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REVIEW

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Optimizing CAR-T cell therapy for solid tumors: current challenges and potential strategies

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Abstract

Chimeric antigen receptor (CAR)-T cell therapy demonstrates substantial efficacy in various hematological malignancies. However, its application in solid tumors is still limited. Clinical studies report suboptimal outcomes such as reduced cytotoxicity of CAR-T cells and tumor evasion, underscoring the need to address the challenges of sliding cytotoxicity in CAR-T cells. Despite improvements from fourth and next-generation CAR-T cells, new challenges include systemic toxicity from continuously secreted proteins, low productivity, and elevated costs. Recent research targets genetic modifications to boost killing potential, metabolic interventions to hinder tumor progression, and diverse combination strategies to enhance CAR-T cell therapy. Efforts to reduce the duration and cost of CAR-T cell therapy include developing allogenic and in-vivo approaches, promising significant future advancements. Concurrently, innovative technologies and platforms enhance the potential of CAR-T cell therapy to overcome limitations in treating solid tumors. This review explores strategies to optimize CAR-T cell therapies for solid tumors, focusing on enhancing cytotoxicity and overcoming application restrictions. We summarize recent advances in T cell subset selection, CAR-T structural modifications, infiltration enhancement, genetic and metabolic interventions, production optimization, and the integration of novel technologies, presenting therapeutic approaches that could improve CAR-T cell therapy's efficacy and applicability in solid tumors.

Keywords CAR-T cell therapy, Solid tumors, Challenges of cytotoxicity, Restriction in application, Novel technologies

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Background

Adoptive T cell therapy, including chimeric antigen receptor (CAR) T cells, has achieved significant success in targeted cancer cell eradication [1, 2]. Although the FDA has approved CAR-T cell therapies for certain hematological malignancies, a significant proportion of patients relapse within one year after infusion [3]. Moreover, their limited efficacy in solid tumors presents ongoing challenges [4, 5]. Nevertheless, the majority of patients, particularly those with solid tumors, continue to exhibit resistance to CAR-T cell therapy. Several challenges persist in treating solid tumors with CAR-T cells, including insufficient cytotoxicity, poor infiltration, tumor microenvironment suppression, high costs, and safety concerns [6]. Although some CAR-T products are approved for use with chemotherapy or monoclonal antibodies to enhance engraftment [7], none are yet approved for treating solid tumors [8]. Key considerations in optimizing CAR constructs include enhancing their fitness, persistence, and anti-tumor efficacy [9, 10]. Like natural cytotoxic T lymphocytes (CTLs), CAR-T cells can induce tumor cell death via perforin/granzyme, death receptor, and cytokine-mediated pathways [11–13] (Fig. 1). Several innovations in fourth and next-generation CAR-T cells have significantly enhanced their efficacy [14] through optimized T cell subsets, CAR structure refinement, genetic and metabolic interventions, and combination therapies, yielding promising results [15, 16]. Meanwhile, ongoing efforts to develop allogeneic and in-vivo CAR-T therapies show promise for future applications [17]. Integrating advanced technologies and new platforms may enhance the implementation of CAR-T therapies in solid tumor management [18].

Consequently, this review thoroughly examines emerging strategies that enhance the anti-tumor efficacy of CAR-T cells in solid tumors. These strategies include selecting specific T cell subsets, modifying structures, enhancing infiltration into the tumor microenvironment (TME), and applying genetic and metabolic interventions. Additionally, it covers improvements in CAR-T cell production and the use of novel technologies and platforms to reduce time and costs. Furthermore, we explore the challenges of applying CAR-T cell therapy to solid tumors by analyzing relevant preclinical studies and

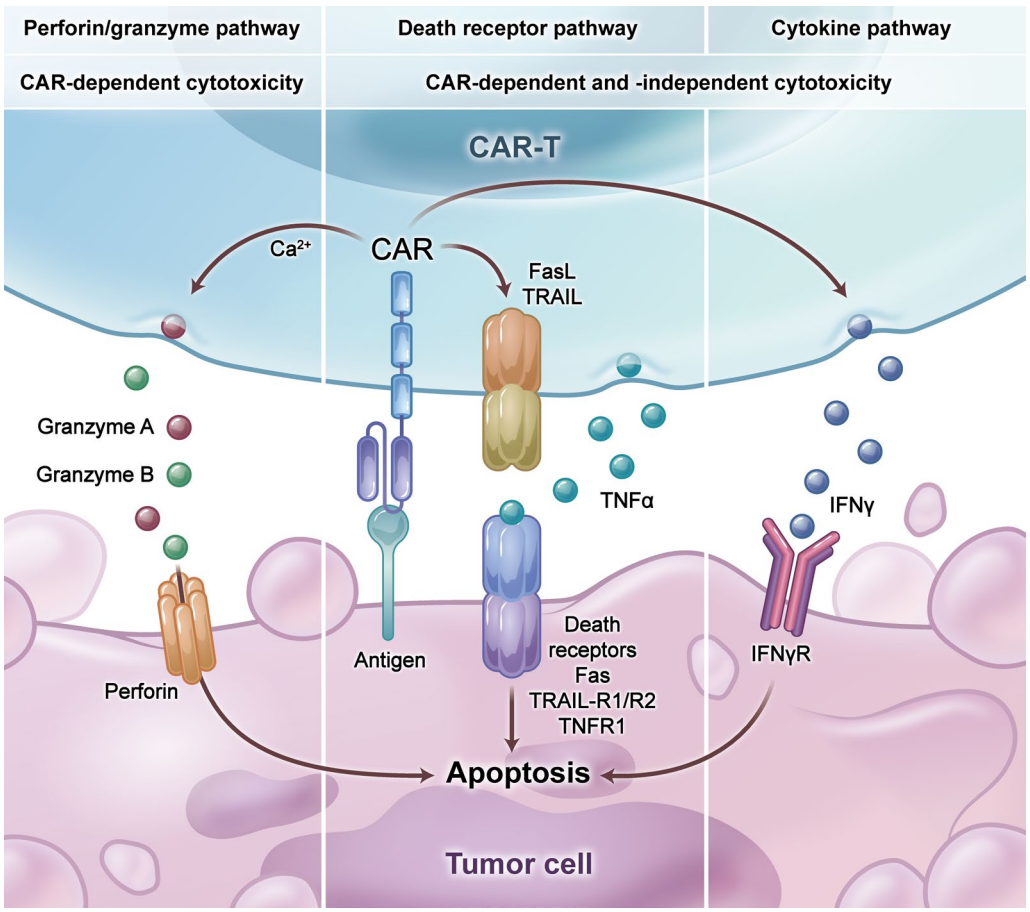

Fig. 1 Killing mechanisms of CAR-T cell

clinical trials. This review aims to propose potential strategies to overcome challenges and enhance the efficacy and applicability of CAR-T cell therapies in treating solid malignancies.

CAR-T cell kill the targeted tumor cell through three pathways: (1) perforin and granzymes pathway; (2) death receptor pathway (FasL/Fas, TRAIL/TRAIL-R1/2, and TNFα/TNFR1); (3) cytokine-mediated pathway (IFNγ).

Selection of T cell subsets

The exploration of alternative cellular sources for CAR generation is increasingly attracting interest [19]. T cells are categorized into subsets based on specific surface molecules they express. These phenotypic distinctions correlate with the migratory and functional properties of each T cell subset [20]. Studies indicate that various T cell phenotypes in CAR-T therapy play distinct roles in cytotoxicity and safety [21]. Consequently, a potential strategy to boost CAR-T therapy efficacy in tumor eradication involves selectively choosing or modifying an appropriate T cell subset during manufacturing (Fig. 2, Table 1).

$CD4^+$ CAR-T cells exhibit heightened production of IFN-γ to enhance cytotoxicity against tumor cells, while $CD8^+CD161^+$ CAR-T cells release granzyme and perforin, in tumor cells, CAR-γδT cells secrete cytokines and chemokines, CAR-NKT cells produce granzyme B and perforin to induce tumor apoptosis, and CAR-TN/SCM cells increase persistence and eliminated exhaustion and memory phenotype.

