Hematology/Oncology and Stem Cell Therapy

Volume 15 | Issue 3 Article 11

Next-Generation Chimeric Antigen Receptor T Cells

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ORIGINAL RESEARCH REPORT

Next-Generation Chimeric Antigen Receptor T-cells

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Abstract

The U.S. Food and Drug Administration (FDA) approved 6 CAR T cell (CAR-T) products, including tisagenlecleucel (tisa-cel), axicabtagene ciloleucel (axi-cel), brexucabtagene autoleucel (brexu-cel), lisocabtagene maraleucel (liso-cel), idecabtagene vicleucel (ide-cel), and ciltacabtagene autoleucel (cilta-cel) in the last 5 years. CAR T-cell therapy significantly improved outcomes for patients with B-cell non-Hodgkin lymphoma (NHL) and multiple myeloma (MM). However, recurrence and progression may occur after the initial response due to multiple mechanisms (Zeng and Zhang, 2022) [1]. Furthermore, CAR T-cell therapy is not broadly utilized in solid tumors due to various barriers. This review discusses the evolution of CAR T-cell therapies and how the "younger-generation" CAR T cells counteract these challenges to potentially broaden their applications in the future.

1. T-cell activation

In a mature and naïve T cell, the T-cell receptor (TCR)-CD3 complex is composed of a genetically-diverse αβ (or γδ) TCR heterodimer noncovalently associated with the invariant CD3 dimers CD3ϵγ, CD3ϵδ, and CD3ζζ [2]. The TCR mediates recognition of antigenic peptides bound to MHC molecules (pMHC), whereas the CD3 molecules transduce activation signals to the T cell [2]. This signal alone is insufficient for full T-cell activation. T cells that only receive the first signal become anergic and do not proliferate in response to antigens. The second signal required is induced when a costimulatory receptor, such as CD28 and 4-1BB (CD137), on the T cells, binds to its cognate ligand on the antigen-presenting cell (APC). A third signal promoting productive T cell responses is provided by cytokine signaling [3]. The essential features of TCR are similar to those of immunoglobulins (Igs). Like Ig heavy and light chains, TCR α and β chains consist of a variable (V) amino-terminal region and a constant (C) region. However, TCR can only bind a peptide presented by a major histocompatibility complex (MHC) molecule on an APC, while an Ig can bind free antigens [4,5].

2. First-generation CARs

To study the underlying mechanism responsible for the difference TCR and Ig functions, Kuwana et al. constructed a type of T cell that carries chimeric receptors composed of Ig-derived V regions and TCR-derived C regions in 1987. The chimeric receptors functioned independently to activate these T cells without MHC molecules when the targeted antigen was present [4]. In 1989, Gross et al. demonstrated that the CAR T cells specifically killed antigen-bearing target cells across strain and species barriers. Moreover, such T cells responded to immobilized antigen−protein conjugates, bypassing the need for cellular processing and presentation [6]. Similar effects were observed in vivo by Becker et al. using transgenic mice [7]. To improve transduction efficiency, Eshhar et al. developed a single-

Received 22 May 2022; revised 22 June 2022; accepted 29 September 2022.
Available online 15 December 2022

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https://doi.org/10.56875/2589-0646.1035

chain approach based on the single-chain variable region of antibodies (scFv). Such scFv domains, which join the antibody's heavy and light variable (VH and VL) gene segments with a flexible linker, have been proven to exhibit the same specificity and affinity as the natural antigen-binding (Fab) fragment [8]. These CAR T cells, which are composed of scFv and CD3ζ, are considered first-generation CAR T cells. Owing to the lack of costimulation, full activation of these T cells cannot be achieved, thereby leading to the development of second generation CAR T cells.

