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CAR T-cell therapy: Blessing of 21st century

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ABSTRACT

More than twenty years of research on cellular immunotherapy has recently resulted in the development of genetically-modified T cell products that express synthetic chimeric antigen receptor (CAR) with specificity toward the cell-surface tumor antigens. Recent studies have demonstrated promising response rates after infusion of these cells in patients with B-cell precursor and mature B-cell neoplasms, including acute lymphoblastic leukemia, diffuse large B-cell lymphoma, and plasma cell myeloma. Given the satisfactory evidence of their outstanding therapeutic benefit, CD19-targeted CAR T cells have become the first genetic engineering element approved by the United States Food and Drug Administration to treat patients for whom other promising options are unavailable. While clinicians are widely using CAR T-cell therapies, the two most common toxicities are cytokine-release syndrome and CAR T cell-related neurotoxicity/encephalopathy syndrome. Moreover, some studies have explained that the relapse after receiving CAR T-cell therapy is caused by acquired resistance due to genetic mutation or splicing variants, leading to the loss or diminished surface expression of the target molecule in neoplastic cells. To overcome these caveats and achieve therapeutic success, restless efforts are ongoing toward the development of next-generation CAR T cells with more sophisticated design.

Key words: *Chimeric Antigen Receptor T cells*, *Diffuse Large B-cell Lymphoma*, *Cytokine-Release Syndrome*, *Acquired Resistance*

INTRODUCTION

For years, the main modalities for treating cancer have been surgery, chemotherapy, and radiation therapy. As a result, the progression of cancer in some cases has been effectively prevented but that is not enough. Currently, targeted therapy, which includes the use of immune checkpoint modulators, has been rapidly established as critical to eliminate cancer cells. For more than two decades, advanced research on cellular immunotherapy has resulted in the development of cancer-specific adoptive T-cell therapy. Chimeric antigen receptor (CAR) T cells are genetically modified to express synthetic receptors that redirect T cell specifically toward the antigen that is abundantly expressed over the surface of the tumor cells. These genetically-modified cells selectively fight against cancer cells that express specific antigens (Figure 1)[1]. In Japan, CAR T-cell therapy is currently available for B-cell precursor acute lymphoblastic leukemia and aggressive B-cell lymphomas, particularly for diffuse large B-cell lymphoma (DLBCL)[2]. DLBCL, the most common subtype of malignant lymphoma, accounts for 36% of all malignant lymphoma occurring in Japan[3]. Approximately 30–40% of DLBCL patients exhibit relapse or fail salvage chemotherapy[4]. Some patients can be treated with more intensive chemotherapy with autologous or allogeneic hematopoietic stem-cell transplantation (HSCT). However, a considerable portion of patients are ineligible for such therapies. Therefore, before CAR-T therapy, there was no promising and effective therapy for these patients.

In this review, we discuss the CAR structure, different generations of CAR T cells, several clinical trials of CAR T cells for DLBCL and multiple myeloma, and the efforts to overcome the limitations of CAR T-cell therapy. We then summarize the toxicities associated with this therapeutic modality, with an emphasis on cytokine-release syndrome (CRS) and CAR T cell-related neurotoxicity (NT)/encephalopathy syndrome, which are life-threatening. These toxicities are unique and atypical compared to those associated with traditional chemotherapy and small- molecule inhibitors. Therefore, clinicians and healthcare workers involved in CAR T-cell therapy must understand the characteristics and adequate management of CAR T cell-associated toxicities.

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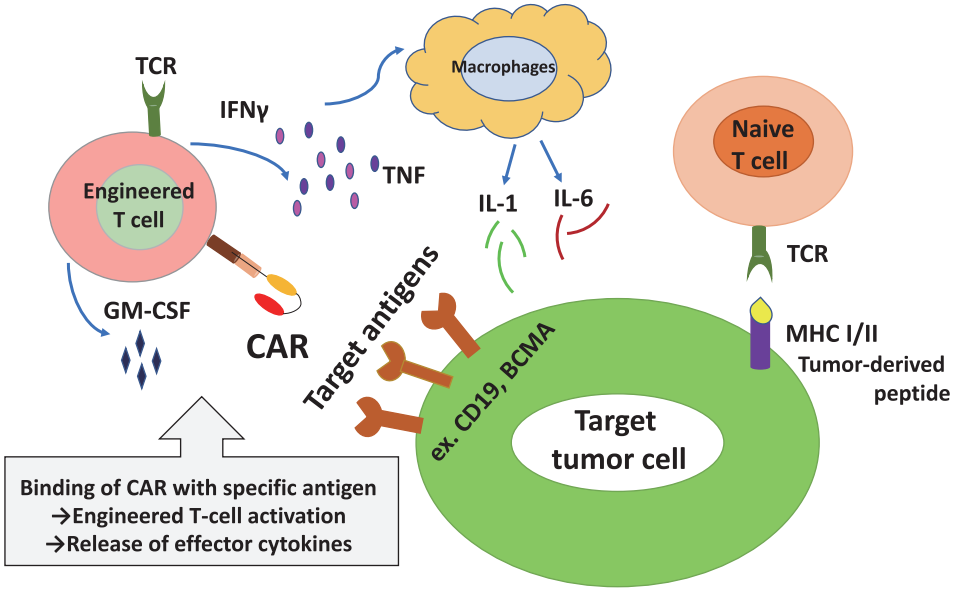

