Current status and hurdles for CAR-T cell immune therapy

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Abstract
Chimeric antigen receptor T (CAR-T) cells have emerged as novel and promising immune therapies for the treatment of multiple types of cancer in patients with hematological malignancies. There are several key components critical for development and application of CAR-T therapy. First, the design of CAR vectors can considerably affect several aspects of the physiological functions of these T cells. Moreover, despite the wide use of γ-retrovirus and lentivirus in mediating gene transfer into T cells, optimal CAR delivery systems are also being developed and evaluated. In addition, several classes of mouse models have been used to evaluate the efficacies of CAR-T cells; however, each model has its own limitations. Clinically, although surprising complete remission (CR) rates were observed in acute lymphoblastic leukemia (ALL), lymphoma, and multiple myeloma (MM), there is still a lack of specific targets for acute myeloid leukemia (AML). Leukemia relapse remains a major challenge, and its mechanism is presently under investigation. Cytokine release syndrome (CRS) and neurotoxicity are life-threatening adverse effects that need to be carefully treated. Several factors that compromise the activities of anti-solid cancer CAR-T cells have been recognized, and further improvements targeting these factors are the focus of the development of novel CAR-T cells. Overcoming the current hurdles will lead to optimal responses of CAR-T cells, thus paving the way for their wide clinical application.

Keywords: Cancer, Chimeric antigen receptor T cells, Immune therapy

Studies conducted since the past few decades have revealed the critical roles played by immune cells in cancer surveillance and development. Of the diverse immune cell types, T cells have been the focus of cancer immunology because of their ability to specifically recognize cancer cells via T cell receptors (TCRs) through peptide-major histocompatibility complexes (pMHC) and by triggering strong immune responses. Therefore, the aim of several researchers dedicated to developing cancer immune therapeutic strategies has been to reactivate tumor antigen-specific T cells or redirect bystander T cells to recognize certain antigens expressed by tumor cells with high specificity. The development of checkpoint blockage therapies and cancer vaccines as well as adoptive cell transfer (ACT) technology represents the major translational achievements of the fundamental findings in this field of research.

In recent years, the clinical application of chimeric antigen receptor T (CAR-T) cells has achieved major success in the treatment of several types of hematological malignancies. In 2017, the U.S. Food and Drug Administration approved CAR-T cell therapy for the treatment of relapsed or refractory (R/R) acute lymphoblastic lymphoma (tisagenlecleucel) and diffuse large B-cell lymphoma (axicabtagene ciloleucel) and diffuse large B-cell lymphoma (axicabtagene ciloleucel). Meanwhile, there is an increase in the number of CAR-T-Clinical trials being registered and ongoing at ClinicalTrials.gov. These breakthroughs and progresses emphasize the significant value of ACT therapy and encourage its further development. In this review, our primary aim is to provide a brief introduction on the design of CAR vectors, CAR gene delivery, mouse modeling, and current clinical results of CAR-T cell therapies, and to summarize the hurdles faced and potential solutions for these aspects.

1. THE DESIGN OF CAR VECTORS

CARs are recombinant membrane receptor molecules that are generally expressed on the surface of T cells. The extracellular
domain of CAR contains monoclonal antibody-derived single-chain variable fragments (ScFv) that mediate binding with tumor-associated antigens (TAAs) and a space/hinge domain typically derived from the FC fragment of IgG to extend ScFv from the T cell membrane. To locate CAR on the cell membrane, a transmembrane fragment follows the extracellular domain. The intracellular domains contain CD3ζ and co-stimulatory domains, which together mimic the canonical first and second signals to induce T cell activation. Upon encountering target tumor cells, CAR binds with TAA and induces the activation of CD3ζ and co-stimulatory signals, followed by triggering of downstream phosphorylation cascades, which then activate T cells to proliferate and elicit effector functions such as cytotoxicity and cytokine secretion. The cytotoxicity of CAR-T cells depends on the secretion of perforin and granzyme and the expression of Fas/Fas ligand, which can directly lyse target cells or mediate apoptosis. IFN-γ, which can upregulate MHC expression by tumor cells, also drives the polarization of M1 macrophages. Secretion of the lymphocyte growth factor IL-2 supports the robust proliferation of CAR-T cells, whereas GM-CSF promotes the activation and expansion of myeloid cells to induce systemic immune responses. The first-generation CAR-T cells utilize only CD3ζ to activate T cells without incorporating a co-stimulatory domain, resulting in poor in vivo antitumor efficacy of these cells. The second-generation CAR-T cells, which generally utilize CD28 or 4-1BB endoplasmic dominans as a co-stimulatory signal, have demonstrated remarkable efficacies in patients with leukemia. Furthermore, the third-generation CAR-T cells incorporated with another co-stimulatory domain together with CD28 or 4-1BB, such as ICOS, CD40, OX40, TLR2, and DAP10, have been reported and functionally evaluated in preclinical models or clinical trials.