3. Second-generation CARs

Second-generation CARs contain the scFv and CD3ζ components present in the first-generation together with a costimulatory domain, which markedly increases T-cell proliferation and interleukin (IL)-2 secretion [3,9]. All current commercial CAR T-cell products, which utilize either a CD28 or 4-1BB costimulatory domain, belong to this category [3]. Axi-cel and brexu-cel contain a CD28 costimulatory domain, while tisa-cel, liso-cel, ide-cel, and cilta-cel contain the 4-1BB costimulatory domain [3]. CD28 and 4-1BB differ in expression patterns and intracellular signaling. However, current data from clinical trials do not demonstrate clear superiority of either CD28 or 4-1BB-costimulated CARs for controlling B-cell lymphomas or B-cell acute lymphoblastic leukemia (B-ALL) [3]. In addition to CD28 and 4-1BB, there are other costimulatory molecules employed within chimeric antigen receptors, such as ICOS (CD278), OX40 (CD134), CD27, CD40, CD40L, and TLRs [10].

4. Third-generation CARs

CD28 and 4-1BB signal through different pathways. CD28 signaling occurs immediately after TCR engagement before subsequent activation of the 4-1BB pathway [11]. Stimulation of CD28 results in faster and enhanced protein phosphorylation, resulting in a rapid increase in effector T-cell phenotype and function. In contrast, 4-1BB CAR T cells preferentially expressed T-cell memory-associated genes and exhibited more sustained anti-tumor activity [12]. CAR T-cell activity can be further enhanced by incorporating two costimulatory domains in tandem, which is a characteristic of third-generation CAR T cells [10]. In a clinical trial designed by Ramos et al., two CD19-specific CAR T-cell products were prepared in parallel from autologous peripheral blood mononuclear cells collected from 16 patients with B-cell non-Hodgkin lymphoma. The first construct was transduced with a second-generation CAR containing CD28 sequences alone, and the second was transduced with a third-generation CAR containing both CD28 and 4-1BB. Both products were reinfused simultaneously to the same patient after ex vivo expansion [11]. The third-generation CAR T cells had superior expansion and longer persistence than the second-generation CAR T cells. This difference was most striking in patients with a low disease burden resulting from depleted normal CD19+ B-cells due to prior therapies. It was presumed that fewer CD19+ B-cells provided less stimulation to CAR T cells, which benefited from additional costimulation [11].

5. Fourth-generation CARs

Optimal T-cell activation and proliferation require multiple signals, including T-cell receptor (TCR) engagement (signal 1), costimulation (signal 2), and cytokine engagement (signal 3) [13]. However, first-generation CAR T cells only transduce signal 1 via the scFv domain, whereas second- and third-generation CAR T cells contain scFv and costimulatory domains to transduce signals 1 and 2. Although they can transiently produce IL-2 after the initial antigen stimulation, these CAR T cells generally lack the long-term ability to sufficiently activate signal 3 [14]. Nonetheless, signal 3 plays an essential role in T-cell activation and expansion. Costimulatory cytokines, such as IL-15, may lower the activation threshold in T cells [14].

In hematologic malignancies (HM), lymphodepletion provides the first crucial source of cytokines, and has proved essential for the initial burst of CAR T-cell expansion. Moreover, lymphoid organs and the bone marrow, which are the main pathologic location of HM, are rich in both costimulatory molecules and cytokines. CD19 CAR T-cell engagement of B-ALL blasts and normal B-cells in the bone marrow brings them into contact with homeostatic cytokines produced by the fibroreticular cells within the bone marrow stroma [14]. However, the solid tumor microenvironment (TME) lacks such favorable conditions. Additionally, there are other contributing factors disobliging CAR T cells in solid tumor treatment, such as the physical barriers of the solid tumors and the solid TME characterized by oxidative stress, nutritional depletion, and hypoxia [15]. In addition, tumor cells express ligands of inhibitory immune checkpoints such as programmed death-1 ligand (PD-L1/L2) and recruit inhibitory immune cells such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) to interfere with the cytotoxic function of effector T cells [16]. Cytokine engagement (signal 3) may partially reverse tumor immune inhibition.

