

REVIEW

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Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients



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Abstract

Acute myeloid leukemia (AML) is a serious, life-threatening, and hardly curable hematological malignancy that affects the myeloid cell progenies and challenges patients of all ages but mostly occurs in adults. Although several therapies are available including chemotherapy, allogeneic hematopoietic stem cell transplantation (alloHSCT), and receptor-antagonist drugs, the 5-year survival of patients is quietly disappointing, less than 30%. alloHSCT is the major curative approach for AML with promising results but the treatment has severe adverse effects such as graft-versus-host disease (GVHD). Therefore, as an alternative, more efficient and less harmful immunotherapy-based approaches such as the adoptive transferring T cell therapy are in development for the treatment of AML. As such, chimeric antigen receptor (CAR) T cells are engineered T cells which have been developed in recent years as a breakthrough in cancer therapy. Interestingly, CAR T cells are effective against both solid tumors and hematological cancers such as AML. Gradually, CAR T cell therapy found its way into cancer therapy and was widely used for the treatment of hematologic malignancies with successful results particularly with somewhat better results in hematological cancer in comparison to solid tumors. The AML is generally fatal, therapy-resistant, and sometimes refractory disease with a disappointing low survival rate and weak prognosis. The 5-year survival rate for AML is only about 30%. However, the survival rate seems to be age-dependent. Novel CAR T cell therapy is a light at the end of the tunnel. The CD19 is an important target antigen in AML and lymphoma and the CAR T cells are engineered to target the CD19. In addition, a lot of research goes on the discovery of novel target antigens with therapeutic efficacy and utilizable for generating CAR T cells against various types of cancers. In recent years, many pieces of research on screening and identification of novel AML antigen targets with the goal of generation of effective anti-cancer CAR T cells have led to new therapies with strong cytotoxicity against cancerous cells and impressive clinical outcomes. Also, more recently, an improved version of CAR T cells which were called modified or smartly reprogrammed CAR T cells has been designed with less unwelcome effects, less toxicity against normal cells, more safety, more specificity, longer persistence, and proliferation capability. The purpose of this review is to discuss and explain the most recent advances in CAR T cell-based therapies targeting AML antigens and review the results of preclinical and clinical trials. Moreover, we will criticize the clinical challenges, side effects, and the

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different strategies for CAR T cell therapy.

Keywords: Acute myeloid leukemia, Adoptive cell therapy, Chimeric antigen receptor T cells, Hematological malignancy, Target antigen

Introduction

AML is a severe hematological malignancy, which although commonly occurs among adults, also is ranked second most common childhood leukemia. AML is an aggressive and heterogeneous cancer that affects the blood and bone marrow. It is characterized by deviated function and proliferation of immature white blood cells (WBCs). At this type of malignancy, the hematopoietic stem cells (HSCs) are persuaded to proliferate in an uncontrollable manner followed by the overproduction of immature WBCs [1, 2]. To date, a wide variety of therapeutic strategies have been developed for AML including chemotherapy, target therapy, and immunotherapy-based treatments [3].

The main choice of treatment to reach a full remission and hampered chance of relapse is chemotherapy which itself is accompanied by alloHSCT. However, the prognosis of refractory/relapsed AML has remained poor and its 5-year survival rate is around 27% [2, 3]. Unfortunately, the treatment strategy comprised of chemotherapy and alloHSCT suffers from some serious disadvantages such as severe and long-term toxic effects on healthy/non-cancerous tissues/organs, restrictions in eliminating the leukemic stem cells, and failed outcomes due to weakness in targeted therapy [4, 5].

Some immunotherapy approaches have emerged with effective applications and interestingly they achieved more successful results when administered in hematological malignancies and solid tumors [6]. Over the last decade, it was illustrated that immunotherapy-based strategies are attractive for AML patients resistant to chemotherapy. These novel therapies can specifically target the antigens on leukemic stem cells and leukemic blasts so less toxicity is achieved by using the therapy [7].

Vaccine therapy, monoclonal antibodies, checkpoint inhibitors, stem cell transplantation, and CAR T cell therapy are some of the immunotherapy techniques with promising outcomes in the treatment of AML [7].

Several new therapeutic agents based on antibodies have been developed targeting key molecules of AML. For instance, gemtuzumab ozogamicin (Mylotarg®; Pfizer) is a humanized mAb linked to an anti-tumor antibiotic, calicheamicin, and so is considered as an antibiotic-antibody conjugate. It was approved by the United States Food and Drug Administration (FDA) for treatment of relapsed AML in adults and children with confident therapeutic results [8]. The ADC treatment alongside other antibody-based therapies such as bispecific T cell engager (BiTE) antibodies achieved successful outcomes.

Therefore, recent advances provide an impressive breakthrough in the treatment of hematological malignancies (e.g., AML). Engineered CAR T cells are one of the novel therapy methods and also a kind of adoptive cell therapy (ACT) [9, 10]. A special type of engineered autologous T cell expressing recombinant receptors specific for target tumor antigens is called a CAR T cell. CAR is composed of the antigen-binding region and functional part of T cell [11]. CAR T cells have many advantages including MHC-independent antigen recognition, acting more specific than TCR, programmable to recognize any tumor antigens, higher proliferation, longer persistence, manageable cytotoxicity capacity, and the capability of preventing tumor escape; therefore, it is a superior therapeutic choice in cancer treatment [12, 13]. For instance, the anti-CD19 CAR T cell is one of the commonly used CAR T cells which has exhibited strong and long-lasting anti-tumor activity in acute lymphocytic leukemia (ALL). It is the first CAR T cell approved by U.S. FDA [14].

In this review, we will discuss the current therapeutic approaches for AML with a special focus on the CAR T cell therapy, structure, generation, and function of CARs, trials on the CAR T cells for the treatment of AML, possible challenges and toxicity of CARs, as well as the suggestions for improving the CAR T cells' safety and efficacy and for decreasing the toxicity.

Therapeutic approaches for AML

Chemotherapy

Chemotherapy is an aggressive approach performed by the injection of anti-cancer (cytotoxic) drugs to kill growing cells in the body. It has been shown that chemotherapeutic drugs are persistent in the bloodstream, so can affect the organs, tissues, and immune cells. Although chemotherapy kills rapidly dividing cells, its harmful effects on healthy tissues are inevitable. The most important side effect of chemotherapy is the weakening of the immune system, which reduces the ability to fight infection, bleeding, and fatigue. Chemotherapy is the main treatment for AML. Common methods of chemotherapy include intravenous (IV) injections. Chemotherapy for AML is divided into three stages: induction, post-remission, and consolidation. The chemical drugs most commonly used to treat AML are a combination of cytarabine with daunorubicin or idarubicin. However, some drugs such as fludarabine, 6-thioguanine (6-TG), methotrexate (MTX), and azacitidine can be used [15–17].

Antigen targeted therapy

Hedgehog (Hh) signaling pathway

Hh is a key pathway required for the survival of leukemia stem cells which can be disturbed by specific inhibitors. Glasdegib, an oral Hh pathway suppressor, has demonstrated a strong suppression effect on the proliferation of AML cells [18, 19]. The promising responses and good tolerability of Glasdegib were observed in the phase I trial study [20].

Fms-like tyrosine kinase 3 (FLT3)

FLT3 is a transmembrane tyrosine kinase and its mutant forms are mostly overexpressed in AML [21]. Midostaurin is one of the important preventing agents against FLT3, which has shown beneficial effects on repressing the AML and increasing the survival rate especially when it is used along with chemotherapy agents [22].

Isocitrate dehydrogenase 1,2 (IDH1/2)

Ivosidenib and Enasidenib are other chemical agents used to restrain the functionality of IDH1 and IDH2 mutant forms (in cancerous cells), respectively. The mutant forms cause a cascade of harmful intracellular changes such as enhanced 2-hydroxyglutarate production, an oncometabolite that inhibits the enzymes with an important role in histone methylation. The subsequent changes in methylation processes cause transcriptional dysregulation and therefore resulted in the promotion of proliferation and blockade of differentiation of myeloid cells. The drugs targeting IDH1 and IDH2 reverse these harmful changes and therefore cause therapeutic effects in AML patients [23].

B cell leukemia/lymphoma-2 (BCL2) and P53

Survival of leukemic blasts (myeloblasts) is mainly associated with the presence of anti-apoptotic proteins termed B cell leukemia/lymphoma-2 (BCL2). Venetoclax is a BCL-2 homology 3 (BH3)-mimetic compound that returns the apoptosis capability in target BCL-2-mutant cells and therefore it can induce apoptosis in targeted cancerous cells [24, 25]. In addition, it was known that the overexpressed mutant form of P53 in AML cells can cause cancer development. APR-246 is a novel therapeutic molecule and one of the powerful blockers of P53 mutant form and can mediate the P53 refolding and reactivation to induce the apoptosis/cell cycle arrest in tumor cells [26].

E-selectin

E-selectin is an overexpressed cell adhesion molecule on leukemic blasts. Uproleselan (GMI-1271) is an E-selectin blocker that can increase the anti-cancer chemotherapy response against AML [27].

Polo-like kinase-1 (PLK1)

PLK1 is a key mitosis regulator and helps the cell for the right passage through mitosis processes. It is thought that PLK1 has a role in DNA modification and replication procedures. Volasertib and Rigosertib are important suppressors of PLK1 and developed for the treatment of AML [28, 29].

Cyclin-dependent kinases (CDKs)

CDKs, the members of the protein kinases family, are also important target molecules in AML. CDKs have critical roles in cell cycle regulation, cell differentiation, mRNA preparation, and transcription regulation. CHK1 protein kinase overexpression in AML is correlated with limited responses to chemotherapy and a shorter duration of survival. Prexasertib is a CHK1 inhibitory agent that can restrict the replication processes [30]. Moreover, FDA granted palbociclib as the CDK4/6 inhibitory agent for the treatment of breast cancer. It has a good potential to be used as a therapy in other cancers such as AML [31].