2. DELIVERY OF CAR VECTORS INTO T CELLS

A typical technical strategy for manufacturing autologous CAR-T cells with viral vectors primarily includes the following steps: enrichment of PBMCs from peripheral blood by apheresis, magnetic isolation of T cells, activation of T cells, viral transduction, expansion, and harvesting and freezing of cells. Although the reliability of γ-retrovirus and lentivirus has been extensively confirmed, novel gene delivery approaches are still being developed to reduce the possibility of oncogenic mutations caused by stochastic viral integration, enhance the efficiency of large gene transfer, and simplify the manufacturing processes. Currently, the most commonly used gene delivery approach for CAR-T cell manufacture is the replication of defective γ-retrovirus and lentivirus mediated by gene integration, although some disadvantages are associated with this approach. First, both these viruses are produced by the transient transfection of virus-encoding plasmids into packaging cell lines; theoretically, replication-competitive retro/lentivirus (RCR/RCL) could be generated by the recombination of genes encoding the viral genome and structural components. Therefore, the current viral packaging system uses three to four plasmids to separately encode these components. However, this requires the preparation of large amounts of plasmids, which is labor-intensive. Moreover, because of the instability of the virus and its short half-life period, the harvested viral supernatant must be quickly used or cryopreserved. Furthermore, impurities such as plasmid DNA and nucleic acids and proteins released by packaging cells must be detected and removed, which drastically increases process complexity and economic cost.

Another method to deliver CAR into T cells is the transposon/transposase system, which uses electroporation to transfer plasmids into cells and mediates CAR integration with its transposase activities. Both sleeping beauty and PiggyBac (PB) systems have been evaluated for CAR gene transfer. Compared with retroviruses and lentiviruses, this approach can significantly reduce the complexity of the manufacturing process and avoid the potential risks caused by RCR/RCL. However, cell viability is more seriously affected by this approach as both electroporation and exogenous plasmids could act as inducers of cell death. Thus, electroporation of mRNA encoding the CAR into the cytoplasm of T cells has been demonstrated to increase cell viability and reduce the genotoxicity and side effects caused by constitutive CAR expression. Clinical trials of mesothelin-specific CAR-T cells based on this strategy are ongoing (NCT01355965). More recently, the CRISPR-CAS9 gene editing system has been employed for CAR gene transfer. This approach allows the integration of the CAR gene into specific sites, such as the TCR α constant (TRAC) locus; thus avoiding the generation of oncogenic mutations potentially caused by stochastic integration. Meanwhile, the CRISPR-CAS9 system has also been used to generate universal CAR-T cells. The TCR and β-2-microglobin coding genes are disrupted in these cells to compromise graft-versus-host responses and prevent cells from being attacked by host T cells in alloergic situations.

3. MOUSE MODELS FOR EVALUATING CAR-T CELLS

In general, studies evaluating the antitumor activities of CAR-T cells use both in vitro cell culture-based assays and in vivo tumor-bearing mouse models. In vitro assays are used to assess certain aspects or functions, such as cytokine secretion, deregulation, and cytotoxicity, as well as proliferation upon target cell stimulation. These assays can indicate the recognition capability of CAR but are not as helpful for evaluating efficacy and predicting the patient’s response. The tumor-bearing mouse models are at least able to provide a physiological or pathological environment that is similar to the human body to a certain degree. To date, mouse models used for evaluating the efficacy of CAR-T cells can be classified into four types: syngeneic, transgenic, xenograft, and humanized models.

3.1. Syngeneic Models

Syngeneic mouse models typically use immunocompetent mice and murine tumors and T cells. The advantage of using these models is that the composition and the functions of immune system in these models are normal. Because CAR-T cells can trigger systemic immune responses that involve multiple types of immune cells to mediate antitumor activities and adverse effects, such as CRS and neurotoxicity, syngeneic mouse models are extremely useful to study the interaction between CAR-T cells and other host immune cells, such as macrophages and myeloid-derived suppressor cells (MDSC), and analyze the synergy between CAR-T cells and other immunostimulating drugs. However, considering the subjective differences in mouse and human biology and the differences in target antigens and CAR sequences, these models cannot model human CAR-T cells targeting human cancer.