Fourth-generation CAR T cells take an additional step to incorporate a transgenic cytokine sequence, fulfilling the lack of signal 3 in the older generations and counteracting the immunosuppressive microenvironment in solid tumors. Added to the basic CAR T structure of scFv and costimulatory domains is a transgenic cytokine sequence. The cytokine expression is induced by the NFAT transcription factor and subsequently the NFAT/IL-2 minimal promoter upon T-cell activation [17]. Inducible expression of the transgenic cytokine can either provide stimulation in an autocrine fashion to sustain survival and expansion of CAR T cells or in a paracrine fashion to modulate the immune cell environment, compensating for the immunosuppressive TME [17]. Because the cytokine will not be produced until T-cell activation by a specific tumor antigen, the delivery is more specific than most other delivery methods and carries fewer systemic side effects. A higher level of the cytokine can be achieved because T cells are converted into living factories actively producing the cytokine, in contrast to other delivery models that administer a certain amount, such as intratumoral injections [17]. CAR T cells can also be engineered to reduce the expression of certain cytokines.

Based on the mechanisms, fourth-generation CAR T cells are also called T cells redirected for antigen-unrestricted cytokine-initiated killing (TRUCKs). Future potentials of TRUCKs may include secretion of more than one cytokine to combine their capacities with the secretion of other therapeutic proteins, such as cancer-directed monoclonal antibodies, immune checkpoint inhibitors, and pro-drug converting enzymes [17].

6. Fifth-generation CAR or next-generation CAR

While TRUCKs focus on delivering cytokines, fifth-generation CAR T cells focus on how to interpret the cytokine signals. Multiple designs have been developed. Kagoya et al. developed a CAR that encoded a truncated cytoplasmic domain of IL-2Rβ and a STAT3-binding YXXQ motif together with scFv targeting CD19, CD3z, and CD28 domains [13]. This generation of CAR T cells showed better proliferation and cytokine polyfunctionality compared to second-generation CAR T cells, suggesting a key role of STAT3 in suppressing terminal differentiation of T cells [13]. Many other fifth-generation CARs were designed via the fusion of IL-4 receptor ectodomain and another cytokine receptor's endodomain as IL-4 can be produced by tumor cells and is a soluble inhibitory/Th2-polarizing cytokine that is partially responsible for the immunosuppressive TME [18–20]. Many researchers have generated CARs in which the IL-4 receptor exodomain was fused to the IL-7 receptor endodomain. IL-7 helps maintain a Th1 phenotype in effector cells and augments their proliferation and cytotoxic functions as opposed to IL-4. The fusion of IL-4 and IL-7 receptors therefore inverted the effects of tumor-derived IL-4 so that the proliferation and activation of tumor-directed cytotoxic T cells were enhanced rather than inhibited, resulting in superior antitumor activity [19,20]. Cytokine-driven expansion can also be utilized in CAR T cell manufacturing. A CAR named T1E28z is currently in phase 1 and 2 clinical trials (ClinicalTrials.gov number, NCT01818323). T1E28z is directed against ErbB dimers in head and neck squamous cell carcinoma and is co-expressed with 4αβ [21]. 4αβ is a chimeric cytokine receptor that was engineered by fusion of the IL-4 receptor α (IL-4Rα) ectodomain to the βc subunit used by IL-2 and IL-15. Addition of IL-4 to T-cells that express 4αβ resulted in STAT3/STAT5/ERK phosphorylation and exponential proliferation, mimicking the actions of IL-2 [18]. Reproducible IL-4-dependent expansion and enrichment of CAR T cells under good manufacturing practice was achieved from both patients and healthy donors [21].

7. Safety switches

As much as we strive for efficacy, we need to maintain a sophisticated balance between efficacy and safety. One innovative strategy is to include a "safety switch" to "turn off" CAR T cells when they cause life-threatening adverse events or to limit long-term "on-target off-tumor" toxicities, such as B-cell aplasia caused by CD19 CAR T cells.

