Chimeric Antigen Receptor T Cells: Clinical Applications, Advances and Challenges

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Abstract Chimeric antigen receptor (CAR) T cells have emerged as a potential groundbreaking treatment for patients with advanced B-cell and other hematologic malignancies. CAR T cells recognize and eliminate tumor cells via cytotoxic killing, independent of the major histocompatibility complex. They are predominantly used in the treatment of many leukemias and lymphomas, such as acute lymphoblastic leukemia, chronic lymphocytic leukemia, Non-Hodgkin lymphoma, and multiple myeloma, via the administration of CD19-targeted or BCMA-targeted CAR T cells respectively. Although there is strong clinical data to support the efficacy of this therapy, toxicity, relapse, and a lack of its broad application for solid tumors have emerged as challenges. In this section, we will highlight the application of CAR T cells in treating hematologic malignancies, as well as their application in solid tumors. Here, we will review the engineering of CAR T cells, clinical data on CD19 and BCMA CAR T cells, and limitations of these therapies. Additionally, we will discuss the development of novel approaches to engineer CAR T cells, identify target antigens, increase their effectiveness and mitigate toxicity. These advances will allow for progress of this therapy and help to overcome the hurdles that are currently present in the use of CAR T cells.

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1 T Cell Engineering With CAR T Cells

The genetic engineering of T cells with a chimeric antigen receptor (CAR) enables adoptively transferred T cells [1–5] to recognize specific tumor targets. These synthetic receptors have a structure that is analogous to the canonical components that are essential for T-cell signaling. CARs have an antigen-binding domain, the single chain variable fragment (scFV), which consists of the immunoglobulin VH and VL [6]. The CD3 zeta chain mediates the activating property of CARs, whereas the costimulatory properties are executed by co-receptors such as CD28 and 4-1BB. Hence, the CAR mediates antigen recognition, T-cell activation, and costimulation [4]. Of note, CARs are distinct from physiologic T-cell receptors in that these molecules do not need peptide processing or HLA expression for antigen binding. CAR T-cell engineering has evolved over time and there are now products that are denoted as fourth generation CAR T cells. The generations of CAR T cells are outlined in Fig. 1. These CART-cell products may utilize costimulatory receptors, such as CD28, 4-1BB, CD134, or CD137. Of note, the fourth generation CARs are the most novel iteration and use a domain referred to as TRUCK or T-cell Redirected to Universal Cytokine Killing. This specific generation is supported by activated T-cell nuclear transcriptional signals, which allows them to secrete specific cytokines such as IL-12 into the tumor microenvironment. This signaling also aids in the recruitment and activation of other immune cells to ensure a robust immune response [7].

Most CAR T-cell studies have utilized retroviral or lentiviral vectors as a mechanism to incorporate CAR cDNA into the T-cell genome [4]. Here we review the use of autologous CAR T cells, which are generated from the patient's peripheral blood T cells, engineered to express the CAR, and re-infused following the administration of conditioning chemotherapy [8–12] as illustrated in Fig. 2. The use of donor-derived or alternative cell sources for CAR T cells is outside the scope of this section.

2 CD19 CARs Targeting B-Cell Malignancies

CD19, an antigen expressed on normal B-cell as well as several B-cell malignancies, is the most common CAR target. Clinical studies of CD19-targeted CAR T cells have demonstrated that they are effective against CD19 malignancies [13–21]. For patients with B-cell malignancies who relapse following chemotherapy, treatment options and the potential for cure are limited. The three main B-cell malignancies that are treated with CD19 CAR T cells include, B-cell Acute Lymphocytic Leukemia (B-ALL), Chronic Lymphocytic Leukemia (CLL), and Non-Hodgkin Lymphoma (NHL). In B-ALL, there is a rapid outgrowth of cancerous immature B-cells that take over the bone marrow and blood stream [18]. CLL, on the other hand, is the most common leukemia in adults, and the main form is a slowly

