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REVIEW

CAR T-cell immunotherapy: a powerful weapon for fighting hematological B-cell malignancies

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Abstract **The current standard of care in hematological malignancies has brought considerable clinical benefits to patients. However, important bottlenecks still limit optimal achievements following a current medical practice. The genetic complexity of the diseases and the heterogeneity of tumor clones cause difficulty in ensuring long-term efficacy of conventional treatments for most hematological disorders. Consequently, new treatment strategies are necessary to improve clinical outcomes. Chimeric antigen receptor T-cell (CAR T) immunotherapy opens a new path for targeted therapy of hematological malignancies. In this review, through a representative case study, we summarize the current experience of CAR T-cell therapy, the management of common side effects, the causative mechanisms of therapy resistance, and new strategies to improve the efficacy of CAR T-cell therapy.**

Keywords CAR T cells; hematological malignancies; review

Case

A 57-year-old Chinese man presented with worsening pain in the bones of his chest and waist. A chest computerized tomography (CT) scan revealed severe damage on the bones widely involving the ribs, scapulae, clavicles, sternum, and thoracic vertebra. In addition, a pathological fracture on the T7 lumbar vertebra was observed by magnetic resonance imaging. Subsequent tests showed an increase in the level of monoclonal immunoglobulin IgD and λ in serum and a 34% increase in the number of plasmablasts in the bone marrow (BM). He was diagnosed with multiple myeloma (MM) and stratified to be ISS III stage. This patient was initially administrated with a standard induction therapy, which included bortezomib, doxorubicin, and dexamethasone (PAD). Four cycles of this regimen did not bring the disease to full control because not only M protein and BM plasma cells reduced moderately, but also his forehead presented a growing plasmacytoma as confirmed by pathological examination. Thus, a second scheme, in which bortezomib was combined with lenalidomide, cyclophosphamide, and dexamethasone (VRCD), was applied. The patient obtained partial remission with four cycles of VRCD, which was continued for an additional 7 cycles, followed by a 6-month oral administration of lenalidomide. One year after his best response to VRCD, the disease progressed. He tried several salvage treatments, including VRCD with the addition of etoposide, melphalan plus lenalidomide, and dexamethasone, but the effects were limited (BM plasma accounted for 22% and M spike level was 11 g/L). Meanwhile, his forehead plasmacytoma was even bigger. In 2016, several US Food and Drug Administration (FDA) approved drugs such as daratumumab, pomalidomide, and ixazomib were considered, yet they had not been in the market of the Chinese mainland.

Preface

Over the past decades, the overall survival of patients with hematological malignancies has been greatly improved. The advances in clinical outcome are largely contributed

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by a rapid development of novel therapeutic modalities, such as oncoprotein-targeting drugs, immune modulating agents, and epigenetic adjusting medicine. However, we are still distant from achieving a cure because a number of patients still face relapsed/refractory (r/r) dilemma. The above-mentioned case typically represents a current bottleneck in treating highly aggressive blood disease, which fails to respond to or is highly compromised with standard of care. Therefore, developing a precise and curative medical approach is necessary.

With the comprehensive understanding in tumorigenesis and the evolution in biological technologies, immunotherapy rapidly grows to be a cancer-killing power, which might get over the hurdle of therapy resistance. This type of approach makes use of innate functionality of immune cells to eliminate cancer cells by targeting tumor antigen on malignant populations or inhibiting immune checkpoint on immune cells [1]. The pioneer product, monoclonal antibody, has successfully prolonged the life span of non-Hodgkin lymphoma (NHL) and MM; thus, it was included in the clinical practice guidelines [2]. With this advancement, chimeric antigen receptor T-cell (CAR T) therapy, a new concept to train patient self-immune cells to be a "living drug" by incorporating the variable domain of a monoclonal antibody onto T lymphocyte [3], is emerging to draw a revolutionized landscape of cancer immunotherapy.

Clinical practice of anti-CD19 or anti-BCMA CAR T cells

The initial success of this gene-modified therapeutic approach mostly comes from CD19, BCMA targeting CAR-transduced T cells.

By using CD19 CAR T cells, a number of patients with r/r B cell lineage malignancies, such as chronic lymphoid leukemia (CLL), acute lymphoblastic leukemia (ALL), and NHL, acquired a second chance of lives, and some obtained a durable remission without detectable tumor cells [4–15]. These clinical trial data are summarized in Tables 1 and 2. Given the outstanding efficacious and tolerated features, four anti-CD19 CAR T-cell products, including tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, and lisocabtagene maraleucel, have received FDA designations for the treatment of r/r B-cell precursors, namely, ALL and/or B-cell NHL. Tisagenlecleucel, a currently unique product for the treatment of pediatric ALL, can achieve 80% complete remission at its early response [5]. In addition, tisagenlecleucel and axicabtagene ciloleucel showed 40%–50% durable response in the long-term follow-up studies of r/r NHL patients [12,13]. The other two authorized agents have a distinct manufacturing from the routine approach used in the two above-mentioned products. Lisocabtagene maraleucel, a second approved CAR T-cell product developed by Kite Pharma, shares the same construct as axicabtagene ciloleucel, but during T cell processing, the circulating tumor cells are removed, which decreases the risk of activation and exhaustion of CAR T cells before infusion. This improvement is necessary for leukemia and mantle cell lymphoma [15]. Moreover, brexucabtagene autoleucel developed by Juno Therapeutics adopts an innovative manufacturing process, which yields a final CAR T-cell product with a defined ratio of $CD4^+$ and $CD8^+$ T cells [14].

In the BCMA CAR T-cell setting, many manufacturers are involved in the innovation of an appropriate product for treating MM, a commonly recognized incurable hematological disease that universally expresses BCMA antigen on its plasmablasts. Remarkable achievements of anti-BCMA CAR T-cell therapy have brought a curable hope to MM [16–26]. The clinical trial results are collectively shown in Table 3. Two leading products, idecabtagene vicleucel (Bluebird Bio/Celgene) and ciltacabtagene autoleucel (Nanjing Legend/Janssen), have accomplished multi-institutional trials with a remarkable overall response of 73%–95% [22,24]. Recently, idecabtagene vicleucel was authorized by the US FDA for the treatment of adult patients with r/r MM after four or more prior lines of therapy, being the first product to be on the market in the class of CAR-redirected cellular therapy against BCMA.

Studies of CAR T cells against other targets

The CAR T cells targeting to other novel antigens are explored to evaluate their efficacy and safety in lymphoid malignancies. Anti-CD22 CAR T cells were highly efficacious in anti-CD19 CAR T-cell-resistant B-ALL and large B cell lymphoma patients, particularly in those with CD19 antigen reduced or lost on tumor cells [27,28]. CD20- and CD30-targeting CAR T agents have good tolerance and substantial anti-lymphoma activity [29,30]. κ.CAR T cells, which were investigated in patients with r/r κ^+ NHL/CLL, also showed some tumor killing capacity [31]. In MM, CAR T cells directed against CD44v6, APRIL, CD56, CD229, CD70, and GPRC5D are evaluated in preclinical studies, whereas clinical trials on CAR Ts targeting CD38, light chain, SLAMF7 (also known as CS1), CD138, and integrin 7 are underway. Remarkable results have been obtained with anti-GPRC5D, -SLAMF7, and -integrin 7 CAR T cells [32–34].