$CD4^+$ T cells

Infusing a specific ratio of $CD4^+$ CAR-T cells enhances cytotoxic capacity and produces a synergistic effect, attributed to their increased bystander effect [22] and persistence [23] compared to $CD8^+$ CAR-T cells [24, 25]. A clinical trial with patients receiving a 1:1 ratio of $CD4^+$ to $CD8^+$ CAR-T cells demonstrated enhanced anti-tumor efficacy [26]. Subsequent studies suggest that IFN-γ upregulation contributes to this enhanced efficacy [22]. Lisocabtagene maraleucel (liso-cel), a CD19-targeting autologous CAR-T therapy, has demonstrated promising outcomes, achieving high response rates and minimal severe side effects at specific $CD4^+$ and $CD8^+$ T cell

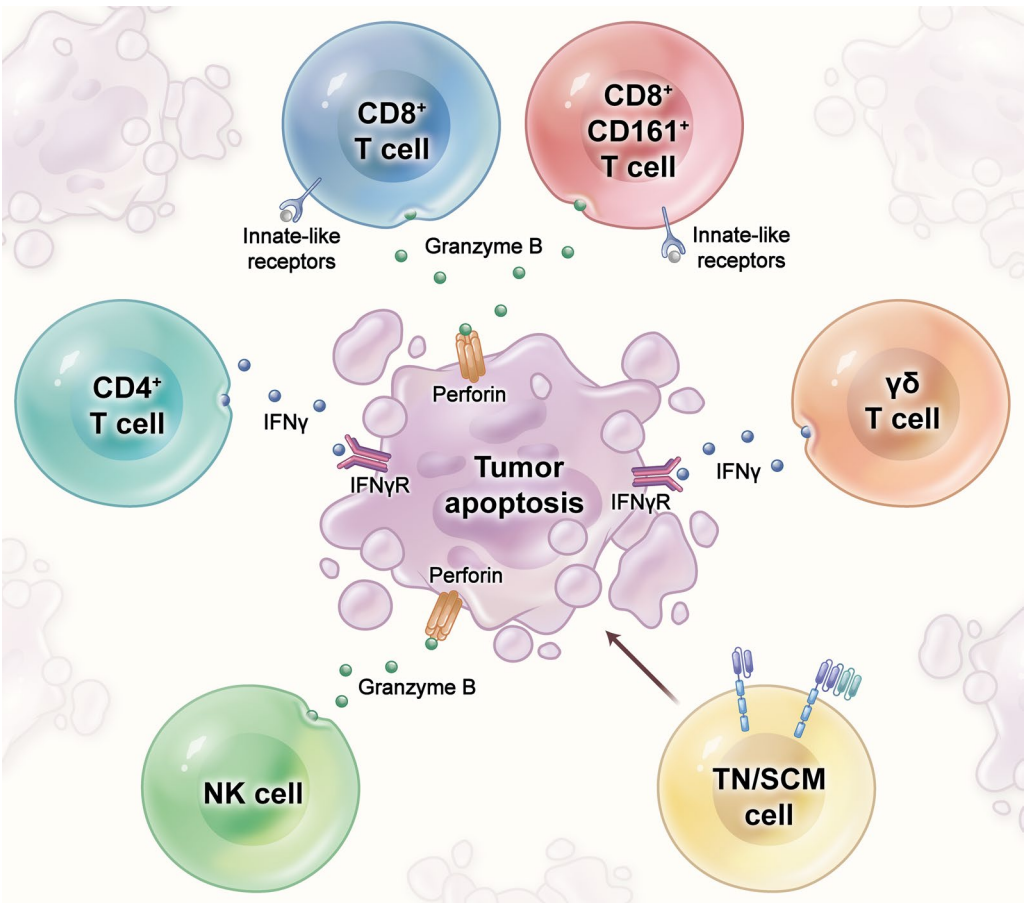

Fig. 2 Subsets of CAR-T cells

Table 1 Subsets of CAR-T cells

Subset name	Replace subset	Function	Secreted cytokines
$CD4^+$ T	$CD8^+$ T	Bystander effect and stronger persistence characteristics	IFN-γ
$CD8^+CD161^+$ T	$CD8^+$ T	Identify various T cell subsets and have a more robust ability to kill foreign cells	Granzyme, perforin
γδ T	αβ T	Mitigate cancer cells and act through increased production of cytokines and chemokines, as well as their role of antigen-presenting cells in activating αβ T cells	Increased production of cytokines and chemokines
NKT	Ordinary CAR-T	Increased levels of perforin and granzyme B and have an enhanced anti-tumor effect	Perforin and granzyme B
Naive/stem memory T (TN/SCM)	Unselected T cell	Advantages of increased persistence and eliminated exhaustion and memory phenotype	–

doses [27]. Nevertheless, further clinical investigations are needed to assess liso-cel's efficacy compared to other products, regardless of T cell subset differences, focusing on cytotoxicity and bystander effects in vivo.

$CD8^+CD161^+$ T cells

$CD8^+CD161^+$ T cells have been identified as a promising starting cell population for CAR-T cell therapy. CD161 distinguishes various T cell subsets, notably a polyclonal subset expressing CD8 αβ T cells, known for enhanced cytotoxic and memory characteristics [28]. Konduri et al. conducted a comprehensive study on a unique T-cell subset within the context of CAR-T cell therapy. Their research revealed that isolated human $CD8^+CD161^+$ T cells exhibited increased cytotoxicity against foreign cells compared to $CD8^+CD161^-$ T cells. The study indicated that $CD8^+CD161^+$ T cells, transduced with CAR and showing increased expression of granzyme B, perforin, and innate-like receptors, significantly enhanced anti-tumor protection and survival [29].

γδ T cells

To improve the effectiveness and safety of CAR-T cells, researchers are exploring γδ T cells as substitutes for αβ T cells, attributed to their lower risk of graft-versus-host disease (GvHD) and unique major histocompatibility complex (MHC)-independent mechanisms. Studies have shown that CAR-γδ T cells can effectively target cancer cells, including bone metastatic prostate, breast, and gastric cancer cells, through enhanced cytokine and chemokine production, and by acting as antigen-presenting cells that activate αβT cells [30–33]. Furthermore, the combined effect of zoledronate (ZOL), which is approved for pathological fractures in metastatic castrate-resistant prostate cancer and found to activate CAR-γδT cells independently of CAR, and the endogenous Vγ9Vδ2 T cell receptor enhances γδT cell function, with ZOL pretreatment showing significant efficacy [32]. This observation indicates that the TCR signaling pathway plays a significant role in enhancing the cytotoxicity of CAR-γδT cells [32]. Multiple researchers are refining CAR-γδT cells by engineering Vδ1 T cells, a γδT cell subset with a 4-1BB/CD3ζ and GPC-3 CAR to boost their naïve memory phenotype, resulting in increased proliferation, cytokine production, and cytotoxic activity against hepatocellular cancer cells in vitro [34].

NKT cells

An additional strategy to enhance both effectiveness and safety in CAR-T processing is to incorporate natural killer T (NKT) cells as foundational elements. NKT cells are a specialized small population of T cells that play a critical role in connecting the innate and adaptive immune system. It is known that NKT cells, despite having a TCR, do not react with peptide loaded onto MHC, but instead react to lipid antigens presented on the MHC-I-like molecule CD1d. Unlike MHC molecules which present peptides bound into a groove on their surface, CD1d has two deep pockets in which the acyl chains of lipid antigens can bind leaving the head group presented to the NKT cell TCR [35]. NKT cells can be stimulated through lipid antigens presented on CD1d and show increased cytotoxicity, better safety profiles, and lower GvHD incidence compared to conventional CAR-T cells [19, 36–38]. Compared to standard CAR-T cells, CAR-NKT cells exhibit superior anti-tumor efficacy by activating apoptosis pathways via increased perforin and granzyme B levels [37]. IL-15, a pro-survival cytokine essential for T-cell memory [39], also plays a crucial role in the development and homeostatic maintenance of NKT cells, protecting them from hypoxia. Its transgenic expression in adoptively transferred NKTs significantly boosts their cytotoxic activity [40]. Consequently, co-expressing IL-15

with any costimulatory domain can increase CAR-NKT abundance and enhance cytotoxicity in vitro [41].

TN/SCM cells

Moreover, a study on naive/stem memory T cells (TN/SCM) engineered with CAR demonstrated significant antineoplastic activity in hematopoietic stem/precursor cell-humanized mice. This approach provided increased persistence, reduced exhaustion and memory phenotypes compared to unselected counterparts [42]. The application of CAR- TN/SCM cell therapy has been shown to enhance both the efficacy and safety of CAR-T treatments, with reduced side effects. Recent studies have reported promising results for CD19 and CD19/20 CAR-T cells derived from TN/SCM cells, showing enhanced activity and safety in adults with relapsed/refractory B-ALL and NHL [43, 44].

Overall, this discussion highlights the selection of T cell subsets as innovative platforms to enhance the antitumor efficacy of CAR-T cell therapy. This is evidenced by $CD4^+$ CAR-T cells enhancing cytotoxicity through increased IFN-γ production, $CD8^+CD161^+$ CAR-T cells targeting HER-2 with specific cytokines, CAR-γδT cells secreting cytokines and chemokines, CAR-NKT cells producing granzyme B and perforin to induce apoptosis, and CAR-TN/SCM cells showing significant antineoplastic activity as a novel therapeutic strategy.

