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Chimeric antigen receptor T-cell therapy: design improvements and therapeutic strategies in cancer treatment

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ABSTRACT

Objectives: To summarize important findings from research on chimeric antigen receptor (CAR) T-cell immunotherapy in cancer. We discuss CAR design, cell products, toxicity management, heterogenous solid tumors and allogeneic transfer.

Methods: A review of literature was conducted. The available literature was selected on original research, state-of-the-art design, relevance to the objective and journal impact factor.

Results: First-generation CARs provide patient T cells with tumor-specific antigen recognition. Second- and third-generation CARs incorporate costimulatory domains for enhanced T-cell persistence and antitumor activity. Fourth-generation CAR T cells (TRUCKs) include a cytokine production cassette, and hold promise in the treatment of heterogenous solid tumors. Transduced cell phenotype and subset composition are important factors. Suicide genes and safety switches are designed to decrease potential toxicity. Multi-specific CAR T cells can address heterogenous tumors. Allogeneic, off-the-shelf CAR T cells might reduce the production delay.

Conclusion: CAR T cells have revolutionized the immunotherapeutic treatment of cancer: exciting results in refractory and relapsed B-cell malignancies have been published. Neurologic complications, solid tumor management and allogeneic constructs require further research. In conclusion, further design adjustments will enable CAR T cells to decisively reshape the field of cancer immunotherapy.

KEYWORDS

CAR T cell; chimeric antigen receptor; cancer immunotherapy; B-cell malignancy; adoptive cell therapy

Introduction

The immune system is a key driver behind tumor elimination, as shown by the increased incidence of cancer in patients with a defective immune system. Furthermore, infusion of autologous tumor-infiltrating lymphocytes (TILs) can induce regression in metastatic melanoma [1]. However, high-affinity T cells recognizing cancer cells may be absent due to negative selection in the thymus or may be exhausted by the immunosuppressive tumor microenvironment. Cancer immunotherapy aims to redirect and augment patient T cells. Chimeric antigen receptors (CARs) combine the functionality of antibodies produced by B cells with the antitumor efficacy of various T-cell subsets. Therefore, CARs provide T cells with specific tumor cell recognition in a major histocompatibility complex (MHC)-independent fashion. In this review, we will discuss novel advances in CAR T-cell design, important therapeutic challenges and possible solutions.

Results

CAR T-cell design

The first-generation CAR incorporates an extracellular, single-chain variable fragment (scFv) of monoclonal antibodies (Figure 1(a–b)) linked by a hinge/spacer (Figure 1(c)) to a transmembrane domain (Figure 1(d)) which is connected to an intracellular signaling domain, typically CD3ζ (Figure 1(1–3)). CAR constructs are potentially immunogenic, resulting in host-versus-graft reactions. Therefore, humanization of murine components (e.g. scFv) is recommended. CAR-transduced T cells acquire a MHC-independent, high-affinity, tumor-specific antigen (TSA) recognition resulting in potent antitumor activity. First-generation CAR T cells were hampered by limited post-infusion expansion and clinical activity. Therefore, second- and third-generation CARs were developed, in order to increase efficacy, proliferation and persistence in vivo [2].

Second-generation CARs incorporate a costimulatory domain linked to CD3ζ, allowing for potent T-cell activation. It remains unclear which costimulatory domain is preferable as costimulatory molecules have distinct properties. For instance, CD28 enhances the cytolytic potential of T cells and early tumor clearance, whereas inclusion of 4-1BB (CD137) increases T-cell persistence and decreases exhaustion. Therefore, two costimulatory signals are incorporated in third-generation CAR T cells: CD28 4-1BB ligand CAR T cells display improved persistence and antitumor activity [3,4]. However, this may
Clinical protocol and clinical results

During a 2- to 4-week process, autologous T cells are first collected through leukapheresis and subsequently manipulated in vitro. To increase transduction efficiency, the cell cycle is induced by interleukin-2 (IL-2), sometimes combined with other cytokines. The CAR construct can be inserted by lentiviral or retroviral transduction, or electroporation [7]. The T cells carrying the CAR are isolated based on the expression of a marker gene that is incorporated in the construct.