Figure 1 Mechanism of action of CAR T cells. Physiologically, tumor-derived peptides presented on the major histocompatibility complex (MHC) I/II molecules are recognized by the T-cell receptor (TCR) complex on naïve or memory T cells, which react with those antigen, and help antigen-presenting cells to engulf malignant cells. The engineering of the CAR T cells allows to bypass this process because the expression of CAR endow T cells with an ability to directly recognize and destroy tumor cells. The co-stimulatory domain of CAR stimulates the engineered T cell and thus these cells release inflammatory cytokines like granulocyte-macrophage colony stimulation factor (GM-CSF), tumor necrosis factor (TNF) and interferon-γ (IFN-γ) upon subsequent activation. These cytokines activate tissue-resident macrophages to proliferate and to secrete interleuken-1 (IL-1) and interleuken-6 (IL-6), which sometimes predispose patients to the development of CRS and neurotoxicity along with lysis of tumor cells. After binding of CAR with specific antigen on tumor cells, co-stimulation and CD3ζ signaling lead to engineered T cell activation and release of several cytokines.

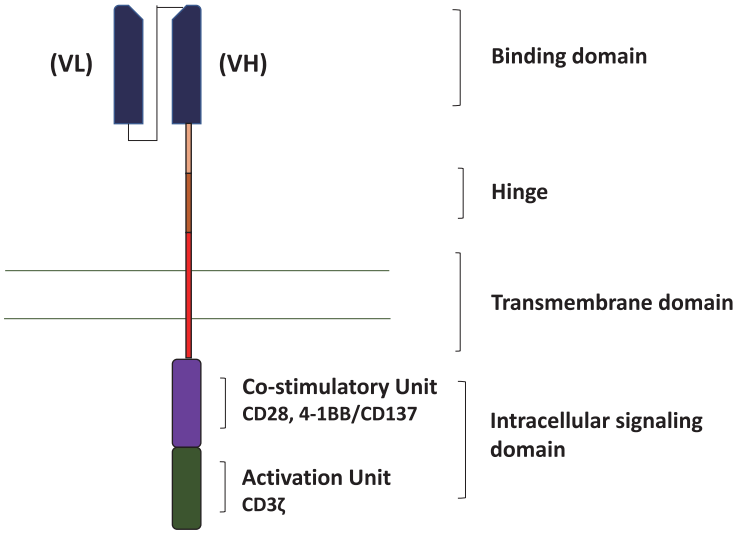

Figure 2 The structure of CAR. The CAR has four important elements: an antigen-binding domain, a hinge, a transmembrane domain, and an intracellular signaling domain. VH = immunoglobulin heavy chain variable region; VL = immunoglobulin light chain variable region.

Components of CAR T cell

In general, the CARs structure has four important elements: an antigen-binding domain, a hinge, a trans-membrane domain, and an intracellular signaling domain (Figure 2). By editing the CARs design, researchers are trying to achieve the best efficacy.

Antigen recognition and binding domains

The primary function of CARs is to identify the target antigen expressed on the tumor cells and guide CAR-expressing lymphocytes to the specific antigen. An antigen-binding domain is derived from variable heavy (VH) and variable light (VL) chains of the monoclonal antibodies joined together by a flexible linker leading to the generation of a single-chain variable fragment (scFv)[5]. Researchers are currently trying to employ a specific ligand, instead of scFv. For example, juvenile myelomonocytic leukemia is characterized by exclusive genetical abnormalities in the granulocyte-macrophage colony-stimulating factor receptor (GMR,

