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Perspective

CAR-T in cancer therapeutics and updates

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ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy has emerged as a groundbreaking approach in cancer treatment, utilizing the immune system's capabilities to combat malignancies. This innovative therapy involves extracting T-cells from a patient's blood, genetically modifying them to target specific cancer cells, and reinfusing them back into the patient's body. The genetically modified T-cells then seek out and eliminate cancer cells, offering a promising therapeutic strategy. Since its initial approval in 2017, CAR-T therapy has witnessed remarkable advancements and updates. Notably, CAR-T therapy, which was initially developed for hematological malignancies, has expanded its scope to target solid tumors. Currently, clinical trials are underway to explore the efficacy of CAR-T therapy in treating various solid tumors, such as lung cancer, breast cancer, and ovarian cancer. These trials hold great potential to revolutionize cancer treatment and provide new hope to patients with challenging-to-treat solid tumors. In this mini-review, we present an overview of CAR-T therapy's mechanisms, emphasizing its role in targeting cancer cells and the potential therapeutic benefits. Additionally, we discuss the recent progress and updates in CAR-T therapy, particularly its application in treating solid tumors, and highlight the ongoing clinical trials aimed at broadening its therapeutic horizon. The evolving landscape of CAR-T therapy signifies a promising direction in cancer therapeutics, with the potential to revolutionize the treatment of both hematological and solid tumor malignancies.

1. Evolving landscape of CAR-T therapy in B cell malignancies

In the 1980s, Zelig Eshhar's groundbreaking research aimed to overcome the challenges of T-cell recognition of antigens restricted by human leukocyte antigen (HLA)/major histocompatibility complex (MHC). Among the notable efforts in this direction, Gross et al. made a significant breakthrough by demonstrating the expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity.[1] This achievement involved ligating genomic DNA sequences of variable domain of heavy chain (VH) and variable domain of light chain (VL) from the anti-2,4,6-trinitrophenyl (TNP) Sp6 hybridoma to either one of the gene fragments encoding the constant region of α or β TCR chains. The resulting chimeric TCR genes endowed T cells with the ability to recognize antigens with antibody-like precision, leading to responses against cells bearing TNP haptenic groups on their surfaces, as evidenced by IL-2 production or cytolytic activity.

Building upon these findings, Eshhar and colleagues designed the first chimeric receptor by covalently linking the single-chain variable fragment (scFv) from antibody variable region with the γ chain or ζ chain of T cell receptors.[2] This innovative approach facilitated the expression of these chimeric molecules in T cells, conferring potent responses to the targeted antigen. Further advancements by Finney et al. involved the addition of the CD28 co-stimulatory domain to the chimeric receptor molecules, demonstrating the provision of both primary and co-stimulatory signaling in T cells from a single gene product. Additionally, Tammana et al. explored the role of co-stimulatory signaling domains, such as 4-1BB and CD28, in CD19 chimeric antigen receptor (CAR)-T cell therapy and suggested that inclusion of 4-1BB co-stimulatory molecules seemed more critical than CD28 in this context.[3]

As a result of these foundational developments, the basic structures of CAR-T cells were established. Kochenderfer et al., within Rosenberg's group, reported the first treatment protocol of lymphoma using CD19 CAR-T cells. This pioneering approach involved generating CAR-T cells through retrovirus-mediated CD19 CAR transfection of peripheral blood mononuclear cells (PBMCs) obtained from apheresis, followed by lymphocyte depletion with cyclophosphamide and fludarabine before CAR-T infusion, along with a course of IL-2 administration.[4] This treatment resulted in 32 weeks of partial remission in the patient. Concurrently, June's group investigated the therapeutic potential of CD19 CAR-T cells in B-cell acute lymphocytic leukemia (ALL), which ultimately resulted in U.S. Food and Drug Administration (FDA) approval for the treatment of B-cell ALL and lymphoma. As per the FDA website (fda.gov), a total of five indications have been approved for CAR-T therapy treatments so far, reflecting the growing number of conditions that can be effectively addressed using this innovative approach. Below is a compilation

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Table 1
Current FDA-approved chimeric antigen receptor–engineered T-cell treatments and prevalence of adverse events.