A cyclophosphamide and/or fludarabine lymphodepleting regimen (Figure 2(a)) is administered to the patient prior to CAR T-cell therapy. Lymphodepletion is believed to decrease competition for stimulatory cytokines and diminish inhibition by regulatory T cells, thereby promoting CAR T-cell proliferation and activation. In addition, lymphodepletion may lessen host-versus-graft disease due to the potential immunogenicity of murine CAR components. However, depletion of the tumor due to preparative chemotherapy could reduce availability of targets required for CAR T-cell recognition, stimulation and subsequent expansion. Moreover, toxicity needs to be considered when selecting preparative regimens.

The most successful responses to CD19 CAR T-cell therapy have been observed in B-cell acute lymphoblastic leukemia. In addition, encouraging results have been achieved in B-cell non-Hodgkin lymphoma and B-cell chronic lymphocytic leukemia. Moreover, the value of CAR T-cell therapy in other hematologic and solid tumors is being investigated. For example, clinical trials with B-cell maturation antigen (BMCA)-targeting CAR T cells have shown encouraging results against multiple myeloma [8]. Early clinical data on CAR T-cell therapy in acute myeloid leukemia (AML) patients have been published (reviewed recently), despite the fact that all identified targets on AML are also present on normal cells [9]. Disappointing results of CAR T-cell therapy in solid tumors are attributable to specific limitations, in particular the immunosuppressive microenvironment, tumor heterogeneity and growth (limited CAR T-cell migration and infiltration into the center of the tumor). In conclusion, further advancements are required to solve current challenges, paving the way for CAR T-cell therapy in solid tumors. Possible solutions include TRUCKs to activate immune-mediated killing of tumor cells in spite of the immunosuppressive microenvironment, and multi-specific CAR T cells for the treatment of heterogenous solid tumors.

Cell types for transduction

Typically, all T cells obtained by leukapheresis are used for CAR-transduction. However, this heterogeneous CD4/CD8 T-cell population consists of phenotypically and functionally distinct subpopulations, and the composition and availability of the peripheral T-cell population differs between patients. The function of specific subpopulations requires additional investigation in order to determine the optimal composition for CAR T-cell therapy. It is currently known that naive and central memory T cells have increased proliferation capacity compared to effector (memory) T cells. Furthermore, central and stem memory T cells may display improved efficacy in vivo [10].

Figure 1. CAR design.

The extracellular domain containing a single-chain variable fragment (scFv) similar to monoclonal antibodies (mAbs) is connected by a hinge to a transmembrane domain. The intracellular domain consisting of CD3ζ can be linked to additional costimulatory domains such as CD28 or 4-1BB. Second- and third-generation CARs incorporate, respectively, one or two costimulatory domains.

Legend: scFv [a, b]; hinge/spacer [c]; transmembrane domain [d]; CD3ζ [1]; costimulatory intracellular domains [2,3]; cytokines [4]; costimulatory ligand [5]; variable light chain (VL); variable heavy chain (VH).
CD8 T cells typically show the most efficacious cytotoxic potential. Antitumor activity in vivo is further enhanced by CD4 T-cell presence, likely due to assisting CD8 T cells in addition to direct CD4 T-cell cytotoxicity. Therefore, administration of CAR T cells with a defined CD4:CD8 ratio can increase antitumor activity (as shown in vitro) [11].

γδ T cells provide endogenous MHC-independent recognition of metabolic and stress ligands which may counter downregulation of surface molecules. Safety may be superior to αβ T cells due lower cytokine secretion, with similar cytolytic capacity and anticancer effect. Moreover, allogeneic transfer of γδ T cells is unlikely to induce graft-versus-host disease, as noncancerous host cells should not be attacked due to absence of an allogeneic αβ T-cell receptor (TCR) on γδ T cells, as was shown in the results of haploidentical, αβ/CD19-depleted grafts [12].

Natural killer (NK) cells have a relatively short lifespan, display innate MHC-independent targeting and diminished cytokine release relative to αβ T cells. NK cells are not susceptible to MHC-mediated graft-versus-host disease, enabling allogeneic off-the-shelf transfer. Transfer of CAR-expressing, alloreactive NK cells (typically NK-92 cell line) is currently evaluated for safety, optimal dose and efficacy in patients with relapsed or refractory tumors [13,14].