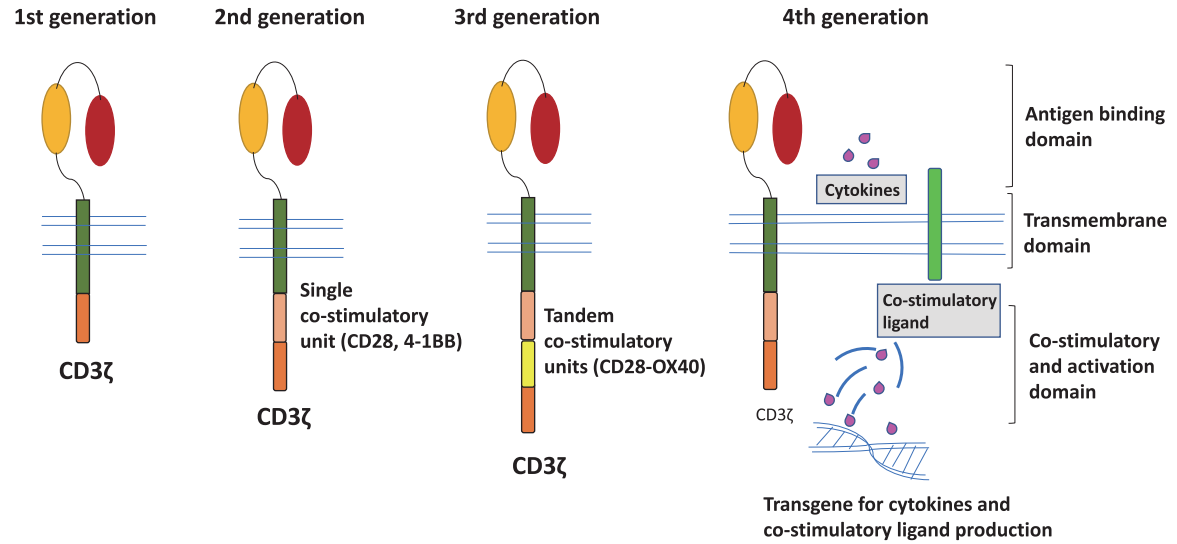

Figure 3 Different generation of CARs. The first-generation CAR only had CD3ζ signaling domain, whereas the second-generation CAR contains a co-stimulatory domain derived from CD28 or 4-1BB in addition to CD3ζ signaling domain. The third-generation CAR designing is composed of tandem two co-stimulatory domains, where first one is either CD28 or 4-1BB domain and second one is CD28, 4-1BB or OX40 domain. The fourth-generation CAR comprises of some additional genes for the production of extra-cytokines or some of the CARs contain additional structure known as co-stimulatory ligand.

CD116). Nakazawa et al. constructed GMR-targeted CAR T cells by replacing the anti-CD19 scFv portion with a full-length granulocyte-macrophage colony-stimulating factor using a piggyBac transposon plasmid encoding CD19 CAR[6]. This resulted in inducing cytotoxicity on the CD34+ fraction of juvenile myelomonocytic leukemia cells, but not on normal hematopoietic stem cells. Many other groups have reported scFv alternatives such as nanobodies, cytokines, and peptides.

Hinge and transmembrane domains

Between the extracellular antigen-binding domain and intracellular signaling domain, there is a connector known as hinge, which is derived from CD8, IgG1, and IgG4. Several studies have elucidated that hinge design is critical for successful binding to the target antigen[7]. CAR rooted in T cells through the transmembrane domain is composed of CD28, CD3ζ, CD8α or CD4 , with each component manifesting characteristics individually or in combination.

Intracellular signaling domain

The intracellular signaling domain usually consists of two parts; i) an activation domain and ii) a co-stimulatory domain. The activation domain is responsible for T cell activation for cytolytic activity, and extensive research has proven CD3ζ to be a good candidate for providing the immunoreceptor tyrosine-based activation motif in CARs. However, different studies suggest that CD3ζ alone is not sufficient to generate persistently effective T cell response. Therefore, researchers have been looking for a co-stimulatory domain that can upmodulate function of CAR T cells (Figure 3). Until now, CD28 or 4-1BB (CD137) have been recognized as the most efficient co-stimulatory domain: they have higher response rates that lead to FDA approval for generating CAR T cells[8,9]. CARs that contain the CD28 region are prone to differentiate into effector memory T cells, whereas those containing the 4-1BB region differentiate into central memory T cells[10]. Other reported co-stimulatory domains are OX40 (CD134), inducible T cell co-stimulator (ICOS), CD27, and killer cell immunoglobulin-like receptor 2DS2 (KIRS2DS2), all of which have shown effective results in the preclinical test.

Generation of CAR T cells

First-generation CARs were designed to produce activated T cells that were enabled for specific cytolysis in tumor cells, but were unable to maintain sustained persistence and proliferation. Therefore, scientists redesigned the receptors that triggered both T-cell activation and co-stimulation, resulting in a longer life span and proficient T-cell function[11]. These improved cells are known *as second-generation* CAR T cells. The second-generation CARs yielded satisfactory expansion, and the presence of the 4-1BB increased the lifespan along with better therapeutic benefit. Hence, investigators prefer utilizing second-generation CAR T cells in clinical trials to achieve the desired outcome.

The need for better CAR T cell performance motivated scientists to construct *third-generation* CARs. They designed a CAR by engineering two co-stimulatory domains within their cytoplasmic tail. A tandem combination of CD28 and 4-1BB domains or CD28 and OX40 domains is common among the lists. Compared to their second-generation counterparts, third-generation CARs showed better or similar activity *in vitro* and *in vivo*.