Tumor antigens targeted by the above-mentioned conventional CAR T-cell therapy are "cell-surface" antigens. However, many potential tumor-associated antigens (TAA), such as tissue differentiation antigens, cancer-germline antigens, and neoantigens, are located in the intracellular compartment [35]. Recently, the development of T cell receptor (TCR)-like CAR T cells, which bear a

Table 1 Major clinical trials of CAR T-cell therapy for relapsed/refractory B-ALL and CLL

Study	Disease	Number of cases infused	Dose	Lymphodepletion	MRD-CR for ALL or CR for CLL	Relapse rate	$CD19^-$ relapse rate	Proceeding to allo-HSCT (*n*)	Median follow-up (month)	High-grade/ severe CRS	High-grade/ severe neurotoxicity	Reference and NCT number
University of Pennsylvania and Children's Hospital of Philadelphia	B-ALL	30	$(0.76–20.6)\times10^6$ cells/kg	Flu/Cy Cy/VP CVAD-B CVAD-A Cy Etoposide/ cytarabine Clofarabine	79% (22/28)	26% (7/27)	43% (3/7)	3	7	27% (8/30)	NR	[4] NCT01626495 NCT01029366
ELIANA trial	B-ALL	75	$(0.2–5.4)\times10^6$ cells/kg	Flu/Cy Etoposide/ cytarabine	81% (61/75)	36% (22/61)	68% (15/22)	8	13.1	47% (35/75)	13% (10/75)	[5] NCT02435849
Memorial Sloan Kettering Cancer Center	B-ALL	53	$(1–3)\times10^6$ cells/kg	Flu/Cy Cy	67% (32/48)	61% (25/41)	16% (4/25)	17	29	26% (14/53)	42% (22/53)	[6] NCT01044069
National Cancer Institute	B-ALL	51	DL1: 1×10^5/kg DL2: 3×10^6/kg	Low dose Flu/Cy FLAG Ifosfamide/ etoposide High dose Flu/Cy	55% (28/51)	29% (8/28)	63% (5/8)	21	18.7	14% (7/51)	6% (3/51)	[7] NCT01593696
Fred Hutchinson Cancer Research Center	B-ALL	53	DL1: 0.2×10^6/kg DL2: 2×10^6/kg	Cy/etoposide Cy Cy/Flu	85% (45/53)	49% (22/45)	27% (6/22)	18	30.9	19% (10/53)	23% (12/53)	[8] NCT01865617
Seattle Children's Hospital	B-ALL	43	DL1: 0.5×10^5/kg DL2: 1×10^6/kg DL3: 5×10^5/kg DL4: 10×10^6/kg	Flu/Cy Cy Cy/ etoposide	93% (40/43)	45% (18/40)	39% (7/18)	11	9.6	23% (10/43)	21% (9/43)	[9] NCT02028455
University of Pennsylvania	CLL	14	$(0.14–11)\times10^8$ cells	Bendamustine Pentostatin/Cy Flu/Cy	29% (4/14)	0%	0%	0	19	43% (6/14)	7% (1/14)	[10] NCT01029366
Fred Hutchinson Cancer Research Center	CLL	24	DL1: 2×10^5/kg DL2: 2×10^6/kg DL3: 2×10^7/kg	Flu/Cy Cy Flu	17% (4/24)	NR	NR	NR	NR	8% (2/24)	25% (6/24)	[11] NCT01865617

ALL, acute lymphoblastic leukemia; CLL, chronic lymphoid leukemia; DL, dose level; Flu, fludarabine; Cy, cyclophosphamide; allo-HSCT, allogeneic hematopoietic stem cell transplantation; NR, not reported.

Table 2 Outstanding clinical trials of anti-CD19 CAR T-cell therapy for B cell NHL

	ZUMA-1	JULIET	TRANSCEND NHL001	ZUMA-2
Product	Axicabtagene ciloleucel (Yescarta) (KTE-C19)	Tisagenlecleucel (CTL019)	Lisocabtagene maraleucel (JCAR017)	Brexucabtagene autoleucel (KTE-X19)
Costimulatory	CD28	4-1BB	4-1BB	CD28
NCT number	NCT02348216	NCT02445248	NCT02631044	NCT02601313
Reference	[12]	[13]	[14]	[15]
Number of cases infused	108	111	269	74
Disease	Cohort 1: DLBCL Cohort 2: PMBCL, tFL	DLBCL, tFL	DLBCL NOS, HGBCL, tFL, transformed iNHL, PMBCL	MCL
Lymphodepletion	Flu/Cy	Flu/Cy Bendamustine	Flu/Cy	Flu/Cy
Dose infused	2×10^6 cells/kg	$(0.1–6)\times10^8$ cells	DL1: 0.5×10^8 cells DL2: 1×10^8 cells DL3: 1.5×10^8 cells	2×10^6 cells/kg
CD4:CD8	Not specified	Not specified	1:1	Not specified
Number of complete response (evaluable cases)	59 (101)	37 (93)	136 (256)	40 (60)
Median follow-up (month)	27.1	14	12	12.3
High-grade/severe CRS	11%	22%	2%	15%
High-grade/severe neurotoxicity	32%	12%	10%	31%

NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; tFL, transformed follicular lymphoma; NOS, not otherwise specified; HGBCL, high-grade B-cell lymphoma; iNHL, indolent NHL; MCL, mantle cell lymphoma; DL, dose level; Flu, fludarabine; Cy, cyclophosphamide.

TCR-like antibody moiety, aims to target antigenic peptides restricted by the major histocompatibility complex (pMHC) on tumor cells [35,36]. This therapeutic approach faces two major challenges that limit its clinical application. First, target cells often express a low density of pMHC, thereby resulting in low TCR-like-CAR–pMHC interaction. The second issue is the requirement for fine tuning the affinity of TCR-like CAR T cells to avoid overstimulation [35]. Studies have been designed to optimize TCR-like affinity for a better antigenic specificity [36].

Case (continued)

This patient was enrolled in Legend-2 phase I trial (NCT03090659) in April 2017. The screening examinations showed a high BCMA frequency of 93.2% on BM plasmablasts by flow cytometry, a high-risk cytogenetics of del(17p) by FISH testing, and extramedullary involvement on the forehead by whole-body PET-CT scanning. Autologous leukocyte apheresis was performed for the manufacture of CAR T cells. After 1 month, three splitting infusions of ciltacabtagene autoleucel (a total of 1.05×10^6/kg body weight) were administrated on day 0, 2, and 6, following to a three consecutive lymphodepletion therapy of fludarabine and cyclophosphamide from day −5 to day −3. On day 7, patient's temperature started to rise, reaching the highest of 40.9 °C on day 10. The oxygen saturation was 97%, but the systolic blood pressure was only 84 mmHg. In addition, laboratory tests indicated a grade 3 hepatic impairment with an increase of transaminitis and grade 3 leukopenia. Moreover, circulating CAR T cells demonstrated robust expansion with a dramatic increase in the level of IL-6 and IL-2R in serum. His acute side reaction was categorized as grade 3 cytokine release syndrome (CRS). He received tocilizumab and other essential supporting care and recovered 2 days later, in which the symptoms were relieved, and the positive parameters gradually turned normal. One month after the onset of CAR T-cell infusion, flow-based BM minimal residual disease (MRD) and serum/urine M-protein immunofixation were negative. The forehead plasmacytoma shrank significantly. PET-CT showed no soft tissue tumor involvement around day 120. In the following 8 months, this case maintained a stringent complete response (CR) status. Circulating CAR T cells persisted for at least 9 months. Meanwhile, his serum polyclonal immunoglobulins persisted in low levels. This long-term adverse event caused him to be vulnerable to infectious diseases. Thus, he was intravenously supplemented with gammaglobulin once a month.