Vaccine therapy

A vaccine is a prominent example of active immunotherapy which can trigger the immune system responses against a specific antigen. Immune responses can be elicited by various types of vaccines such as dendritic cell (DC) vaccines, peptide-antigen vaccines, and DNA vaccines. For AML, Wilms' Tumor-1 (WT-1) antigen was used to generate a well tolerable vaccine, which can induce the antigen-specific T cell responses and elicit a moderate but acceptable level of therapeutic responses [32]. Moreover, OCV-501, an HLA peptide derived from WT-1 protein was used to design an antigen-based vaccine for AML. It was evaluated in phase 2 clinical trial and the treatment was well tolerated by elderly AML patients [33]. In addition, various DC vaccines have been developed for the treatment of AML. One of the DC vaccines emanated from AML cell lines was called DCP-001 and was evaluated in a phase I clinical trial study [34]. Also, another DC vaccine which is comprised of WT1 mRNA-electroporated DCs was evaluated against AML in a phase II trial study. The vaccine decreased the commonly occurred relapse during the remission period after AML chemotherapy [35]. Also, another DC vaccine was developed by fusing patient-derived AML cells and autologous dendritic cells, making a hybridoma capable of effectively stimulating the immune system and acts as a personalized anti-cancer therapy. The vaccine was assessed in a third phase clinical trial [36].

Monoclonal antibody therapy

Various monoclonal antibodies (mAbs) targeting different antigens of tumor cells are widely used as the

immunotherapy approaches for AML and other types of cancer. As an example, an anti-CD33 mAb called gemtuzumab was fused to an anti-tumor antibiotic, calicheamicin; a complex comprised of gemtuzumab and calicheamicin, the conjugate, was called gemtuzumab ozogamicin [37]. This complex has been also approved and administered for non-chemo-eligible relapsed AML patients, and it showed good toxicity against leukemic cells. Moreover, other trial studies have been conducted to appraise the gemtuzumab efficacy when administered simultaneously (as combination therapy) with other chemotherapy drugs like decitabine (NCT00882102), cytarabine (NCT02473146), and azacitidine [38]. Another example of antibody-drug conjugate therapy is the vadastuximab talirine, an anti-CD33 mAb conjugated to pyrrolbenzodiazepine, a highly potent DNA binding agent for the treatment of AML [39]. Moreover, there are also some other mAbs under development such as CSL360, IMG632, and SGN-CD123A which target the CD123, an overexpressed antigen in AML cells [40–42].

Bispecific antibody therapy

Bispecific antibodies (BAB) composed of two distinct variable domains, one specific for a tumor antigen and the other specific for a CD3 receptor on the T cell, have been developed in recent years with some superiority in comparison to monospecific antibodies. BABs can detect the tumor cell and simultaneously activate the killing mechanisms in T cells by engaging them towards the detected tumor cell. An anti-CD3/CD33 bispecific antibody (AMG330) and an anti-CD3/CD123 antibody named flotetuzumab are two good examples of bispecific antibodies, both evaluated for the treatment of AML. Compared to old-fashioned mAbs, BABs have some advantages such as increased safety, more efficacy in tumor cell killing, and reduced exhaustion of the immune cells which is particularly characterized by deletion or inhibited functions of T cells [43, 44].

Checkpoint inhibitors

Checkpoint inhibitors are used to prevent the negative modulation of the immune system. Checkpoints are proteins/receptors on immune cells particularly T cells and in the case of challenges with the tumor cells, they send an “off” signal to T cell and prevent initiation of killing mechanisms. The checkpoint proteins keep the T cells in check and prevent the overactivation of the immune system. Also, there are partner proteins on tumor cells that can bond to checkpoints and make the T cells deactivated, so the tumor cells can escape from immune system anti-tumor responses. CTLA4 and PD-1 are overexpressed checkpoints in AML patients. These checkpoints have been the subject of the development of novel mAb-based therapies. Currently, there are two

mAbs named ipilimumab and nivolumab blocking the checkpoints CTLA4 and PD-1, respectively [45, 46]. PD-1 blocking along with adoptive cell therapy have shown effective anti-tumor responses. Furthermore, higher anti-tumor toxicity was reported in combinational therapies such as nivolumab-ipilimumab and azacitidine-nivolumab [47, 48]. There is also an approach in clinical trials for AML by which affect T cell by PD1 inhibitors such as nivolumab and pembrolizumab along with macrophages by targeting CD47 such as magrolimab. This approach along with hypomethylating agent administration has been under clinical investigations [49].

Allogeneic hematopoietic stem cell transplantation (alloHSCT)

alloHSCT is one of the beneficial and curative post-remission approaches which is used in various types of cancers particularly hematological malignancies like AML. alloHSCT is done by collecting and transplanting the matching stem cells from a donor into the patient. To completely eradicate tumor cells, patients receive alloHSCT after undergoing a conditioning therapeutic regimen such as chemotherapy or radiation therapy. Then, over the engraftment process, the production of new blood cells is reestablished and restarted by transplanted stem cells. HLA differences between the donor and the recipient can activate the immune system response against the cells, so the closer the tissue “match” between the donor and recipient, the more likely they are to “take” the transplant cells. For most patients with recurrent AML, allogeneic HSCT is preferable to autologous HSCT, for returning sick cells to a patient after treatment may mean returning some leukemia cells to them. Donor cells are more beneficial because of GVL. In this regard, donors’ immune cells recognize and attack to remaining leukemia as soon as injected, which does not happen in autologous HSCT [50, 51]. Clinical investigations revealed a significant survival rate among AML patients after receiving alloHSCT. However, there are some drawbacks or side effects when using the alloHSCT method such as graft-versus-host disease (GVHD), relapse, increased mortality, and infection [52–54]. GVHD is one of the most serious complications of allogeneic HSCTs which happens when the donor’s immune system attacks the recipients’ tissues. Symptoms are including skin rashes, nausea, diarrhea, and jaundice [55]. Although allogeneic HSCT usually needs a younger and healthy candidate, alloHSCT-induced graft versus leukemia (GVL) has been shown to increase disease-free survival in elderly patients with AML. Currently, 22% of allogeneic HSCT procedure have been done on patients older than 60 years; however, alloHSCT has been performed on only 6% of AML patients over the age of 60 in the USA, indicating that hematology units are

reluctant to consider an allogeneic HSCT as a treatment option for patients with AML [56].

CAR T cell therapy

CAR T cell structure

ACT is a novel and potent immunotherapy-based technique with encouraging results in the treatment of cancer. Also, CAR immune cells particularly T cell, which is generated by genetically engineering T cells, is one of the ACT approaches with promising results. The first proposal for using CARs was presented by Eshhar et al. in 1989 to direct the T cells to target the specific antigens [11]. CAR T cell therapy is a great breakthrough inefficient therapy of blood cancer and even solid tumors particularly to prevent relapse [57, 58]. CAR is expressed as a recombinant receptor composed of the antigen-binding region (ScFv) and a signaling/empowering part on the patient's T cells. CARs are designed by using part of mAbs and can recognize only one specific antigen epitope. Therefore, CAR T cell engineering is implemented first by isolation of patients' T cells, next by incorporating the CARs into the T cells, expansion of newly generated CAR T cells, and then re-infusion of newly engineered cells into the patient's body [59, 60].

A CAR T cell construct is composed of four main parts as follows (Fig. 1): (1) an antigen recognizing extracellular domain called ScFv which itself is comprised of light and heavy variable chain of a monoclonal antibody. (2) Spacer or hinge

region which provides flexibility and is usually made of either IgG-based part such as CH2, CH3, CH2CH3, or even hingeless or Ig-based hinges from naive T cell molecules such as CD8 or CD28. The ScFv and hinge together make the ectodomain part of CAR. (3) Transmembrane part of CAR is placed through the membrane and its structure is usually derived from CD3- ζ , CD4, CD8, or CD28 molecules. (4) Intracellular signaling domain is mainly the cytoplasmic CD3- ζ domain although it can be accompanied by some co-stimulatory molecules such as CD28, 4-1BB, or OX40. Moreover, some other immunoreceptor tyrosine-based activation motifs (ITAMs) such as the Fc receptor for IgE- γ domain have been evaluated as the intracellular activating domain but with less efficacy compared to CD3- ζ [61, 62]. The transmembrane domain and intracellular domain together form the endodomain of CAR. To enforce the naive T cell to generate CARs, CAR transgene is transferred into the naive T cells via various methods such as viral vectors [63], gene editing techniques [64], mRNA electroporation [65], and liposomes [66].