3.2. Transgenic Models

Given that syngeneic mouse models do not express human TAAs, transgenic human TAA-expressing immunocompetent
mice have been developed. In this model, CAR-T cells are produced by modifying murine T cells with human extracellular domains and murine intracellular domains. This model can reflect the off-target effect of CAR-T cells that may recognize TAA expression in normal tissues. Furthermore, transplantation of murine cancer cell lines that stably express human TAs in this model enables the recognition of human TAA by human ScFv and induces systemic immune responses elicited by mouse immune cells.36–38

3.3. Xenograft Models

The successful construction of severe combined immunodeficient mice (SCID mice) that possess defects in the development of T and B cells allows xenotransplantation of human tissues and cells, including human cancer cell lines, tumor tissues of patient-derived xenografts, and immune cells. In recent decades, SCID mice have been continuously upgraded to generate NOD-SCID and NOD-SCID IL2Rγ−/− mice. The former carries a defect in the innate immune system, and the latter was introduced with an IL-2 receptor γ mutation to completely block its lymphocyte development, including NK cells. This genetic modification drastically enhances the efficacy for the transplantation of human xenografts.39,40 Application of these mouse models enables the functional evaluation of human CAR-T cells against human cancer, and they have also been recognized as a common platform for the development of CAR-T cells targeting novel target antigens or disease types.

However, despite the advantages of xenograft mouse models, there are certain drawbacks. First, these models have a compromised immune system, owing to which they cannot model the interplays and crosstalk of CAR-T cells between other host immune cells.41 In addition, the native TCR expressed by human T cells can recognize murine xeno-antigen-MHC complexes, thereby leading to hyperactivation of these infused T cells and finally the induction of xenogenic graft-versus-host disease (xeno GVHD) in these mice.42,43 Because of this xenoactiviation, the growth kinetics and tissue distribution of infused CAR-T cells in these mice may not be able to simulate that of human conditions, and the lethality of xeno GVHD can interfere in cancer, resulting in death, thereby leading to an inaccurate evaluation of efficacy.

3.4. Humanized Models

To construct human immune system in the immunodeficient mice, the NOD-SCID IL2Rγ−/− mice can be transplanted with human CD34-positive hematopoietic stem and progenitor cells (HSPCs) to reconstitute human lymphoid and myeloid cells in these mice.44 Human cancer cells can be subsequently transplanted into these mice after successful reconstitution. CAR-T cells produced from the same donor of HSPCs (e.g., isolating both T cells and HSPCs from a cord blood sample) can be administered to these mice.43,44 Moreover, the construction of a BLT mouse model (co-transplantation with human bone marrow, fetal liver, and thymus in immunodeficient mice) can better mimic normal human T and B cells allows xenotransplantation of human tissues and cells, including human cancer cell lines, tumor tissues of patient-derived xenografts, and immune cells. In recent decades, SCID mice have been continuously upgraded to generate NOD-SCID and NOD-SCID IL2Rγ−/− mice. The former carries a defect in the innate immune system, and the latter was introduced with an IL-2 receptor γ mutation to completely block its lymphocyte development, including NK cells. This genetic modification drastically enhances the efficacy for the transplantation of human xenografts.39,40 Application of these mouse models enables the functional evaluation of human CAR-T cells against human cancer, and they have also been recognized as a common platform for the development of CAR-T cells targeting novel target antigens or disease types.

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4. CLINICAL APPLICATIONS AND HURDLES OF CAR-T CELL THERAPY

Table 1 summarized the information regarding the target antigens and disease types from the published results of clinical trials of CAR-T cell therapies. More importantly, we will discuss the current status and hurdles of CAR-T cell therapies for both hematological and solid malignancies reflected by the current clinical results, and summarize those findings from preclinical studies which aim to improve the performance of CAR-T cells.

Table 1

| Target antigens of CAR T-cells in the published clinical trials | Diseases | References |
|---------------------------------------------------------------|----------|------------|
| CD19 CD19 B-ALL, CLL, NHL, MM                                 | 46–56    |
| CD22 CD22 B-ALL                                               | 57       |
| CD33 CD33 AML                                                  | 58       |
| CD13 CD13 AML                                                  | 61       |
| CD34 CD34 AML                                                  | 61       |
| CD30 CD30 Hodgkin lymphoma                                     | 62,63    |
| Kappa light chains CD19, GC, MM                               | 64       |
| CD10 CD10 AML                                                  | 65       |
| CA3X CA3X B-ALL, CLL, NHL, MM                                 | 66       |
| CEA CEA Metastatic gastrointestinal cancers                    | 66,68    |
| FRA FRA Ovarian cancer                                         | 69       |
| MS/LT MS/LT PDAC, MPM                                          | 70,72    |
| HER2 HER2 GBM, sarcoma, biliary tract cancer, pancreatic cancer| 73,74    |
| CD2 CD2 Melanoma, GBM                                          | 75       |
| CD133 CD133 Cholangiocarcinoma                                 | 81,82    |
| MUC1 MUC1 Seminal vesicle cancer                               | 83       |