Treatment title	Target	Underlying malignancy	Grade ≥ 3 cytokine release syndrome, %	Grade ≥ 3 neurotoxicity, %	Other common grade ≥ 3 adverse events, %
Tisagenlecleucel (Kymriah, Novartis Pharmaceuticals)	CD19	B-cell ALL	77	22	Febrile neutropenia (34)
		DLBCL	74	19	Febrile neutropenia (17), anemia (59)
		FL	53	6	Febrile neutropenia (13)
Axicabtagene ciloleucel (Yescarta, KitebPharma)	CD19	LBCL	9	25–31	Febrile neutropenia (31), anemia (8)
		FL	8	21	Febrile neutropenia (41)
Brexucabtagene autoleucel (Tecartus, Kite Pharma)	CD19	Mantle cell lymphoma	18	37	Neutropenia (37), anemia (17)
		B-cell ALL	26	35	Neutropenia (12)
Lisocabtagene maraleucel (Breyanzi, Juno Therapeutics)	CD19	LBCL	4.1	12	Febrile neutropenia (8)
Idecabtagene vicleucel Abecma (Abecma, Bristol Myers Squibb and bluebird bio, Inc.)	BCMA	Multiple Myeloma	9	4	Febrile neutropenia (17), HLH/MAS (4–8)
Ciltacabtagene autoleucel (Carvykti, Janssen Biotech, Inc. and Legend Biotech)	BCMA	Multiple Myeloma	5	11*	Prolonged neutropenia (30), HLH/MAS (1)

* The incidence of grade 5 (fatal) is 2%.
Abbreviations: ALL, acute lymphocytic leukemia; BCMA, B cell maturation antigen; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HLH/MAS, hemophagocytic lymphohistiocytosis/macrophage activation syndrome; LBCL, large B-cell lymphoma.

of CAR-T products that have received FDA approval, along with their respective indications for CAR-T treatment (Table 1).

2. Advances in B-cell malignancy treatment with CAR-T cell therapy

B lymphocytes, a vital component of the immune system, fulfill crucial roles, including antibody production and pathogen elimination.[5] These cells can differentiate into plasma cells, responsible for generating large quantities of circulating antibodies in the bloodstream and lymphatic system. Besides antibody production, B cells also exhibit antigen presentation capabilities and regulatory functions. They can act as antigen-presenting cells (APCs), initiating immune responses by presenting antigens to other immune cells like T cells. Additionally, B cells can modulate immune cell activity through cytokine production. However, individuals with B cell deficiencies can lead relatively normal lives with immunoglobulin replacement therapy,[6] which provides the necessary antibodies to bolster the immune system's ability to combat infections.

In the context of B cell malignancies, CAR-T cell therapy offers a targeted approach to eliminating cancerous B cells by focusing on pan-B markers such as CD19 or CD20. While effective in treating these cancers, CAR-T therapy may inadvertently deplete normal B cells, including those responsible for immunoglobulin production. As a consequence, patients become susceptible to infections, necessitating immunoglobulin replacement therapy to reinforce the immune system and prevent infections.[7]

A notable side effect of CD19 CAR-T cell therapy is neurotoxicity, which manifests as a range of neurological symptoms, including confusion, agitation, delirium, seizures, and in severe cases, cerebral edema and coma.[8] Neurotoxicity associated with CAR-T therapy is thought to be linked to the expression of CD19 on mural cells of the blood-brain barrier and the release of cytokines, resulting in brain inflammation.[9] Close monitoring of patients undergoing CAR-T therapy for neurotoxicity signs is crucial, enabling prompt symptom management and prevention of serious complications.[8]

CAR-T cell therapy targeting B-cell maturation antigen (BCMA) has shown promising outcomes in treating multiple myeloma, a type of blood cancer affecting plasma cells.[10] Plasma cells are specialized within the B-cell lineage for antibody production against infections. However, treatment-related neurotoxicities have been observed in some patients.[11] In the CARTITUDE-1 study, 5% of multiple myeloma patients treated with ciltacabtagene autoleucel (cilta-cel), a BCMA-targeted CAR-T cell therapy, reported movement and neurocognitive treatment-emergent adverse events (MNTs). Strategies implemented during the cilta-cel development program, such as enhanced bridging therapy, aggressive treatment of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), early symptom detection through handwriting assessments, and extended monitoring/reporting time for neurotoxicity, reduced the incidence of MNTs from 5% to <1% across the cilta-cel program. These findings support the favorable benefit-risk profile of cilta-cel for multiple myeloma treatment.[12] Nevertheless, conflicting evidence regarding BCMA expression in the human brain exists, warranting further investigation.[13]