The process of CAR T cell development and its various generations

Different types of CAR T cells have been developed over time in four generations (Fig. 2). They were distinguished mainly by the variations in co-stimulatory molecules. So, CAR generations are distinguished by enhanced persistence,

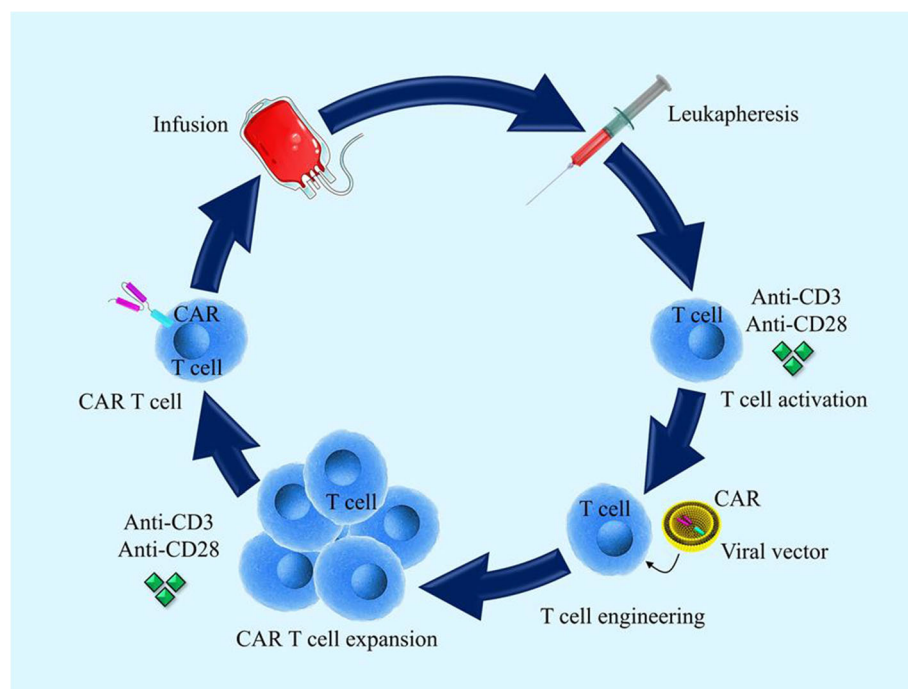


Fig. 1 Isolation, engineering, proliferation, and administration of CAR T cells. In the first stage, T cells are isolated from patients through leukapheresis and then harvested and activated. After that, the engineering and construction of the CARs are done in T cells. After the proliferation of the produced CAR T cells, the cells are re-injected into patients

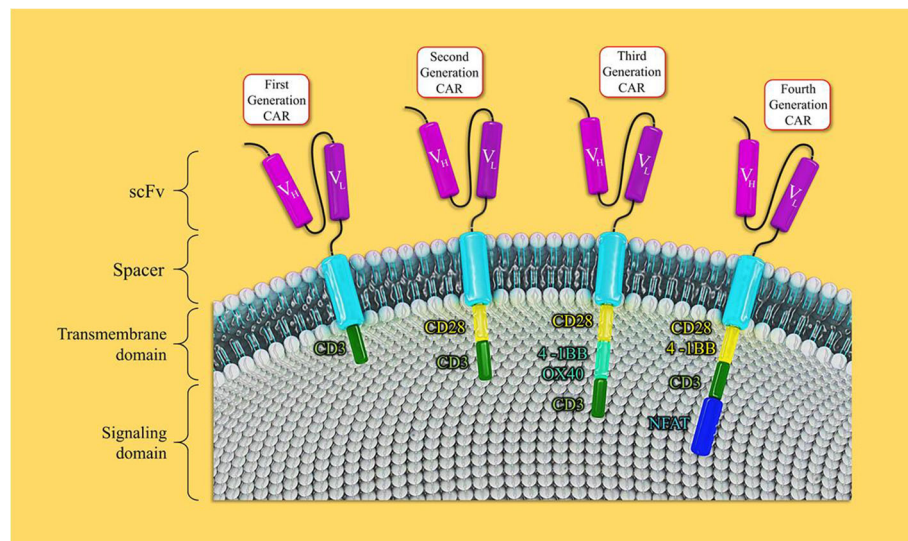


Fig. 2 Different generations of CAR T cells. CAR signaling and applications are updated with each new generation. The first generation consists of an intracellular domain (CD3 ζ chain), the second generation of CAR T cells has been composed of the addition of CD28 to CD3 as co-stimulatory molecules. In the third generation of CAR T cells, OX-40, a second co-stimulatory molecule, has been added. In the fourth generation, NFAT has been added to the induction of cassette containing the IL-12 gene promoter. NFAT, nuclear factor of activated T cells

proliferation, and killing activity. All generations comprise the scFv region and CD3 ζ intracellular signaling domain as the base parts. Second generation has also one co-stimulatory domain and the 3rd generation has two co-stimulatory domains in addition to base parts [12]. Fourth generation of CAR T cells termed T cell Redirected for Universal Cytokine Killing (TRUCKs) is the latest developed generation of CAR T cells with the capability of constitutively releasing cytokines (IL-12, IL-15, IL-18, and IL-2) or other biological factors to strengthen the anti-tumor activity. TRUCKs are armored with nuclear transcription factors. The armored with CAR-inducible transgenes capable of encoding various cytokine and mediators [67, 68].

CAR T cell function

In contrast to the classical T cells with recognizing the antigens by T cell receptor (TCR), chimeric antigen receptor (CAR) in engineered CAR T cells has a role in antigen recognition. As well, CAR T cells can identify broader types of antigens in the independence pathway of MHC. Antigen recognition through ScFv followed by intracellular signaling activates the CAR T cell against target cells. After activation, the CAR T cell specifically exerts its functionality through the secretion of anti-tumor cytokines, perforin, and granzymes into the tumor microenvironment (TME) [69, 70]. Therewith, immune system surveillance, recruitment of other immune cells, tumor cell elimination, and inhibition of tumor relapse can be increased by using CAR T cell as a living drug.

CAR T cell therapy in hematological malignancies

CAR T cell therapy with hopeful success results has been developed in various hematological malignancies without significant efficacy in solid tumors. Immunosuppressive conditions of the solid tumor microenvironment, antigen heterogeneity, and other related hurdles reduce the CAR T cell efficacy in the context of solid tumor tissue [58]. CAR T cell-based therapies with manageable cytotoxicity and high efficacy are widely utilized in hematological cancers such as acute and chronic forms of leukemia, lymphoma, and multiple myeloma [13].

In hematological cancers, CD19 is a commonly used target molecule in generating CAR T cells. Several done or undergoing clinical studies have been conducted to evaluate the CD19-CAR T cell therapeutic function in relapsed/refractory (R/R) leukemias and lymphomas [71–73]. Encouragingly, U.S. Food and Drug Administration (FDA) has approved CD19-CAR T cell so-called Kymriah (CTL019) due to the efficient therapeutic findings, and safety for adults and children with acute lymphoblastic leukemia (ALL) [14]. Lately, CD19-CAR T cell efficacy has been also validated for R/R diffuse large B cell lymphoma (DLBCL) and R/R follicular lymphoma (FL), which highlighted the remarkable and tolerable anti-tumor function [74–76].

CD22 another overexpressed antigen on B cell cancers can be targeted by the CD22-CAR T cell. The impressive anti-tumor function of CD22-CAR T cell was reported in relapsed cancer after CD19-CAR T cell therapy or in eradicating the ALL tumor cells [77].

Moreover, various studies affirmed the CD20-CAR T cell safety and efficacy in R/R Non-Hodgkin's lymphoma (NHL), follicular- and mantle cell lymphomas [78, 79]. Additionally, treatment of chronic lymphocytic leukemia (CLL) as another common hematological malignancy was investigated by using the engineered CAR T cell against CD19 or the tyrosine-protein kinase transmembrane receptor [80, 81]. Another useful CAR T cell therapy in B cell malignancies is the anti- κ/λ CAR T cell which has demonstrated the cytotoxicity against malignant B cells without effects on normal cells [82]. Hopefully, CAR T cell therapy has pointed to the encouraging results in multiple myeloma (MM), a bone-marrow-derived refractory hematological malignancy. Surprisingly, in clinical trial studies, anti-CD138 CAR T cells and anti-BCMA CAR T cells designed for MM represented the powerful and well-tolerated activity against tumor cells. Conversely, CD19-CAR T cells in MM revealed a non-significant function against myeloma cells due to the lower expression of CD19 antigen [83, 84].

CAR T cell therapy and potential targets in AML

In AML, due to the difficult identification of target antigens, CAR T cell therapy is encountered various challenges and no approved study exists related to CAR T cell therapy for AML yet. Therefore, increasing the understanding of the AML microenvironment can be contributed to discovering the enthusiastic and proper target antigens (Table 1). Based on this, several clinical studies have been developed to investigate the CAR T cell therapeutic function against predisposed antigens such as CD7, CD33, CD38, CD44, CD70, CD123, CLL, FLT3L, FR β , Le-Y, LILRB2, NKG2D, PR1, and WT1. In this review, various types of CAR T cells against candidate antigens have been described (Table 2).

CD7-CAR T cell

CD7 is a transmembrane glycoprotein expressed by T cells, NK cells, and cord blood myeloid progenitors with a co-stimulatory role in B and T cell lymphoid development interactions. CD7 is also expressed by leukemic cells like AML (30%) but not by healthy myeloid cells. So, it can be a potential candidate for eradicating tumor cells selectively with no toxicity on normal cells [107]. For engineering CAR T cells, CD7 removal requires limiting the T cell fratricide due to the CD7 expression by T cells [108].

In two studies by Gomes-Silva et al. [85, 108], CD7-CAR T cell was engineered against CD7⁺ tumor cells in a xenograft model of AML. Before CAR T cell generation, the CD7 gene of primary activated T cells was removed by CRISPR/Cas9 strategy. Then, the second generation of CD7-knockout (CD7^{KO}) CD28-CD3 ζ -CD7-CAR T cell was designed by using the ScFv derived

from the anti-CD7 antibody. Astonishingly, findings manifested the high cytolytic effects against AML include notable elimination of primary AML blasts and leukemia colony-forming cells, no toxicity on healthy and erythroid cells, and a high concentration of IFN- γ . Moreover, reduction of tumor burden indicated that CD7-CAR T cell prevents systemic leukemia progression. In consequence, CD7-CAR T cell can be a potent treatment for refractory or relapsed AML.

