# REVIEW ON ENGINEERED T CELLS DIRECTED AGAINST TUMORS

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## ABSTRACT

Human body have T cells in the immune system which protect the body from infection by pathogens and clear mutant cells through specific recognition by T cell receptors (TCRs). In Cancer immunotherapy, these T cells boosts the antitumor efficacy of T cells by release inhibition of Immune checkpoints. It also expands the adaptive immunity by promote the adoptive transfer of T cells. These T cells are genetically equipped with chimeric antigen receptors (CARs) or TCRs . It shown phenomenol effectiveness in treating some hematological malignancies. Whereas the efficacy of Engineered T cells in treating solid tomors is far from satisfactory. In this review, we are going to summarize how the genetically engineered T cells are developed and what are the recent studies now investigated for genetically engineered T cells for Tumor immunotherapy and also discuss about the strategies for improving the performance of these T cells for the fight against Tumors

## INTRODUCTION

Our immune system carries the two most important cells which are named as T cells and B cells. T cells plays an important role in cell mediated adaptive immunity. These T cells have the component named as TCR which helps them to identify the antigens, it was found in 1980s. By this recognition of antigens they laying the foundation for cancer immunotherapy.

In cancer immunotherapy our body's own immune system was fight with cancerous cells. This therapy was formed as an annual scientific breakthrough in 2013 by Science magazine and it has exhibited the promising antitumor efficacy in recent years[5–7]. Cancer immunotherapies are categorized into three, they are (i) immune checkpoint inhibitors (ICIs), (iI) adoptive cell therapies (ACTs), and (III)tumor vaccines[8,9].By this way of cancer therapy most of patients get relieved from cancer. This T cells only show the limited efficacy agains tumor cells. By cloning of TCR gene of the TIL, it is ease to endow T cells with defined specificity by transferring the cloned TCR gene. By engineering the T cells with Viral vectors to express the TCR gene with defined specificity obtained by transferring the cloned TCR gene. But the usage of engineered T cells have some limitations like HLA restriction, side effects, etc.,

Chimeric antigen receptor modified T (CAR-T) cells are genetically engineered to express CAR molecules that targets the suface antigens on cancer cells and other immune cells, it also face the some limitations of TCR-T cells. Before we use these T cells we should investigate this T cells in Preclinical and clinical studies, because they may exhibit dramatic efficacy in treating hematological malignancies. These moderate effects have been obtained from the treatment of cancer. In this review we are going to summarize the recent investigations of genetically engineered T cells, and mosty we focusing on CAR construct optimization clinical efficacy, and strategies to overcome resistance and other limitation. We also going to give an outlook for the future application of genetically engineered T cell to tumor therapy.

## RATIONALE FOR THE EMERGENCE OF GENETICALLY ENGINEERED T CELLS

T cells have the ability of autoimmune tolerance after the positive selection of thymocytes. These T cells participate in cell mediated immunity. By inducing the dendritic cells, the specificity of transferred T cells has been enhanced. For the treatment of hematological malignancies, the transfer of allogeneic T cells is an important strategy to induce cancer elimination[44], but it damage the normal tissue and visceral organs in recipients, resulting in graft-versus-host disease (GVHD)[45,46]. The prevention of GVHD by T cell depletion or host-specific allogenic T cell elimination has been proven to be effective and to improve long-time survival[47–49].

The major type of T cells called as effector T cells leave the blood and invade into tumor tissue and attack tumor cells. Rosenberg and his colleagues initiate the Pioneering clinical trials using expanded TILs which is used for the treatment of melanoma and other tumors which demonstrate the adoptive transfer of autologous. TILs is beneficial in retrogress primary tumor cells and limit metastasis. For the treatment of cancer immunotherapies, the adoptive transfer of TILs has been demonstrated to be one of the most crucial and it also used for the treatment of melanoma and several other tumors[10].

## GENETICALLY ENGINEERED T CELLS FOR TREATING HEMATOLOGICAL MALIGNANCIES

### USE OF CAR-T CELLS FOR TREATING HEMATOLOGICAL MALIGNANCIES

After the development of CARs, CAR-T cell-based immunotherapy was utilized in the treatment of various diseases like lymphoma, leukemia, myeloma, and other hematological malignancies (Table 1)[21]. CAR-T therapy is beneficial in the treatment of hematological malignancies and it also exhibits controllable and tolerable toxicity.

CD19 CAR-T cells are the most frequently used for tumor therapy, and by using them hundreds of clinical trials has been happened. It functioning unobtrusively and broadly expressed CD19 in B- cell lymphomas or leukemias

From the most recently updated data , the trial of CD19 CAR-T therapy which has 75 evaluable patients were participated in the study. From that 81% of the patients achieved 3-month overall remission and 76% of patients achieved 12- month from overall survival (OS; the median OS have 19 months). Additionally, CTL019 has been tested in R/R chronic lymphocytic leukemia (The CLL is 17 evaluable patients; overall response rate (ORR) has recorded as 53%, complete response (CR) rate was measured as 35%), In follicular lymphoma (14 evaluable patients, ORR was recorded as 79%, and then CR was 71% in the duration 6 months), In multiple myeloma (MM) (the evaluable patients was 10 , from them 1 has the cytokine release syndrome

(CRS) and 2 of them possess longer progression-free survival).