Fourth-generation CAR T cells were engineered to express cytokines or co-stimulatory ligands, in addition to harnessing the benefit of second or third-generation CAR T cells. The purpose was to increase the lifespan and improve the expansion efficiency of the generated T cells. However, it also led to the development of toxicity

due to the uncontrolled expansion of CAR T cells. To minimize sever toxicities, a new approach of incorporating "suicidal gene" was implemented[12].

Toxicities associated with the CAR T-cell therapy

The two types of adverse effects reported by clinical trial investigators while using CD19 CAR T-cell therapy are cytokine-release syndrome (CRS) and CAR T cell-related neurotoxicity (NT) or encephalopathy syndrome[13].

Cytokine release syndrome (CRS)

T cells are activated via their T cell receptor (TCR) interaction with the tumor cells, and the interaction leads to the secretion of inflammatory cytokines like interleukin (IL)-1, IL-2, IL-6, IL-10, IL-15, interferon-γ, and tumor necrosis factor as reported by several groups[13,14]. Owing to the releasing of inflammatory cytokines, patients develop some unique symptoms, such as fever, fatigue, hypotension and hypoxia, chills, tachycardia, hypogammaglobulinemia, and prolonged cytopenia. Severe cases of multi-organ failure and death have also been reported. Identifying symptoms early is considered the best treatment approach for CRS. Simultaneously, an appropriate grading system will help effectively manage patients. Therefore, a grading system was developed by the CAR T-cell therapy-associated TOXicity (CARTOX) working group[15]. Features such as hemodynamic stability, degree of hypoxemia, organ failure, and other comorbidities are considered most important for the grading. Special attention should be given to the patients in grades 3 and 4, along with grade 2 suffering from other comorbidities. Patients in extreme situations require special measures, such as vasopressors, management in the ICU, cytokine antagonist therapy, and steroids. Since IL-6 is crucial in the pathogenesis of CRS, the IL-6 receptor blocker tocilizumab and anti-IL-6 antibody siltuximab have been proven to be the best treatment regimens to control the adverse events effect[16]. Previous reports suggested that elevated C-reactive protein levels in the sera of the patients are considered an alternative marker for the onset of CRS; thus supportive management is suggested[17].

CAR T cell-related neurotoxicity

After the infusion of CAR T cells, the symptoms of NT sometimes overlap with CRS[15]. Earliest identified symptoms of NT are confusion, inappropriate language, reduced writing capability, headache, and other symptoms such as aphasia, disorientation, motor weakness, incontinence, increased intracranial pressure, and papilloedema. Similar to CRS, early identification and adequate grading are strongly recommended to improve patient management. CAR T cell-related adverse effects usually resolve within a few weeks but can be fatal in the most severe cases requiring intensive care management. As the symptoms and signs of each type of toxicity may overlap, it is important for clinicians to recognize the potential complications as the toxicity can be life-threatening and can lead to death under certain circumstances. Researchers have also reported that NK-92 CAR-infused patients experience lower incidences of CRS and NT. While the development of grade 3 or 4 CRS or NT after CAR T-cell infusion is common, a clinical trial by Tang et al. demonstrated relatively lower CRS and NT development with CAR NK cell-therapy for acute myeloid leukemia[18]. After the injection of 1×10^9 irradiated CAR NK-92 cells targeting CD33 in two patients, grade 1 CRS was observed, which restored to normal the following day. Another patient received 5×10^9 cells suffered from a high fever that subsided within two days (NCT02944162). Although adverse effects seemed manageable, the tumors relapsed later.

Clinical trials of CAR T-cell therapy for B-cell lymphoma

In 2015, the first CAR T cell study organized by the National Cancer Institute (NCI) reported its efficacy and safety in nine patients suffering from refractory $CD19^+$ B-cell lymphomas[19]. The results of four patients with complete response (CR) and two patients with partial response (PR) among seven evaluated patients after CAR T cell infusion encouraged the researchers to investigate the therapy further. In addition, the robust achievement of single-center studies have encouraged the investigators to conduct multicenter trials in various academic settings.

Axicabtagene ciloleucel (Axi-cel, Yescarta)

In the SCHOLAR-1 retrospective non-Hodgkin lymphoma study, investigators defined the best response to B-cell lymphoma as the progression of disease after the first line of treatment or second-line of chemotherapy, or if the disease relapsed in less than 1 year after prior high-dose chemotherapy with autologous HSCT. This study revealed 26% of an objective response rate (ORR), 7% of CR rate, and 6.3 months of median overall survival[20]. To evaluate the CAR T cells response over the conventional therapy, ZUMA-1 was the first multicenter study to justify the use of autologous anti-CD19 CAR T cells for the treatment of relapsed or refractory large B-cell lymphoma[21,22]. Axicabtagene ciloleucel (YESCARTA: Kite Pharma, Inc) is a second-generation CAR T cell containing two co-stimulatory domains synthesized from human CD3ζ and CD28 for CAR T-cell activation against the CD19 antigen, which is expressed on the surface of the tumor.