CD33-CAR T cell

CD33 as another potential target antigen is a transmembrane protein of the sialic acid-binding immunoglobulin-like lectin (SIGLEC) family with regulatory effect on leukocytes in immune responses. CD33 is expressed on normal progenitor cells, myeloid cells, and more than 90% of AML cells possessing diagnostic and therapeutic capabilities [109].

The recombinant humanized anti-CD33 antibody conjugated calicheamicin so-called gemtuzumab ozogamicin (GO) is used for AML treatment as an only approved drug. Clinical findings of using GO against CD33 illustrated the potential of CD33 as an attractive and possible target antigen for AML [110]. Some in vivo and in vitro studies have shown the sustained substantial anti-tumor activity of CD33-CAR T cell against AML cells, significant tumor eradication, and maintenance of T cell persistence during the cytotoxicity [93, 111, 112].

In a phase I trial study conducted by Wang et al. [113], the safety and efficacy of CD33-CAR T cells were assessed in relapsed and refractory AML patients. They administered a total of 1.12×10^9 autologous T cells with 38% anti-CD33 CAR expression. As a consequence, CD33-CAR T cells displayed notable cytolytic functions against CD33⁺ blasts such as remarkable tumor degradation in the early stage as well as high maintenance of CAR T cell number and cytotoxicity. However, disease progression, pancytopenia, high fever, and CRS toxicity were observed as adverse effects due to the production of a high level of proinflammatory cytokines. Based upon this, safety measures should be considered to reducing the CD33-CAR T cell.

In a preclinical study, to increase the CAR T cell safety, Kenderian et al. [86] generated the transiently expressed mRNA-modified second-generation CD33-CAR T cell by using the ScFv derived from GO. The study findings disclosed that CD33-CAR T cells can strongly eliminate the human AML cells and myelodysplastic syndrome blasts in mouse xenografts. The reported in vivo and in vitro results of assessing the CD33-CAR T cells were the significant proliferation of CAR T cell, prominent anti-tumor activity, increased level of cytokine production, degranulation, and leukemia burden reduction. Additionally, they suggested that CD33 gene eradication through the CRISPR/Cas9

Table 1 AML target molecules

Target antigens	Type of molecule	Role	On normal cells	On HSCs	On LSCs	On AML blasts	References
CD7	Ig superfamily/ Glycoprotein	B and T cell lymphoid development, transmembrane protein	T, NK cells, and myeloid progenitors	No	Yes	Yes	[85]
CD33	The protein of the SIGLEC family	Transmembrane receptor	Progenitor, myeloid, and kupffer cells	Yes	Yes	Yes	[86, 87]
CD38	Glycoprotein	Cyclic ADP ribose hydroxylase, a transmembrane protein	B, T, NK cells	No	Yes	Yes	[88, 89]
CD44v6	Glycoprotein	Transmembrane protein	Keratinocytes	No	Yes	Yes	[90]
CD70	Glycoprotein from the TNF family	Transmembrane protein	T and B cell	No	Yes	Yes	[91, 92]
CD123	Type I cytokine receptor of IL-3	IL-3 receptor α subunit	Myeloid progenitors, DC and, basophils	Yes	Yes	Yes	[92–94]
FLT3	Type III cytokine receptor	Tyrosine kinase receptor	Neurons, testis	Yes	Yes	Yes	[95, 96]
CCL1	Glycoprotein	Transmembrane receptor	Myeloid, lung, epithelial cells	No	Yes	Yes	[97, 98]
LeY	Glycosphingolipid (fucosyltransferase)	Blood group Ag	Intestinal epithelial cells	Yes	Yes	Yes	[99, 100]
FRβ	Folate-binding protein receptor	Folate delivery	Myeloid cells	No	Yes	Yes	[101]
LILRB4	Leukocyte Ig-like Receptor-B family	Inhibitory receptor role in immune tolerance	Monocytes	No	Yes	Yes	[102]
NKG2D	C-type lectin-like receptor protein	Activator receptor	NK, NKT, Ty δ , Th, and CTL	No	Yes	Yes	[103, 104]
PR1	Proteinase protein	HLA-presented antigens	Neutrophils	No	No	Yes	[105]
WT1	Zinc-finger DNA binding protein	Transcription factor	Kidney endometrium and testis cells	No	No	Yes	[106]
mLPA	Methyl-lysophosphatidic acid	CD1c-restricted T cell antigen	MO, DC, B and T cells	No	Yes	Yes	1
IDH1(R132)	Isocitrate dehydrogenase 1	Glyoxylate bypass, tricarboxylic acid cycle	Hepatocytes, cytotrophoblasts	Yes	Yes	Yes	2,3,4
IDH2(R140)	Isocitrate dehydrogenase 2	Glyoxylate bypass, tricarboxylic acid cycle	Distal tubular cells, cytotrophoblasts	Yes	Yes	Yes	5
NPM1^{mut}	Nucleophosmin 1 mutant	Biogenesis of ribosomes, Chaperone, Host-virus interaction	Low cell and tissue type specificity	No	Yes	Yes	6,7
NOTCH2	NOTCH signaling molecule isoform 2	Developmental processes	Non-lymphoid progenitor cells, Paneth cells	Yes	Yes	Yes	8
MUC1	Glycoprotein	Protective function cell signaling	Surface of most simple epithelia and Treg cells	Yes	Yes	Yes	9, 10
CD96	Member of immunoglobulin superfamily	Adhesion of activated T and NK cells	T cells and NK cells	No	Yes	Yes	11
PRL3	Protein tyrosine phosphatase type Iva member 3	Reinforcing PI3K/Akt activation	Cardiomyocyte, neutrophil, non-classical monocyte	Yes	Yes	Yes	12, 13
IL12RB1	Interleukin 12 receptor beta 1	Cytokine signaling	T cells, Kupffer cells, B cells	Yes	Yes	Yes	11

Abbreviations: AML acute myeloid leukemia; HSCs hematopoietic stem cells; LSCs Leukemic stem cells; Ig immunoglobulin; NK Natural killer cell; SIGLEC sialic acid-binding immunoglobulin-like lectin; ADP adenosine diphosphate; TNF tumor necrosis factor; FLT3 Fms-like tyrosine kinase 3; CCL1 c-type lectin-like molecule-1; LeY Lewis Y; FR β folate receptor β ; LILRB leukocyte immunoglobulin-like receptor B4; NKG2D Natural killer group 2 D; PR1 proteinase; WT1 Wilms Tumor 1; DC dendritic cell; mLPA methyl-lysophosphatidic acid; IDH1 isocitrate dehydrogenase 1; NPM1^{mut} nucleophosmin 1 mutant; NOTCH2 NOTCH signaling molecule isoform; MUC1 mucin1; PRL3 protein tyrosine phosphatase type Iva member 3; IL12RB1 interleukin 12 receptor beta 1

Table 2 CAR T cell against AML target antigens

Target antigen	Clinical trial ID	Phase	Disease	Institution
CD33	NCT03126864	I	R/R AML	University of Texas MD Anderson Cancer Center, Houston, Texas, United States
	NCT02799680	I	R/R AML	Affiliated Hospital of Academy of Military Medical Sciences, Beijing, Beijing, China Chinese PLA General Hospital, Beijing, Beijing, China
	NCT01864902	I/II	R/R AML	Biotherapeutic Department and Pediatrics Department of Chinese PLA General Hospital, Hematological Department, Affiliated Hospital of Changzhi Medical College, Beijing, Beijing, China
	NCT02944162	I/II	R/R AML	PersonGen BioTherapeutics (Suzhou) Co., Ltd., Suzhou, Jiangsu, China
	NCT03291444	I	R/R AML, MDS; ALL	Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China
	NCT03473457	n.a.	R/R AML	Southern Medical University Zhujiang Hospital, Guangdong, Guangdong, China
	NCT03222674	I/II	AML	Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong, China
CD38	NCT03291444	I	R/R AML, MDS; ALL	Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China
	NCT03473457	n.a.	R/R AML	Southern Medical University Zhujiang Hospital, Guangdong, Guangdong, China
	NCT03222674	I/II	AML	Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong, China
CD123	NCT03585517	I	AML	Xian Lu, Beijing, China
	NCT03114670	I	Recurred AML after alloHSCT	Fengtai District, Beijing Shi, China
	NCT03556982	I/II	R/R AML	307 Hospital of PLA, Beijing, Beijing, China
	NCT02623582	I	R/R AML	Abramson Cancer Center of the University of Pennsylvania, Philadelphia, Pennsylvania, United States
	NCT02159495	I	R/R AML	City of Hope Medical Center, Duarte, California, United States
	NCT03672851	I	R/R AML	Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China
	NCT03766126	I	R/R AML	University of Pennsylvania, Philadelphia, Pennsylvania, United States
	NCT03291444	I	R/R AML, MDS; ALL	Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China
	NCT03473457	n.a.	R/R AML	Southern Medical University Zhujiang Hospital, Guangdong, Guangdong, China
	NCT03796390	I	R/R AML	Hebei Yanda Ludaopei Hospital Langfang, Hebei, China
	NCT03222674	I/II	AML	Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong, China
UCAR T123	NCT03190278	I	R/R AML	Dana-Farber Cancer Institute Boston, Massachusetts, United States Weill Medical College of Cornell University New York, New York, United States, MD Anderson Cancer Center Houston, Texas, United States
	NCT01864902	I	R/R AML, high-risk AML	Weill Cornell Medical College, New York, New York, United States MD Anderson Cancer Center, Houston, Texas, United States
CD123/ CLL1	NCT03631576	II/III	R/R AML	Fujian Medical University Union Hospital, Fuzhou, Fujian, China
CD33/ CLL1	NCT03795779	I	R/R AML, MDS, MPN, CML	The General Hospital of Western Theater Command Chengdu, China Peking University Shenzhen Hospital Shenzhen, China
CCL1	NCT03222674	I/II	AML	Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong, China Shenzhen Genomimmune Medical Institute, Shenzhen, Guangdong, China Yunnan Cancer Hospital & The Third Affiliated Hospital of Kunming Medical University & Yunnan Cancer Center, KunMing, Yunnan, China
Lewis Y	NCT01716364	I	Myeloma, AML, MDS	Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
WT1	NCT03291444	I	R/R AML, ALL, MDS	Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China
CD7/ NK92	NCT03018405	I/II	R/R AML	PersonGen BioTherapeutics (Suzhou) Co., Ltd., Suzhou, Jiangsu, China
NKG2D	NCT02203825	I	AML, MDS-RAEB, MM	Dana-Farber Cancer Institute, Boston, Massachusetts, United States
	NCT03018405	I/II	R/R AML, AML, Myeloma	Tampa, Florida, United States Buffalo, New York, United States Brussels, Belgium Brussels, Belgium Ghent, Belgium