**Table 1. Application of engineered T cells in clinical trials for treating hematological malignancies**

| Type of the arm | Target/construct | Phase | No. of patients/disease | Efficacy |
|---|---|---|---|---|
| Tisagenlecleucel, CTL019 | CD19-(4-1BB)-(CD3-zeta) | Phase II | 75, RR-ALL children and young adults | OR 81% (3 months) OS 76% (12 months) |
| Tisagenlecleucel, CTL019 | CD19-(4-1BB)-(CD3-zeta) | Phase II | 17, R/R CLL | ORR, 53%; CR, 35% |
| Tisagenlecleucel, CTL019 | CD19-(4-1BB)-(CD3-zeta) | Case series | 14, FL | ORR, 79%; CR, 71% |
| Tisagenlecleucel, CTL019 | CD19-(4-1BB)-(CD3-zeta) | Phase I after ASCT | 10, MM | CRS, 10%; longer progression-free survival 20% |
| JCAR017 | CD19-(4-1BB)-(CD3-zeta) | Phase II | 68, R/R DLBCL | ORR 75%; CRR 37% |
| Axicabtagene ciloleuce, KTE-C19 | CD19-(CD28)-(CD3-zeta) | Phase II | 111, R/R DLBCL | ORR, 82%; CR, 40% |
| CD20 CAR-T | CD19-(CD3-zeta) | Phase I | 7, FL and MCL | PR, 14.2%; CR, 28.5% |
| CD20 CAR-T | CD20-(4-1BB)-(CD3-zeta) | Phase II | 11, R/R NHL, primarily DLBCL | ORR 82%; CRR 55% |
| CD22-CAR T | CD22-(4-1BB)-(CD3-zeta) | Phase I | 21, RR-ALL children and young adults | ORR, 53% |
| bb2121 | BCMA CAR-T | Phase I | 20, R/R-MM | ORR 89%; RR 100% |

| | | | | |
|---|---|---|---|---|
| LCAR-B38M | BCMA CAR-T | Phase I | 19, R/R-MM | ORR 100%; 32% MRD-negative CR, and 32% nCR |
| κ or λ light chain | κ-directed CAR | Phase I | 9, NHL/CLL | PR 11% |
| NY-ESO-1-LAGE-1 | Antigens NY-ESO-1 and LAGE- | Phase I/II (with ASCT) | 20, MM | 70% CR or nCR |
| WT1 TCR-T Bispecific antibodies | Antigen WT1 | Phase I/II (with) | 12, AML | 66% CR |
| Blinatumomab | CD19-CD3 | Phase II | 21, RR-DLBCL | ORR 43%; CRR 19% |
| CAR-NK | Cd19-(NK-92) | Registered clinical trials | CD19-positive B cell malignancies | Unpublished |
| CAR-NK | Cd33-(NK-92) | Registered clinical trials | AML | Unpublished |
| CAR-NK | Cd7-(NK-92) | Registered clinical trials | CD7-positive leukemia or lymphoma | Unpublished |
| CAR-NK | CD19-(cord blood) | Registered clinical trials | CD19-positive leukemia or lymphoma | Unpublished |

The Product named as axicabtagene ciloleucel which exhibits the robust efficacy of treatment of R/R diffuse large of B cell lymphoma (DLBCL). The phase II trials shows the Overall response rate of 82%, and the CR rate was measure as 40% at the duration of 15.2 moths. The Juno therapeutics produce a product named as JCAR017, which results dramatic Overall Response Rate was 75% and the CR value was 37% in the duration of 6 months. From the Overall report, CD19 CAR-T therapy demonstrate promising effects of therapeutic and safety, which illustrates the CD19 CAR-T and it has advantages for the hematological malignancies treatment, exclusively for B cell malignancies.

| Table 2. Published clinical studies using CAR-T cells for treating solid tumors | | | | | |
|---|---|---|---|---|---|
| Targeted antigen | Disease | Vector | CAR generation | Sponsor | NCT identifier |
| FRa | Ovarian cancer | Retrovirus | First | National Cancer Institute | NA |
| Mesothelin | Pancreatic cancer | mRNA | Second | University of Pennsylvania | NA |
| c-MET | Breast cancer | mRNA | Second | University of Pennsylvania | NCT01837602 |
| EGFRvIII | Glioblastoma | Lentiviral | Second | University of Pennsylvania | NCT02209376 |
| CEACAM5 | CRC | Retrovirus | First | The University of Manchester | NCT01212887 |
| CEA | CRC | Lentivirus | Second | Third Military Medical University | NCT02349724 |
| HER2 | Glioblastoma | | Second | Baylor College of Medicine | NCT01109095 |
| GD2 | Neuroblastoma | Retrovirus | First | Baylor College of Medicine | NCT00085930 |
| CD133 | HCC, CRC, pancreatic cancer | Lentivirus | Second | Chinese PLA General Hospital | NCT02541370 |