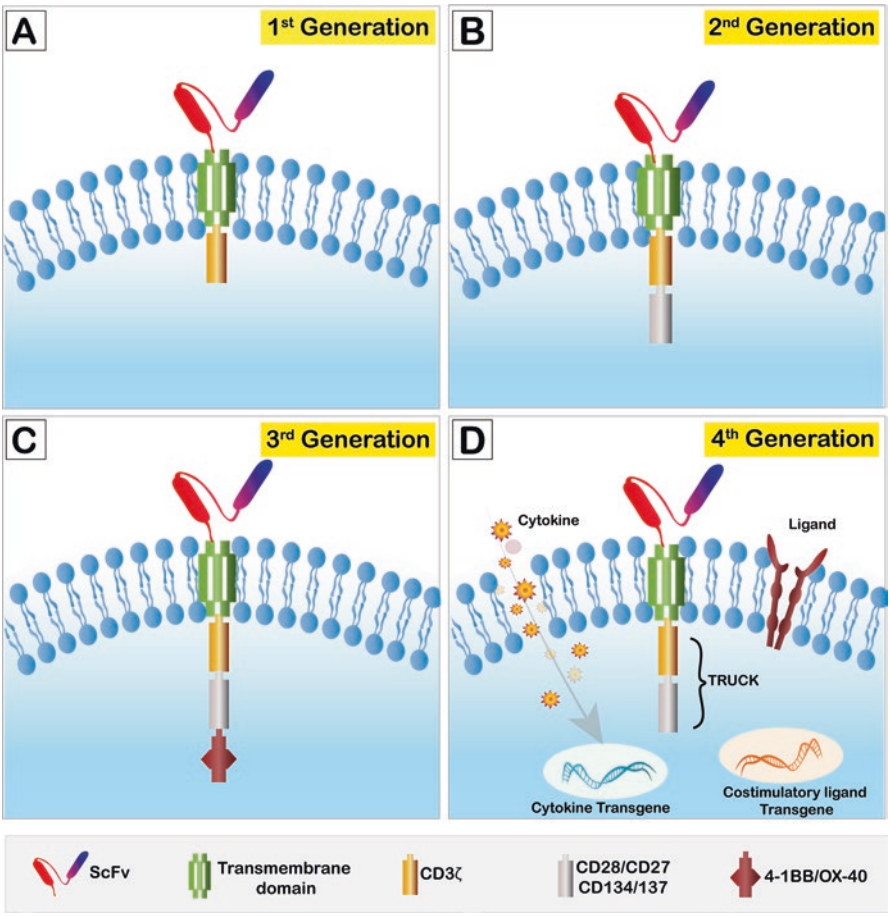

Fig. 1 Progressive generations of CAR T cells. (**A**) The first generation of CARs have a CD3ζ chain along with the scFV with linkers and a transmembrane domain. This generation of CAR T cells lack a costimulatory domain. (**B**, **C**) The second and third generations have one or two co-stimulatory domains, respectively, that induce enhanced proliferation, decreased terminal differentiation and higher activation of the T cells. (**D**) The fourth generation of CAR T cells are engineered with T cells redirected to Universal Cytokine Killing (TRUCK). These cells are designed with an inducible cytokine transgene cassette and additional receptors for the co-stimulatory ligand transgene

progressing outgrowth of more mature cancerous B cells [22]. Finally, NHLs are malignancies of B, T and Natural Killer Cells (NK) that typically infiltrate lymphoid and hematopoietic tissues. The cancerous cells of NHL can arise from either immature or mature lymphocytes. Several CD19 CAR T-cell clinical trials have focused on targeting the mature B-cell NHL neoplasms, which include Diffuse Large B-cell Lymphoma (DLBCL), Primary Mediastinal B-cell Lymphoma, and Follicular Lymphoma (FL). As noted in Table 1, the clinical outcomes of recipients