Abbreviations: R/R relapsed/refractory, AML acute myeloid leukemia, ALL acute lymphoblastic leukemia, MDS myelodysplastic syndrome, CML chronic myeloid leukemia, MPN myeloproliferative neoplasm, alloHSCT allogeneic hematopoietic stem cell transplantation, RAEB refractory anemia with excess blasts, MM multiple myeloma

gene-editing strategy as a CD33 knockout (KO) HSPC contributes to increasing the safety of CAR T cells. In this regard, the results of a study demonstrated that the combined administration of CD33-CAR T cell with CD33 KO HSPC led to targeting the AML cells specifically and reducing the myelotoxicity [114].

CD38-CAR T cell

CD38 as an AML target antigen is a type II transmembrane glycoprotein expressing on AML blasts but not on normal human hematopoietic stem cells. CD38 The decreased level of CD34 and increased level of CD38 contribute to progenitor cell differentiation [115]. To engineer CD38-CAR T cells against AML cells, the intensity and number of CD38 should be increased due to the 83% responsibility of CD38 expression in AML cells. Based on this, All-trans retinoic acid (ATRA) as a therapeutic factor of acute promyelocytic leukemia (APL) treatment has the ability to inducing CD38 expression on AML cells [115, 116].

In a study, Yoshida and colleagues engineered CD38-CAR T cells to target leukemia cells. They showed that ATRA increased the CD38 expression on AML cells, and subsequently, remarkable cytotoxicity of CD38-CAR T cell combined with ATRA was observed in eliminating the tumor cells [88].

CD44v6-CAR T cell

CD44v6 termed as a variant 6 isoforms of the hyaluronic acid CD44 receptor and class I membrane glycoprotein is overexpressed in AML and other hematological malignancies. CD44v6 expression was also observed on circulating monocytes; however, CD44v6 has shown a low expression on healthy cells and no expression on progenitors, HSCs, and resting T and B cells [117–119]. CD44v6 expression is required for tumor cell growth and is related to poor prognosis of AML and multiple myeloma (MM). So, CD44v6 can be a potential target antigen for AML therapy [90].

In a study by Casucci et al. [90], a second-generation CD44v6-CAR T cell was generated by using the ScFv derived from a humanized anti-CD44v6 antibody to targeting CD44+ AML cells safely and effectively. It has been reported that CD44v6-CAR T cells eradicated the CD44+ tumor cells efficiently along and produced the anti-tumor cytokines. Based on published results, CD44v6-CAR T cells eliminated the tumor cells selectively; however, monocytopenia was observed as only hematologic toxicity of CD44v6-CAR T cell so-called on-target/off-tumor toxicity on circulating monocytes. To overcoming this side effect, nonimmunogenic inducible Caspase 9 (iC9) [120] and thymidine kinase [121] suicide genes co-expressed in CD44v6-CAR T cells were applied to ablating CAR T cell efficiently.

CD70-CAR T cell

CD70 is a ligand for CD27 identified as a type II transmembrane glycoprotein, a member of the TNF family. CD70 expression is upregulated on APCs and can be expressed on T and B cells which are triggered by stimulatory factors. CD70 expression has been evidenced on AML bulk cells and leukemic stem cells (LSC) but not on normal hematopoietic stem cells (HSCs) [91, 92].

In a preclinical study conducted in the AML xenograft model, a second-generation CD27-CD3 ζ -CD70-CAR T cell was generated and validated. Strong capability of proliferation, robust potential cytolytic function in eliminating the tumor cells, increased level of TNF- α and IFN- γ production, and no toxicity on healthy HSCs are the reported applications of the CD70-CAR T cell [92]. These promising outcomes suggest the applicability of CD70 antigen as a proper choice for developing CAR T cell against AML.

CD123-CAR T cell

Another proper target antigen for AML cell therapy is the IL-3 receptor α subunit (IL3R α) named CD123. CD123 overexpression has been evidenced on leukemic stem cells (LSCs) and AML blasts and no significant expression on normal hematopoietic stem cells [122]. In a clinical study, anti-CD123 neutralizing monoclonal antibody demonstrated insufficient efficacy against AML [123].

Gill and colleagues engineered a second-generation CD123-CAR T cell to target CD123+ AML cells in the xenograft model. Interestingly, strong anti-tumor cytotoxicity, establishing memory T cells, proliferation, persistence, degranulation, and effector cytokine production were reported as results of using CD123-CAR T cell [94].

In a study, Thokala et al. engineered CD123-CAR T cells by using different chains of VL and VH from various CD123 specific monoclonal antibodies (mAbs) instead of only one specific antibody. Findings revealed the CD123-CAR T cell cytotoxicity against CD123+ AML cells with no effect on CD123- B cell lymphoma cells and tumor burden reduction. Concerning myelotoxicity of CD123 like CD33, it remains a problem with targeting this antigen for AML therapy. Surprisingly, CAR T cells with ScFv composed of VL and VH from various mAbs presented the low off-tumor toxicity and lysis effect on healthy hematopoietic stem cells compared to CAR T cells with VL and VH chains of only one mAb [124].

FLT3-CAR T cell

One of the most common mutant genes responsible for about 30% of AML patients is a tyrosine kinase receptor so-called FMS-like tyrosine kinase-3 (FLT3) [125]. Two frequent mutant types of FLT3 are internal tandem

duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations accounted for 24% and 7% of AML patients respectively [126, 127]. FLT3 kinase activated by ITD and TKD augments AML progression by initiating the PI3K/Akt, Raf/MEK/ERK, and JAK/STAT5 signaling pathways [128]. In particular, AML patients possessing FLT3 have shown poor prognosis and clinical outcomes. Allogeneic HSCT is the only operative treatment available for FLT3+ AML cases [129]. However, FLT3 as a capable AML target antigen can be targeted by developing new targeted therapy approaches to improve AML treatment.

In a preclinical study [95], a second-generation FLT3-4-1BB-CD3-CAR T cell was engineered by using the anti-human FLT3 antibody-derived ScFv to targeting the FLT3+ tumor cells. According to in vitro investigations, IFN- γ and IL-2 were produced by the FLT3-CAR T cell encountering AML cell lines. Withal, inhibition of leukemia cell proliferation was observed by in vivo evaluation of FLT3-CAR T cell function. Toxicity assessment of FLT3-CAR T cell in xenograft model indicated no significant toxicity to multipotent myeloid progenitors (MMPs) and hematopoietic stem cells (HSCs) along with equivalent toxicity to common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs) describing the less hematologic toxicity of FLT3-CAR T cell.

In another preclinical study, Wang et al. [130] engineered FLT3L-4-1BB-CD3 ζ -CAR T cell to assessing its functionality against FLT3L+ leukemia cells. As a result, prominent anti-tumor cytotoxicity of CAR T cell against FLT3+ leukemia cells as well as the influential killing of the ITD type FLT3 cells compared to the wild type FLT3 cells was observed by using the CAR T cell. Therewith, AML xenograft model survival was lengthened by the effect of FLT3L CAR T cell. FLT3L-CAR T cell indicated less off-tumor toxicity on healthy progenitor and hematopoietic stem cells.

CLL1-CAR T cell

C-type lectin-like molecule-1 (CLL1) as an inhibitory receptor is a type II transmembrane glycoprotein that can be a potential candidate for AML CAR T cell therapy. CLL1 expression accounts for 92% in AML cases, which is overexpressed on differentiated myeloid cells and AML blasts. As well, CLL overexpression has been evidenced on leukemic stem cells (LSCs) but not on normal HSCs which proposes the less off-tumor toxicity of CLL1-CAR T cell. Currently, targeting CLL1 by specific monoclonal antibody has revealed an effective therapeutic function against AML along with reducing tumor burden [131, 132].

In a previous study, the third generation of CLL1-CAR T cell composed of anti-CCL1 ScFv fused to CD28, 4-1BB, and CD3 signaling domains was generated. This

anti-CCL1 CAR T cell showed the in vitro and in vivo strong functionality against CLL1+ tumor cells with a wide production of effector cytokines and chemokines including GM-CSF, TNF- α , IFN- γ , and IL-13 [97].

Moreover, two other related studies reported the results of using second-generation CAR T cells to targeting CLL1+ AML cells with sparing normal myeloid precursor cells. Increased persistence and cytotoxicity, efficient tumor elimination, anti-inflammatory cytokine production, and managing tumor relapse were reported as results of CLL1-CAR T cell activity [98, 133]. Importantly, it has been reported that using the inducible caspase 9 (iC9) strategy transduced into T cell along with the CAR gene increased the safety of CLL1-CAR T cells by controlling its function. This approach contributed to inhibiting the CAR T cell overactivity by triggering apoptosis [134].