Clinical trials focusing on CD19/CD20 CAR-T cell therapy for B-cell lymphoma have demonstrated promising results. Meta-analysis of data collected up to July 2019 from 17 relevant studies showed an overall response rate (RR) of 63% (95% confidence interval [CI], 41%–85%) and a complete response rate (CRR) of 39% (95% CI, 25%–54%).[14] In our own unpublished research data, we have developed CD20/CD19 bispecific CAR-T cells and conducted investigator-initiated trials, where more than 20 patients achieved an overall response rate (ORR) of over 90% and a CRR of over 80%.[15]

In summary, CAR-T cell therapy has revolutionized the treatment of B-cell malignancies. However, it is crucial to manage the potential consequences of this therapy on the immune system, such as depletion of antibody-producing cells, through appropriate medical interventions like immunoglobulin replacement therapy. Close monitoring for neurotoxicity and further research on BCMA expression in the brain will enhance the safety and efficacy of CAR-T cell therapy for B-cell malignancies.

3. Trials of CAR-T therapy for myeloid cell malignancies

Acute myeloid leukemia (AML) is a complex disease with diverse molecular alterations, requiring multiple therapeutic approaches for long-term disease control.[16] AML cells often express antigens associated with myeloid cells, such as CD123, CD33, CD38, and CLL1. These markers can be utilized for disease identification, diagnosis, and targeted therapy development. While CAR-T cell therapy has shown remarkable success in treating B-cell malignancies, its effectiveness in treating AML has been more limited.

CD123 CAR-T therapy targets CD123/IL-3Rα, which is overexpressed in AML and other hematological malignancies, including the

leukemia-initiating cell compartment.[17] CD123 is also expressed on normal myeloid cells and vascular endothelium.[18] Moreover, interferon (IFN) γ and tumor necrosis factor (TNF) α can upregulate CD123 expression on endothelial cells and hemopoietic cells *in vitro*.[19] The use of CAR-T cells targeting CD123 in AML raises concerns about potential myeloablation, myelotoxicity, and the development of capillary leak syndrome.

Clinicaltrials.gov lists 28 registered clinical trials investigating the safety and effectiveness of CD123 targeting in AML using CD123 CAR-T, along with one trial using CAR-NK CD123. Among the autologous CAR-T trials, three are currently active but not recruiting, and four are still recruiting patients. Many of these trials have exceeded their completion dates without verified status for over two years or have been withdrawn or terminated. Only one trial has completed safety and efficacy assessments.

Additionally, three trials have explored CAR-T CD123 with allogeneic T cells to generate universal CAR (UCAR)-T against CD123 for the treatment of CD123-positive AML or blastic plasmacytoid dendritic cell neoplasm (BPDCN). Limited information is available about these trials, but news reports on Phase I studies of UCART123 by Cellectis indicate that an elderly patient with BPDCN died after receiving UCAR123 CAR-T cells, experiencing severe cytokine release syndrome, capillary leak syndrome, and lung infection. Another patient with AML experienced grade 4 capillary leak syndrome but recovered after three days. However, in updated reports of CD123 CAR-T trials, there have been no observations of capillary leak syndrome.

Sallman et al.[20] reported on UCART123v1.2 treatments in 16 AML patients, and clinical benefits were observed in four patients, with one achieving complete remission (CR) and experiencing no significant myelotoxicity. Budde et al.[21] reported trials conducted by the City of Hope National Medical Center in California involving autologous CD123 CAR-T cell therapy for seven AML patients. All six patients in the AML cohort had refractory AML following allogeneic hematopoietic stem cell transplant (allo-HSCT). Two patients with CR had proceeded to second allo-HSCTs, while the remaining patients did not experience promising benefits. One patient with BPDCN continued to be in CR for 60 days post-CAR-T transfusion. Naik et al.[22] reported the treatment of autologous CD123-CAR-T cells in pediatric patients with AML. In the dose escalation of dose level 2, clinical benefits without any adverse infusion events were observed. One patient had blast reduction percentage without CR, one had no response, and one achieved CR.