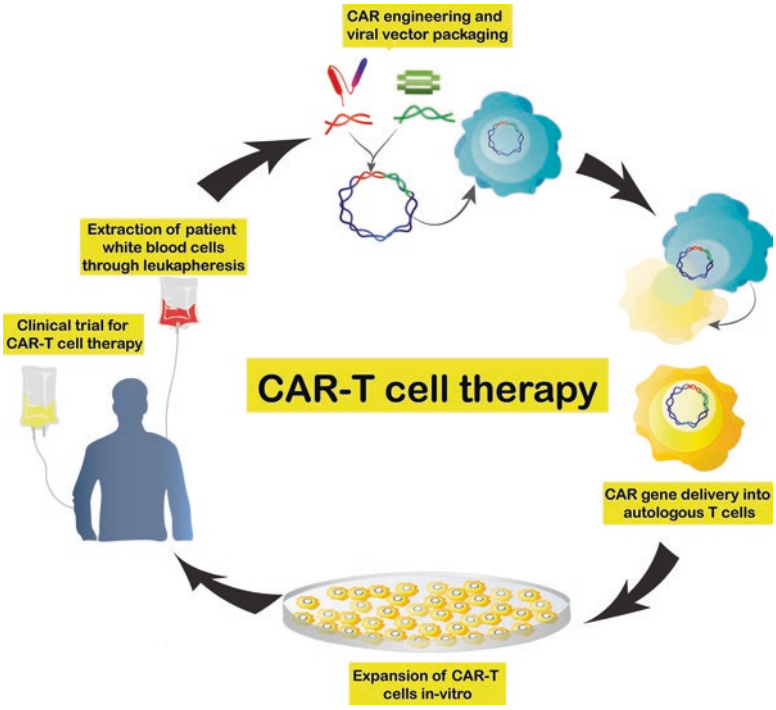

Fig. 2 The workflow of engineering CAR T-cell therapy. The treatment begins with isolating patient T cells by a process called leukapheresis. Once the T cells are isolated from the patient's blood, they are enriched and activated. The selected antigen CARs are transduced using a lentiviral or retroviral vector and introduced into the autologous patient's T cells for reprogramming. These newly engineered CAR T cells expand in vitro in the laboratory. Following adequate expansion, the CAR T cells are re-introduced into the patient's blood stream intravenously

Table 1 Summary of CD19 CAR T cells

Disease	Clinical Outcome (%)
Relapsed B-cell Acute Lymphocytic Leukemia [18, 20, 21, 23–26]	CR: 70–90
Relapsed Chronic Lymphocytic Leukemia [22, 26]	CR: 25–50 ORR: 50–80
Relapsed Non-Hodgkin Lymphoma [13, 26, 27]	CR: 33–60 ORR: 60–70

of CD19 CAR T cells vary by disease. Patients with B-cell ALL who receive CD19 CAR T cells have the highest complete response (CR), followed by patients with Non-Hodgkin lymphoma and chronic lymphocytic leukemia.

The clinical efficacy of CD19 CAR T cells ultimately led to the FDA approval of two CD19-targeted CAR T-cell products. Tisagenlecleucel (Kymriah™) is approved for patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia who are refractory or in second or later relapse. This cellular product uses

4-1BB for its co-stimulatory domain and is delivered via a lentiviral vector [26]. Axicabtagene ciloleucel (Yescarta™) is approved for adult patients with specific types of relapsed or refractory non-Hodgkin lymphoma (DLBL, primary mediastinal, and FL) after two or more lines of systemic therapy. This cell therapy uses CD28 for its co-stimulatory domain and is delivered by a retroviral vector [27].

3 CD19 CAR T-Cell Toxicity

Despite the therapeutic efficacy of CD19 CAR T-cell therapy, there are several potential toxicities to consider. First, CD19 CAR T-cell therapy presents with on-target off-tumor toxicity that presents as B-cell aplasia. B cell aplasia causes hypogammaglobulinemia that can be treated with intravenous immunoglobulin replacement therapy. Second, cytokine release syndrome (CRS) is the most frequent life-threatening complication that may occur due to the release of cytokines from CAR tumor killing [4]. Classical CRS presents with symptoms including, fever, fatigue, nausea, vomiting, diarrhea, rashes, acute kidney injury, delirium, hallucinations, hypotension, and even severe multiple organ failure [28]. Finally, neurotoxicity or immune effector cell (IEC)-associated neurotoxicity syndrome (ICANS) is another potential serious toxicity that may occur following CAR T-cell therapy [29]. ICANS may present with delirium, headache, encephalopathy, aphasia, lethargy, difficulty concentrating, agitation, tremor, seizures, and, rarely, cerebral edema [30]. Both CRS and ICANS are assessed clinically with a Grade of 1 to 4 depending upon the severity of the patient's symptoms and the symptoms are treated based upon the corresponding grade [31].