For the production of axicabtagene ciloleucel, peripheral blood mononuclear cells are collected from the patients by a process called apheresis. Mononuclear cells are activated with anti-CD3 antibody and recombinant human IL-2. T cells are then transduced in a replication-incompetent retroviral vector expressing the anti-CD19 CAR transgene. The transduced T cells are expanded by cell culture and cryopreserved for delivery to the patients. The upper threshold for infusion is 2×10^8 CAR T cells. Before infusion, lymphodepleting chemotherapy, such as cyclophosphamide 1,500 mg/m^2 and fludarabine 90 mg/m^2, are prescribed and used in fractionated doses on the 5th, 4th, and 3rd day before infusion. It was

reported that when the patient was infused with axicabtagene ciloluce l following chemotherapy, CAR T cells expanded and reached the maximum number within 2 weeks after infusion. The decline in number of cells was observed for following 3 months.

In phase I of ZUMA-1, a total of seven patients were evaluated after a 1-month trial. In four patients, the ORR was 71% and CR was 57%, and 12 months after axicabtagene ciloleucel infusion, three patients exhibited CR. Severe toxicities including NT, which were resolved by supportive treatment, and a fatal case of CRS were also reported in this study[21]. In phase II of ZUMA-1, of the 111 patients selected for leukapheresis, 101 patients received axicabtagene ciloleucel and were selected for intention-to-treat analysis[22]. Surprisingly, in this trial, CAR T cell manufacturing success rate was 99%. Because of the disease progression or severe adverse reactions, nine patients were unfit to receive an axicabtagene ciloleucel infusion. The median time starting from apheresis to final axicabtagene ciloleucel delivery was 17 days. The investigator reported an ORR of 83%, CR of 58%, and PR of 25%. At a median follow-up of 27.1 months, ongoing response and CR were observed in 39% and 37% of the patients, respectively. The median duration of response for all patients was 11.1 months, except in the patients who achieved CR, and 24 months survival proportion was 51%. Considering the adverse effects of CRS, tocilizumab was used both as a single dose and as multi-dose, and 25% of the patients suffered from CRS for more than 14 day. Headache, tremors, encephalopathy, aphasia, delirium, and dizziness were the common neurological symptoms, and supportive care was the treatment of the choice.

Therefore, ZUMA-1 phase II outcomes are exciting and encouraging compared to the SCHOLAR-1 study. Based on this evidence, Axi-cell was approved by the FDA on October 17, 2017 to treat patients with relapsed and refractory large B cell lymphoma[9].

Tisagenlecleucel (CTL019, Kymirah)

Tisagenlecleucel or CTL019 is another form of second-generation anti-CD19 CAR T cells in which the scientist of the University of Pennsylvania used the 4-1BB co-stimulatory domain. Based on the prior experience of efficacy in patients with refractory leukemia[23]. A total of 38 patients with CD19+ DLBCL or FL were enrolled in a single-center study[24]. The eligibility criteria for DLBCL were disease relapse after autologous HSCT, whereas eligibility criteria for FL was disease progression within < 2 years after receiving immunochemotherapy as second-line treatment. Finally, 28 patients received treatment, and 10 patients could not receive therapy due to rapid disease progression. The primary endpoints were the ORR and CR rates. Among all patients, 64% showed a response, with 43% showing CR in DLBCL and 71% in FL. The median follow-up was 28.6 months, and 57% of all the patients remained progression-free. The median response duration was not reached during the follow-up. The median progression-free survival (PFS) did not reache the cut-off date for data collection.

The success of this single-center phase II trial encouraged a pivotal phase II study to observe the safety and efficacy of tisagenlecleucel in adult patients with relapsed or refractory DLBCL. An international phase II trial named JULIET was conducted in 10 countries, and centrally manufactured cryopreserved materials were supplied globally[24,25]. Non-eligible criteria included age less than 18 years, presence of primary mediastinal B cell lymphoma, involvement of the central nervous system (CNS), and a prior history of anti-CD-19 therapy. In this trial, 51% of patients had refractory disease with a minimum of three lines of anticancer therapy, and 49% had prior received autologous HSCT. The median age of the trial participants was 56 years. A total of 167 patients were enrolled in the JULIET trial, and 115 patients received tisagenlecleucel infusion (four patients did not receive infusion by data cut-off). In this study, bridging chemotherapy was recommended, and 92% of patients received it. The median time from infusion to data cut-off was 19.3 months. Of the 99 evaluable patients (≥ 3 months of follow-up), the best ORR was 54% with CR and PR of 40% and 13%, respectively. The 12- and 18-months OS rates of all patients were 48% and 43%, respectively. The median response duration in responders was not reached. The median OS of all patients was 11.1 months. The 12- and 18-month relapse-free rates were 64%. Outpatient infusion of CTL019 was feasible and was administered to 26 patients, and 20 (77%) remained as outpatients for more than three days. CTL019/Kymirah was the first CAR T cell approved by the FDA on August 30, 2017[8].