## USE OF GENETICALLY ENGINEERED T CELLS FOR TREATING SOLID TUMORS

Genetically engineered T cells have long been employed to treat solid tumors[17,30]. The clinical efficacy of this type of treatment is far from satisfactory compared with that achieved in treating hematological malignancies[24]. Considerable research has been conducted in an attempt to enhance the antitumor activities of CAR-T and TCR-T cells, and different strategies aiming to determine the efficacy and safety of CAR-T therapy are being tested in clinical trials for the treatment of cancers, such as breast cancer, sarcoma, and neuroblastoma (Table 2). The first clinical application of CAR-T therapy for cancer treatment was the use of CAR-T cells recognizing carbonic anhydrase IX (CAIX) for the treatment of metastatic renal cell carcinoma, which showed moderate antitumor activity[30]. Other results of clinical trials that used genetically engineered T cells to treat solid tumors have been barely comparable to those achieved with CAR-T therapy for leukemia and lymphoma.

The most commonly used targets for CAR-T therapy are surface antigens, such as carcinoembryonic antigen (CEA) for colorectal adenocarcinoma, fibroblast activation protein for malignant pleural mesothelioma, diganglioside GD2 for neuroblastoma, glioblastoma, melanoma, and osteosarcoma, human epidermal growth factor receptor 2 (HER2) for HER2-

positive sarcoma, mesothelin for pancreatic cancer, IL-13 receptor α (IL-13Rα) for glioma, and mutant αvβ6 integrin for pancreatic tumors. TCR-engineered T cells always target the p-HLA complex.

**T cell trafficking to tumor sites**

To enhance the efforts of CAR-T cell trafficking have been made. Investigators were use the chemokine receptors to modiy CAR T cells.

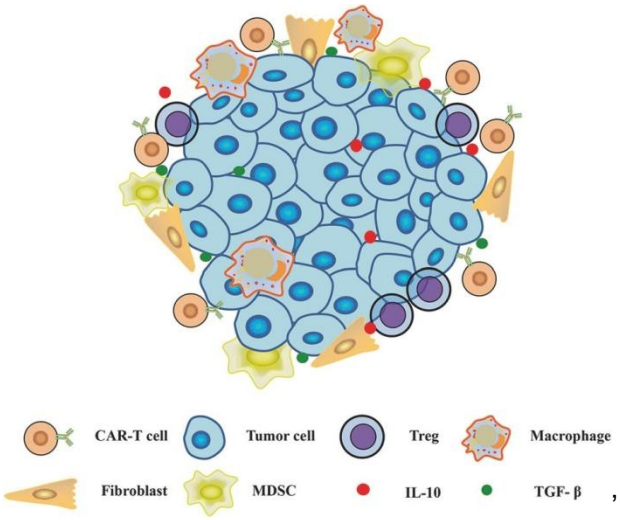


Fig. 1 Immunosuppressive microenvironment in solid tumors.

## IMMUNOSUPPRESSIVE MICROENVIRONMENT

In solid tumors, CAR-T cells face a hostile tumor microenvironment (TME), even though CAR-T cells are able to migrate to tumor sites. Solid tumors are usually infiltrated by an abundance of immune- suppressor cells, including M2 tumor-associated macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) and B cells, which protect malignant cells from the antitumor activity of the immune system. In addition, immunosuppressive cytokines and inhibitory immune checkpoints play a crucial role in tumor pathogenesis and metastasis and limit the therapeutic potential of cancer immunotherapies.

## EVOLUTION OF GENETICALLY ENGINEERED T CELLS

The first clinical application of CAR-T cells was for the treatment of metastatic renal cell carcinoma by the infusion of CAR-T cells recognizing CAIX. CAIX CAR-T cells successfully targeted CAIX- expressing tumor cells but also recognized CAIX-expressing normal tissues, resulting in so-called “on-target, off-tumor” toxicity and grade 2-4 enzyme disturbances. The antitumor efficacy of CAIX CAR-T was moderate, mainly due to the construction of a CAR with an intracellular CD3ζ motif, which failed to induce robust in vivo antitumor effects after engaging

with tumor cells. To obtain ideal antitumor efficacy and prevent severe adverse effects, the CAR construct was optimized. The CAR molecule consists of four parts: the extracellular scFv, the transmembrane domain, the costimulatory domain and the CD3ζ signal domain, which are intracellular.

## OTHER CHALLENGES FOR ENGINEERED T THERAPY

Genetically engineered T cells have benefited patients in the treatment of tumors, but other obstacles beyond the issues discussed above have challenged the application of genetically modified T cells, even in the treatment of hematological malignancies.

- Failure of CAR-T cell generation
- Relapse after CAR-T therapy
- Side effects

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