4 Challenges With CD19 CAR T-Cell Therapy

Although the introduction of T-cell engineering has created new strategies to target malignancies that have failed 2 or more other treatment regimens, relapse rates remain high. In B-ALL, approximately 30–60% of patients relapse after CAR treatment, and among those, 10–20% are CD19-negative, suggesting antigen escape by the tumor cells [32]. With regards to CD19-positive relapse, the key mechanism for CAR failure is poor persistence of the CAR T cells [33]. Several approaches to overcome these challenges have been investigated, including the use of dual-targeting CAR T therapy and the development of CAR constructs with the capacity for increased persistence in patients. These CAR construct advances have included alterations to the transmembrane, extracellular and intracellular signaling domains [32].

5 BCMA CAR T Cells for Multiple Myeloma

Multiple Myeloma (MM) is the second most common hematologic malignancy in the United States. This disease is characterized by the expansion of malignant plasma cells in the bone marrow and associated with excessive production of monoclonal antibodies in the blood and urine of patients. Additional clinical findings include osteolytic bone lesions and immunodeficiency both of which limit the length and quality of life [34]. Treatment with proteasome inhibitors (PI) and immunomodulatory drugs (anti-CD38 and anti-SLAMF7 drugs) has significantly increased progression free and overall survival in MM patients in the newly diagnosed and relapsed/refractory setting. Both of these immunomodulatory drug targets, however, are highly expressed on normal tissues especially hematopoietic lineages and immune effector cells [35, 36]. Thus, other MM specific targets must be explored for long-term usage. Additionally, overall survival of patients with relapsed disease after PI and immunomodulatory drug treatments is quite low. Accordingly, more efficacious therapies and novel strategies are urgently needed in order to develop curative therapies.

Excitingly, B-cell maturation antigen (BCMA), a transmembrane glycoprotein in the tumor necrosis factor receptor superfamily 17 (TNFRSF17), is expressed at significantly higher levels in all MM malignant cells but not on other normal tissues besides mature plasma cells (PC) [37]. BCMA itself is only induced in late memory B-cells committed to the PC lineage and is present on all PCs [38, 39]. Consequently, BCMA-targeted CAR T cells were developed to treat patients with MM. Early clinical trials have already shown significant clinical activity in patients with relapsed/refractory MM who have undergone at least three prior treatments, including a proteasome inhibitor and an immunomodulatory agent treatment. As of 2019, four Phase 1 dose-escalation clinical studies were completed, three are open and recruiting and two are still in the preclinical stages. Of the four completed Phase 1 trials, of which all use lentiviral delivery of the vector, three utilize 4-1BB as their co-stimulation domain and one uses CD28 for co-stimulation. One Juno-sponsored trial, has a construct, EGFRt/BCMA-41BBz, that incorporates the suicide gene EGFRt [40]. Many of the newer BMCA CAR T-cell constructs in the preclinical phases have begun to include suicide genes or inactivation switches. The details of these Phase 1/2 and preclinical trials are summarized in Table 2.

6 Challenges With BCMA CAR T-Cell Therapy

The application of CAR T-cell therapy in cancer treatment for MM still faces several challenges and clinical limitations including the persistence and survival of CAR T cells, toxicity of conditional chemotherapy or the CAR T-cell therapy itself, and disease progress due to antigen escape. There is limited data to assess the duration of the benefits of BCMA CAR T-cell therapy. To overcome some of these