Table 3 Clinical trials of anti-BCMA CAR T-cell therapy for MM

Manufacturer	NCI	UPenn	MSKCC	MSKCC	Legend Biotech	Legend Biotech	Legend Biotech	Poseida Therapeutics	Bluebird Bio	IASO Biotherapeutics	CARsgen Therapeutics
Study site, reference (NCT number)	NCI [16] (NCT02215967)	UPenn [17] (NCT02546167)	MSKCC [18] (NCT03070327)	MSKCC [19] (NCT03430011)	XJTU [20] (NCT03090659)	Multi-sites in China [21] (NCT03090659, ChiCTRONH-17012285)	Multi-sites in the US [22] (NCT03548207)	Multi-sites in the US [23] (NCT03288493)	Multi-sites in the US [24] (NCT03361748)	HZUST [25] (ChiCTR-1800018137)	Multi-sites in China [26] (NCT03716856, NCT03302403, NCT03380039)
Ag-binding domain (species)	scFv (murine)	scFv (human)	scFv (human)	scFv (human)	Bispecific variable fragments of heavy-chain Ab (lama)	Bispecific variable fragments of heavy-chain Ab (lama)	Bispecific variable fragments of heavy-chain Ab (lama)	Centyrin^{TM} (human)	scFv (murine)	scFv (human)	scFv (human)
Suicide gene	None	None	EGFRt	None	None	None	None	Peptide activated by Rimiducid	None	None	None
Lymphodepletion	Flu/Cy	None or Cy	Cy or Flu/Cy	Flu/Cy	Cy	Cy or Flu/Cy	Flu/Cy	Flu/Cy	Flu/Cy	Flu/Cy	Flu/Cy
BCMA expression required	Yes	No	Yes	No	Yes	Yes	No	No	No	Yes	Yes
No. of evaluable patients	16	25	11	44	57	17	97	34	128	18	24
No. of prior therapies	10	7	6	6	3	5	6	7	6	4	5
High-risk cytogenetics	40%	96%	82%	NA	NA	38%	NA	NA	35%	39%	NA
CAR T dose	9×10^6/kg	$(10–500)\times10^6$	$(72–818)\times10^6$	$(300–600)\times10^6$	$(0.07–2.1)\times10^6$/kg	$(0.21–1.52)\times10^6$/kg	$(0.5–1.0)\times10^6$/kg	$(0.75–15)\times10^6$/kg	$(150–450)\times10^6$	$(1.0–6.0)\times10^6$/kg	$(50–180)\times10^6$
ORR	81%	48%	64%	91%	88%	88%	95%	57%	73%	100%	88%
⩾CR	13%	8%	NA	39%	68%	76%	56%	NA	33%	72%	79%

MM, multiple myeloma; Flu, fludarabine; Cy, cyclophosphamide; scFv, single-chain variable fragments; NCI, National Cancer Institute; UPenn, University of Pennsylvania; MSKCC, Memorial Sloan Kettering Cancer Center; XJTU, Second Affiliated Hospital of Xi'an Jiao Tong University; HZUST, Huazhong University of Science and Technology; ORR, overall response rate; CR, complete response; NA, not available.

Conditioning regimen

Similar to other trials, this case received a combination preconditioning therapy before CAR T-cell infusion. Lymphodepletion therapy prior to adoptive T-cell transfer can promote the reaction against tumors because it can enhance antitumor activity through removing endogenous cellular elements and setting free cytokines to augment T cell function [37]. The available data from many clinical trials further demonstrated the importance of lymphocyte depletion before the infusion of CAR T cells and showed that lymphocyte depletion enhanced *in vivo* activity and persistence of CAR T cells. At present, fludarabine plus cyclophosphamide is a commonly used regimen, but compared with the therapeutic efficacy of the group having cyclophosphamide alone, the addition of fludarabine or the absence of conditioning application did not show a significant difference [17,21]. This finding indicated that fludarabine, even the lymphodepletion regimen, might not be absolutely required for CAR T-cell clinical activity.

Cytokine release syndrome

In clinical trials, the occurrence of an immune activation resulting in broad clinical manifestations associated with elevated inflammatory cytokines, known as CRS, is the most prevalent adverse event following CAR T-cell infusion [38]. Tumor burden and pre-existing infection before CAR T-cell therapy are key factors associated with increased risk of CRS [39]. After CAR T-cell infusion, other important factors, such as a peak of CAR T-cell expansion, cytokine levels, and endothelial activation, contribute to the occurrence and severity of CRS [39,40]. The assessment and grading of this toxicity vary considerably across clinical trials and institutions. The American Society for Transplantation and Cellular Therapy recently proposed a consensus grading for CRS and neurotoxicity [41]. Clinical symptoms of CRS vary from mild signs, such as fever to life-threatening systemic manifestations, for example, multiorgan dysfunction.

In the management of CRS, cytokine blockade by antibody is the key measure to effectively reverse the acute systemic inflammatory reaction. Tocilizumab and siltuximab, targeting to IL-6 receptor and IL-6, are recommended by NCCN for CRS treatment [42]. In patients who are resistant to anti-IL-6 agent, etanercept, a tumor necrosis factor-α inhibitor, has been clinically proven to be highly effective without interfering with CAR T-cell response [43]. Recent experiments have revealed that monoclonal antibody targeting other cytokines, for example, anakinra for IL-1 [44] and lenzilumab for GM-CSF [45], could be efficient in preventing CAR T-related CRS in murine models. Apart from cytokine inhibitors, corticosteroids are also recommended for severe CRS.

Immune effector cell-associated neurotoxicity syndrome

Clinical experience with anti-CD19 CAR T cells shows that neurotoxicity (formerly known as cytokine-related encephalopathy and currently designated as immune effector cell-associated neurotoxicity syndrome (ICANS)) represents another major adverse effect of CAR T-cell therapy. High mortality caused by neurotoxicity resulted in a halt of the phase II ROCKET trial and a discontinuation of JCAR015 by the FDA. The frequency of ICANS varied across studies, ranging from 13% [5] to 78% [46] in BCP-ALL, from 21% [13] to 67% [12] in NHL, and approximately 35% in CLL [10,11]. In anti-BCMA CAR T-cell therapies in MM, the frequency of neurotoxicity was significantly different between ciltacabtagene autoleucel (2%) [20] and idecabtagene vicleucel (18%) [24]. The causative pathophysiology of ICANS was unclear, but this syndrome might be related to endothelial injuries induced by inflammatory cytokines resulting from CNS invasion by CAR T cells [47]. CD19-expressing human brain mural cells, which help maintain blood–brain barrier integrity, were recently considered as a key contributor to neurotoxicity of CAR T-cell therapy [48]. This finding provided insights into the high incidence of CNS invasion in B-cell ALL patients. The design, manufacturing [49], and dose [50] of infused CAR T cells might also be related to ICANS.