Lisocabtagene maraleucel (Liso-cel, Breyanzi, JCAR017)

Lisocabtagene is a second-generation 4-1BB co-stimulatory domain-containing CAR T cells that use a 1:1 ratio of CD4 and CD8 T cells[26]. The manufacturing process involves a separate collection of CD4+ and CD8+ T cells by leukapheresis. Individual cell activation, transduction, and expansion are done. The outcome of the procedure, as mentioned earlier, is the production of higher number of memory T cells compared to more differentiated T-cell component and hardly detectable CD19+ cells.

The TRANSCEND 001 is a multicenter, multi-cohort study that has an extra relaxation for patient selection rather than selecting patients suffering only from relapsed or refractory large B-cell lymphomas[27]. B-cell lymphomas patients with diverse histological conditions were enrolled, such as patients with decreased cardiac function or low renal function and who already had a secondary malignant site in the CNS. Owing to the disease control process, bridging therapy was permitted when lisocabtagene maraleucel preparation was ongoing, and fludarabine and cyclophosphamide are used as the conditioning regimens. In this study, 97% of the patients received at least two lines of systemic therapy, and 67% had a chemotherapy-refractory disease. As the study intended to observe the treatment effect in various types of patients, 3% of the patients enrolled had a secondary

Table 1 Some features of multicenter studies with CD19 CAR T-cell therapy

Study Variable	ZUMA-1 (Locke et al.)	JULIET (Schuster et al.)	TRANSCEND (Abramson et al.)
scFv	FMC63	FMC63	FM63
Co-stimulatory domain	CD28	4-1BB (CD 137)	4-1BB (CD137)
Vector delivery	Retrovirus	Lentivirus	Lentivirus
Defined cell	No	No	Yes, CD4:CD8 fixed ratio
Number of patients enrolled/infused	111/101	167/111	344/294
Median follow up	27.1 months	19.3months	18.8 months
CAR T dose	2.0×10^6 cells/kg	Median, 3.1×10^8cells	DL1 5.0×10^7 cells DL2 1.0×10^8 cell
Lymphodepleting regimen	Flu 30 mg/m^2 × 3 days Cy 500 mg/m^2 × 3 days	Flu 25 mg/m^2 × 3 days Cy 250 mg/m^2 × 3 days or B 90 mg/m^2 × 2 days	Flu 30 mg/m^2 × 3 days Cy 300 mg/m^2 × 3 days
Complete response	58%	40%	53%
ORR	83%	52%	73%
CRS	93%	58%	42%
NT	64%	21%	30%

DL, dose level; Flu, fludarabine; Cy, cyclophosphamide; B, bendamustine; ORR, overall response rate; CRS, cytokine-release syndrome; NT, neurotoxicity

CNS involvement. According to the data cut-off for this analysis (Aug 12, 2019), 344 patients, with a median follow-up of 18.8 months, were selected for the leukapheresis process. Among 344 patients, 269 patients received at least one dose of lisocabtagene maraleucel and were considered eligible for the efficacy analysis. The ORR was 73% with CR of 53%. The PFS and overall survival at one year were 44% and 58%, respectively. Lisocabtagene maraleucel was detected in the patient blood one year after the infusion. Similar to other CD19 CAR T cells, lisocabtagene maraleucel infusion resulted in the development of CRS and NT. A total of 42% of the patients experienced adverse CRS effects, and grade 3 or worse situations were reported in 2% of the cases. Similarly, NT developed in 30% and grade 3 or worse events were observed in 10% of the cases. Other adverse effects included neutropenia, anemia, thrombocytopenia, fatigue, nausea, and headaches. The characteristics of the three CAR T-cell products for DLBCL are reviewed in Table 1.

Clinical trials of CAR T-cell therapy for plasma cell myeloma

As CD-19 CAR T cells reflected evidence of tremendous achievement in the treatment of B-cell lymphoma, experts in plasma cell neoplasms or multiple myeloma turned their attention towards CAR T-cells. B-cell maturation antigen (BCMA), a member of tumor necrosis factor receptor superfamily 17 (TNFRSF17)[28], has been identified as one of the best candidates for designing CAR specifically targeting relapsed or refractory multiple myeloma (RRMM). BCMA is ideal because it is abundantly expressed on malignant plasma cells and mature B cells, but not in the other tissue of the body, except that a very low level of expression is observed in hematopoietic stem cells.