NKG2D CAR-T therapy utilizes the NKG2D receptor expressed by NK cells and subsets of T cells, including CD8+ T cells and $\gamma\delta$ T cells. This receptor serves as a recognition receptor for detecting and eliminating transformed and infected cells, participating in the genesis of inflammatory diseases.[23] NKG2D CAR-T cells can recognize a diverse range of NKG2D ligands expressed by target cells, making it a potential therapy for various cancers. Clinicaltrials.gov lists over 20 trials involving NKG2D CAR-T cells, targeting cancers such as AML and solid tumors. However, NKG2D ligands are not expressed on AML leukemic stem cells, resulting in only temporary remission with NKG2D CAR-T treatment.[24] Inhibition of PARP1, which is overexpressed in NKG2D leukemic stem cells, has been explored to induce the expression of NKG2D ligands on CD34+ AML cells, potentially enhancing NKG2D CAR-T treatment in combination with PARP1 inhibitors. NKG2D is expressed in certain healthy cells, including activated T cells, gut epithelium, and bronchial epithelial cells.[25] The expression of NKG2D ligands in activated T cells can lead to fratricide of NKG2D CAR-T cells.[26] Clinical trial results of Celyad NKG2D CYAD-1 showed objective responses in three out of 12 evaluable patients with relapsed or refractory AML. Two responders proceeded to allo-HSCT, maintaining durable ongoing remissions. However, CYAD-01 investigation has been discontinued in clinical studies due to clinical futility. Another study investigated an optimized short hairpin RNA (shRNA) technology to modulate NKG2D ligand expression on CYAD-01 cells, resulting in CYAD-02 with increased expansion during manufacturing, higher engraftment one week after injection, and improved survival in an aggressive AML mouse model.[27] Clinical trials of CYAD-02 in relapsed/refractory AML or myelodysplastic syndrome patients are currently ongoing.

4. GD2 CAR-T therapy for neuroblastoma treatment

Neuroblastoma is the most common extracranial solid tumor in children and is often a high-risk and potentially lethal disease. However, significant progress has been made in improving the 5-year survival rate of patients with metastatic neuroblastoma through clinical trials incorporating various treatment modalities, such as high-dose chemotherapy with autologous stem cell transplantation, differentiating agents, and immunotherapy.[28]

Gangliosides are carbohydrate-containing sphingolipids that are present in normal tissues, making them unsuitable targets for cancer therapy in most cases. However, the disialoganglioside GD2 subtype of ganglioside has limited expression in normal tissues but is overexpressed in a wide range of tumors, including neuroblastoma.[29] The use of anti-GD2 antibodies has been approved by the FDA for the clinical treatment of neuroblastoma. Mejzener et al.[30] conducted a study where they treated four patients, three with H3K27M diffuse intrinsic pontine glioma and one with spinal cord diffuse midline glioma, with GD2 CAR-T cells. The CAR-T cells were administered to all four patients via intravenous infusion, and three of them also received additional local administrations via Ommaya reservoir intracerebroventricularly. Encouragingly, three out of the four patients showed clinical and radiographic improvement.

Del Bufalo et al.[31] reported their findings from a study involving 27 patients with relapsed or refractory high-risk neuroblastoma treated with GD2-CART01. These children, who had undergone extensive prior treatment, received GD2-CART01 therapy. Out of the 27 patients, 17 demonstrated a response to the treatment, resulting in an overall response rate of 63%. Among the responders, nine achieved a complete response, and eight achieved a partial response. However, it is important to note that severe hematologic toxic effects, including anemia, neutropenia, and thrombocytopenia, were observed in all patients, in addition to the well-recognized cytokine release syndrome associated with CAR-T therapy in hematopoietic malignancies. Martinez et al.[32] reported the expression of GD2 in bone marrow mesenchymal stromal cells, which may provide a hypothetical explanation for the observed severe hematological toxic effects and cytokine release syndrome in GD2-CART01 therapy.

5. Targeting tumor-associated antigens with affinity-tuned CAR-T cells

Monoclonal antibodies have been successful in identifying a very limited number of tumor-specific antigens in most cancer cells, which can be targeted by CAR-T therapy.[33] However, a significant proportion of overexpressed proteins in cancer, known as tumor-associated antigens (TAAs), include auto-proteins such as EGFR, p53, hTERT, and carbonic anhydrase IX.[34] To selectively target cancer cells with higher levels of TAAs, an affinity-tuned CAR-T approach has been developed.[35] This involves constructing a panel of ErbB2 or EGFR CARs using known mutations found in the single-chain fragment variable (scFv) sequences. By analyzing the expression of ErbB2 or EGFR on different cancer cell lines using flow cytometry, a diverse collection of tumor cell lines with varying expression levels of these proteins has been compiled. Lower affinity scFvs in ErbB2 CARs have demonstrated the ability to distinguish between tumor cells expressing low and high levels of ErbB2, while not recognizing physiological levels of the antigen. These affinity-reduced ErbB2 CAR-T cells have shown effectiveness in eliminating tumors *in vivo* while significantly reducing toxicity against tissues expressing physiological levels of ErbB2. Similarly, the data on CAR-T targeting EGFR

highlights the enhanced therapeutic index achieved through affinity-tuned scFvs. In a review by Mause et al.[36], the advancements in affinity-tuned antibodies and their role in improving the function and selectivity of CAR-T cells were discussed.