LeY-CAR T cell

Lewis Y (LeY) is a carbohydrate tumor-associated antigen related to blood-group members. Its expression has been reported by various epithelial-derived cancers and less expression by healthy tissues [134]. Several studies have noticed the relationship between LeY antigen and progression of solid tumors and hematological malignancies. Due to LeY expression on early myeloid progenitor cells, it can be a proper targeting choice for AML treatment [99]. Hence, to target tumors, T cells like engineered CAR T cells have been developed to redirect against LeY tumor cells.

In a preclinical study, Peinert et al. [99] generated the anti-LeY CAR T cell and then investigated its functionality against AML and MM (multiple myeloma) cells. According to the reported results, engineered LeY-CAR T cells specifically targeted the LeY+ cells by producing the IFN- γ .

To conduct clinical trial studies for AML and MM adoptive cell therapy by the CAR T cell, Neeson et al. [135] engineered the CD28-CD3z-LeY CAR T cell by using the autologous T cells based on LeY-T ex vivo transduction and expansion protocol. CD8+ modified T cells showed a cytolytic response against LeY+ tumor cells and high-level production of IFN- γ .

FR β -CAR T cell

Membrane-associated folate receptor β (FR β) is a member of proteins bounded to folic acid and has a role in folate transporting. FR β has an overexpression on some nonepithelial origin malignancies and less expression on most of the normal cells. Its expression was evidenced on myeloid-lineage hematopoietic cells and accounts for expression on 70 % of primary AML cells [136]. Various targeted therapies have been developed as hopeful treatment strategies for malignancies like AML and by

targeting the FR family. All-trans retinoic acid (ATRA) elevates the FR β expression on myeloid leukemia cells but not on negative receptor cells which increased the folate-conjugated drug potential as a treatment in a pre-clinical study [137, 138].

In a preclinical study [101], the first anti-FR β -CAR T cell (m909) was engineered to targeting AML cells in vitro and in vivo. The effective cytolytic function of m909 CAR T cell was observed against FR β + cells in vitro and regression of AML tumor cells in vivo with no toxicity on HPSCs. Moreover, they presented the enhancement functionality of CAR T cells due to the elevated expression of FR β in the presence of ATRA. In another study [101], a high-affinity (HA) anti-FR β ScFv-CAR T cell was generated for AML treatment. The strong anti-tumor function and potential lyse myeloid-lineage target cells without toxicity on HSCs were observed through using HA-FR β -CAR T cells in vitro and in vivo.

Consequently, FR β can be an encouraging and proper target antigen for AML CAR T cell therapy powerful activity in combination with ATRA and without toxicity on normal cells.

LILRB4-CAR T cell

Monocytic AML (M5) is a common type of AML in children including 20% of AML cases along with poor outcomes. LILRB4 as a potential target antigen in AML belongs to the family of the leukocyte immunoglobulin-like receptor-B family. LILRB4 expression was reported on healthy monocytic cells and monocytic AML cells in all stages [139].

In a preclinical study, 41BB-CD3 ζ -anti-LILRB4-CAR T cell was engineered by using the humanized ScFv to target LILRB4 AML cells specifically. Interestingly, applying powerful cytotoxicity to AML cells in vitro, decrement of xenograft model tumor burden in vivo, and reduction of off-tumor toxicity by sparing normal progenitors and HSPCs were found as results of using LILRB4-CAR T cell [102]. These results have illustrated the potential capacity of LILRB4-CAR T cell therapy in targeting the AML selectively and specifically.

NKG2D-CAR T cell

Natural killer group 2D (NKG2D) known as an activator receptor with homodimer and hexamer form is expressed on CD8+ T cells, a small amount of CD4+, $\gamma\delta$ T cells, NK cell, and NKT cell. NKG2D has a co-stimulatory role on T cells in its native form which depends on TCR antigen recognition. NKG2D has a wide spectrum of ligands that overexpressed encountering DNA damage, infections, and malignant transformation [140, 141]. Broad expression of NKG2D ligands was evidenced in various hematological malignancies such as AML and MM, and solid tumors with no expression on healthy cells [142, 143]. Based on this, NKG2D

was identified as a potential target candidate for CAR T cell therapy in clinical applications.

Previous preclinical studies in murine models revealed that NKG2D-CAR T cell led to the induction of host protective immune responses and effective eradication of tumor cells in ovarian cancer, multiple myeloma, and lymphoma. Also, it has been demonstrated that NKG2D along with its various ligand expression could suppress the growth of tumor cells [103, 104, 144].

In a phase I clinical trial study conducted by Baumeste et al. [145], autologous first-generation NKG2D-CD3 ζ -CAR T cell was engineered and validated in AML and myelodysplastic syndrome (MDS) patients. For safety and efficacy evaluation, single intravenous administration of NKG2D-CAR T cell was performed by a dose of 1×10^6 up to 3×10^7 T cells. As a result, in one AML patient, an objective clinical response with a high level of IFN- γ production was reported without any high-grade toxicities. Interestingly, it has been reported there are no long-term toxicities like myeloablation and B cell aplasia, infusion toxicity, autoimmunity, and CRS.

PR1-CAR T cells

In the intracellular process, leukemia-associated-antigens and neoantigens are presented to T cells by HLA-II molecules. TCR-mimic (TCRm) CARs have been developed to targeting the HLA-presented antigens [10].

One of the important leukemia-associated antigens that arose from Proteinase 3 and neutrophil elastase is Proteinase 1 (PR1), HLA A2-restricted nonameric peptide. These proteinases are normally expressed in neutrophils azurophilic granules and are overexpressed on myeloid leukemia blasts [146]. To targeting PR1+ AML cells, Ma et al. developed the anti-PR1/HLA-A2-second-generation CAR T cell by using the ScFv derived from anti-PR1/HLA-A2 TCR-like antibody so-called h8F4. As a consequence, the PR1-CAR T cell exhibited the effective avidity to the PR1+ target cell and preferentially targeted the human AML cell lines and primary AML blasts with high cytotoxicity in vitro. Also, outcomes of evaluating the CAR T cell off-target toxicity have illustrated that leukemia progenitor cells were preferentially suppressed by anti-PR1/HLA2-2-CAR T cell rather than normal hematopoietic progenitors [105]. The successfully reported findings of the h8F4-CAR suggesting the potential of endogenous self-antigens for targeting by CAR T cell in AML.

WT1-CAR T cells

Wilms Tumor 1 (WT1) as an oncogenic, zinc-finger transcription factor is another HLA A2-restricted intracellular target antigen in AML. WT1 has an important role in the various cellular processes such as organ development, differentiation, proliferation, and apoptosis.

Normally, WT1 has less expression by the bone marrow, kidney, gonads, and spleen; however, its overexpression has been proved in various hematological malignancies like AML and CML, and several solid tumors including glioblastoma, mesothelioma, ovarian cancer, and gastrointestinal cancers. Indeed, the poor prognosis of AML and lymphoid leukemia patients is strongly related to WT1 overexpression of WT1 on tumor cells [147–149].

In a study conducted by Rafiq et al. [106], WT1-CAR T cell as another TCRm CAR was developed and validated by using the HLA-A*02:01-a peptide that arose from WT1 antigen. In practice, WT1⁺/HLA-A*02:01⁺ primary tumor cells or cell lines were distinguished and lysed by WT1-CAR T cell. Based on this, prominent cytotoxicity and significant secretion of IL-2 and IFN-γ were observed against primary AML cells or cell lines.

CAR T cell improvement strategies, side effects, and challenges

Despite the more beneficial effects of CAR T cells, their proliferation, persistence, and anti-tumor functions may decrease encountering some challenges in hematological malignancies or solid tumors (Fig. 3).

Improvement strategies

CAR T cell delivery

Intravenous infusion (Systemic delivery) and intracranial infusion (local delivery) are both CAR T cell delivery strategies that have a significant role in increasing the safety and efficacy of CAR T cell therapy.

Accordingly, specifically targeting the CD19, CD20, CD30, CD33, and BCMA antigens expressing on various B cell lymphomas has reported improved outcomes in the intravenous administration of CAR T cell [150, 151]. In contrast to hematological malignancies, intravenous infusion has demonstrated an inefficient effect on solid tumors. Findings revealed that in solid tumors, intravenous administration has led to CAR T cell accumulation in non-tumoral organs and trafficking disturbance into tumor sites [152, 153]. Undesirably, blood systemic adverse effects have mostly been caused by intravenous infusion of CAR T cells in hematological malignancies or solid tumors.

Encouragingly, successful results of CAR T cell local delivery have been evidenced in solid tumors. For an instance, efficient tumor remission was observed by intracranial infusion of IL13Rα-CAR T cells in glioblastoma [154].