Safety switches, or "suicide" genes, have been applied to other cellular therapies for decades, such as the herpes simplex virus thymidine kinase (HSV-TK) suicide gene. Administration of donor T-cells expressing the HSV-TK with an allogenic bone marrow transplant could allow, if graft-versus-host disease (GVHD) was to occur, a selective in vivo depletion of these T cells by the use of ganciclovir [22,23]. However, the application of HSV-TK in CAR-T is restricted due to technical disadvantages [24]. Activation of HSV-TK by ganciclovir is relatively slow, requiring 3 days to have a complete effect in vitro. Moreover, the viral thymidine kinase (TK) gene product has intrinsic immunogenicity that may cause transduced cells to be rejected by the host immune system in immunocompetent individuals. Additionally, if ganciclovir is used to treat cytomegalovirus infections, the transduced cells will be undesirably depleted [25]. In 2005, Straathof et al. described a new

suicide system for engineered human T cells with an inducible Caspase 9 (iC9). Caspase 9 is a late-stage apoptosis pathway molecule and iC9 can be induced by a chemical inducer of dimerization (CID) [24]. This system, after being further developed, is now named CaspaCIDe®. It consists of an iC9 gene together with the small-molecule, bio-inert CID drug, AP1903. The iC9 gene contains the intracellular portion of the human caspase 9 protein fused to a drug-binding domain derived from human FK506-binding protein. Intravenous administration of AP1903 produces cross-linking of the drug-binding domains of this chimeric protein, which in turn dimerizes and activates caspase 9, resulting in cellular apoptosis [25]. The iC9 suicide system was proven to be efficient in killing both CAR T cells and CAR + tumor cells [25,26].

In contrast to an intrinsic safety switch, which is transduced into T cells as part of the CAR, pharmacologic control can work as an extrinsic safety switch on CAR T cells. The most investigated molecule is the tyrosine-kinase inhibitor dasatinib. Dasatinib suppresses T-cell activation via inhibition of proximal TCR signaling kinases. Given the similar manner in which TCRs and CARs transduce intracellular signals, dasatinib would hypothetically suppress CAR T-cell activation and function [27]. When treated with dasatinib, a dose-dependent suppression of cytotoxicity, cytokine secretion, and proliferation of CAR T-cells was observed in vitro. Interestingly, Dasatinib-treated CAR T cells regained function within hours of drug removal after short- and long-term ($\leq$10 days) treatment. Mice with CAR T cells suppressed by dasatinib experienced no toxicity, demonstrating suppression of both antitumor activity and induction of inflammatory cytokines in a xenograft model [27]. The fully and rapidly reversible suppression is a crucial advantage over the iC9 suicide system as it offers the option to switch on the CAR T-cells after toxicities subside and can be switched off and on multiple times. Additionally, the dose of dasatinib can be titrated to achieve partial or complete inhibition of CAR T-cell function [28].

8. The gating strategies

Several gating strategies are under investigation, which includes "AND", "NOT", and "OR" strategies. "AND" gating strategy aims to engineer CAR T cells to activate only in response to target cells expressing two antigens concurrently, enabling discrimination between tumor cells expressing antigen pairs versus healthy tissue expressing only one of the targets [29]. "NOT" strategy, on the other hand, aims to target antigen 1 only in the absence of antigen 2 by incorporating the intracellular domain of either CTLA-4 or, more effectively, PD1 on the CAR targeting antigen 2 [29]. Both "AND" and "NOT" strategies aim to discriminate between tumor cells and normal cells and thus decrease "on-target off-tumor" toxicity.

The "OR" strategy however aims to increase the sensitivity of CAR T-cells. Dual- or multi-targeted "OR" CARs can be generated using the so-called "adaptor'' CARs, which have an extracellular domain that can bind a variety of binders with different antigen specificities [29].

9. Off-the-shelf CAR T-cell products

Currently, autologous CAR T cells are the mainstay of CAR T cell therapy. However, they carry certain risks and disadvantages, such as an extremely high cost, risk of manufacturing failure, and delay in availability owing to logistical complexities [30]. The biological characteristics of autologous T cells are also negatively impacted by the previous lines of treatment [30]. Off-the-shelf, or allogeneic, CAR T cells may potentially address these issues, as they are healthier, readily available, and can be readministered multiple times. The production to scale will likely reduce the cost. The major obstacles requiring investigation are the risks of rejection and GVHD. A comprehensive review by Bi et al. thoroughly covers this separately in this issue.

Conflict of interest

None.

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