual manufacturing process.

3.1.2. Timing

It takes an average of 3 weeks to manufacture and ship auto-CAR T cells, valuable time that many advanced patients with HM do not have. In one study, 11% of patients with B-NHL did not receive the intended auto-CAR T therapy due to rapid disease progression and clinical deterioration [21]. In another study of children and young adults with B-ALL, 7.6% of patient died before receiving auto-CART treatment [4]. Allo-CAR T is immediately available for rapidly progressing diseases.

3.1.3. Cost

The FDA has approved auto-CAR T products for lymphoid malignancies and MM with a tag price of $373,000 to $475,000 [22,23]. The high cost of auto-CAR T is not sustainable and restricts the access to this potentially curing treatment in the US and globally. Allo-CAR T is easily scalable, and, if proven to be as efficacious, will definitely be less expensive than auto-CAR T products.

3.2. Cons

Allo-CAR T is still in its early development phase and requires large clinical trials to test its safety and efficacy. Human leukocyte antigen (HLA) mismatch between a healthy donor and the patient can trigger an immune response, potentially leading to graft versus host disease (GVHD) and CAR T rejection. Strategies to mitigate these disadvantages are ongoing.

3.2.1. GVHD

In allo-HCT studies, αβ T cells have an important role in the development of GVHD [24]. The T cell receptor (TCR) in allo-CAR T cells can recognize the recipient antigen via HLA molecules and subsequently activate CAR T cells to target recipient tissues, causing GVHD [25]. Two main strategies to mitigate the risk of GVHD are being studied.

1. Avoiding the use αβ T cells. Natural killer (NK) cells, NK T cell and γδ T cells do not possess TCRs and are unlikely to induce GVHD. *In vivo* studies of these cells have shown a potent cytotoxic effect against glioblastoma and lymphoma [26–28]. A phase II clinical trial of CAR NK cells for patients with B-NHL is ongoing at our institution (NCT05020015). In 2020, Liu et al. reported 11 patients with B cell lymphoid malignancies treated with allo-CAR NK therapy, and no GVHD was reported in any of these cases [29].
2. TCR gene editing. Since αβ T cells play a vital role in GVHD, interference of TCR expression on αβ T cells has been widely adopted in allo-CAR T therapy. The αβ TCR is a heterodimer with an α chain encoded by a single gene, *TRAC*, and a β chain encoded by two genes. The deletion of the *TRAC* to disrupt TCR expression is currently the most commonly used strategy in allo-CAR T manufacturing. Gene editing tools such as zinc finger nucleases, transcription activator-like effector nucleases (TALEN), and mega TAL nucleases have been reported to successfully knock out the *TRAC* gene [30–32]. In addition to these tools, CRISPR-Cas9 is being widely used in current clinical trials. It uses a CAR construct to create a knock-out in the *TRAC* locus, which simultaneously introduces the CAR structure to effector cells and interrupts TCR expression in effector cells. This approach has also shown other advantages, such as absence of constant excessive T cell activation, greater antitumor potency, and low risk of mutagenic insertions [33].

3.2.2. Rejection

Rapid allo-CAR T rejection by the recipient immune system threatens the survival and efficacy of this modality. To prevent rejection, two main strategies are under investigation.

1. Elimination of HLA class I expression by targeting HLA-A or beta2-microglobulin (B2M), which is required for HLA-I expression [34,35]. Without HLA-I expression, the host T cells cannot recognize allo-CAR T cells as foreign cells. One preclinical study used CRISPR/Cas9 to double knock out *TRAC* and *B2M*, which showed promising efficacy and safety [36]. However, activated T cells also express HLA Class II, which triggers rejection [37]. Targeting HLA class II expression on allo-CAR T cells can potentially mitigate rejection. However, reduced HLA expression on allo-CAR T cells does not protect them from the host NK cells.
2. Intensifying lymphodepletion. In one study, researchers knocked out CD52 on allo-CAR T and added an anti-CD52 antibody, alemtuzumab, to the lymphodepletion regimen [38]. This selective lymphodepletion approach potentially neutralizes the host T cells without affecting the allo-CAR T cells.

4. Current clinical experiences

The clinical experience with allo-CAR T therapy is limited, and currently, there are many ongoing clinical trials. Qasim et al. reported the administration of a single dose infusion of universal CAR19 (UCART19) T cells to two infants with relapsed refractory B-ALL. Both infants achieved molecular remission within 28 days and went on to receive allo-HCT [39]. Benjamin et al. treated 21 patients with B-ALL with UCART19; 67% achieved complete response after 28 days, 2 patients developed acute grade 1 skin GVHD, and no rejection was reported [40]. The results of the ALPHA2 study in relapsed/refractory B-LBCL were presented in the 2021 ASCO and ASH meetings. In 15 patients who received ALLO-501A, the overall complete response rate was 50%, and no GVHD or rejection was reported [41,42]. However, a chromosomal abnormality was later found in one patient, and the trial was withheld by the FDA. Both UCART19 and ALLO-501A use TALEN technology to disrupt expression of TRAC and CD52 to prevent GVHD and rejection. With the current limited clinical experience, few manageable GVHD cases were observed, and no acute rejection was reported in allo-CAR T therapies. Meanwhile, NK cells have been designed to express anti-CD19 CARs to overcome CAR T therapy limitations. In 2020, Liu et al. reported their clinical experience of using anti-CD19 CAR NK cells to treat B-NHL or chronic lymphocytic leukemia. Eleven patients received CAR NK cells with no occurrence of GVHD, CRS or ICANS, and seven achieved complete remission within 30 days of infusion [29]. Although limited, the clinical experience of allo-CAR T therapy to date

seems very promising, and there are many large-scale ongoing trials addressing its safety and efficacy.

5. Conclusion and future direction

With six FDA-approved CAR T products to date, CAR T therapy is the hotspot for relapsed refractory B cell HMs. However, this transformative therapy has several limitations, such as the inability to collect lymphocytes in 5–10% of patients, long manufacturing lead time, and prohibitive cost. Allo-CAR T can potentially address most of the drawbacks of auto-CAR T therapy, albeit with its own challenges—that is, rapid rejection and risk of GVHD. Gene editing technology to knock out *TCR*, *HLA-I*, and *CD52* and the use of HLA-independent effector cells (NK cells, NK T cells, and γδ T cells) are being employed to mitigate allo-CAR T drawbacks.

The early phase of clinical allo-CAR T data is promising, and large phase II clinical trials are currently underway.

Conflict of interest

The authors declare no conflict of interest.

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