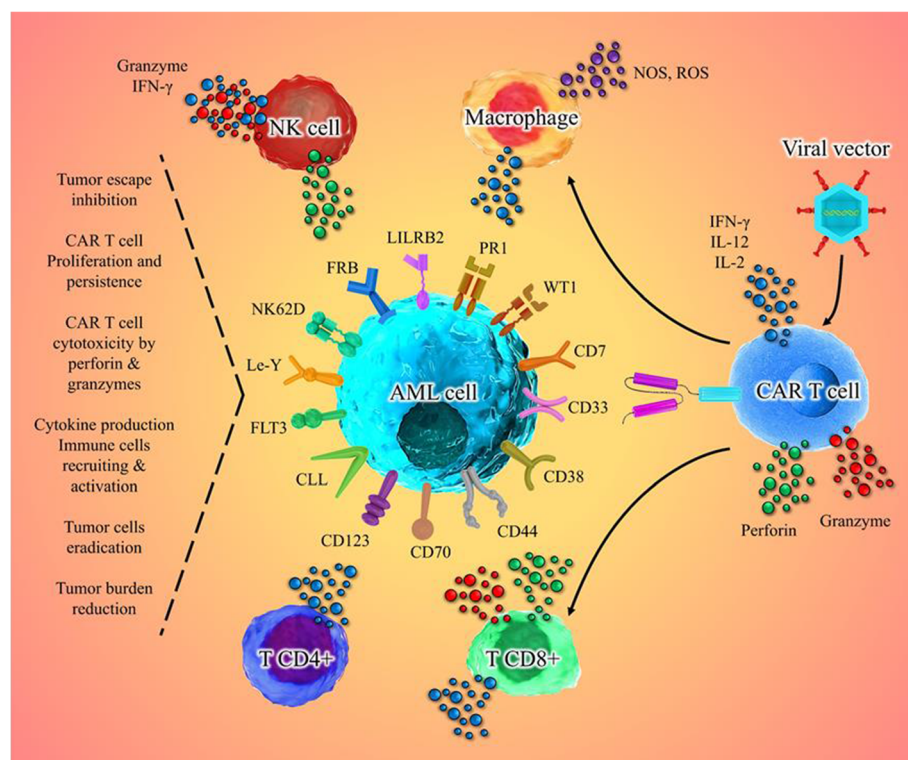


Fig. 3 Implications of the association between the CAR T cell and cancer cells in AML. The production of a CAR T cell in patients with AML leads to its identification and binding to TAA or TSA on the surface of the cancer cell and has a variety of effects, including activation of macrophages to produce ROS and NOS, activation of NK cells to secrete perforin and granzyme, and secretion of cytokines that strengthen the body's immune system, which eventually kills tumor cells

CAR T cell production

Appropriate concentration (10^8 – 10^9 cells) of CAR T cell requires impressive efficacy. However, some challenges led to insufficient CAR T cell production. Ex vivo increasing number of CAR T cells is an important phase of CAR T cell manufacturing process after engineering can be improved the function quality of produced cells. For this reason, non-significant expansion of engineered T cells may lead to the insufficient response of CAR T cells.

To expand the CAR T cells, synthetic APCs and stimulatory cytokines like IL-2, IL-7, and IL-15 along with anti-CD3 and anti-CD28 antibodies can be used as expansion trigger factors [155, 156].

Further, patients' lymphopenia arose from chemotherapy as another major reason for CAR T cell failure production. Importantly, different types of infused T cells impressed the anti-tumor efficacy of CAR T cell therapy. As an instance, central memory T CD8⁺ cells have remarkably indicated the effective cytotoxicity against tumor cells [157–159].

CAR T cell persistence and efficacy

Poor persistence or powerless activity of CAR T cell may be related to different causes such as the weak structure of CARs, absence of co-stimulatory molecules, ex vivo and in vivo insufficient expansion or activation, inappropriate infusion strategy, and patients' lymphopenia. On this ground, some strategies can be used to achieving long persistence and potent anti-tumor activity which are highlighted as two major important features of successful CAR T cell treatment. As described previously, CAR T cells' persistence, proliferation, and efficacy are promoted from the first generation to the third. Using the third generation of CAR has represented the intense anti-tumor response in comparison to first and second generation [73]. Furthermore, incorporating CD28 or 4-1BB stimulatory domains in CAR structure is a prosperous manner of overcoming poor persistence of CAR T cell and augmenting its functionality [160, 161]. Considerably, the safe and impressive response of CAR T cell, induction of T cell expansion, and high production of IFN- γ have been observed through the use of CD27 co-stimulatory factor [162]. TLR2 is another beneficial co-stimulatory molecule that impressed CAR T cell anti-tumor activity by strengthening the T cell proliferation as well as inflammatory cytokine production [163]. Similarly, the CAR T cells possessing MyD88/CD40 domains have illustrated the potential cytotoxicity against tumor cells [164]. Consequently, CAR T cells' survival and efficacy can become better by armoring CAR T cells with various powerful co-stimulatory molecules or modifying optimized CAR T cells.

Cancer relapse after CAR T cell therapy

Tumor antigen escape, antigen losing, and the short survival of CAR T cells are the possible reasons for cancer relapse; however, principal mechanisms related to relapse are unclear. Despite the successful results of CD19-CAR T cell therapy in various hematological malignancies, cancer relapse has been reported in some patients. Based on this, uncontrollable tumor cell growth, CD19 antigen escape by deletion, and CD19 masking by CAR T cell led to the CD19 inaccessibility which is protected from CAR T cell attack [165–167]. To overcome this challenge, rising frequent responses of the immune system, using other immunotherapy strategies, and promoting CAR T cell expansion and activation can be useful.

Autologous and allogeneic CAR T cells

Using the autologous or allogeneic T cells as another principal challenge of CAR T cell therapy has remained unclear. The engineer of autologous CAR T cell is identified as a safe cell therapy with restricted autoimmune responses and GVHD. However, using these types of cells encounters some disadvantages which include high costs and time, elaborate types of equipment, and specialized experts. Disappointingly, the insufficient number of T cells in some patients with lymphopenia and unsuccessful choice for serious disorders are the other challenges of using autologous T cells [168]. Contrarily, allogeneic T cells have more advantages including low-time and low-cost engineering procedures, producing high-quality and high-quantity CAR T cells, and noticeable treatment. Encouragingly, the GVHD effect of using allogeneic T cells can be inhibited through the inactivation of related genes, and also, they can be safely acted after allogeneic HSCT [169].

Tumor immune-escape strategies

Tumor cells have various strategies to escape and protect themselves from the immune system which generates different challenges for therapeutic strategies like CAR T cell therapy. As an example, in AML malignancy, AML blasts evade the immune system by recruiting various strategies to include impediment of NK cell cytotoxicity, reduction of tumor antigen expression, enhancement of activated T cell exhaustion, upregulation of anti-apoptotic proteins, upregulation of T cell's inhibitory ligands like PD-L1, generation of metabolically and immunosuppressive microenvironment, expansion of T-reg cell number, suppression of immunosynapse configuration, and losing the HLA molecules [170–172]. For this reason, various development strategies should be established to overcoming the tumor cells' immune escape, increasing the effectiveness of CAR T cell therapy by engineering smart generation modified T cells, and improving the CAR T cell therapeutic efficacy in combination with other cancer treatment strategies.

Side effects

Cytokine release syndrome (CRS)

Same as other therapeutic approaches, some side effects also have been reported in CAR T cell therapy. One of the major disadvantages of CAR T cells is related to cytokine production phenomena such as macrophage activation syndrome, hemophagocytic lymphohistiocytosis, and cytokine release syndrome (CRS). CRS may occur in patients with great tumor burden and due to the robust activity of CAR T cells or administration of a high dose of CAR T cell [173]. Strong production of cytokines such as IL-6, IFN- γ , IL-1 β , GM-CSF, and TNF- α during the CAR T cell function induces the CRS response followed by fevers, hypoxia, myalgia, hypotension, vascular leakage, and neurological disorders [174].

On-target/off-tumor toxicity

“On-target/off-tumor” toxicity is another fundamental side effect of CAR T cell that entailed normal tissue devastation which is mediated by targeting antigen-expressing both on tumor cells and healthy cells. CD19 [175], mesothelin [176], Her2 [177], and CAIX [178] are examples of target antigens that led to on-target/off-tumor toxicity through the CAR T cell therapy. Normal B cell damage, so-called B cell aplasia, is the most common example of on-target/off-tumor toxicity caused by anti-CD19 CAR T cell [179]. As well, cardiopulmonary injury due to the destruction of cardiac and pulmonary epithelial is another example that is caused by the anti-Her2 CAR T cells [180].

Anaphylaxis

The major population of engineered CAR T cells is composed of the murine ScFv. Based on this, these types of CAR T cells encountering cellular and humoral rejection caused by murine protein immunogenicity which led to the anaphylaxis outbreak. Acute anaphylaxis as an immediate interaction occurs because of the host immune system reaction after recognizing the foreign component has entered [181].

Neurotoxicity

Neurotoxicity is nervous system toxicity accompanied by expressive aphasia, delirium, seizure, and confusion symptoms. The incidence etiology of neurotoxicity is unclear; however, it may happen by inflammation derived from cytokine overproduction which has been mostly reported in patients treated by the CD19-CAR T cell [182].

Insertional oncogenesis

Besides, insertional oncogenesis is another side effect that comes about through the transferring of CAR transgene into T cells by lentiviral or retroviral vectors which can increase the induced malignant transformation risk.

Fortunately, the incidence rate of insertional oncogenesis is less; however, considering accurate and safe measures is required in the CAR T cell engineering procedure [183].

Hematological toxicity

Hematological toxicity as another adverse effect of CAR T cell therapy is caused after allogeneic hematopoietic stem cell transplantation (alloHSCT) and CAR T cell administration. Neutropenia, anemia, and thrombocytopenia are the reported examples of hematopoietic system toxicities after CAR T cell therapy. Normal HSPC destruction by CAR T cell due to the presence of common antigen on malignant cells and HSPCs like CD33 is identified as hematological toxicity in AML patients [184, 185].

CAR T cell therapy challenges regarding their toxicity

CAR T cell toxicity management

To mitigate the toxicity and improve the clinical application and efficacy of CAR T cell therapy, various approaches have been developed. Some strategies are used to control the CAR T cell activity with switch-off mechanisms to avoid the CAR T cell overactivity, and some of them have been developed to decrease mentioned side effects derived from CAR T cells. In addition, using the high-affinity ScFv, humanized or human-derived ScFv rather than murine, utilization of local delivery instead of intravenously, and optimizing the CAR T cell manufacturing and infusion process can be improved the clinical therapeutic applications of CAR T cell [182, 186].