The production of BCMA-targeted CAR T cells bb2121 or idecabtagene vicleucel was supported by Bluebird Bio and Celgene. The eligibility criteria for the phase 1 clinical trial (NCT02658929) were > 30% presence of plasma cells in the bone marrow along with a history of at least three previous lines of therapies[29]. In the phase 1 trial, leukapheresis was conducted in 36 enrolled patient; however, three patients were excluded from the trial due to disease progression. All patients were observed until disease progression. According to the investigator, the ORR was 85%, and CR or stringent CR was reported in 45% of the patients. The median PFS was 11.8 months while the median duration of response was 10.9 months. In terms of safety evaluation, approximately 97% of the patients showed signs of ≥ grade 3 adverse events. At least 76% of the patients experienced CRS, and 6% had grade 3 CRS. Neurotoxicity was reported in 42% of patients, where one patient developed grade 4 toxicity at 11 days post-infusion. Neutropenia, leukopenia, anemia, and thrombocytopenia were the common hematological toxic effects.

The above results motivated the investigators to proceed with the phase 2 KarMMa trial (NCT 03361748). After enrolling 140 patients, 128 patients were finally subjected to BCMA CAR T-cell therapy[30]. The medium follow-up period was 13.3 months, where a response was observed in 73% of patients and a CR or better response was observed in 33% of the

patients. A minimal residual disease-negative condition was defined as having less than 10^{-5} nucleated cells: 26% of all patients revealed this condition, and 79% of the patients achieved CR or better response. One noteworthy outcome was median PFS, which was 8.8 months; 12.1 months when the dose was 450×10^6 cells, and 20.2 months for the patient who had a complete or stringent response. Regarding safety, 99% of the patients had grade 3 or 4 adverse effects. Unusual symptoms such as hypogammaglobulinemia and infections developed after eight weeks of infusion. Hematological events like neutropenia (91%), anemia (70%), and thrombocytopenia (63%) topped the list. A total of 84% of the patients had the CRS, and only 5% showed grade 3 or more. Eighteen percent of the patients exhibited neurological effects, and only 3% of patients had symptoms of grade 3 or higher. All these findings of the phase 2 KarMMa trial led to the FDA approval (March 2021) of idecabtagene vicleucel (ide-cell) for the treatment of adult patients with RRMM who had already experienced at least three prior lines of therapies.

Challenges for the CAR T-cell therapy

Combatting CAR T-cell resistance or exhaustion to tumor

Although the CAR T cells are an innovative discovery for relapsed and refractory B cell neoplasms, many groups have reported a relapse or disease persistence after CAR T-cell therapy. Analysis of the mechanism indicated anticipated escape of the antigen by a mutation in the CD19 gene or the development of its splice variant[31–33]. Other mechanisms can be explained by switching to a genetically related but phenotypically alteration (lineage switch) and downregulation of target surface antigen to levels required for CAR T-cell activation[34]. A strategy targeting several antigens has been adopted during the CAR design to overcome the tumor escape phenomenon. In B-cell malignancies, other molecules, such as CD20 and CD22, are also co-expressed along with CD19. A study reported the development of bispecific CD19/CD20 CARs and preclinical results showed significant action on B-cell malignancy and CD19-positive tumor cells[35]. Before designing a bispecific CAR, it is essential to confirm that the two or multiple antigens are co-expressed on the tumor but not over the normal tissues. Dai et al. used autologous CD19/CD22 CAR T cell-therapy in six adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia[36]. Conditioning chemotherapy was administered before bispecific CAR T-cell infusion, and minimal residual disease (MRD) negativity was reported in all six patients. The longest MRD-negativity was achieved in one patient for up to 11 months. Grade 1 or 2 CRS, but no NT occurred. However, 5 months later, in one patient out of six patients, the reemergence of blast cells that did not express CD19 and exhibited the diminished binding to CD22 was observed.

Programmed cell death ligand (PD-L1) expression is another probable mechanism of tumor resistance. PD-L1 expression has been approved as a biomarker for non-small-cell lung carcinoma by the FDA. Therefore, programmed death 1 (PD-1) inhibition has received attention as an attractive approach among other multiple strategies to counter resistance mechanisms. In 2017, pembrolizumab (anti-PD-L1) therapy was approved by the FDA for several groups of the tumor. Cherkassky et al. used a preclinical mouse model of pleural mesothelioma to determine if PD-1 acted as a component of mesothelin-targeted CAR T-cell exhaustion and how CAR T cells counteracted those inhibition mechanisms[37]. They reported that blockage of the PD-1/PD-L1 pathway using PD-1 antibody, cell-intrinsic PD-1 shRNA, or a PD-1 dominant negative receptor could help regain the effector activity of CD-28 CAR T cells.