CD19 = chronic lymphocytic leukemia; CD22 = platelet aggregation; CD33 = myeloid cells; MS/LT = myelodysplasia; FRA = ovarian cancer; MS/LT = proteinase, MPM = pancreatic adenocarcinoma; HER2 = breast cancer; CD2 = melanoma, GBM; CD133 = melanoma, GBM; MUC1 = breast cancer; CEA = pancreatic adenocarcinoma; RCC = renal cell carcinoma.

Therefore, human cytokine transgenic immunodeficient mice were developed to facilitate the reconstitution and differentiation of myeloid cells from HSPCs. This model has been used to mimic CRS caused by CD19-specific CAR-T cell therapy.45

4.1. Acute Lymphoblastic Leukemia

CD19-specific CAR-T cells containing either CD28 or 4–1BB co-stimulatory domain have shown interesting clinical outcomes in R/R B-cell acute lymphoblastic leukemia (B-ALL), generally with complete remission rates of >70%. Patients typically received a preconditioning regimen comprising cyclophosphamide and fludarabine to deplete lymphocytes and reduce the leukemia burden before CAR-T infusion to induce better expansion and persistence of CAR-T cells. CRS and neurotoxicity were the major life-threatening adverse effects caused by CAR-T cell-triggered immune responses.53,84,85 Administration of tocilizumab, an IL-6R antagonistic mAb, has been widely used to alleviate CRS. Moreover, a safe and potent anti-CD19 CAR-T cell therapy was reported recently, as indicated by the fact that no CRS or neurological events were observed and the CR rate remained at >50%.86
However, despite the remarkable CR rate, up to 50% of CAR19 T cell-treated patients suffered from leukemia relapse within 1 year after therapy and were generally resistant to the secondary infusion of the same CAR-T cell product. In some patients, relapse occurred because of the antigen loss of CD19, and the subsequent infusion of CD22 CAR-T cells was able to elicit immune responses in these cases. Dual-antigen targeting CAR-T cells may also be helpful in the prevention of antigen loss caused by leukemia relapse.

Nevertheless, the relapsed tumor cells retain CD19 expression in the majority of patients with relapse, and the mechanisms underlying these relapses remain elusive. It is speculated that CAR molecules are immunogenic and can induce host immune responses, which can mediate elimination of infused CAR-T cells. In fact, Turtle et al found that host anti-CAR immune responses are mediated by CD8 T cells in some patients. The application of humanized ScFv may enhance CAR-T cell persistence and reduce the relapse rate. Vaccination has been reported to improve the persistence of CD19 CAR-T cells in patients; however, the relapse rate remains high. Furthermore, clinical trials using 4-1BB co-stimulated CAR-T cells have reported better duration of CAR-T cells compared with that of CD28. It has also been suggested that 4-1BB induces T cell exhaustion to a lower extent compared with induced by CD28, and this effect contributes to superior T cell persistence. However, the choice between CD28 and 4-1BB remains controversial, and a recent study demonstrated better efficacy of CD28-calibrated CAR-T cells. Moreover, Ruella et al reported that the transduction of single leukemia cells induced resistance to CAR-T cell therapy. More recently, Hamieh et al found that trogocytosis, the process of antigen transfer from target cells to the surface of CAR-T cells and mediate tumor escape; their findings further indicate the complexity and the unknown mechanisms underlying leukemia relapses.

4.2. Acute Myeloid Leukemia

Numerous clinical studies aimed at reproducing the success of CD19-specific CAR-T cells in ALL, CD33, CD123, NKG2D, and Lewis Y-specific CAR-T cells have been reported thus far. Although responses were observed for CD33 and CD123 CAR-T cell therapies, the myeloablative effect caused by these CAR-T cells has hindered their application and the impact prognosis in patients.

In contrast, NKG2D chimeric receptor T cells and Lewis Y-specific CAR-T cells exhibited no significant toxicity but limited responses. In a recent preclinical study, Kim et al developed a novel technology with genetic inactivation of CD33 in hematopoietic stem cells to enable the application of CAR-T cell therapy for the treatment of AML. Targets that are more specific are urgently required to be confirmed to elicit substantial responses with acceptable adverse effects for patients with AML. CCL1, FLT3-ITD, CD44v6, CD7, and folate receptor β are targets that have already been evaluated in preclinical stages, and their clinical outcomes are worth looking into.