CAR-T structure modification (Fig. 3)

(1) Novel targets of CAR-T cell therapies in solid tumors LunX, CLDN6, EphA2,, VEGFR-2, tmTNF-α, B7-H3, TAG72 and dual targets CD123/CLL1, CD19/CD22, PRDM1/NR4A3, TAG72/CD47, CD276/FGFR4; (2) VHH and Costimulatory modification: IL15 and IL21 co-stimulation, C3aR co-stimulation, IL12 integration, 4-1BB and optimized CD28 co-stimulation and PD1/CD28 chimeric-switch receptor (CSR); (3) overcoming immunosuppressive TME: IFN-γ expression, blocking PD-1/PD-L1 immune checkpoints, overexpress chemokines, using bispecific T-cell engagers, tTF-NGR expression; the secretion of anti-CD40 antibodies, CAR-T cells release a CD47-SIRPα blocker CV1, PD-1-TREM2 scFv-secreting CAR-T cells, IL15 and IL21 co-stimulation.

Novel targets

The efficacy of CAR-T cells in solid tumors is limited by off-target effects due to the heterogeneity of solid tumors and the absence of specific tumor antigens [45]. To target solid tumors effectively, it is crucial to identify ideal antigens that are exclusively expressed in tumor tissues. Targeting various stages of tumor development, such as proliferation, invasion, migration, angiogenesis, metastasis, and apoptosis, could enhance T cell attachment to solid tumors and increase cytotoxicity to eradicate tumor cells.

LunX, known to activate the Erk and JNK signaling pathways by directly interacting with the 14-3-3 protein, has been identified as a facilitator of tumor growth, metastasis, and invasion [46]. Hu et al. identified high levels of LunX expression in NSCLC cell lines using immunofluorescence techniques. Consequently, LunX-CAR T cells were generated and co-cultured with LunX-positive cell lines to assess if they could elicit stronger responses than CD19-CAR-T cells. The results showed that LunX-CAR-T cells significantly increased IFN-γ, IL-2, and TNF-α levels in the presence of LunX-positive tumor cells ($p < 0.001$), suppressed lung cancer cell growth, and enhanced survival in a PDX mouse model [47].

The oncofetal antigen Claudin 6 (CLDN6) is highly expressed in germ cell tumors (GCT), epithelial ovarian cancer (EOC), and endometrial carcinoma, while being nearly absent in healthy tissues. Adrian et al. discovered that esophageal adenocarcinoma (EAC) and gastric adenocarcinoma (GAC) with elevated CLDN6 levels upregulate pathways related to tumor proliferation, including cell cycle progression, DNA replication, and receptor/extracellular matrix interactions [48]. In the recent phase 1/2 BNT211-01 trial, CLDN6 CAR-T cells demonstrated significant efficacy in relapsed/refractory CLDN6-positive solid tumors, particularly in germ cell tumors, which showed the highest objective response rate. Furthermore, treatment with CLDN6-specific CAR-T cells maintained manageable safety profiles, even at high doses [49].

Targeting key angiogenic factors can significantly suppress tumor growth. The receptor tyrosine kinase (RTK) EphA2 is overexpressed in several solid tumors but minimally in normal lung tissues [50]. Based on this, EphA2-specific CAR-T cells were constructed, and their anti-tumor effects evaluated in a xenograft SCID Beige mouse model of EphA2-positive NSCLC. Ultimately, tumors in mice treated with EphA2-specific CAR-T cells showed a decreasing trend correlated with increased IFN-γ expression [51]. Vascular endothelial growth factor 2 receptor (VEGFR-2), another RTK, is abnormally expressed on tumor endothelial cells. Zhong et al. reported on KDR-CAR-T cells, modified with a CAR targeting VEGFR-2/KDR, that could inhibit tumor angiogenesis. KDR-CAR-T cells effectively targeted and killed lung cancer KDR-A549 xenografts, accompanied by increased IFN-γ and granzyme B secretion, highlighting their potent efficacy against lung cancer and other vascularized solid tumors [52]. Additionally, B7-H3 is commonly present on solid tumor cells and associated blood vessels [53]. Zang et al. developed a new generation of CAR-T cells (TOP CAR) modified with a unique monoclonal antibody targeting B7-H3, enhancing T cell

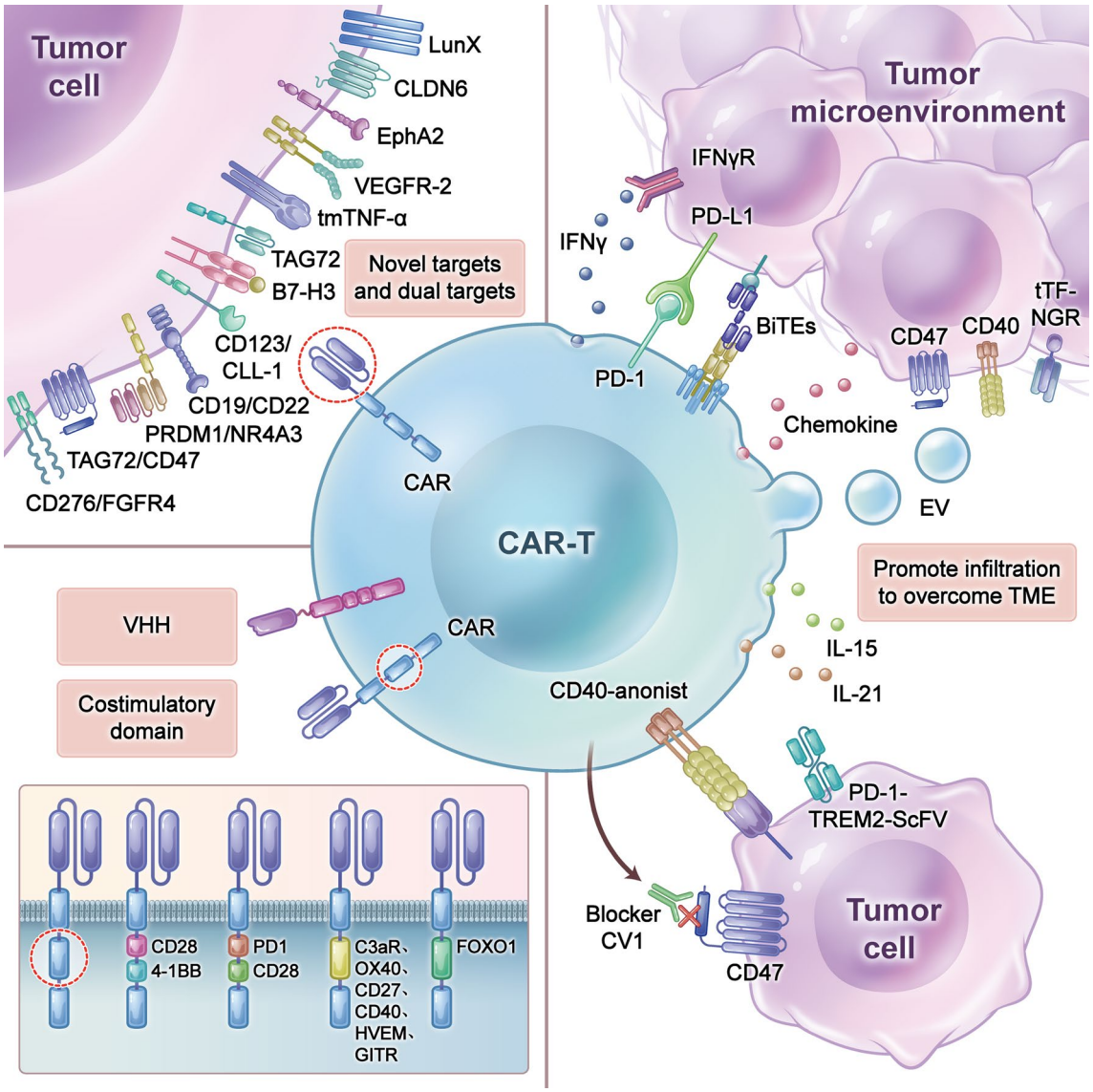

Fig. 3 CAR-T structure modification to enhance killing potential

attachment to solid tumors and improving anti-tumor effects [54].

Additionally, transmembrane tumor necrosis factor-alpha (tmTNF-α), a type II membrane protein, can be hydrolyzed into secreted tumor necrosis factor-alpha (sTNF-α), leaving a residual N-terminal fragment on the cell surface [55]. The expression of tmTNF-α is upregulated in over 60% of ductal breast cancer (BC) patients and is positively associated with tumor size and lymph node metastasis, primarily due to its anti-apoptotic functions mediated by the tmTNF-α/NTF-ERK-GST and tmTNF-α/NTF-NF-κB pathways [56]. Ba et al. designed CAR-T cells specifically targeting tmTNF-α and observed that these cells showed increased cytotoxicity against tmTNF-α-expressing BC cells, along with enhanced secretion of IFN-γ and IL-2 in vitro, leading to significant tumor regression [57].