Therefore, recipients of CAR T-cell therapy with a central nervous system (CNS) disease or a history of seizures should receive antiseizure prophylaxis with levetiracetam. Treatment of ICANS is basically supportive. Anti-IL-6 therapy is administered if ICANS occurs concomitantly with CRS. Patients with $\geqslant$ grade 2 ICANS, not associated with CRS, or who are refractory to prior tocilizumab, require corticosteroids [41]. According to evidence-based guidelines [41,47] and our clinical experience, immediate administration of small and repeated doses of methylprednisolone (0.5 mg/kg) or dexamethasone (5 mg) intravenously every 8 or 12 h is beneficial. At present, the treatment of corticosteroid-refractory cases has no therapeutic consensus. Treatment with siltuximab and anakinra might be effective because of their direct effects on circulating cytokines.

Other adverse events related to CAR T-cell therapy

Hemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory syndrome characterized by massive immune cell activation leading to severe multi-organ injury, severe infection associated with agranulocytosis, and hemorrhage caused by thrombocytopenia (less than 20 000/μL) [51]. An early detection is crucial for effective treatment of HLH. Tocilizumab and corticosteroids should be promptly administered, but if this treatment fails in 48–

72 h, then immunosuppressive therapy, in accordance with the HLH-2004 protocol, should be initiated [47].

In rare cases of MM with high tumor burden or extramedullary plasmacytoma, effective CAR T-cell treatment could cause tumor lysis syndrome (TLS). The clinical presentation of TLS ranges from typical signs of acute renal injury to atypical tumor bleeding caused by rupture of vessels within the tumor or a respiratory distress syndrome, which results from tumor lysis in the chest cavity [21]. Multi-disciplinary emergency care is needed for patients at high risk for TLS.

In addition, late adverse events have been identified, which must be given attention. Common late adverse events are prolonged cytopenia and iatrogenic immunoglobulin deficiency, which make patients prone to repeated serious infections, particularly pulmonary infections. Therefore, supportive care with granulocyte colony-stimulating factor (for patients with neutrophils less than 800/μL) and regular immunoglobulin infusion are important in the first 6 months after CAR T-cell treatment [21]. Other long-term adverse events, such as secondary malignancies, immune-related phenomenon, and graft-versus-host disease (GVHD) in patients with previous allogeneic hematopoietic stem cell transplantation (HSCT), have also been described [52]. Physicians experienced in treating hematologic malignancies can usually handle these long-term complications. However, late-onset neurologic/psychiatric disorders are difficult to identify and address [53]. A systematic follow-up is needed for evidence-based therapy.

The advances in our understanding and the treatment of CAR T-cell-related adverse events, particularly CRS and ICANS, have led to improved outcomes. However, in some cases, the health deterioration caused by these adverse events can be fast. Fatal CRS and neurotoxicities have occurred during almost all stages in CAR T-cell therapy. Further understanding of the events at the molecular, cellular, and organism levels, careful monitoring of patients and early intervention are essential to minimize these risks.

Case (continued)

In June 2018, this patient proceeded with his 1-year follow-up examination. He did not present any symptoms at that time. However, immunofixation detected monoclonal immunoglobulin IgD and λ in serum, which was the same as it was before. M spike was 1.7 g/L. Flow-based BM MRD turned positive, showing 0.6% plasmablasts with a group of original immunophenotypic markers ($CD38^+CD138^+CD56^-CD19^-$). Whole-body PET-CT found a localized soft tissue plasmacytoma on the left chest wall, and no other extramedullary lesions were observed. Anti-CAR T-cell antibody was negative in serum. BCMA density on the tumor cells was 84.6%. Therefore, this case was diagnosed as BCMA-positive relapse. In addition, he received another anti-MM CAR T-cell agent as salvage in August 2018. However, highly severe and toxic effects occurred on the 16th day after drug infusion. The patient eventually died of pulmonary hemorrhage and respiratory failure.

Mechanisms of CAR T-cell resistance

CAR T cells have demonstrated their therapeutic benefits in patients with high-risk hematological malignancies. However, therapy resistance is rising as a major obstacle. Based on our current knowledge, three main causative mechanisms of resistance have been identified: (1) impaired T-cell function, (2) tumor microenvironment "barrier," and (3) tumor antigen modulation (Fig. 1).

Impaired T cell function

A number of studies have identified critical quality attributes in apheresed T cells, which impact response to CAR T-cell therapy in hematologic malignancies [54–57]. Autologous T cells in these patients display a wide variability in anti-tumor potency, which not only poses a challenge in the treatment of various indications, but also offers an opportunity to extract critical parameters separating effective from ineffective products, even at the pre-manufacturing stage. Uncovering the uncertain activity of T cells possessed in each case must ensure a good-quality product. Analysis among patients with B-cell leukemia demonstrated that T cells, which were more enriched in early memory phenotype, tended to be more proliferative and cytotoxic [54–58]. In particular, a high proportion of $CD8^+$ early memory T lymphocyte population in aphereses of CLL cases predicted a durable remission [55]. Notably, $CD8^+$ T cells from CLL patients were considered as a functional defect attributed to T cell exhaustion compared with healthy donors [59]. Altered cytokine production and high expression of inhibitory receptors largely contributed to the reconstruction of T cells in CLL patients [59–61]. In addition, CLL cells downregulated CD40 ligand on $CD4^+$ T cells. CD40 ligand was considered as an enhancer for tumor-targeting capability of T cells [62].

Apart from innate function, antigen dose, and homing, the foreign species of origin of the antigen recognition domain in the CAR construct provides an extrinsic way of inhibiting CAR T cells. The humoral or cellular immunogenicity was reported in a number of clinical trials using CAR T cells with either murine-derived scFv fragment [51,63–65] or alpaca-derived receptor [20,21]. Anti-CAR antibodies or cellular-type response induced by host