4.3. Lymphoma

The response rate of CD19-specific CAR-T cells in patients with lymphoma (primarily DLBCL and transformed follicular lymphoma, t-FL) is generally in the range of 50% to 80%, and the CR rate is 40% to 60%. A high relapse rate (>60%) has also been observed with long-term follow-up. Except for the abovementioned mechanisms responsible for leukemia relapse, lymphoma tissue microenvironment can suppress the activity of CAR-T cells through the expression of PD-L1 or through the recruitment of tumor-associated macrophages to mediate relapse. Co-targeting these targets with CAR-T cells may be effective to overcome this immune suppression.

4.4. Multiple Myeloma

Multiple myeloma is plasma cell-derived cancer and has the ability to produce immunoglobulin, which results in tissue damage. Despite significant improvement in the prognosis of patients through targeted therapies, including proteasome inhibitors, epigenetic modulators, immune-stimulating drugs, as well as monoclonal antibodies, MM remains largely incurable. B-cell maturation antigen (BCMA), a membrane receptor expressed during B-cell differentiation into plasma cells, is highly specific for MM cancer cells. Furthermore, bb2121, a BCMA CAR-T cell therapy that targets R/R MM, was reported to achieve an 85% objective response rate and a 45% CR rate according to a recent publication, thereby demonstrated the feasibility of its wide clinical application.

4.5. Solid Tumors

In contrast to the surprising performance of CAR-T cell therapy in hematological malignancies, its clinical application for the treatment of solid cancers remains challenging. Recently published phase I trials have demonstrated that rare CR can be induced in patients with solid cancers. The tested disease types, efficacy, toxicity, and the available targets have been intensively reviewed. It was suggested that factors such as the immunosuppressive tumor microenvironment, poor T cell infiltration and persistency, tumor antigen heterogeneity, and off-target toxicities are major obstructions that limit the efficacy of CAR-T cells against solid cancers. To overcome these impediments, several researchers have attempted to enhance the effector functions, persistency, and alleviation of T cell exhaustion further modification or optimization of CAR with other signaling domains, such as 4-1BB ligand, CD27, CD40 and its ligand, OX-40, ICOS, TLR2, DAP10, JAK-STAT, and cytokines, including IL-7, IL-12, IL-15, IL-18. Notably, Adachi et al reported a significant synergy between the cytokine IL-7 and the chemokine CCL19 in enhancing CAR-T cell infiltration into the tumor tissue.

The tumor microenvironment generally comprises immunosuppressive cells such as MDSC and immunosuppressive molecules such as PD-L1 and TGF-β, which dampen T cell responses. Targeting MDSC by administering all-trans-retinoic acid (ATRA), poly I:C, or infusing chimeric NKG2D receptor NK cells has demonstrated synergistic activities with CAR-T cell therapies. Meanwhile, targeting immunosuppressive molecules such as PD-1/PD-L1 and TGF-β reported improved the potency of CAR-T cells against certain solid cancers. A recent study also revealed that the transcription factor NR4A is a protein that limits CAR-T cell functions, and its inhibition improves the potency of CAR-T cells. Furthermore, oncolytic viruses (OV) have been suggested to stimulate systemic immune responses and destroy solid tumor microenvironments; therefore, combining the therapeutic strategy of OV with CAR-T cells could have significant potential in treating solid cancers.

It has been demonstrated that primary cancer cells possess considerable genetic and phenotypic heterogeneities, and thus escape from the attack of CAR-T cells against single targets. Induction of host immune responses by CAR-T cells may help prevent this. In addition, dual or multispecific CAR-T cells may be used as a strategy to overcome antigen
escape. However, the off-target effect may also be amplified because of the potential expression of individual antigens on normal tissues.\(^1\) A well-designed study conducted by Roybal et al described a precision novel design of tumor recognition by T cells with combinatorial antigen-sensing circuits,\(^15\) which may prevent the induction of off-target responses.

### 5. CONCLUSIONS AND PERSPECTIVES

The success of CAR-T cell therapy has been inspiring in the field of cancer immunology, especially in treating B-cell-derived hematological malignancies. However, the manufacturing processes, including the CAR delivery method, need to be further improved. Animal models are being developed and upgraded to better mimic the human body and human cancers. Further research must focus on challenges such as the relapse of B-cell leukemia/lymphoma/MM, lack of available targets for AML, and the low efficacies in treating solid cancers. Adverse effects, including CRS and neurotoxicity, should be considered, better modeled, and rationally intervened, while preserving normal antitumor activities. Participation and cooperation of both researchers and physicians could contribute to the step-by-step resolution of these challenges to improve prognosis for a wide range of patients with cancer.