In addition to previously discussed topics, researchers are identifying more targets that influence tumor growth. For instance, CAR-T cells targeting TAG72 have shown efficacy in advanced ovarian cancer preclinical animal models [58]. These findings underscore the significant potential of CAR-T cells that target specific antigens in treating solid tumors.

Dual targets

Furthermore, dual-target CAR-T cells expand the cytotoxic range beyond that of single-target CAR-T cells

and enhance anti-tumor efficacy in vitro experiments. Notably, CAR-T cells co-cultured with dual-target counterparts produced significantly higher levels of IFN-γ compared to those cultured with single-target CAR-T cells [59, 60]. Of those, CAR-T cells targeting CD123/CLL-1 outperform single-targeted counterparts in killing leukemia cells by releasing higher level of cytokines. Similarly, bispecific CAR-T cells targeting CD19/CD22 exhibit enhanced cytotoxicity against B-cell malignancies [61, 62]. Clinically, the tandem CD19/CD22 CAR-T group showed significantly greater efficacy than the CD19 CAR-T group [63].

Flexible design of dual-target CAR-T cells can significantly enhance targeting accuracy of tumor cells and reduce off-target toxicity to non-tumor tissues. Consequently, dual-target CAR-T cell therapy can improve efficacy against solid tumors and extend patients' survival. Recently, an increasing number of studies have reported on dual-target strategies specific to solid tumors. CAR-T cells targeting both PRDM1 and NR4A3 have been shown to counteract exhaustion of tumor-infiltrating T cells and enhance antitumor responses [64]. CAR-T cells targeting both TAG72 and CD47 demonstrated enhanced cytotoxicity against ovarian cancer cells [65]. Dual CAR-T cells targeting CD276 and FGFR4 effectively cleared Rh4 tumors in mouse models [66]. Overall, dual-target CAR T cells possess promising therapeutic potential.

Costimulatory domain

The inclusion of costimulatory domains in CAR is crucial for stimulating and proliferating CAR-T cells, with ongoing research aimed at identifying the most effective costimulatory molecules. To enhance CAR-T cell therapy efficacy in solid tumors, researchers have introduced novel costimulatory domains, cytokine genes, and cytokine receptors into T cells, improving their persistence and antitumor functionality [67].

The most common costimulatory domains, CD28 and 4-1BB, are critical for CAR-T cells. CD28-costimulated CARs release more cytokines and show superior antitumor efficacy compared to 4-1BB-costimulated CARs [3, 68]. However, CD28 co-stimulation has been associated with increased susceptibility to exhaustion and reduced persistence, with a higher incidence of neurological toxicities, although these effects are not solely attributable to CD28 signaling. Long AH's research suggests that reduced persistence results from gene expression related to exhaustion, and modifying the CD28 costimulatory domain could alleviate this issue [69]. Consequently, Roselli et al. developed CD19-specific CAR-T cells that include 4-1BB and a mutant CD28 (featuring only the PYAP signaling motif, mut06). The results revealed that co-stimulation with both 4-1BB and optimized CD28 significantly enhanced CAR-T cells' antitumor activity by improving the central memory phenotype, boosting proliferation, and increasing recruitment of lymphocyte-specific protein-tyrosine kinase to the CAR [70]. These approaches have also been applied in ovarian cancer studies [71].

The insertion of the C3aR domain into CAR and the development of BB-ζ-C3aR CAR-T cells targeting CD19 or BCMA have shown significant cytolytic activity against these tumor cells in vitro. In vivo studies in ALL and multiple myeloma (MM) xenograft mouse models have demonstrated that BB-ζ-C3aR CAR-T cells effectively reduce tumor burden and enhance overall survival (OS). Mechanistically, BB-ζ-C3aR CAR-T cells tend to induce cell death and inhibit the Th17 phenotype of regulatory T cells (Tregs). These findings indicate that C3aR stimulation boosts CAR-T cell efficacy by suppressing Th17 amplification and preventing memory T cell invasion of tumor cells [72].

Unlike conventional costimulatory signals, which are activated alongside T cell receptor signals during T cell activation, a novel costimulatory signal has been developed to operate independently of T cell receptors, allowing for autonomous T cell stimulation. Researchers screened several stimulatory receptors, including ICOS, CD27, CD40L, and OX40, and found that OX40 offers the most significant amplification effect for CAR-T cells. OX40, a member of the TNF superfamily, is a critical T cell costimulatory signal. Upon binding to its ligand OX40L (also known as CD252), three OX40 protein molecules assemble into a trimeric complex, which then forms a hexametric structure. This complex initiates downstream signaling pathways such as NF-κB, PI3K, and AKT, resulting in sustained activation that enhances cytokine production, prolongs survival, and increases the cytotoxicity of effector T cells [73].

CD27 is expressed on $CD4^+$ and $CD8^+$ T cells, NK cells, and B cells, while its ligand, CD70, is found on antigen-presenting cells like dendritic cells and macrophages. Activation of CD27 on T cells initiates downstream signaling that activates NF-κB, leading to T cell activation, differentiation, and clonal expansion. CD27 stimulated CAR-T cells demonstrate enhanced antigen-stimulated effector function and increased expression of anti-apoptotic proteins, prolonging survival in a human ovarian cancer cell-mouse model compared to original CAR-T cells [74].

CD40 is present on myeloid-derived antigen-presenting cells such as dendritic cells, granulocytes, and macrophages. Its ligand, CD40L, is predominantly found on activated T cells and NK cells. The interaction between CD40 and CD40L on antigen-presenting cells triggers signals that stimulate and proliferate cytotoxic T cells. To

enhance costimulatory signaling, CD40 CAR-T cells are engineered with additional molecules like CD79A in the B-cell signaling domain, activating downstream pathways such as NF-κB, NFAT, and AP-1. Compared to CD28 or 4-1BB costimulated CAR-T cells, CD79A/CD40 CAR-T cells showed higher NF-κB and p38 activity and greater proliferation when co-cultured with CD19-expressing cells in vitro. In a mouse model using Raji tumor cell vaccination, CD79A/CD40-stimulated CAR-T cells showed superior antitumor activity and proliferation compared to those stimulated by CD28 or 4-1BB [75].

HVEM and GITR have recently been incorporated into CAR-T cell constructs. HVEM is expressed on various immune cells, such as T cells, B cells, NK cells, and dendritic cells. The interaction of HVEM with its ligands, LIGHT or LTα, activates and proliferates T cells, while its binding to BTLA inhibits T cell activation. The structure and function of HVEM in CAR-T cells have been documented in only one report so far. HVEM G2 CAR-T cells show greater anti-tumor activity and increased production of proinflammatory cytokines like IL-2, TNF-α, and IFN-γ compared to CD28 or 4-1BB G2 CAR-T cells [76]. Targeted CD19 GITR G2 CAR-T cells significantly inhibit Raji tumor cell growth in the body. Additionally, GITR co-stimulation in T-cell lymphoma and melanoma models shows enhanced cytotoxicity compared to DAP-10, CD28, 4-1BB, ICOS, and OX40 co-stimulated CAR-T cells in vivo [77].

Additionally, several studies have shown that modifying TME increases CAR-T cell efficacy in gastric cancer [78]. Chen et al. discovered that c-Met CAR-T cells released higher levels of cytokines like IFN-γ, TNF-α, IL-6, and IL-10 than Mock T cells, while cMet-PD1/CD28 CAR-T cells further increased IFN-γ and TNF-α secretion and reduced IL-6 compared to c-Met CAR-T cells [79]. In the cMet-CAR context, the cMet-PD1/CD28 CAR incorporates a PD1/CD28 chimeric-switch receptor (CSR) that converts the inhibitory PD-1 signal into an activating CD28 signal, enhancing immunosuppression. Consequently, the PD1/CD28 CSR enhances the long-term antitumor effects and killing ability of cMet-CAR-T cells [80]. Moreover, CAR-T cells targeting c-Met showed no significant off-target toxic effects on normal organs [81]. Another study found that CD19-PD-1/CD28-CAR-T cells, a new anti-CD19 CAR-T cell therapy, elicit a potent and lasting anticancer response and are effective post-CD19-CAR-T failure in relapsed/refractory DLBCL [82].

The efficacy of CAR-T cells' immune attack is often reduced by tumor-induced immunosuppressive signals, including inhibitory cytokines such as IL-4, IL-10, and TGFβ. Interleukins (ILs) are cytokines that play a critical role in CAR-T cells' anti-tumor processes. Previous studies have shown that IL-12 accumulates specifically in tumor tissues, alleviating suppression by tumor stroma and enhancing IFN-γ release [83]. Hombach et al. engineered IL-12 into the outer part of the CAR, preserving the functionality of both the CAR and IL-12. Interestingly, IL-12-CAR-T cells exhibited antigen-independent and HLA-E restricted cytotoxic abilities, capable of killing cancer cells regardless of the presence of a CAR-targeted antigen [84]. Additionally, IL-4 released by tumor cells within the TME can counteract T cell effector functions, whereas IL-15 can enhance the antitumor activity of CAR-T cells [85].