Inhibition of CRS and Neurotoxicity

To inhibit the incidence of CRS, anti-inflammatory corticosteroid drugs like dexamethasone as a first choice and cytokine blockade antibodies can be used. Tocilizumab or sarilumab as an anti-IL-6 receptor antibody can be administered to suppressing the effects of IL-6 production [187, 188]. Moreover, ibrutinib has a role in reducing inflammatory cytokine production like IFN- γ [189]. Anakinra as an IL-1 receptor pharmacological antagonist and lenzilumab as an anti-GM-CSF blockade antibody reduce the CRS and neurotoxicity by neutralizing these cytokines [190, 191].

Inhibition of anaphylaxis

Intending to overcome anaphylaxis toxicity, efforts for using the humanized or human ScFv are undergoing, to improve the survival and function of CAR T cells [192].

Inhibition of hematological toxicity

In hematological malignancies, the incidence of hematological toxicity is possible due to the CAR T cell activity. To overcome this toxicity, common antigens between leukemic cells and HSPCs should be removed from HSPCs by gene-editing

methods like CRISPR/Cas9 system and then used for alloHSCT. As an example, in a CD33⁺ AML patient, alloHSCT with CD33^{negative} HSPCs along with anti-CD33-CAR T cell therapy can eradicate the CD33⁺ blasts without effect on CD33^{negative} HSPCs which results in reducing the hematopoietic toxicity [114, 193]. Collectively, combinational therapy of alloHSCT and CAR T cell therapy as a novel therapeutic strategy would be improved cancer treatment like AML.

Inhibition of “on-target/off-tumor” toxicity and CAR T cell overactivity

Various controllable strategies have been developed to switch off the CAR T cell, optimize the CAR T cell function, and prevent the CAR T cell overactivation which mitigated the CAR T cell-derived side effects like on-target/off-tumor toxicity. Accordingly, development strategies include the mRNA electroporation delivery system, suicide genes, CRISPR/Cas9 gene-editing strategy, and smart or multi-targeted CAR T cells (“Advanced generations of CAR T cell” section).

mRNA electroporation CAR T cell transient delivery systems like mRNA electroporation strategy as well as transposon/transposase are used to limit CAR T cell expression and activation. A biodegradable CAR like RNA-CAR123 is an example of using an mRNA electroporation mechanism that gradually decays the CAR T cell expression and activation in AML cases [193].

Suicide genes Employing the suicide genes allows decaying the CAR T cell activity selectively which may be critical to prevent the toxicity. The herpes simplex virus thymidine kinase was the first validated suicide gene in human trial studies which elicits the CAR T cell destruction by catalyzing the ganciclovir phosphorylation as an acyclic nucleoside analog. However, immunogenicity caused by herpes simplex virus thymidine kinase is identified as a limitation of this strategy [194]. Fas and caspase 9 (ICasp9) termed as death molecules are the dimerize suicide genes used to depleting CAR T cells selectively. A small-dimerizing molecule like AP1903 causes the Fas or caspase 9 dimerization followed by downstream caspase activation and the apoptotic stimulation in modified T cells [195, 196].

Eliminating genes Eliminating genes are known as another mechanism of CAR T cell-selective exhaustion. To induce the CAR T cell death, CD20 or EGFR identified as cell surface antigens can be used in CAR structure. Then, the infusion of CD20 (rituximab) or EGFR (cetuximab) mAbs trigger the CAR T cell death through their interaction with mentioned eliminating genes [197, 198].

CRISPR/Cas9 genome editing system An important gene-modifying strategy with a high potential operation is Clustered Regularly Interspaced Short Palindromic Repeats CRISPR/CRISPR-Associated 9 (Cas9). This strategy is used to developing the next-generation CAR T cells with high efficacy and safety by genome manipulation of antigens, enzymes, checkpoints, and cytokines [61, 64].

Advanced generations of CAR T cell

Nowadays, to improve the CAR T cell efficacy and safety, next-generation CAR T cells have been developed so-called smart, programmable, or multi-targeted CAR T cells. These smart CARs are including Tandem CAR, Dual CAR, Universal CAR, SUPRA CAR, SynNotch CAR, TRUCKs, Split CAR, physiological CAR, and iCAR which have various roles in targeting multiple antigens on tumor cells, controlling CAR T cell function, and switching off CARs [58, 61, 199]. As a suggestion, smart CARs possessing the mentioned capabilities can be employed in AML CAR T cell therapy and decrease the related side effects as described above. Concerning variously identified target antigens on AML blasts, multi-targeted CARs like Tandem, dual, SUPRA, or universal CARs can be used to targeting two or more antigens concurrently which co-expressed on tumor cells without on-target/off-tumor toxicity on normal tissues [200].

In a study by Cartellieri et al. [201], a flexible CAR platform so-called universal CAR T cell was engineered to target CD33 and/or CD123 AML blasts in vitro and in vivo. Universal CAR regulating the CAR T cell function by on/off switching mechanism. As a result, universal CAR T cell lyses the AML blasts and AML cell lines potentially by dual-targeting the CD33 and CD123 antigens. Interestingly, no on-target/off-tumor toxicity and xenogeneic graft-versus-host disease were reported in using the universal CAR. In practice, two or multiple antigens targeted simultaneously can be reduced the risk of tumor escape, on-target/off-tumor toxicity, and increased the specificity of antigen targeting.

Considering the lack of proper specific targets of AML, new potential targets can be discovered to improve the AML treatment. Hence, in a study by Perna et al. [200], the discovery and analysis of AML new target antigens were conducted by combining the proteomic and genomic resulted from data from AML patients and healthy volunteers to finding the ideal targets of AML for CAR T cell therapy. As a consequence, four hopeful and potential target antigens were found to include ADGRE2, CCR1, CD70, and LILRB2 which could be multiple targeted by engineering appropriate smart CARs. More than that, they predicted identified antigen overexpression on the majority of AML blasts along with limited expression on normal cells and

activated T cells, expression in a majority of AML patients, and less off-tumor toxicity on healthy cells. Consequently, recognizing the new target antigens in AML and establishing the powerful smart CAR T cells can hopefully increase the success rate of CAR T cell therapy in AML patients.

Concluding remarks

In the last decades, the importance of cancer treatment has led to developing immunotherapy-based approaches [7]. T cell equipped with CAR named CAR T cell is one of the potential adoptive cell therapy evolved for solid tumors and hematological malignancies and indicated the hopeful remission [11]. Interestingly, the hopeful effectiveness of CAR T cells has been reported from several preclinical and trial studies. Various CAR T cells have been engineered for different targets of malignancies in which the FDA-approved CD19-CAR T cells as a common and effective type emphasizes the utilization of CAR T cell therapy. Lastly, multiple CAR T cells have been designed for diverse extracellular and intracellular antigens of AML with encouraging results such as potent cytotoxicity, high persistence, and proliferation, increased cytokine production, less toxicity on normal cells, and prevention of tumor immune escape. Nonetheless, the same as other cancer treatment approaches, there are some challenges and toxicity related to the CAR T cell which can be overcome by CARs modifying or armoring strategies. In AML, to overcome the tumor immune-escape challenge and improve the CAR T cell functionality, some strategies can be provided such as avoiding the T-reg cell expansion, upregulating the proapoptotic proteins, increasing the tumor antigens expression, and blocking the inhibitory checkpoints. Moreover, designing the CAR T cell through high-quality and accurate procedures would improve the proliferation, persistence, and performance of CAR T cells to overcome the related challenges. Also, to prevent the common side effects of CAR T cell which include CRS, on-target/off-tumor toxicity, anaphylaxis, neurotoxicity, insertional oncogenesis, and hematological toxicity, some efficient strategies need to be discovered. Based on this, CRISPR/Cas9 known as a potent gene-editing strategy was used in alloHSCT and CAR T cell therapy to decrease the hematological and on-target/off-tumor toxicity by knocking out the related genes [193]. Additionally, the switching-off mechanisms like mRNA electroporation, suicide, or eliminating genes as a potent controllable mechanism of CAR T cell overactivity and programmable or multi-targeted CAR T cells as powerful armoring CAR T cells have been developed to enhance the safety, efficacy, and cytotoxic specificity [58, 61].

In conclusion, since there is no approved CAR T cell therapy approach for AML, discovering the predisposed and potential AML target antigens and performing the various strategies to improving the CAR T cells' safety and efficacy can lead to a breakthrough in AML CAR T cell therapy.

Abbreviations

CAR: Chimeric antigen receptor; GvHD: Graft-versus-host disease; CAR-NK: CAR-transduced NK cells; NK cells: Natural killer cells; ICIs: Immune checkpoint inhibitors; ACTs: Adaptive cell therapies; NHL: Non-Hodgkin lymphomas; DLBCL: Diffused large B cell lymphoma; FL: Follicular lymphoma; ALL: Lymphoblastic leukemia; MM: Multiple myeloma; AML: Acute myeloid leukemia; CRS: Cytokine release syndrome; CTLs: Cytotoxic T lymphocytes; NCRs: Cytotoxicity receptors; SHP-1: SH-2 containing protein tyrosine phosphatase; ITIMs: Immunoreceptor tyrosine-based inhibitory motifs; hESCs: Human embryonic stem cells; SCGM: Stem Cell Growth Medium; bNHL: B cell non-Hodgkin's lymphoma; RCC: Renal cell carcinoma; CAIX: Carbonic anhydrase IX; EpCAM: Epithelial cell adhesion molecule; ICAS9: Inducible caspase 9

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