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### REFERENCES

\[\text{[1]}\] June CH, O’Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science 2018;359(6382):1361–1365.
\[\text{[2]}\] Liu B, Song Y, Liu D. Clinical trials of CAR-T cells in China. J Hematol Oncol 2017;10(1):166.
\[\text{[3]}\] June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med 2018;379(1):64–73.
\[\text{[4]}\] Johnson LA, June CH. Driving gene-engineered T cell immunotherapy of cancer. Cell Res 2017;27(1):38–58.
\[\text{[5]}\] Lim WA, June CH. The principles of engineering immune cells to treat cancer. Cell 2017;168(4):724–740.
\[\text{[6]}\] Guedan S, Calderon H, Posey AD Jr, Maus MV. Engineering and design of chimeric antigen receptors. Mol THER Methods Clin Dev 2019;12:145–156.
\[\text{[7]}\] Lee YH, Kim CH. Evolution of chimeric antigen receptor (CAR) T cell therapy: current status and future perspectives. Arch Pharm Res 2019;42(7):607–616.
\[\text{[8]}\] Benmaberek MK, Karches CH, Cadilla BL, Lesch S, Endres D, Kobold S. Killing mechanisms of chimeric antigen receptor (CAR) T cells. Int J Mol Sci 2019;20(6):pii: E12883.
\[\text{[9]}\] Dotti G, Gottschalk S, Savoldo B, Brenner MK. Design and development of therapies using chimeric antigen receptor-expressing T cells. IMMUNOL Rev 2014;237(1):107–126.
\[\text{[10]}\] Sadelain M, Brentsens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Discov 2013;3(4):388–398.
\[\text{[11]}\] Hombach AA, Abken H. Costimulation by chimeric antigen receptors revisited the T cell antitumor response benefits from combined CD28-OX40 signaling. Int J Cancer 2011;129(12):2935–2944.
\[\text{[12]}\] Guedan S, Posey AD Jr, Shaw C, et al. Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. JCI Insight 2018;3(1).
\[\text{[13]}\] Lai Y, Weng J, Wei X, et al. Toll-like receptor 2 costimulation potentiates the antitumor efficacy of CAR T cells. Leukemia 2018;33(3):801–808.
\[\text{[14]}\] Zhao R, Cheng L, Jiang Z, et al. DNAx-activating protein 10 costimulation enhances the anti-tumor efficacy of chimeric antigen receptor T cells. Oncoimmunology 2019;8(1):e1509173.
\[\text{[15]}\] Vormittag P, Gunn R, Ghorashian S, Veraitch FS. A guide to manufacturing CAR T cell therapies. Curr Opin Biotechnol 2018;53:164–181.
\[\text{[16]}\] Pucopo NJ, Mueller KP, Das A, et al. Bioengineering solutions for manufacturing challenges in CAR T cells. Biotechnol J 2018;13(2):1700095.
\[\text{[17]}\] Gomes-Silva D, Ramos CA. Cancer immunotherapy using CAR-T cells: from the research bench to the assembly line. Biotechnol J 2018;13(2):1700097.
\[\text{[18]}\] Milone MC, O’Doherty U. Clinical use of lentiviral vectors. Leukemia 2018;32(7):1529–1541.
\[\text{[19]}\] Cornetta K, Duffy L, Turtle CJ, et al. Absence of replication-competent lentivirus in the clinic: analysis of infused T cells products. Mol THER 2018;26(1):280–288.
\[\text{[20]}\] Gandara C, Affleck V, Stoll EA. Manufacture of third-generation lentivirus for preclinical use, with process development considerations for translation to good manufacturing practice. Hum Gene Ther Methods 2018;29(1):1–15.
\[\text{[21]}\] Kennedy A, Cribbs AF. Production and concentration of lentivirus for transduction of primary human T cells. Methods Mol Biol 2016;1448:85–93.
\[\text{[22]}\] Bishop DC, Xu N, Tse B, et al. PiggyBac-engineered T cells expressing CD19-specific CARs that lack IgG1 Fc spacers have potent activity against B-ALL xenografts. Mol THER 2018;26(8):1883–1895.
\[\text{[23]}\] Manuri PV, Wilson MH, Maini SN, et al. piggyBac transposase/transposase system to generate CD19-specific T cells for the treatment of B-lineage malignancies. Hum Gene Ther 2010;21(4):427–437.
\[\text{[24]}\] Monje T, Miksy C, Gogshirv T, et al. Enhanced CAR T cell engineering using non-viral Sleeping Beauty transposon transposition from minicircle vectors. Leukemia 2017;31(1):186–194.
\[\text{[25]}\] Krebsera P, Singh H, Huws MH, et al. Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells. J Clin Invest 2016;126(9):3363–3376.
\[\text{[26]}\] Tipanje J, VandenDriessche T, Chaak MH. Transposons: moving forward from preclinical studies to clinical trials. Hum Gene Ther 2017;28(11):1087–1104.
\[\text{[27]}\] Beatty GL, Haas AR, Maus MV, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res 2014;2(2):112–120.
\[\text{[28]}\] Beatty GL, O’Hara MH, Lacey SF, et al. Activity of mesothelin-specific chimeric antigen receptor T cells against pancreatic carcinoma metastases in a phase I trial. Gastroenterology 2018;155(1):29–32.
\[\text{[29]}\] Esquema J, Mansilla-Soto J, Gaviarids T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 2017;543(7643):113–117.
\[\text{[30]}\] Da X, Park JJ, Du Y, et al. One-step generation of modular CAR-T cells with AAV-CrH1. Nat Methods 2019;16(3):247–254.
\[\text{[31]}\] Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Cancer Res 2017;23(7):2255–2266.
\[\text{[32]}\] Zhao J, Lin Q, Song Y, Liu D. Universal CARs, universal T cells, and universal CAR T cells. J Hematol Oncol 2018;11(1):132.
\[\text{[33]}\] Avanzi MP, Yeku O, Li X, et al. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell Rep 2018;23(7):2130–2141.
\[\text{[34]}\] Wang LC, Lo A, Scholler J, et al. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. Cancer Immunol Res 2014;2(2):154–166.
\[\text{[35]}\] Weiss T, Weller M, Guckenberg M, Sentman CL, Roth P. NKG2D-based CAR T cells and radiotherapy exert synergistic efficacy in glioblastoma. Cancer Res 2018;78(4):1031–1043.
\[\text{[36]}\] Maridiana S, John LB, Henderson MA, et al. A Multifunctional role for adjuvant anti-4-1BB therapy in augmenting antitumor response by chimeric antigen receptor T cells. Cancer Res 2017;77(6):1296–1309.
\[\text{[37]}\] Pegram HJ, Lee JC, Hayman EG, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood 2012;119(18):4133–4141.
\[\text{[38]}\] Chmielewski M, Hahn O, Rappl G, et al. T cells that target carcinoembryonic antigen eradicate orthotopic pancreatic carcinomas without inducing autoimmune colitis in mice. Gastroenterology 2012;143(4):1095–107.e2.
\[\text{[39]}\] Xu C, Li X, Liu P, Li M, Luo F. Patient-derived xenograft mouse models: a high fidelity tool for individualized medicine. Onclet Lett 2019;17(1):3–10.
Siegler EL, Wang P. Preclinical models in chimeric antigen receptor-engineered T-cell therapy. Hum Gene Ther 2018;29(5):534–546.