In summary, the CAR construct is central to CAR-T cells, critically determining their cytotoxicity and antitumor efficacy, particularly in treating solid tumors with significant phenotypic diversity. By developing novel targets or optimizing costimulatory domains, CAR-T cells can be enhanced to have higher specificity, improved antitumor capabilities, and increased effector molecule secretion.

Disruption of key genes in CAR-T cell (Fig. 4)

(1) AS1842856 suppress FOXO1 in tumor cell to control tumor growth; (2) Knocking out SHP-1 increase expression of TNF-α, IL-2, IFN-γ, thus increase cytotoxicity; (3) CRISPR-Cas9 targeting cbl-b alleviates CAR-T exhaustion, diminish tumor progression,upregulate TNF-α and IFN-γ, thus increase cytotoxicity. (4) The presence PIK3R3 in CAR-T cells led to a significant enhancement of AP-1 and NF-κB signaling pathways, interleukin-2 (IL-2) production, and efficacy in tumor eradication; (5) The introduction of specific mutations, such as PIK3CD, into CAR-T cells resulted in the development of enhanced CAR-T cells with increased anti-cancer capabilities; (6) The CAR into the TET2 gene may possess enhanced proliferation capacity, anti-tumor efficacy, and longevity. (7) NOTCH1 signaling upregulates antigen response and $CD4^+$ and $CD8^+$ CAR-T cells, as well as effector cytokines.

Modifying CAR-T cells by targeting genes and regulation of gene expression involved in cellular proliferation, differentiation, exhaustion, and cytokine production is a growing research area. The approaches include gene editing techniques like CRISPR-Cas9 and pharmacological agents that activate or inhibit signaling molecules [86, 87]. Several research is ongoing into disruption of key genes in CAR-T cells.

FOXO1 acts as a transcription factor and plays a crucial role in enhancing CAR-T cell activity by regulating the expression of various genes, affecting the behavior and functions of CAR-T cells. Recent studies have shown that increasing of FOXO1 expression can make CAR-T cells behave like stem cells and enhance their cytotoxicity

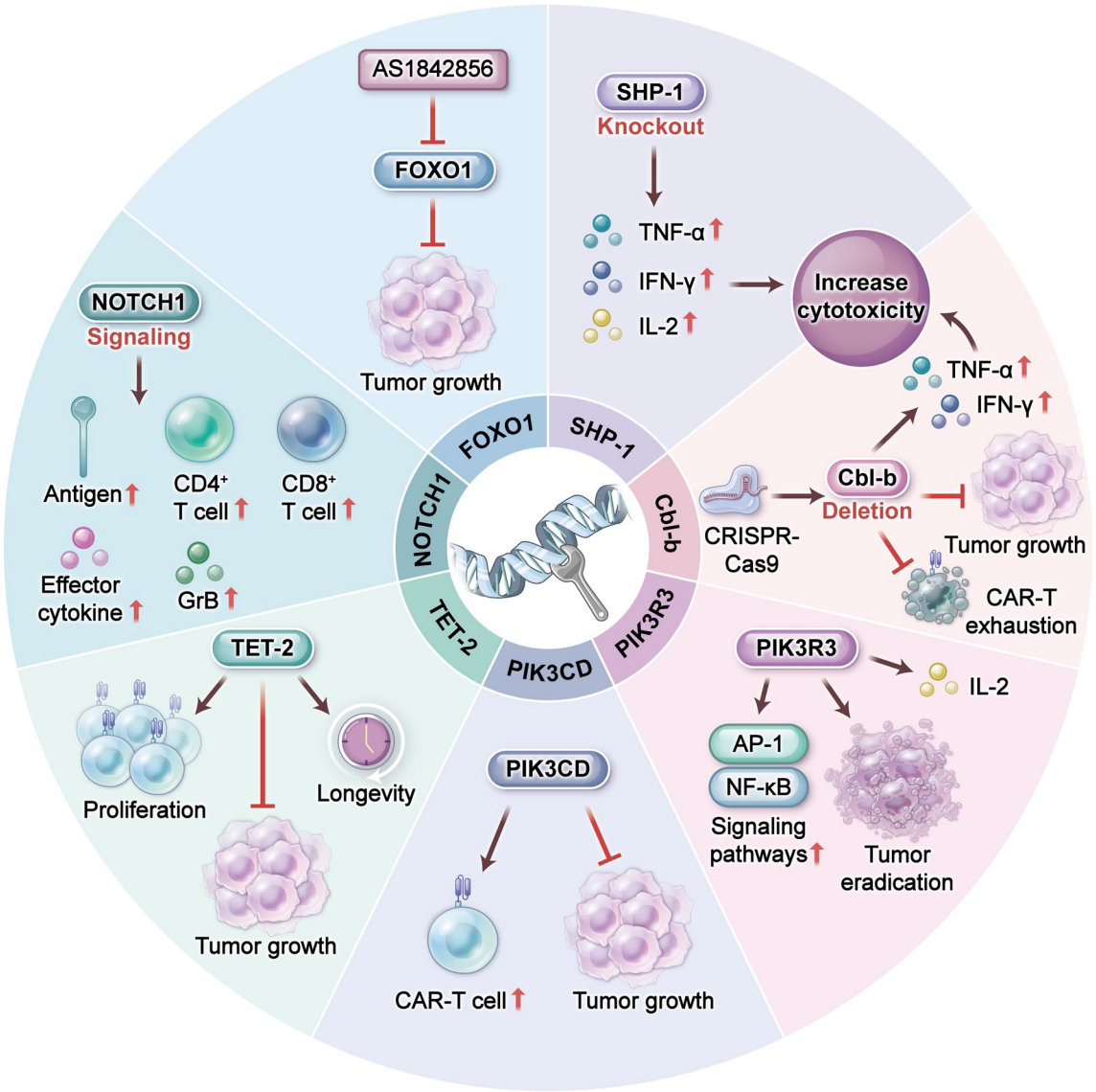

Fig. 4 Disruption of key genes in CAR-T cell

in vivo [88]. It has demonstrated that FOXO1 promotes a stem-like phenotype in CAR-T cells, enhancing their persistence and maturation into more effective cancer-fighting cells. Moreover, CAR-T cells with high FOXO1 expression exhibited superior cytotoxicity, maintaining effectiveness for extended periods post-injection in mice and improving efficacy against solid tumor [89]. Based on the encouraging results, researchers plan to initiate clinical trials to verify the efficacy and safety of high FOXO1 CAR-T cells for cancer treatment within the next two years. AS1842856 is an inhibitor that binds to the transcription factor FOXO1 [90]. Suppressing FOXO1 by AS1842856 in T cells has been linked to the development of stem cell memory characteristics, increased proliferation and migration, and enhanced cytotoxic abilities [90].

Additionally, considering the critical role of IL15 in T cell differentiation, research shows that CAR-T cells with elevated levels of IL-15 and FOXO1 exhibit enhanced longevity and increased efficacy in tumor suppression. In vivo, genetically modified CAR-T cells significantly reduce tumor size by over 30% and increase survival rates by 50% compared to conventional CAR-T cells. Ongoing efforts are focused on initiating multiple clinical trials to implement the dual regulation approach of IL-15 and FOXO1 in cancer treatment [91]. Moreover, the cytotoxicity of CAR-T cells enhanced after co-culturing with NOTCH-specific monoclonal antibodies [92]. NOTCH1

agonism directs T cells towards a phenotype with a stronger antigen response, enhanced interaction between $CD4^+$ and $CD8^+$ CAR-T cells, and increased the secretion of effector cytokines [92, 93].

Utilizing CRISPR-Cas9 to target genes related to cbl-b, a member of the RING family of E3 ubiquitin ligases and a molecular adaptor, researchers created Cbl-$b^{-/-}$CAR-T cells [94, 95]. Deletion of cbl-b has been shown to reduce exhaustion in CAR-T cells, increase levels of IFN-γ and TNF-α and enhance cytotoxicity [95]. Recent reports have indicated that artificial intelligence has improved the specificity of the CRISPR-Cas9 system, enhancing its clinical therapeutic applications [96].

CAR-T cells with a memory-like phenotype and reduced exhaustion gene expression potentially present prolonged persistence. For example, a single CAR-T cell clone with CAR integrated into the TET2 gene has shown enhanced proliferation, antitumor efficacy, and longevity [97]. Employing strategies that target T cell activation and proliferation is also effective. Knocking out SHP-1, a protein tyrosine phosphatase that inhibits T cell activation and proliferation, in CD133-CAR-T cells has improved apoptosis in CD133-positive glioma cell lines. The study also showed SHP-1 knocking out CAR-T cells displayed enhanced cytotoxicity, with increased release of TNF-α, IL-2, and IFN-γ in vitro [98].