**Challenges and possible improvements**

The efficacy of CAR T cells in the treatment of hematologic malignancies has clearly been demonstrated, but optimization is still needed. We will discuss challenges regarding CAR T-cell production, toxicity and proliferation in vivo, in addition to targeting heterogeneous solid tumors.

**Management of toxicity**

On target/off tumor toxicity may challenge CAR T-cell use. For example, CD19 CAR T-cell therapy administered for the treatment of B-cell malignancies can cause B-cell aplasia with a potential need for immunoglobulin replacement, but this is a manageable toxicity. However, other CAR T cells recognizing targets on normal cells result in less acceptable toxicities. Due to the powerful cytotoxic activity of CAR T cells, massive eradication of cancerous tissue can lead to tumor lysis syndrome potentially leading to –
mostly reversible – adverse effects such as electrolyte disorders and renal dysfunction. Mild cytokine release is observed in the majority of patients which generally resolves with supportive care. However, severe cytokine release syndrome (CRS) can arise, especially in case of high tumor load. It is a serious complication caused by a massive release of pro-inflammatory cytokines such as IL-6 and IFNγ, resulting in fever, hypotension, nausea, respiratory insufficiency and multiple organ failure with need of transfer to an intensive care unit for advanced life support.

Assessment of cytokine and C-reactive protein levels is advisable, whilst dosing schedules consisting of multiple infusions with increasing CART-cell doses could diminish unexpected adverse effects. Severe CRS can be treated with tocilizumab, an anti-IL-6 receptor antibody (Figure 2(b)), whilst administration of corticosteroids (Figure 2(c)) is reserved for the most severe, life-threatening cases, because of important interference with CART-cell function. Neurotoxicity such as convulsion, cerebral edema or coma typically coincides with CRS, with limited responsiveness to tocilizumab [15]. The mechanism of this potentially fatal encephalopathy is not yet completely understood.

To cope with the above-mentioned, potential life-threatening toxicities, a safety switch could be included in the CAR design. For example, the herpes simplex thymidine kinase suicide gene can be incorporated with ganciclovir administration triggering the safety switch (Figure 2(d)), leading to suicide of the cells. Alternatively, CART cells transduced with the inducible caspase 9 (iCasp9) safety switch may undergo apoptosis upon administration of a chemical inducer of dimerization (CID) (Figure 2(e)) which lacks additional clinical indications in contrast to ganciclovir [16,17].

The CAR construct can be separated into several components to limit potential off-tumor toxicity. Activation of each component can subsequently be coupled to recognition of a distinct antigen. Here, costimulatory signaling and CD3ζ-mediated activation can be incorporated separately into two separate CAR constructs which target different tumor antigens (Figure 2(f)). Therefore, full CART-cell activation requires simultaneous engagement of both receptors to eliminate co-expressing tumors, whilst sparing most noncancerous cells which exclusively express one of both receptor ligands [18].

Alternatively, a synthetic antigen-specific Notch receptor can be incorporated to regulate CAR activity, independent from the CAR/TCR pathway. These Notch receptors consist of an extracellular domain (e.g. scFv or nanobody) directed against a tumor antigen, and a transcription factor. When a tumor surface molecule is recognized, the transcriptional domain is cleaved from the receptor, subsequently entering the nucleus to trigger inducible expression of a CAR (Figure 2(g)). The CAR can subsequently engage a different tumor antigen, hence resulting in antitumor activity only if both tumor antigens are present, resulting in less recognition of normal cells [19].

In addition, CARs can be engineered to recognize antigens exclusively present on nontumoral cells (Figure 2(h)) to suppress CAR T-cell cytotoxicity [20]. Furthermore, electroporation of mRNA-modified CARs into T cells can generate transient expression of the CAR in T cells (Figure 2(i)), thereby limiting severe toxicity [21].

Also, an activation switch can provide controllable costimulatory signaling to CAR T cells in vivo. For example, the MyD88/CD40 switch consists of the MyD88 and CD40 signaling domains and a CID-binding domain. Administration of the CID subsequently activates the MyD88/CD40 switch to provide costimulatory signaling, allowing for modulation of CAR T-cell function in vivo (Figure 2(j)). Hence, potent CAR T-cell expansion, antitumor activity and IL-2 release can be induced only if the antigen-specific CAR and MyD88/CD40 switch are both activated simultaneously [22].