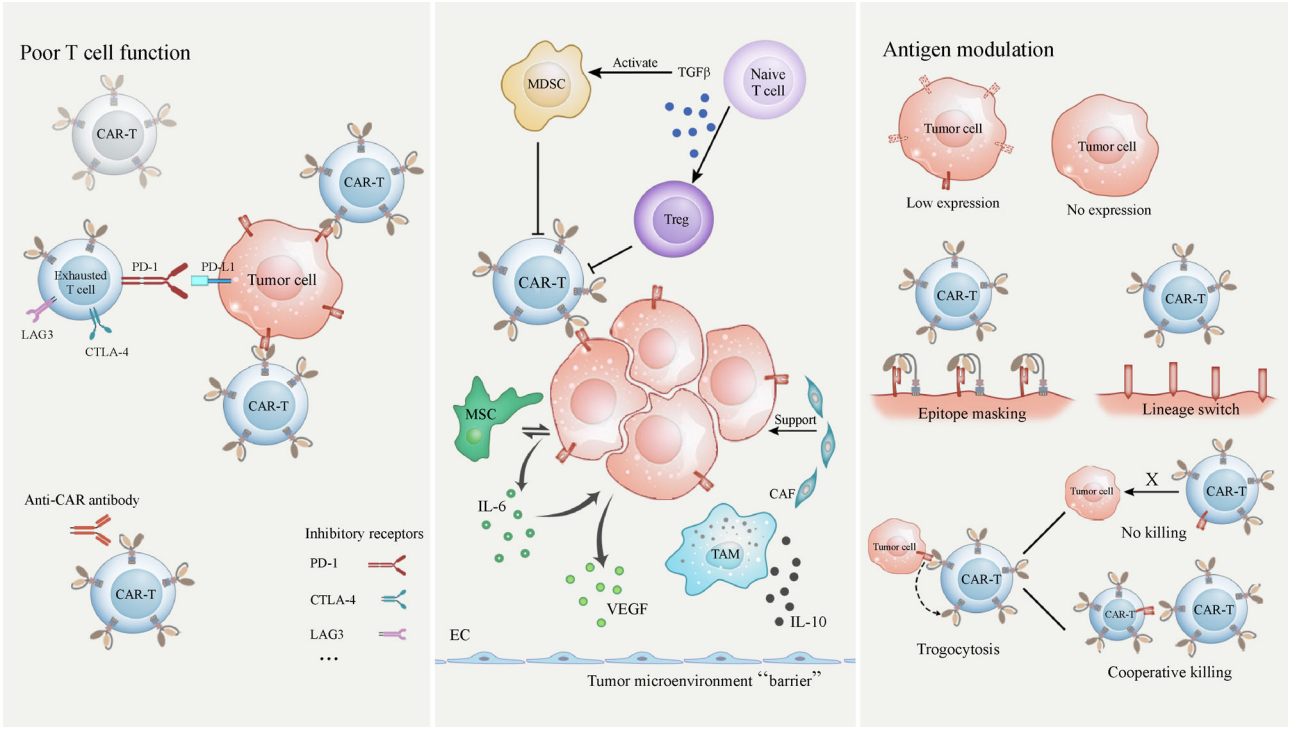

Fig. 1 Graphic illustrations depict the underlying mechanisms of CAR T-cell therapy resistance. From the immune effector perspective, T-cell quality is a key determinant of CAR T-cell cytotoxicity. A number of cases failed in receiving infusion or favorable outcome because of impaired T lymphocyte, which is attributable to T-cell exhaustion or CAR immunogenicity. In tumor setting, the interplay of cellular and non-cellular substances in tumor microenvironment is considered as a barrier in solid tumor CAR T-cell treatment. The variability of tumor antigen expression in cancer cell equally causes difficulties in CAR T-cell recognition and targeting.

immune rejection may limit efficacy of CAR T-cell response, leading to relapse after initial infusion and resistance to reinfusion [21,65]. Fully human CAR is developed to overcome the issues of transgene immunogenicity, showing a positive trend of CAR T-cell function enhancement [25,66,67] and safety improvement [68]. Methods to remove ADA-secreting cells in CAR T-cell treatment are also being considered [21].

Tumor microenvironment "barrier"

Multiple cellular components, including tumor cells, mesenchymal stroma cells (MSCs), endothelial cells (ECs), regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), cooperate and function collectively in the manner of cell-to-cell binding or growth factor/cytokine secretion, thereby constituting a hostile immunosuppressive circumstance for T cells. For instance, the interplay between MM cells and marrow MSCs triggers secretion of IL-6 [69], which, in turn, enhances vascular endothelial growth factor (VEGF) production by MM cells [70,71]. VEGF secretion increases BM angiogenesis via activating ECs and maintaining its survival [72]. This finding also holds true for lymphoma. High VEGF expression was measured in lymphoma and related to a worse prognosis [73]. In addition, VEGF blockade by the tyrosine kinase inhibitor (sunitinib) upregulated chemokines, which was followed by an increased T-cell infiltration [74]. Anti-angiogenic therapy targeting VEGF could normalize tumor vasculature and could be used to modulate CAR T cells to tumor infiltration [75].

Apart from VEGF, inhibitory substances such as the TGFβ, IL-10, and PD-1/PD-L1 axis are produced, and they contribute in this milieu by lowering cytotoxic activity of CAR T cells [76]. TGFβ plays a crucial role in promoting differentiation of naive T cells into Tregs and activating MDSCs. In addition, Tregs and MDSCs suppress the effector T cells with more TGFβ and IL-10 [77,78]. IL-10 promotes differentiation of TAMs, which attracts Tregs via producing CCL22, and further expresses PD-L1 to inhibit activated PD-1$^+$ T cells [79]. In the clinical evaluation setting, the increased number of IL-10-inducible CD8$^+$ Tregs was identified in MM, contributing to tumor immune escape [80]. The presence of MDSCs and TAMs in B cell lymphoma lesions is correlated to a poor overall survival [81–83]. Substantial effectiveness of PD-1 blocking antibodies has been observed in Hodgkin's lymphoma [84,85]. However, PD-1 blockade alone or combined with other immunomodulating agents could not achieve

favorable response in MM [86–88], suggesting a complicated immune disturbance in MM microenvironment hardly reversed by a checkpoint inhibitor, although a significant efficacy of inhibiting PD-L1 in murine MM has been demonstrated in preclinical studies [89,90].

Apart from cellular compartment, extracellular compartment accounts for a high proportion leading to a blockade of T-cell trafficking. Genes associated with extracellular matrix remodeling had high expression in patients with diffuse large B cell lymphoma (DLBCL) who achieved cure compared with poor responders. A high number of infiltrating T cells were also evaluated in DLBCL with persistent remission [91]. Other elements such as cancer-associated fibroblasts (CAFs), which can produce cytokines/chemokines and release proinflammatory and proangiogenic factors, create a supportive MM microenvironment [92,93]. Consequently, targeting fibroblast activation protein in tumor stroma with CAR T cells can inhibit tumor growth and augment host immunity [94].

Tumor antigen modulation

Tumor antigen loss or downregulation was considered as the major cause of relapse after CAR T-cell therapy. Antigen modulation can occur in several ways. First, genetic abnormality of target antigen. Mutations of the CD19 gene were identified in a group of post-CAR T-cell relapse of ALL cases, whose CD19 regions were normal before treatment [95]. Those variations all occurred in the CD19 extracellular transmembrane, thereby leading to a loss of function or loss of binding site of CD19 antigen. In addition, the loss of heterozygosity and alternative splicing of CD19 transcript in the relapsed tumor cells were observed [95,96]. Genetic abnormalities were also found in MM after BCMA CAR T-cell therapy as the underlying mechanism of immune escape. Such abnormalities were acquired by a MM patient by deletion of one allele and a mutation that creates an early stop codon on the second allele following his second infusion [97]. Homozygous deletion of BCMA was identified in another case after anti-BCMA CAR T-cell therapy. Both variations led to irreversible BCMA loss and lack of CAR T-cell proliferation [98]. Second, low-antigen density on tumor cells. Experience from CD22 CAR T-cell therapy implicated that diminished CD22 expression hindered the killing of malignant lymphocytes [27]. Based on the mechanism, trogocytosis was evaluated as a process of reducing tumor antigen and promoting CAR T-cell fratricide by transferring antigen from tumor to T cells [99]. Third, tumor antigen masking. Manufacturing could introduce CAR gene into the contaminating tumor cells unintentionally; thus, exogenous CD19 receptor on B leukemic cells bound to and masked the CD19 epitope, resulting in a tumor escape from CAR T-cell surveillance [100]. Fourth, tumor lineage marker switch. Some B-ALL cases acquired myeloid lineage leukemic cells at the time of relapse after CD19 CAR T-cell therapy in the presence of the same genetic clone, suggesting a partial or complete tumor phenotypic switch under CD19 CAR T-cell selective pressure [101,102]. Fifth, antigen expression regulated by γ-secretase. In MM cells, γ-secretase can directly cleave BCMA and release soluble BCMA (sBCMA), resulting in the decreased expression of BCMA on MM cells and high levels of sBCMA in the blood of MM patients particularly those with RR disease. Meanwhile, the soluble BCMA may serve as a decoy neutralizing BCMA CAR and block the interactions between CAR T cells and BCMA on MM cells, leading to impaired function of CAR T cells [103].