Table 2 Summary of BCMA CAR T cells

BCMA CAR T	Clinical Development Phase	Clinical or Preclinical Details	Toxicities
Anti-BCMA chimeric antigen receptor National Cancer Institute [41, 42]	Phase 1 completed	24 patients, 3 dose escalations ORR: 81% CR: 8% VGPR: 33% PR: 13%	15/16: Grade 4 toxicities CRS, pancytopenia
CART-BCMA Novartis [43]	Phase 1 completed	25 patients, 3 cohorts ORR: 48% CR: 8% PR: 20% VGPR: 20%	CRS, neurotoxicity, Grade 3: 8 (32%) Grade 4: 3 (12%)
LCAR-B38M Nanjing Legend Biotech [44]	Phase 1 completed	57 patients ORR: 88% CR: 68% VGPR: 5% PR: 14%	Grade ≥ 3 toxicities 37/57 patients (65%) CRS: 51 (90%) Grade 3: 4 (7%) 1 patient: neurotoxicity
bb2121 Bluebird Bio Celgene [45]	Phase 1 completed	33 patients, 4 dose cohorts plus a expansion phase ORR: 85%, CR: 45% 6/15 of CRR relapsed	Grade 3 pancytopenia CRS: 76% Grade 1/2: 70% Grade 3: 6% Neurologic toxicities: 42% 3% reversibleGrade 4 neurologic toxicity
EGFRt/ BCMA-41BBz Juno [46]	Phase 1-open/ recruiting	Includes a suicide gene EGFRt	
P-BCMA-101 Poseida Therapeutics [47]	Combined Phase 1/2-open/ recruiting	No transfection, uses mRNA and plasmid DNA for CAR T engineering of T stem cell memory CART A Phase 1, open-label, single ascending dose (SAD), 18 patients ORR: 83% CR: 73% VGPR 5% PR: 17% Safety switch activated by rimiducid	Toxicity: Grade 2 CRS 1 patient

(continued)

Table 2 (continued)

BCMA CAR T	Clinical Development Phase	Clinical or Preclinical Details	Toxicities
Descartes-08 Cartesian Therapeutics [46]	Combined Phase 1/2-open/ recruiting	30 patients $CD8^+$ anti-BCMA CAR T modified by mRNA not transfection Phase 1: dose escalation of the $CD8^+$ BCMA CART Phase 2: treatment with fludarabine and cyclophosphamide	
BCMA CAR Pfizer [48]	Preclinical	Inactivates the TCR alpha chain Contains an intra-CAR rituximab recognition domain to deplete CAR T	
P-BCMA-ALLO1 Poseida Therapeutics [46]	Preclinical	Uses CRISPR to disrupt both the TCR and MHC I expression	

challenges, the field is investigating several strategies to utilize conditioning and combination therapies to aid CAR efficacy and persistence [49]. The combination of BCMA CAR with another antigen targeting CAR T-cell or with other immunomodulatory agents may reduce the risk of relapse due to tumor antigen escape. Additionally, pre-conditioning may deplete T regulatory cells, leading to enhancement of CAR T-cell therapy [50]. Given that one-third of newly diagnosed MM patients are older than 75 years and more than 30% of them are frail, these factors could be barriers to the use of CAR T-cell therapy or the incorporation of the necessary conditioning chemotherapy prior to CAR T-cell therapy [51]. Another issue that needs to be addressed is whether CAR T-cell therapy should move to an earlier line of therapy to avoid only treating MM patients who have more advanced disease and antigen-altered disease states.

7 CARs for Solid Tumors

Although immunotherapy with CAR T cells has achieved success in the treatment of hematological malignancies, the treatment of solid tumors with CAR T cells has been challenging due to the intricacies of solid tumor microenvironments and tumor locations. T cells trafficking to and infiltrating into tumor sites are oftentimes greatly limited by the immunosuppressive microenvironment created by the tumor cells themselves. This limits the ability of the CAR T cells to access the solid tumor milieu and execute their function of killing tumor cells. Furthermore, solid tumors tend to display a large degree of antigen heterogeneity. Many tumors may contain only a subset of cells that express the CAR T target antigen. Even in the setting of a

uniformly expressed tumor antigen, such as the B-cell leukemias and lymphomas discussed above, there is still the possibility of antigen loss or escape [52]. Given these obstacles, strategies have been employed to overcome them, including knock-out of PD-1 in the CAR T-cell, engineering the simultaneous secretion of cytokines or chemokines, and combining CAR T cells with other pharmacologic treatment strategies [53, 54].

8 Challenges With CAR T-Cell Therapy for Solid Tumors

Another complication that limits solid tumor-directed CAR T-cell therapy is immune-related adverse events. These toxicities may occur upon binding of the CAR to antigens on target tumor cells, resulting in the activation of the CAR and the subsequent release of a large quantity of inflammatory cytokines causing CRS which is detailed symptomatically in the CD19 CAR section. Unlike hematological malignancies, most solid tumors share many antigens with normal tissues. This may lead to off-target effects and the destruction of healthy organs by the infused CAR T cells. In order to reduce the risk of this toxicity, more specific antigens for the tumor should be selected. Tumor killing may be improved by utilizing dual-antigen CAR T-cell targeting and modulating the sensitivity of the scFv that comprises the CAR T-cell itself [55].