**CAR T-cell proliferation in vivo**

CAR T-cell expansion can be negatively influenced by the immunosuppressive tumor microenvironment, in addition to host-versus-graft reactions. Inclusion of 4-1BB costimulatory signaling can improve survival and persistence, but may increase adverse effects. Therefore, designs enabling regulation of CAR T-cell proliferation in vivo are preferable.

A TCR targeting a (viral) immunogen can be incorporated in CAR T cells to enable stimulation in vivo (Figure 2(k)). For instance, varicella zoster virus (VZV) CAR T cells incorporate a TCR directed against a VZV immunogen, and a CAR targeting a tumor surface molecule. Therefore, these VZV CAR T cells can be (reactivated through stimulation of the VZV TCR by administration of VZV lyse-packed dendritic cells, at least partially) counteracting the immunosuppressive microenvironment [23,24].

The initial antitumor activity of CAR T cells frequently diminishes in vivo, and CAR T cells may be driven toward terminal differentiation (counteracted by inclusion of 4-1BB costimulatory signaling). The serine/threonine kinase Akt pathway plays an important role in mediating T-cell proliferation and cytokine production. Constitutive Akt expression (Figure 2(l)) can enhance CAR T-cell activation [25]. Moreover, pharmacologic Akt inhibition during CAR T-cell expansion ex vivo can promote a memory phenotype with enhanced antitumor efficacy in vitro [26]. These observations suggest that modulation of the Akt pathway may hold promise for CAR T-cell therapy in the future.

Chimeric programmed cell death 1 (PD1) CD28 receptors (Figure 2(m)) can redirect PD1 ligand recognition to convey T-cell activation instead of
CAR T-cell therapy represent a revolutionary cancer treatment: persisting remissions have been observed in otherwise terminal cancer patients. Furthermore, CAR T cells exemplify a personalized, targeted therapy. Despite promising results, CAR T cells still face several important challenges. First, the powerful antitumor activity of CAR T cells can coincide with severe, possibly life-threatening toxicity. The pathophysiology of these adverse effects requires further investigation, especially the encephalopathy is poorly understood and therefore lacks an efficacious treatment. A variety of safety adaptations have been proposed, such as suicide genes or transient CAR expression. Second, CAR T-cell persistence in vivo requires optimization to achieve lasting protection against tumor relapse. Third, CAR T cells still have a long road ahead before treatment of solid tumors will be optimized enabling efficacy.

Synergistic therapeutics such as immune checkpoint inhibitors can expand the clinical application of CAR T cells but might also further increase on target/off important design limitations. First, CAR T cells incorporating murine parts are susceptible to disruptive, potentially toxic host-versus-graft reactions. Second, targeting two or more tumor antigens to counter antigen escape proves difficult. Mixing of two CAR T-cell lines, each expressing a scFv-based CAR directed against a different tumor antigen, may result in the preferential outgrowth of just one CAR T-cell population. Furthermore, generation of bispecific CAR T cells (incorporating two scFvs in tandem) is hindered by limited packaging size of vectors, and potential cross-pairing between the variable light and heavy chains. Therefore, nanobodies can be used as an alternative to scFv-based extracellular recognition domains. Nanobodies only consist of the variable heavy chain domain, thus eliminating light and heavy chain cross-pairing. Comparatively, these nanobodies are smaller in size which facilitates creation of multidomain constructs, in addition to displaying diminished immunogenicity [39].

Shortage of autologous T cells and high cost

Collection of autologous T cells for transduction can be insufficient due to prior chemotherapy for treatment of the underlying disease. Therefore, allogeneic off-the-shelf CAR T cells might reduce the production delay in patients with active disease (where time is extremely precious) and resolve insufficient collection of autologous T cells [30]. The risk of graft-versus-host disease in case of allogeneic T cells may be alleviated by deletion of the endogenous αβ TCR (Figure 2(r)) [40].

Finally, the transition of CAR T-cell therapy into routine treatment is currently hindered by logistic and financial burdens.

Future challenges and conclusion

CAR T-cell therapy...
tumor toxicity. Allogeneic off-the-shelf CAR T cells and affordable constructs could improve availability in the future. In conclusion, further design adjustments will advance CAR T cells to decisively reshape the field of cancer immunotherapy.

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