Strategies to improve the therapeutic effect of CAR T

The questions that have arisen from the existing results of CAR T cells motivate scientists to pay considerable attention to the study of efficacy improvement. Given the clinical outcomes in hematological lymphoid malignancies, the current solutions to the limitations of CAR T-cell utilization are obtained from two key aspects: (1) T lymphocyte-directed manufacturing and (2) tumor setting-directed modulation.

T lymphocyte-directed manufacturing

CAR construct

All existing CAR constructs utilize the CD3 cytoplasmic domain as a fixed module to deliver a major activation signal. Recent studies identified that increasing CD3 diversity of CAR, that is, incorporation of the CD3ε cytoplasmic domain, yielded a CAR with improved signaling profile and tumor control [104]. The CD3 subunits all contain immunoreceptor tyrosine-based activation motifs and use tyrosine phosphorylation as a functional switch to trigger downstream signaling. Modified ITAM configuration in some CD3 subunits favors persistence of highly functional CARs, balancing the replicative capacity of long-lived memory cells and the acquisition of effective antitumor function [105].

Incorporating cytokine elements into the CAR construct is a potential strategy to enhance engineered T-cell activity. These tumor-targeting cytokines, such as IL-7, IL-12, IL-15, and IL-18, can improve memory T-cell formation and maximize an effective clearance of malignant cells [106–109]. Furthermore, an immunosuppressive tumor microenvironment could be addressed by using CAR T, which secretes IL-12 to enhance the cytotoxic capability of $CD8^+$ T cells, recruit macrophages, and prompt antigen cross-presentation [110].

In targeting different tumor antigens simultaneously or

successively, new CARs are designed to split from the signaling domain of a conventional CAR and combine it with an intermediate molecule to form a universal CAR (UniCAR) [111]. At present, several UniCAR modules have been reported. For example, biotin-binding immune receptor (BBIR) CAR is composed of an extracellular avidin motif linked to an intracellular signaling domain. The switch molecule is a biotinylated antigen-binding motif, that is, a monoclonal antibody (even scFv) or other tumor-specific ligands that selectively bind to avidin within the BBIR [112] (Fig. 2).

A further modified UniCAR is a split, universal, and programmable (SUPRA) CAR system with two components: a leucine zipper-containing universal receptor (zipCAR) linked to the intracellular signaling domain and a separate switch molecule with a cognate leucine zipper linked to an antigen-specific scFv (zipFv) [113] (Fig. 2). In addition, the advantage of these UniCARs resides on their modulability to deal with adverse events during CAR T-cell therapy. In case of severe CRS, withdrawal of the switch molecule can control the off-target effect.

Furthermore, fragment constant gamma-chimeric receptor (Fcγ-CR) is designed for binding to specific TAA-directed mAb [114]. In this system, T cells are engineered to express IgG Fc receptor fragments. Interaction between the Fcγ-CR on T cells and the anti-tumor antibody triggers perforin/granzyme-dependent target cell lysis (Fig. 2).

T-cell subpopulation

To date, most CAR T-cell trials use infused products generated from unselected T cells. Studies have highlighted the importance of distinct T-cell functional subsets (memory and effector) and patient's individual T-cell profile in CAR T-cell therapy [115]. A high CD4:CD8 ratio at the time of leukapheresis induced a clinical response in a phase 1 trial of anti-BCMA CAR T cells for MM [116]. Recently, inhibitors against Akt [117], PI3K [118], bromodomain proteins [119], and glycolysis [55] and the genetic ablation of the key epigenetic regulator TET2 [120] could increase the memory function of CAR Ts, which could enhance the persistence of CAR T cells and improve the overall response rate (ORR) and/or the durability of response.

Source of T cells

The primary determinant to successful CAR T-cell application is the intrinsic fitness of the T cells. Transcriptomic profiling of premanufactured T cells from CLL patients who poorly responded to CAR T-cell therapy revealed signatures of T-cell exhaustion, activation, glycolysis, and apoptosis [55], which might be due to immunosenescence by aging, persistent tumor antigen exposure, and heavy lines of prior chemotherapy [121,122]. Considering that poorly functioning T cells would limit CAR T-cell potential, an alternative strategy would explore the use of healthy, universal donor-derived CAR T cell as an alternative option to address T-cell malfunction and produce effective tumor killers.

Collection and cryopreservation before salvage chemotherapy are recommended in many CAR T-cell centers to obtain autologous healthy lymphocytes.

One alternative approach is the use of healthy UCAR T. Anti-CD19 UCAR T cells achieved molecular remission in two infants with r/r BCP-ALL, who subsequently received allogeneic HSCT [123]. It is crucial to ensure that the TCR/HLA class I loci of allogeneic T cells are fully disrupted by genome-editing technologies using zinc finger nuclease, transcription activator-like effector nuclease, or the CRISPR-Cas9 system. However, UCAR T-cell therapy is still at an early stage with many issues to be resolved.

CAR modification of immune effector cells other than T cells has been developed. Natural killer (NK) cells are

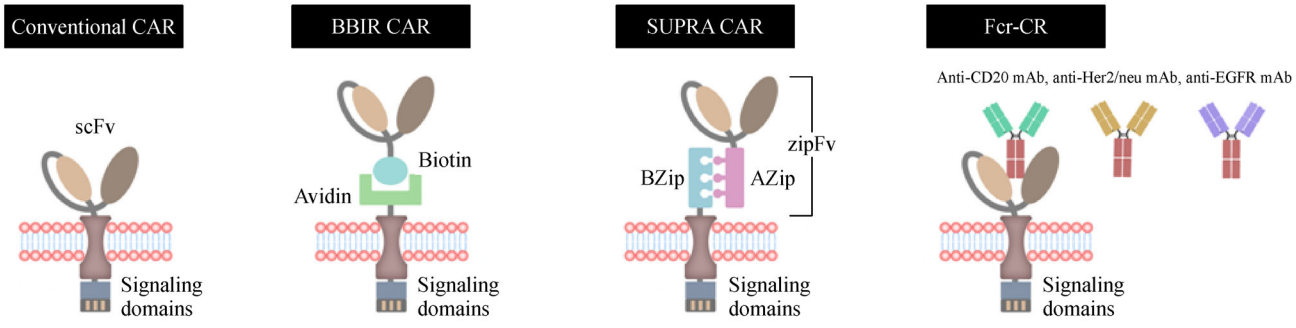

Fig. 2 Structural improvements in newly developed CARs. These innovations aim to guide CAR to target to different tumor antigens simultaneously or successively, thereby getting over the obstacles of antigen escape and targeting failure.

considered as prime candidates, which have innate lymphocytes with an inherent ability to detect and target infected or malignant celles [124,125]. NK cells can be an allogenic treatment, which do not require strict MHC matching, and such cells have no risk of GVHD. The NK cell line NK92 has been used in clinic because it can expand easily and indefinitely. However, its potential *in vivo* tumorigenicity and alloimmune responses limit its application [126,127]. Although the majority of clinical studies of NK cell immunotherapy have used peripheral blood NK cells, several alternative sources of NK cells exist, including BM, human embryonic stem cells (hESCs) [128], induced pluripotent stem cells (iPCSs) [127], and umbilical cord blood [125]. Cord blood NK cells have already entered into clinical trials and demonstrated feasibility and initial efficacy without any major toxic events [125].