Ito R, Takahashi T, Ito M. Humanized mouse models: application to human diseases. J Cell Physiol 2018;233(5):3723–3728.

Ito R, Katano I, Kawai K, et al. Highly sensitive model for xenogenic GVHD using severe immunodeficient NOG mice. Transplantation 2009;87(11):1654–1658.

Jin CH, Xia J, Rahi S, et al. Modeling anti-CD19 CAR T cell therapy in humanized mice with human immune and autologous leukemia. Ebiomedicine 2019;39:173–181.

Norelli M, Camisa B, Barberia G, et al. Monocytode-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. Nat Med 2018;24(6):739–748.

Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 2013;368(5):725–733.

Brentjens RJ, Riviere I, Park JH, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell lymphomas. Blood 2011;118(18):4817–4822.

Garfall AL, Stadtmann EA, Hwang WT, et al. Chimeric antigen receptor T cells against CD19 for multiple myeloma. N Engl J Med 2015;373(11):1040–1047.

Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing anti-B-cell maturation antigen CD22-targeted CAR T cells induce remissions of multiple myeloma in a patient with relapsing/refractory disease. Blood 2015;118(18):4828–4838.

Nelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T cells targeting NKG2D ligands in patients with AML/MDS and advanced refractory acute myeloid leukemia. Mol Ther 2017;25(5):1248–1258.

Katz SC, Burga RA, McCormack F, et al. Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor-modified T-cell therapy for CEA liver metastases. Clin Cancer Res 2015;21(14):3149–3159.

Kandalaft LE, Powell DJ Jr, Cockson G. A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer. J Transl Med 2012;10:157.

Ahmed N, Brawley V, Hegde M, et al. HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase I dose-escalation trial. JAMA Oncol 2017;3(8):1094–1101.

Ahmed N, Brawley VS, Hegde M, et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol 2015;33(15):1688–1696.