Furthermore, several researchers developed PD-1 knockout T cells, and these genetically engineered T cells are tested in clinical trials. Recently, in a phase I first-in-human clinical trial, investigators wanted to determine the safety and feasibility of knocking out three genes in one setting[38]. They used TCR instead of CAR, TRAC (TCR α), TRBC1 (TCR β), and PDCD1 (the gene encoding PD-1), which were knocked out using CRISPR-Cas9 gene editing technology. The purpose of knocking out endogenous TCR is to reduce the CRS-based complication and to identify CRISPR Cas9 technology-related adverse effects. The primary endpoint of the trial was to determine the safety and feasibility of CRISPR-Cas9 gene-edited autologous T-cell infusion. A successful engineered cell production process was conducted in four patients; one patient could not proceed further due to rapid disease progression and another patient was not eligible for further analysis. Lymphodepletion chemotherapy was administered as per the protocol, and 1×10^8 cells /kg body weight were infused in three patients. Two patients had refractory multiple myeloma, and one patient had liposarcoma. According to the clinical observations, CRS was not observed in any patient after the cell infusion. Two patients exhibited a stable disease, and the other patient showed a 50% reduction in abdominal liposarcoma even though other lesions showed signs of progression. Tumor and bone marrow biopsies demonstrated the presence of CRISPR-Cas9-engineered T cells in all three patients; the existence of residual tumor was also noted. The investigators were satisfied with the engraftment of infused T cells, which persisted from 3 to 9 months after infusion.

Barriers to targeting solid tumors

In the case of solid tumors, the application of CAR T-cell therapy is unsatisfactory because the antigens are not distributed homogeneously over the tumor. Other factors that reduce the efficacy of CAR T cells against solid tumors include difficulty in trafficking, extravasation due to excessive vasculature of the tumor, immunosuppressive microenvironment, and excess deposition of the extracellular matrix over tumor-like proteoglycans, fibrous proteins, collagen, etc. Solid tumors are unable to process and present the antigen. To address this phenomenon, Louis and colleagues assessed the effect of CAR T cells on solid tumor-like relapse/refrac-

tory neuroblastoma[39]. They developed first-generation chimeric antigen receptor T cells called EBV-specific cytotoxic T lymphocytes (EBV-CTLs) and activated T cells (ATCs). In a phase I trial, 19 patients were infused with autologous CAR CTLs and CAR-ATCs. After six weeks of infusion, 8 patients showed no disease, 2 showed CR, 4 showed progressive diseases, 2 showed stable diseases, 2 showed tumor necrosis, and 1 showed PR. After four years, the outcomes in 4 patients showed no evidence of disease; 3 were alive with disease; 2 remained in CRs; 10 died of the disease. As first-generation CARs were well tolerated and two patients showed CR. Andras et al. further developed third-generation CAR T cells named GD2-CAR3, where the group targeted GD2 antigen (disialoganglioside) on neuroblastoma[40]. GD2-CAR3 incorporated with CD28 and OX40 co-stimulatory domains to improve cell persistence with or without conditioning and inhibition of PD-L1. Their study comprises three cohorts. Cohort 1 patients received CAR T cells alone, cohort 2 patients received cyclophosphamide and fludarabine conditioning therapy before CAR T-cell infusion. Finally, cohort 3 received fludarabine-cyclophosphamide with a double dose of PD-1 antibody to combat the immunosuppressive tumor microenvironment. The 11 patients who participated in the study were assigned to cohorts 1 and 2 (four patients each) and cohort 3 (three patients). After 6 weeks of infusion, out of 11, 6 patients had PD, and 5 had SD. The median survival time for all 11 patients was 506 days. In cohort 1, diseases in all four patients progressed and they died within 32–506 days. Of the seven patients who received conditioning therapy in cohorts 2 and 3, six survived for 265 to more than 724 days. In cohort 3, out of three patients, two patients achieved CR. Considering CAR T cell-related toxicities, only one patient developed CRS that was spontaneously resolved without IL-6 treatment. No CNS-related toxicities were reported.

CONCLUSION

As immune-checkpoint blockers have opened a new therapeutic window for previously-refractory malignancies, we are now in an era of immunotherapies where treatment strategies are rapidly evolving. The unprecedented success of CD19- or BCMA-directed CAR T-cell therapy has motivated researchers globally to participate in gene-modified cellular therapy. However, challenges remain in overcoming CAR T-cell resistance, particularly among solid tumors. Further identification of the underlying mechanisms is essential for resolving this issue. In this context, introducing genome-editing technology into clinical cellular manufacturing could be a promising direction for improving the safety and efficacy of therapeutic T cells by overcoming these barriers. Several groups have successfully used healthy-donor-derived allogeneic “off-the-shelf CAR-T” in which genome editors inactivate endogenous TCR genes to prevent allogeneic TCR-mediated graft-versus-host disease[41,42], although more intensive efforts are required to resolve safety concern regarding genotoxicity. These technologies will accelerate the development of other cancer-reactive immune cell lines, such as TCR-transduced T cells (TCR-T) and CAR-NK. However, currently, it is vital to recognize that the impact on the health economy is an emerging concern. The exorbitant medical costs amounting to more than 30 million JPY per patient in Japan, required for CAR T therapy, may hinder its dissemination into real-world clinical practice. Therefore, future technological innovations are eagerly awaited to supply high-quality immune cells with lower manufacturing costs.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR’S CONTRIBUTIONS

SC and TI designed the study and SC drafted the original manuscript; NF and TI revised the original draft; TI edited the final manuscript for submission.

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