Early line treatment

As CAR T-cell production becomes more efficient and safer, it is reasonable to consider ranking this strategy in priority among existing treatment options in B-cell malignancies. The optimal sequence of the use of traditional chemotherapy, targeting agents, monoclonal antibody immune therapies, HSCT, and CAR T-cell therapy remains to be determined. We propose that in patients achieving hematological CR and remaining MRD^+ after reinduction, a treatment using CAR T cells could be considered because of its potentially important clinical benefits and relatively moderate CRS risk with low tumor burden.

Tumor setting-directed modulation

Multi-antigen-specific CAR T cells

A dual-targeting/"cocktail" approach may enhance the initial treatment effects while minimizing the risk of relapse [129]. Dual-targeting CAR T-cell therapy refers to a single CAR that is composed of two different scFvs "hand-in-hand" (TanCAR) or two distinct CARs with different scFvs on one single T cell (dual-signaling CAR) [130,131]. An example of the former was the CD19/CD133-TanCAR, which showed a robust cytotoxic effect against $CD19^+CD133^+$ mixed lineage leukemia cells but with only minor activity against normal hematopoietic stem and progenitor cells (HSPCs) *in vivo* [132]. As for dual-signaling CARs, a phase 1 trial of anti-CD19/CD22 CAR T for the treatment of r/r BCP-ALL showed that MRD-negative CR was achieved in all six enrolled patients [133]. Recently, the results of a "cocktail" CAR T approach, in which two single-specific CAR Ts were sequentially infused, were reported. In this study, the sequential infusion of anti-CD19/CD22 third-generation CAR T demonstrated efficacy in 89 patients with r/r B-cell malignancies [134]. In a recent phase 2 trial, 21 patients with r/r MM received an infusion of humanized anti-CD19/BCMA CAR T, and 20 patients had an overall response at a median follow-up of 179 days [135]. Major clinical trial data of dual-targeting/"cocktail" approach are summarized in Table 4 [136–140].

The safety of this approach was also confirmed. Notably, TanCAR may be hindered by the mutual interference between the two divergent receptor structures [141,142] and immunogenicity [141], whereas dual-signaling CAR Ts may encounter problems with viral vector packaging and transduction efficiency [143,144]. Hence, these two approaches need further optimization. By contrast, the "cocktail CAR T" may be a favorable current option, albeit with a relatively high cost. If technical obstacles can be overcome by using approaches such as a rational construct design to eliminate interference, then dual or triple targeting may become an optimal approach.

Tri-specific CAR T cells, a single engineered T cell with three CARs targeting validated antigens, have been developed to broaden the antigen coverage and treat hematologic malignancies. Recently, two variants of a tri-specific CAR targeting BCP-ALL-associated antigens CD19, CD20, and CD22 were designed [145]. The first variant (TriCAR) expressed three monovalent second-generation CARs targeting each antigen individually, whereas the second variant (SideCAR) expressed a monovalent second-generation CD19 CAR and a bivalent CAR containing scFvs targeting CD20/CD22. Both variants demonstrated an improved *in vitro* cytotoxicity against triple-positive ALL cells and reduced CD19-negative relapse when compared with the monovalent CD19 CAR.

Considerable evidence highlights the crucial role of cancer stem cells (CSC) in the evolution and chemoresistance of hematological lymphoid malignancies. Their therapeutic targeting and killing are necessary to overcome relapse from CSC. Comprehensive characterization of CSC might need several specific biomarkers to define this heterogeneous subset in hematological malignancies. If these antigens can be identified, then simultaneously targeting antigens expressed on CSC when targeting the numerous tumor cells could potentially lead to deep remissions and reduce rates of relapse. To date, several investigators have suggested CD19, CD20, or CD38 as a potential stem cell antigen in MM.

Combination of CAR T with other therapeutic modalities

Immune checkpoint inhibitors, particularly PD-1 blocking antibodies, could potentiate the effect of CAR T because the latter increases the expression of inhibitory receptors

Table 4 Major clinical trials of dual-targeting CAR T in hematological B-cell malignancies

Study site	Disease	Targets	Construct of CAR	No. of cases infused	Dose	Best response (CR)	Relapse	Median follow-up (month)	Severe CRS	Severe neurotoxicity	Reference and registered number
Department of Molecular Biology and Immunology, Chinese PLA General Hospital	B-ALL	CD19/CD22	Tandem construct	6	$(1.7–3)\times10^6$/kg	6/6	6/6 1 $CD19^{neg}/CD22^{dim}$	NA	0	0	[133] NCT03185494
Beijing Boren Hospital	ALL	CD19/CD22	Separate constructs by sequential infusions	20	CD19-CAR T: $(3.3–42.8)\times10^5$/kg	20/20	3/20 (1 $CD19^+CD22^+$ relapsed, 1 $CD19^-CD22^{dim}$ relapsed, 1 $CD19^-CD22^+$ relapsed)	18	1/20	1/20	[136]
					CD22-CAR T: $(0.25–47.4)\times10^5$/kg		3/20 (2 $CD19^{neg}$, 1 $CD22^{dim}$)		1/20	1/20	ChiCTR-OIB-17013670
Tongji Hospital, Huazhong University of Science and Technology	B-ALL	CD19/CD22	Separate constructs by sequential infusions	B-ALL 51	CD19-CAR T: $(2.6\pm1.5)\times10^6$/kg in B-ALL; $(5.1\pm2.1)\times10^6$/kg in NHL	ALL CR: 48/50	ALL 24 relapsed (23 $CD19^+CD22^+$ relapsed, 1 $CD19^-/CD22^{dim}$ relapsed)	16.7	19/89	1/89	[134]
	NHL			NHL 38	CD22-CAR T: $(2.7\pm1.2)\times10^6$/kg in B-ALL; $(5.3\pm2.4)\times10^6$/kg in NHL	NHL CR: 18/36	NHL NA	14.4			ChiCTR-OPN-16008526
Bone Marrow Transplantation Department, Great Ormond Street Hospital	B-ALL	CD19/CD22	Bicistronic construct	10	Dose 1: 3×10^6/kg Dose 2: 5×10^6/kg	7/10	3/10 1 $CD19^{neg}/CD22^{dim}$	8	0	0	[137] NCT03289455
Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee	NHL	CD19/CD20	Tandem construct	22	2.5×10^6/kg	14/22	NA	NA	0	0	[138] NCT03019055
Department of Molecular Biology and Immunology, Chinese PLA General Hospital	NHL	CD19/CD20	Tandem construct	28	$(0.5–8)\times10^6$/kg	20/28	4/28 (3 $CD19^+CD20^+$ relapsed, 1 $CD19^-CD20^-$ relapsed)	19.1	4/28	0	[139] NCT03097770
Department of Hematology, the Affiliated Hospital of Xuzhou Medical University	MM	CD19/BCMA	Separate constructs by simultaneous infusions	21	CD19-CAR T (1×10^6 cells /kg) BCMA-CAR T (1×10^6cells/kg)	9 sCR, 3 CR/21	1/21	5.97	1/21	0	[135] ChiCTR-OIC-17011272
Union Hospital, Tongji Medical College, Huazhong University of Science and Technology	MM	BCMA/CD38	Tandem construct	16	0.5, 1.0, 2.0, 3.0 and 4.0×10^6 cells/kg	8 sCR/16	NA	NA	0	0	[140] ChiCTR1800018143

ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; CR, complete response; sCR, stringent complete response; NA, not available.