Feng K, Liu Y, Guo Y, et al. Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers. Protein Cell 2018;9(10):838–847.

Gargett T, Yu W, Dott G, et al. GD2-specific CAR T cells undergo potent activation and deletion following antigen encounter but can be protected from activation-induced cell death by PD-1 blockade. Mol Ther 2016;24(6):1135–1149.

Louis CU, Savoldo B, Dott G, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 2011;118(23):6050–6056.

Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med 2016;375(26):2551–2559.

Tchou J, Zhao Y, Levine BL, et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res 2017;5(12):1132–1161.

Junghans RP, Ma Q, Rathore R, et al. Phase I trial of anti-PSMA designer CAR-T cells in prostate cancer: possible role for interacting interleukin 2T-cell pharmacodynamics as a determinant of clinical response. Prostate 2016;76(14):1257–1270.

Thustlethwaite FC, Gilham DE, Guest RD, et al. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. Cancer Immunol Immunother 2017;66(11):1423–1436.

Guo Y, Feng K, Liu Y, et al. Phase I study of chimeric antigen receptor-modified T cells in patients with EGFR-positive advanced biliary tract cancers. Clin Cancer Res 2018;24(6):1277–1286.

O’Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017;9(399).

Feng KC, Guo YL, Liu Y, et al. Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. J Hematol Oncol 2017;10(1):4.

Wang Y, Chen M, Wu Z, et al. CD133-directed CAR T cells for advanced metastases malignancies: a phase I trial. Oncoimmunology 2018;7(7):e1440169.

You F, Jiang L, Zhang B, et al. Phase I clinical trial demonstrated that MUC1 positive metastatic seminal vesicle cancer can be effectively eradicated by modified Anti-MUC1 chimeric antigen receptor transduced T cells. Sci China Life Sci 2016;59(4):386–397.

Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoblastic leukemia. N Engl J Med 2013;368(16):1509–1518.

Maudie SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014;371(16):1507–1517.

Ying Z, Huang XF, Xiang X, et al. A safe and potent anti-CD19 CAR T cell therapy. Nat Med 2019;25(6):947–948.

Majzner RG, Mackall CL. Tumor antigen escape from CAR T-cell therapy. Cancer Discov 2018;8(10):1219–1226.
Rafig S, Yeku OO, Jackson HJ, et al. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. *Nat Biotechnol* 2018;36(9):847–856.

Kloss CC, Lee J, Zhang A, et al. Dominant-negative TGF-beta receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol Ther* 2018;26(7):1855–1866.

Pan Z, Di S, Shi B, et al. Increased antitumor activities of glypican-3-specific chimeric antigen receptor-modified T cells by coexpression of a soluble PD1-CH3 fusion protein. *Cancer Immunol Immunother* 2018;67(10):1621–1634.

Liu X, Ranganathan R, Jiang S, et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Res* 2016;76(6):1378–1390.

Sukumaran S, Watanabe N, Baigain P, et al. Enhancing the potency and specificity of engineered T cells for cancer treatment. *Cancer Discov* 2018;8(8):972–987.

Chen J, López-Moyado IF, Seo H, et al. NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* 2019;567(7749):530–534.

Rosewell Shaw A, Suzuki M. Oncolytic viruses partner with T-cell therapy for solid tumor treatment. *Front Immunol* 2018;9:2103.

Nishio N, Diaconu I, Liu H, et al. Armed oncolytic virus enhances immune functions of chimeric antigen receptor-modified T cells in solid tumors. *Cancer Res* 2014;74(18):5195–5205.

Guedan S, Alemany R. CAR-T cells and oncolytic viruses: joining forces to overcome the solid tumor challenge. *Front Immunol* 2018;9:2460.

McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell* 2017;168(4):613–628.

Barber A, Rynda A, Sentman CL. Chimeric NGK2D expressing T cells eliminate immunosuppression and activate immune within the ovarian tumor microenvironment. *J Immunol* 2009;183(11):6939–6947.

Kueberuwa G, Kalaitsidou M, Cheadle E, Hawkins RE, Gilham DE. CD19 CAR T cells expressing IL-12 eradicate lymphoma in fully lymphoreplete mice through induction of host immunity. *Mol Ther Oncolytics* 2018;8:41–51.

Tanaka M, Tashiro H, Omer B, et al. Vaccination targeting native receptors to enhance the function and proliferation of chimeric antigen receptor (CAR)-modified T cells. *Clin Cancer Res* 2017;23(14):3499–3509.

Roybal KT, Rupp LJ, Morsut L, et al. Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. *Cell* 2016;164(4):770–779.