In addition, researchers identified variants and mutations with the potential to enhance multiple indicators of T cell activity, crucial for the eradication of cancer cells. The team found loss-of-function mutations in specific regions of genes including PIK3CD and its regulatory subunits PIK3R1, LCK, SOS1, AKT1, and RHOA. Introducing specific mutations, like those in PIK3CD or PIK3R1, into CAR-T cells will enhance the function of cytotoxic [99]. A separate team found that in $CD4^+$ cutaneous T-cell lymphoma, a fusion of CARD11 and PIK3R3, termed CARD11-PIK3R3, occurs. This mutation in CAR-T cells significantly enhanced AP-1 and NF-κB signaling, increased IL-2 production, and improved tumor eradication efficacy both in vitro and in vivo [100].

In conclusion, these findings suggest new strategies for optimizing CAR-T cell therapy by enhancing cell therapy efficacy through regulation of key genes. The results highlight promising avenues for advancing CAR-T cell therapies. Future improvements in CAR-T cell therapy may utilize strategies targeting pathways involved in cellular proliferation, differentiation, exhaustion, and cytokine production.

Variable heavy domain of heavy chain antibodies (VHH)

Recently, VHH single-domain antibody fragments, also known as nanobodies, have been utilized to optimize CAR-T cells [101–103]. ScFv, composed of variable heavy and light chains of monoclonal antibodies connected via a flexible linker, are the conventional antigen-binding domains of CARs. VHH exhibits similar binding affinities to full-sized monoclonal antibodies and possesses characteristics such as stability, solubility, and high-yield expression, can be a superior alternative to scFv [104–106]. A study demonstrated that CAR-T cells releasing anti-CD47 VHH improved innate immune system engagement, increased IFN-γ secretion and enhanced cytotoxicity [102]. Levels of IFN-γ and TNF-α were comparable to those of standard CAR-T cells in studies of VHH-CAR-T cells [103]. Based on the encouraging results, several VHH-based CAR-T studies are in clinical trials [107]. Ciltal-cel, a VHH-based BCMA-targeting CAR-T therapy, has been approved in the USA for patients with relapsed/refractory multiple myeloma [108]. Therefore, VHH-based CAR-T therapies hold promising prospects for clinical application.

Promote infiltration and correcting immunosuppressive TME

TME in tumor is complex and characterized by changes in extracellular matrices, hypoxic regions [109], and immunosuppressive cells including Tregs, MDSCs, and tumor-associated macrophages (TAMs) [10]. TME cells respond to various stimuli from tumor cells and other TME constituents, including growth factors, cytokines, chemokines, and enzymes. These stimuli induce cell polarization, leading to complex interactions within the TME that influence both pro-tumor and anti-tumor processes [110]. The immune-evasive and immunosuppressive nature of the TME significantly reduces the therapeutic efficacy of CAR-T cells in treating solid tumors. Boulch M et al. showed that IFN-γ produced by CAR-T cells can enhance their killing rate and promote tumor regression by interacting with the TME, highlighting the crucial role of IFN-γ and IL-12 in this crosstalk for optimal cytotoxic activity [111]. Therefore, increasing the expression of IFN-γ helps to overcome the immunosuppressive TME and enhance the antitumor efficacy of CAR-T cell therapy.

Immune checkpoints, such as PD-1/PD-L1, play a crucial role in inhibiting T cell proliferation and function [112–114]. EGFR CAR-E27-CCR6 T cells, engineered to express CCR6 and secrete the PD1-blocking single-chain antibody fragment E27, demonstrate potent cytotoxicity and increased secretion of IL-2 and IFN-γ in NSCLC xenograft model [115]. On the other hand, TGF-β secreted by tumor cells in the TME activates Tregs and suppresses cytotoxic function of T cells [116]. Consequently, Chen devised a strategy to boost antitumor immunity by engineering CAR-T cells to release a bispecific trap protein targeting PD-1 and TGF-β concurrently, leading to

reduced inhibitory T-cell signaling and increased effector molecule production, thus enhancing antitumor efficacy while avoiding systemic toxicities [117]. Moreover, Chen et al. observed that c-Met CAR-T cells released more cytokines like IFN-γ, TNF-α, and IL-6 compared to Mock T cells. cMet-PD1/CD28 CAR-T cells, incorporating the PD1/CD28 CSR, further increased IFN-γ and TNF-α secretion while reducing IL-6, indicating enhanced long-term antitumor effects [79, 80].

Nanotechnology has been applied in CAR-T therapy and cancer vaccines [118]. The combination of immune checkpoint inhibitors and nanoparticles has proven to be an effective strategy for enhancing anti-tumor capabilities, highlighting the significant role of nanoparticles in advancing cancer immunotherapy and modifying the TME. Interestingly, CAR-T cells can act as mini drug-stores by producing a specific fusion protein, tTF-NGR, which includes truncated tissue factor (tTF) and an NGR motif (asparagine-glycine-arginine). This fusion protein has pro-coagulatory effects and induces hypoxia, causing tumor cell death when vascular endothelial cells relocate within tumor tissues. GD2-tTF-NGR CAR-T cells specifically target GD2 and release tTF-NGR into the TME. As a result, these CAR-T cells show enhanced infiltration and superior therapeutic activity in human neuroblastoma xenografts compared to control cells expressing inactive tTF-NGR. Furthermore, experiments in vitro validated the mechanism through which hypoxia enhances T cell cytolytic activity [119].

CD40, a member of the TNF receptor superfamily, is predominantly found in antigen-presenting cells (APCs), notably dendritic cells (DCs) [120]. The interaction between CD40 and transiently expressed CD40L on T cells post-TCR stimulation activates DCs and cytotoxic T cells, enhances their recruitment to the tumor, and induces apoptosis in CD40+ tumor cells [121]. Kuhn et al. developed CD40L-expressing CAR-T cells, which remodeled the TME, increased IFN-γ and TNF-α production, and enhanced antitumor efficacy [122]. Using the piggyBac transposon system, Zhang et al. developed meso3-CD40 CAR-T cells targeting mesothelin (MSLN), which also secrete anti-CD40 antibodies. Compared to meso3 CAR-T cells, meso3-CD40 CAR-T cells showed increased secretion of cytokines such as IFN-γ and a more potent cytotoxic effect on target cells [123]. CD47, a ligand frequently overexpressed in various cancers and known for the "do not eat me" signal via SIRPα, plays a key role in immune evasion [124, 125]. A potential therapeutic approach involves using a soluble truncated SIRPα protein variant (CV1) and a high-affinity CD47 blocker to inhibit the CD47-SIRPα signaling pathway [126]. Based on the results, Dacek designed OrexiCAR-T cells to locally release CV1 at the tumor site, treating cancers in conjunction with a monoclonal antibody that targets orthogonally [127].

Triggering TREM2, a member of the immunoglobulin superfamily, contributes to the maturation of TAMs by recognizing ligands like lipids and apoptotic debris in the TME [128]. Consequently, Chen et al. developed PD-1-TREM2 scFv-secreting CAR-T cells that inhibit PD-1/PD-L1 interactions through autocrine signaling These scFvs also blocked TREM2 interactions on TAMs and MDSCs, reducing immunosuppressive cells and boosting T-cell effector and antitumor functions. In a colorectal cancer mouse model, these CAR-T cells showed potent cytotoxicity, marked by increased IFN-γ and TNF-α levels [129].

Interleukins critically influence the proliferation, differentiation, and function of engineered T cells. IL-18 modifies the TME by increasing M1 macrophages, activated dendritic cells, and NK cells, while reducing M2 macrophages and Treg levels. Avanzi et al. engineered CAR-T cells to release IL-18, effectively modulating the TME and significantly enhancing their in vivo expansion, persistence, and survival [130]. Similarly, Glienke developed IL-18-secreting TRUCKs targeting GD2, which showed increased cytokine secretion and specific cytotoxicity towards target cells [131, 132]. IL-15 and IL-21 synergistically improve CAR-T cell efficacy in the TME [133]. NKG2D-CAR-T cells with IL-4R and IL-15R domains convert IL-4R inhibitory signals to IL-15R activation signals, increasing cytotoxicity against IL-4+ pancreatic cell lines [134]. IL-12 accumulates in tumor tissues, alleviating stromal suppression and increasing IFN-γ release [83]. Hombach et al. integrated IL-12 into the CAR's structure, maintaining the functionality of both the CAR and IL-12. Interestingly, IL-12-CAR-T cells showed antigen-independent, HLA-E restricted cytotoxicity, effectively killing cancer cells regardless of the presence of a targeted antigen [84]. Yin identified EGFR variant III and IL13Ra2 in glioma tissues and developed novel BiTE molecules using conformation-specific antibodies targeting EGFR and IL13Ra2 [135]. These BiTE-T cells showed enhanced activation and cytokine synthesis, displaying potent cytotoxicity against glioma targets [136].