To generate low-affinity CAR-binding domains, various strategies have been employed, including error-prone PCR mutagenesis, Kunkel mutagenesis, light chain/complementarity-determining region (CDR) shuffling, site-saturation mutagenesis/deep mutational scan, rational/computational design, display selection of low-affinity binders from extensive antibody variant libraries, and monoclonal screening for antibody clones with reduced affinity. Vedvyas et al.[37] developed AIC100 CAR-T cells targeting ICAM-1 based on the interaction between LFA-1 and ICAM-1. AIC100 CAR exhibits micromolar affinity to ICAM-1, as opposed to nanomolar affinity, to avoid cytotoxicity in normal cells with basal levels of ICAM-1 expression. In a phase I study of AIC100 CAR-T cell treatment in patients with ICAM-1 positive relapsed and/or refractory advanced poorly differentiated and anaplastic thyroid cancer, no dose-limiting toxicities were observed in dose level 1 (10^7) and dose level 2 (10^8). Encouragingly, the first evaluable patient in dose level 2, who had metastatic anaplastic thyroid cancer and had failed multiple lines of therapy, showed an objective and relatively durable partial response, which is unprecedented and highly promising.[38]

6. Dual CAR-T and AND gate recognition of two targets

A groundbreaking strategy for dual targeting of antigens on cells using CAR-T cells was introduced by Kloss et al.[39] The PSMA CAR was designed as a suboptimal T cell activator, while the CD19 CAR acted as a co-stimulatory signal. These signals synergistically worked together to fully activate T cells. However, it is important to acknowledge an inherent limitation where each immunoreceptor tyrosine-based activation motif (ITAM) within the T cell receptor complex exhibited comparable cell killing potential,[40] as supported by unpublished data from our research.

Tousley et al.[41] investigated the CD3 zeta machinery within the CAR structure and identified ZAP-70 and PLC-γ1 as proximal signaling molecules capable of independently initiating CAR T cell signaling. Downstream molecules of ZAP-70, such as LAT and SLP-76, were found to be sufficient for promoting T cell activation, and the pairing of LAT and SLP-76 enabled AND gating. However, there was still observed leakage in CARs with LAT instead of CD3ζ and CARs with SLP-76 alongside CD3ζ. To address this issue, the role of GADS in the GRB2 family was explored, as it binds to LAT and SLP-76, forming a scaffold for PLCγ1. As a result, a true AND gate system was established by deleting the GADS binding sites from LAT and SLP-76, allowing them to serve as the activation domains of the CAR.

7. Localized administration of CAR-T cells for therapy

The approach of administering CAR-T cells through intratumoral injection or in situ delivery within the tumor has garnered significant attention. While systemic distribution of CAR-T cells via intravenous administration has successfully targeted CD19, BCMA, CD20, and GD2, leading to the cure of B cell malignancies and neuroblastoma, it is evident that more sophisticated strategies are required to address the heterogeneity of malignant cells, including cancer stem cells, in most solid tumors. In light of this challenge, intratumoral injection has emerged as a promising strategy to overcome the biodistribution limitations of CAR-T cells in such cases. By combining localized CAR-T cell-mediated cancer cell cytotoxicity with other immune mechanisms, there is potential to induce additional abscopal effects, which can be effective in treating metastatic cancers responsible for over 90% of solid tumor-related deaths.

In the study conducted by You et al.,[42] the intratumoral administration of MUC-1 CAR-T cells was investigated. The CAR-T cells, targeting MUC-1, were directly injected into metastatic lesions of seminal vesicle tumors. This approach demonstrated improved MUC-1 CAR-T cell efficacy, leading to necrosis at the injected sites.

Another innovative approach was developed by Li et al.,[43] who created a scattered porous microneedle patch for the delivery of CAR-T cells within tumors or post-surgical resection cavities. The microneedle patch was fabricated using a mixture of methacryloyl chloride-modified 4-arm-PLGA, triethylene glycol diacetate, and $CaCO_3$ microparticles etched with hydrochloric acid to form pores. This porous microneedle patch exhibited the ability to disperse CAR-T cells, promoting T cell proliferation and enhancing their activity.