(iRs), primarily, PD-1 and Tim-3, after adoptive transfer [146,147]. Recent experiments demonstrated that PD-1 blockade could be safely used with CAR T cells, increasing the efficiency and persistence of CAR T cells [148], whereas modified CAR T cells that secrete PD-1-blocking scFv increased anti-tumor efficacy *in vivo* [149]. In a study of 11 patients with r/r B-NHLs, anti-CD19 CAR T combined with a PD-1 inhibitor was found to be safe, with no dose-limiting toxicities. The ORR and CR rates were 81.8% (9/11) and 45.5% (5/11), respectively [150].

The combination of CAR T with lenalidomide, an immunomodulatory drug that potentiates T cell function and abrogates the suppressive microenvironment, might also bring therapeutic benefits. Lenalidomide induces transcriptional and epigenetic changes in CAR T, leading to increased cell number and Th1 cytokine production, enhanced immunologic synapse formation, and cytotoxicity [151,152]. These properties could directly increase T-cell function even in patients who are refractory to immunomodulatory drugs [153–155].

A recent study in CLL evaluated the combination of ibrutinib, a Bruton's tyrosine kinase inhibitor effective in CLL frontline therapy [156], with a CAR T-cell expressing humanized anti-CD19. This combination achieved CR in 43% of patients and MRD negativity in 78% of patients. This result was more encouraging than those from prior tisagenlecleucel studies in progressive CLL.

Remarkably, the three above-mentioned combinations can restore T-cell function with normalized CD4:CD8 ratios and increase memory T cells, indicating that the synergistic effects of CAR T-cell therapy might be obtained by abrogating the immunosuppressive tumor microenvironment [157].

In reducing the relapse after CAR T treatment caused by the loss or modulation of the antigens on tumor cells targeted by CAR T [95,96,102,158], enhancement of tumor antigen density and CAR-binding affinity can be envisaged [159–161]. Thus, all-trans retinoic acid, interferon-α, and γ-secretase inhibitors can increase the surface expression of target antigens on tumor cells of CAR T-cell-targeted diseases [162,163]. A trial with anti-CD22 CAR T-cell reported that bryostatin-1 could increase CD22 expression, resulting in an improved response [164]. These findings indicated that CAR T in combination with relevant small-molecule drugs may lead to rapid responses and reduce antigen modulation-associated relapse.

Conclusions

CAR T-cell therapy for r/r B-cell malignancies has shown great potential. Several new strategies are emerging to enhance its efficacy and improve control of adverse events (Fig. 3). The most appropriate target antigens should be those selectively expressed on the surface of tumor cells but not on cells of vital organs. In the future, carefully selecting antigen/CAR matches and improving engineering and manufacturing processes should optimize CAR T-cell products to achieve high affinity binding and increase killing power with less adverse effects. In addition, the use of CAR T therapy in a proactive manner, such as in newly diagnosed patients with BCP-ALL, NHL, and MM, may

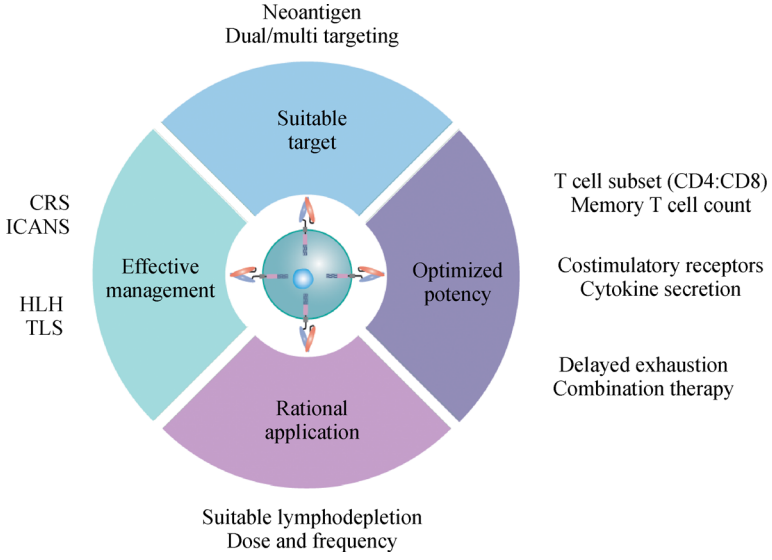

Fig. 3 Current strategies of improving CAR T-cell practical application. From CAR construct optimization to clinical management on adverse events, CAR T-cell immunotherapy is rapidly being advanced to make it more available and accessible to lymphoid hematological malignancies. CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; HLH, hemophagocytic lymphohisticytosis; TLS, tumor lysis syndrome.

lead to more therapeutic benefits while avoiding acute and chronic complications. For BCP-ALL, such a strategy may prompt the transition of CAR T-cell therapy from a "bridging" therapy to a decisive one; thus, many more patients with BCP-ALL can be cured. The approximately 50% long-term survival of patients with r/r NHL treated with CAR T has justified its extension to early-phase cases. In MM, CAR T-cell therapy may challenge the position of auto-HSCT or be sequentially associated with auto-HSCT for patients with poor prognosis to make the disease curable.

In the near future, with the application of CAR T-cell therapy, the adverse effects of CAR T-cell therapy seem to be unavoidable. Therefore, a comprehensive training of interdisciplinary staff, an effective communication, and an appropriate infrastructure are required to ensure safety. These measures should also ensure that research protocols and standard care are appropriately executed, and adequate resources are available to achieve optimal outcomes. Early diagnosis and appropriate management of severe adverse events are key to the success of CAR T-cell therapy. Severe adverse events are largely associated with tumor burden and antigen sensitivity. Based on previously reported clinical experiments, multiply infusion of CAR T cells might be well tolerated and applied to avoid severe CRS compared with a single infusion [21,165]. However, further evaluation of a larger cohort is necessary to determine optimal infusion mode and attenuate infusion-associated SAEs. These findings and our continued evaluation of patients receiving multiple infusions will ensure the dose per infusion and frequency when administering multiple doses of CAR T cells in the future to achieve efficiency.

CAR T-cell treatment should not be considered as an exclusive treatment but rather a weapon that can be integrated into the current standard of care or new treatment modalities in combination with other immune therapies and gene-targeting agents. CAR T-cell therapy may synergize with these treatments to cover heterogeneous clones and provide long-term control or even cure.

Finally, the basic concept of CARs could be further developed for broader use in clinical settings, such as in myeloid malignancies or even some types of solid tumors, by armoring other immune components, including NK cells, B cells, and macrophages. New experimental research and preclinical and prospective clinical trials will explore these possibilities. We strongly believe that CAR T-cell therapy will soon be viewed as a milestone on the road of defeating cancer.

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Compliance with ethics guidelines

Jian-Qing Mi, Jie Xu, Jianfeng Zhou, Weili Zhao, Zhu Chen, J. Joseph Melenhorst , and Saijuan Chen involved in this review writing declare that they have no financial conflicts of interest. This manuscript is a review article and does not contain research protocols requiring approval by the relevant institutional review board or ethics committee.

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