Researchers have explored extracellular vesicles (EVs), including exosomes (< 150 nm) and larger vesicles (150–1000 nm), derived from the cell membrane that carry antigens from parent cells to aid in CAR-T cell development [137, 138]. A study on CD19EV-pretreated CAR-T therapy showed that CD19 EVs enhance CAR-T cell cytotoxicity by promoting their expansion, increasing CD19 expression on their surface, and programming them into effector-memory cells with higher IFN-γ and granzyme B secretion [139].

In summary, optimizing the complex interactions among modified CAR-T cells, endogenous immune cells, tumor cells, and various effector molecules is essential to maximize the efficacy and antitumor effects of novel CAR-T cells in treating solid tumors. Strategies to enhance CAR-T cell therapy include improving tumor-targeting specificity, increasing cytotoxicity, modifying the tumor microenvironment, and preventing tumor escape and relapse, all aimed at boosting the efficacy of CAR-T cells against tumors and advancing their clinical application.

Combinational strategies

How can molecular targeted drugs and CAR-T cells be combined to exert synergistic anti-tumor effects? Studies show that combining molecular targeted drugs and metabolic modulators with CAR-T cell therapy enhances synergistic anti-tumor effects, reduces therapy resistance, and improves cytotoxic efficacy [140].

Combining with metabolic modulators (Fig. 5)

(1) PRODH2 engineering of CAR-Ts to interfere proline metabolism; (2) Suppressing IDO1, Kynureninase and CD38 inhibition to affect tumor glucose metabolism; (3) Blockage of the glutamine/polyamine/hypusine axis to interfere glutamine, a preferable energy source for tumor cells. (4) Inhibiting S1P production by SphK1 stimulation on Tcm cells greatly increased tumor cell damage. (5) INO has been shown to downregulate enzymes associated with glycolysis while upregulating glutaminase and polyamine metabolism enzymes, suggesting a potential for metabolic reprogramming in CAR-T cells. (6) Supplementation with NAD+ can notably enhance the efficacy of T cell-based immunotherapy in combating tumors.

Metabolic processing significantly affects the persistence of CAR-T cells, their ability to overcome adverse TME, and their maintenance of immune characteristics [141, 142]. Potential strategies include modifying substrates involved in energy metabolism and

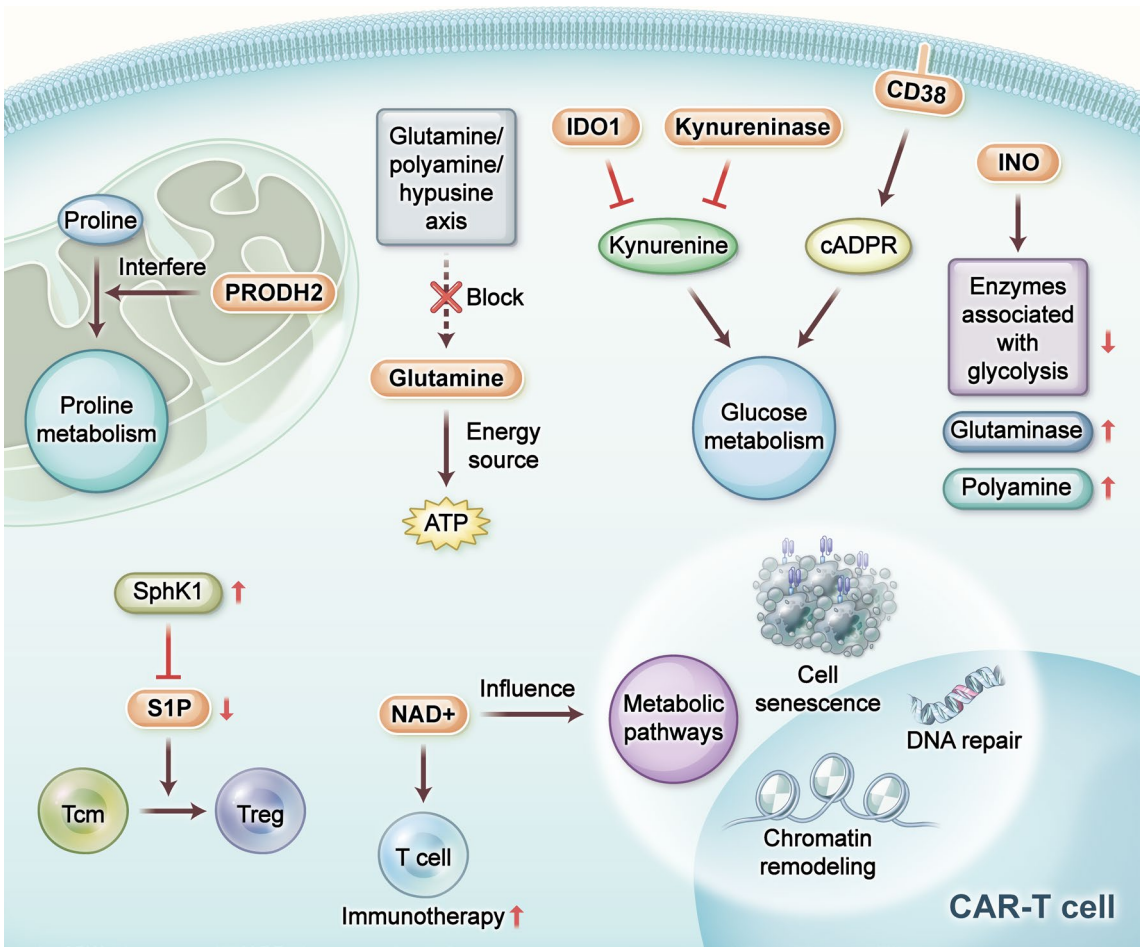

Fig. 5 Metabolic intervention in CAR-T cell

mitochondrial respiration, such as reducing glycolysis and enhancing glutaminolysis and polyamine synthesis.

Intracellular L-arginine metabolism is crucial for cytotoxic and proliferation of CAR-T cell [143, 144]. A study increased L-arginine levels by modulating proline metabolism, involving the enzyme Prodh2 encoded by the PRODH2 gene [145, 146]. Another gain-of-function study involving PRODH2-engineered CAR-T cells showed that PRODH2 knock-in regulates L-arginine, enhancing CAR-T cells' immunomodulatory effects against cancer [143, 146]. Meanwhile, adding sugar nanoparticles to the culture medium allows T cells to assimilate exogenous mitochondria, improving expansion and reducing exhaustion [147].

Targeting glucose metabolism by modifying CAR-T cells to overexpress Kynureninase enhances their ability to break down tumor-derived kynurenine (Kyn), increasing their cytotoxicity in the TME, even at high Kyn concentrations [148]. Kynurenine impairs CD8+ T cell expansion and reduces their secretion of granzyme B and IFN-γ, thereby suppressing CAR-T cell cytokine secretion and cytotoxic activity [148, 149].

Indoleamine 2,3-dioxygenase 1 (IDO1), a key enzyme in kynurenine synthesis, plays a role in tumor immune escape [150]. Combining IDO1 inhibitors with CAR-T cells can improve CAR-T therapies for solid tumors [151–153]. A recent study showed that inhibiting CD38 enzymatic activity enhances CAR-T cell efficacy by suppressing glycolytic metabolism [154].

Additionally, studies have shown that blocking the glutamine/polyamine/hypusine axis [155, 156] can enhance the therapeutic effectiveness of treatments [157]. Since glutamine is a preferred energy source for tumor cells, targeting glutamine metabolism could significantly improve CAR-T therapy [158].

Scientists have verified that inosine (INO) modulates CAR-T cell metabolism by reducing glycolysis and enhancing mitochondrial capacity, glutamine uptake, glutaminolysis and polyamine synthesis [152]. Additionally, inosine can reprogram T cells into a more stem-like phenotype, making it a preferable alternative to glucose-based media for cell culture. Increasing inosine levels in TME can also enhance antitumor capabilities.

Nicotinamide adenine dinucleotide (NAD+) is a coenzyme in REDOX reactions that influences critical cellular processes such as metabolism, DNA repair, chromatin remodeling, and cell senescence. Research has highlighted the crucial role of the nicotinamide phosphoribosyl-transferase-mediated NAD+ salvage pathway in T cell activation. Lower NAD+ levels in T cells impede glycolysis and impair mitochondrial function. Supplementing with NAD+ can significantly enhance the efficacy of T cell-based immunotherapies against tumors [159].