Furthermore, as previously mentioned, Majzner et al.[30] explored the use of an Ommaya reservoir for localized delivery of GD2 CAR-T cells. This localized administration approach holds promise in advancing the effectiveness of CAR-T cell therapy.

8. Allogeneic and universal CAR-T therapy

While the majority of CAR-T cells discussed above are derived from autologous T cells, there exists a cohort of patients for whom such cells may not be viable due to prior chemotherapies, radiotherapies, or disease-related complications. To address this limitation, allogeneic CAR-T cells generated from healthy donor T lymphocytes have emerged as a promising alternative. Successful implementation involves knocking down TCR α/β chains in allogeneic T cells to prevent graft-versus-host disease (GVHD). Simultaneously, the downregulation of the MHC I beta-2 microglobulin (B2M) gene eliminates MHC molecules, reducing the risk of host T cells attacking allogeneic CAR-T cells.[44]

A noteworthy example is UCART19, developed by Cellectis, a universal CAR-T cell product targeting CD19. Employing the TALEN gene-editing method, UCART19 is crafted to disrupt the TCR-α constant chain and CD52 on T cells. Subsequent administration of an anti-CD52 antibody as part of the lymphodepletion regimen enhances efficacy. In a study involving pediatric and adult B-cell acute lymphoblastic leukemia, 67% of patients exhibited a complete response or complete response with incomplete hematological recovery 28 days post-infusion. Notably, patients not receiving alemtuzumab showed no UCART19 expansion or antileukemic activity[45]

The UNIVERSAL trial, reported by Mailankody et al.,[46] explored allogeneic BCMA-targeting CAR-T cells in relapsed/refractory multiple myeloma. T cells with knockout of TCR alpha chain and CD52 were treated with a lymphodepletion regimen. Of the patients treated, 70.8% demonstrated a response, with 45.8% achieving a very good partial response or better and 25% attaining a complete response/stringent complete response. The median duration of response was 8.3 months.

Recognizing concerns associated with autologous CAR-T production—such as prolonged production times, reduced T cell quality, and high costs—there is a growing interest in allogeneic CAR-T therapies. Hu et al.[47] pioneered the use of healthy donor T cells to generate UCAR-T against CD7, incorporating CD7 knockdown to mitigate fratricide effects, and disrupting TCR and HLA II using TRAC and RFX5. In a phase I clinical trial involving 12 patients with T-cell leukemia/lymphoma or CD7-expressing acute myeloid leukemia, the objective response rate was 63.6%, with 4 patients remaining in complete remission after a median follow-up of 10.5 months. Notably, three responding patients were successfully bridged to allogeneic hematopoietic stem cell transplantation.

While these data showcase the promise of allogeneic CAR-T therapy in achieving objective responses and bridging hematological malignancies refractory to standard treatments or rapid progressed diseases to further regiments such as hematopoietic stem cell transplantation or autologous CAR-T therapy, challenges persist. The inherent issues of persistence and the generation of memory CAR-T cells pose ongoing considerations for the viability of allogeneic CAR-T strategies.

In summary, CAR-T therapy has demonstrated remarkable progress and holds great promise in the field of cancer therapeutics, offering new hope to patients with previously untreatable malignancies. Despite the

impressive successes, challenges such as cytokine release syndrome, toxicities management, and the emergence of resistance remain crucial areas for further investigation. Addressing these obstacles will be essential to fully unlock the therapeutic potential of CAR-T therapy. Continued research and ongoing clinical trials exploring novel targets, optimizing CAR design, and enhancing delivery strategies hold the key to advancing the field even further. As the field continues to evolve, collaboration between researchers, clinicians, and industry stakeholders will be vital in harnessing the full power of CAR-T cells to combat cancer. With unwavering dedication and innovative approaches, we can expect a future where CAR-T therapy plays a central role in the fight against cancer, ultimately leading to improved patient outcomes and a brighter, cancer-free world.

Declaration of competing interest

Dr. Zhu, as an author of this manuscript, is an employee and owner of stock in the Cellular Biomedical Group, Inc. presently recognized as Abelzeta Parma. The research described in this manuscript draws upon their own unpublished data involving the development of CD20/CD19 bispecific CAR-T cells and the initiation of investigator-initiated trials. It is important to note that no external financial or personal support has been received from any entity or individual for the preparation, analysis, and discussion of this manuscript.

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