Despite these efforts, there are still no CAR T cells clinically approved for solid tumor treatment. Excitingly, as of 2019, there were more than forty ongoing CAR T-cell clinical trials for the treatment of solid tumors registered in China alone [56]. The antigen targets of these CAR T cells vary, some of which target EGFR (gliomas, colorectal cancers), EpCAM (hepatic, gastric, esophageal, colorectal, prostate cancers), GPC3 (hepatocellular carcinoma, squamous cell lung carcinoma), MSLN (pancreatic, ovarian, endometrial and other mesothelin positive cancers) and MUC1 (pancreatic, hepatocellular, glioma, gastric, colorectal, non-small cell lung cancer and triple negative breast cancer) [57]. A MUC1-targeted CAR T-cell, manufactured by Minerva Biotechnologies, and initiated in September 2019, is a first in human clinical trial in the United States conducted at the Fred Hutchinson Cancer Center. This CAR was engineered to target a truncated form of MUC1 that is highly expressed on breast cancer cells and not as highly expressed on normal tissues. Results from this trial will demonstrate the advancement of solid tumor CAR T-cell technology as we seek to have more clinical studies for other solid tumor antigens.

Furthermore, pre-clinical studies are ongoing to investigate other therapeutic approaches for the treatment of solid tumor with CAR T cells. Tumor heterogeneity in malignancies, such as glioblastoma multiforme (GBM), has proven challenging for treatment with CARs. Peptide-based CARs are being evaluated in order to harness the binding potential of chlorotoxin (CLTX) to tumor cells, given that CLTX binds with higher affinity to tumor cells than any other antigen. CAR T cells bearing the CLTX as the targeting domain demonstrate higher anti-tumor activity both *in vitro* and *in vivo* with minimal off-target effects, which supports this strategy as a

potential treatment for GBM and other solid tumors [58]. Yet another approach has targeted Glypican-3 (GPC3), which is over-expressed in hepatocellular carcinoma (HCC) but not in normal tissues. GPC3-specific CAR T cells are designed with the PiggyBac (PB) transposon-transposase system as opposed to conventional viral vectors. Upon stimulation with the GPC3 antigen, the GPC3 CARs undergo activation and proliferation. Investigators have found that the administration of GPC3 CAR-T cells to HCC xenograft mice results in higher cytokines, such as interferon-γ, and increased cytotoxicity in comparison to mice injected with mock T cells and vehicle controls [59].

9 Future Directions of CAR Therapy

Ongoing research with CAR T cells is focused on strategies to (1) improve CAR T-cell persistence, (2) decrease antigen loss as a mechanism of disease relapse, (3) develop CARs for a wider range of hematologic malignancies as well as solid tumors and, (4) decrease costs of the therapy. Along with the modifications to the engineering of the CAR construct and improvements to the domains of the receptor, CRISPR/Cas9 editing of the CAR has begun to further improve signaling of the CAR. The CRISPR/Cas9 system has been employed to target the genes of inhibitory receptors, such as PD-1 [54], Fas, and HLA-I, to simultaneously delete these genes and limit protein expression of these immune system inhibitors on the CAR itself. Pre-clinical in vivo and in vitro studies with the Fas/HLA-I/CD3 triple deletion CAR have shown that this strategy allows for increased CAR persistence and enhanced immunologic activity with improved cytotoxicity and cytokine secretion from the CAR T cells [60]. Dual targeting strategies for CAR T cells aims to decrease the potential for relapse due to antigen loss by simultaneously targeting multiple antigens, such as CD19 and CD22 [61]. Investigators are actively working to investigate potential antigens to successfully treat diseases ranging from AML [62] and pancreatic cancer [63]. Finally, others are also utilizing CRISPR/Cas9 to develop off-the-shelf CAR T-cell therapies that have the potential to decrease the costs needed for the generation of personalized CAR T cells [63].

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