A study investigated the role of sphingosine-1-phosphate (S1P), a lipid produced by sphingosine kinase 1 (SphK1), in regulating T cell differentiation. The study found that the absence of SphK1 in T cells increased S1P levels, promoting the maintenance of the Tcm phenotype and inhibiting differentiation into Tregs. Researchers used a gene knockout strategy and chemical inhibitors to target SphK1, resulting in significant effects in a melanoma mouse model. Therefore, inhibiting the SphK1/S1P signaling pathway holds clinical potential for enhancing CAR-T cell efficacy [160].

Modifying metabolic pathways in CAR-T cells, tumor cells, and immune cells within the tumor microenvironment presents a promising strategy for enhancing CAR-T cell therapy.

Combining with molecular targeted drugs

Resistance to CAR-T cell immunotherapy can stem from CAR-T cells themselves, the tumor microenvironment, or the cancer cells. Tumor cells can develop "intrinsic resistance" to immune cell killing by CAR-T cells [161]. Strategies have been developed to sensitize tumor cells to death receptor-mediated apoptosis. (Fig. 6).

Combination therapy inhibitors: IAP antagonist, HDAC inhibitor, Serpin 9, SCD1 inhibitors, Qi Yin San Liang San (SLS), Sulforaphane and BCL-2 inhibitor.

Inhibitors of apoptosis proteins (IAPs) are often upregulated in various malignancies to block apoptosis [162]. To address this, scientists have developed a novel approach combining CAR-T cells with IAP antagonists, small molecules that promote the degradation of IAPs. A study found that the IAP antagonist Birinapant increased tumor susceptibility to apoptosis induced by cytokines from CAR-T cells [163].

Histone deacetylase inhibitors (HDACi) enhance the cytotoxicity of CD20 CAR-T cells against B-cell malignancies by increasing TNF-α and IFN-γ secretion [164]. This effect is linked to the broad expression of CD20 in non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), and roughly 40% of precursor B-cell acute lymphoblastic leukemia cases [165]. CD20, a non-glycosylated transmembrane phosphoprotein, plays a key role in B cell development and differentiation, functioning as a calcium channel [166]. HDACi increases CD20 expression in tumors, enhancing the effectiveness of CAR-T cells [167].

Serpin proteinase inhibitor 9 (serpin B9) is known to resist CAR-T cell-mediated tumor killing by binding and neutralizing granzyme B [168, 169]. Unfortunately, serpin B9 is uniformly expressed in B-cell lymphomas, especially in DLBCL and CLL [170]. To address this, scientists

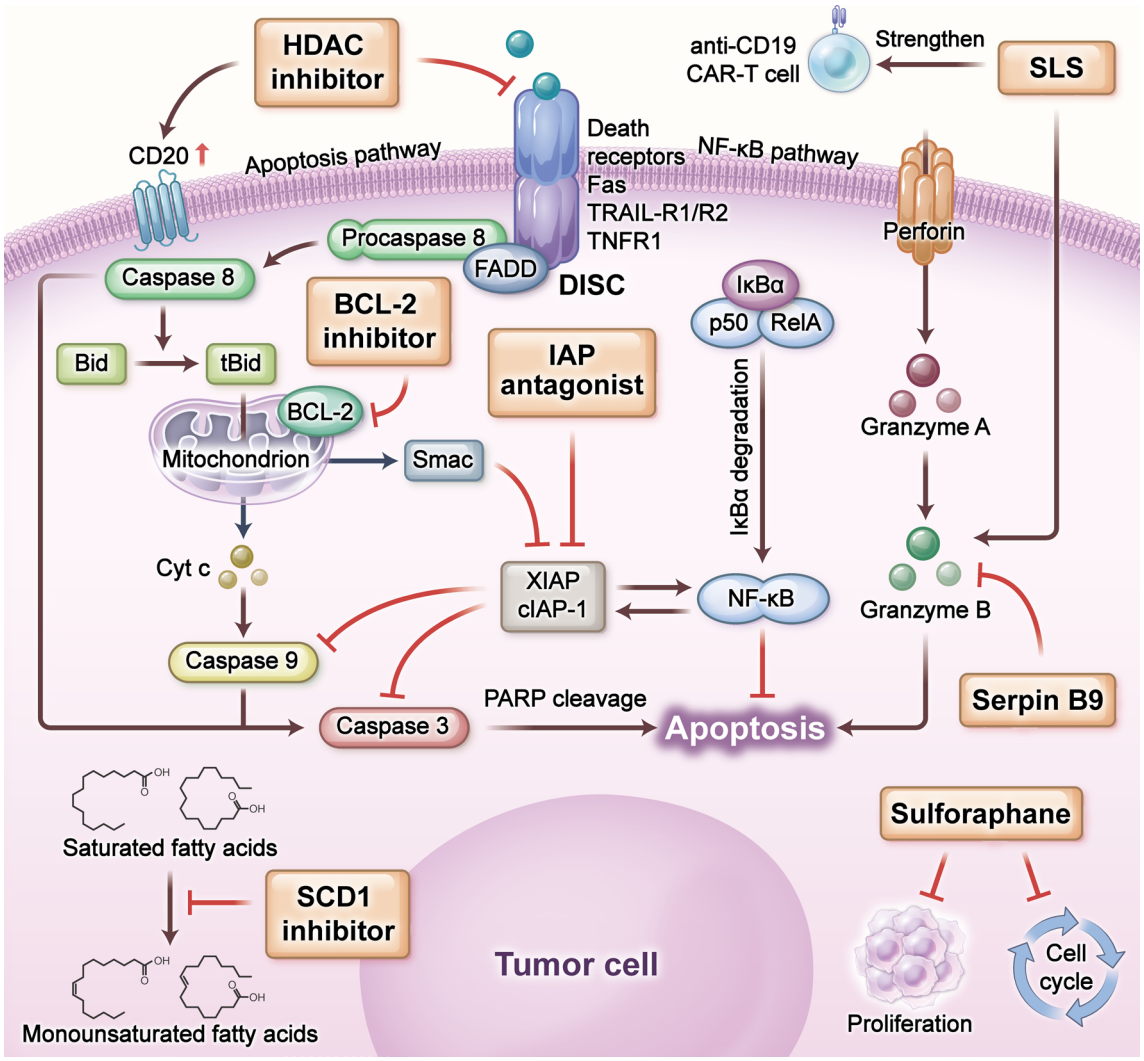

Fig. 6 Molecular targeted drugs in combination with CAR-T cell therapy

silenced serpin B9 expression in tumor cells and observed enhanced CAR-T cell-mediated killing when combined with a serpin B9 inhibitor [170].

Another strategy involves integrating stearoyl-CoA desaturase 1 (SCD1) inhibitors with CAR-T cell therapy. SCD1 inhibitors block the conversion of saturated fatty acids, including palmitic and stearic acids, into monounsaturated fatty acids. Studies have shown that SCD1 inhibitors synergistically enhance the antitumor effects of CAR-T therapy in vivo [171].

Additionally, Chinese medicine formulas and sulforaphane (SFN) can also enhance CAR-T cell cytotoxicity. The formula Qi Yin San Liang San (SLS) strengthens CD19 CAR-T cell function and enhances their ability to target B-cell lymphomas. In vitro studies show that SLS increases granzyme B levels in a dose-dependent manner, partially mediated by IL-17 and apoptosis signaling [172]. SFN, an isothiocyanate found in cruciferous vegetables [173], inhibits tumor cell proliferation and disrupts cell cycle progression through multiple mechanisms [174]. SFN enhances B and T cell abundance [175] and modulates Th1/17 effects by regulating IL-23 and IL-12 production [176]. Research by Shen showed that combining SFN with CAR-T cells increases cytotoxicity and tumor cell lysis by elevating perforin, granzyme B, and IFN-γ levels while suppressing PD-1 expression [177].

A team from the University of Pennsylvania found that combining a BCL-2 inhibitor with overexpressed BCL-2 (F104L) in CAR-T therapy produced a stronger therapeutic effect against B-cell lymphomas. This approach offers a novel method for creating CAR-T cells resistant to pro-apoptotic molecules, amplifying their antitumor efficacy [178].

Table 2 Novel strategies or CAR designs and combinations to enhance cytotoxic efficiency of CAR-T cells therapy in solid tumors

Cancer type	Target	Gene	Metabolism	Combined strategies	Secreted design/ costimulatory domain	Study (year of publication)
Breast cancer	TmTNF-α					Ba et al. (2021)
Hepatocellular carcinoma			IDO1		PD1/CD28 CSR Soluble IL-15 IL21-IL15	Patra et al. (2023) Qin et al. (2023) Makkouk et al. (2021) Batra et al. (2020)
Pancreatic adenocarcinoma			N-glycans		IL18	Greco et al. (2022) Harrer et al. (2022)
Glioblastoma		SHP-1		IAP antagonist	BiTE-secreting bivalent	Liu et al. (2023) Song et al. (2022) Yin et al. (2022)
Gastric cancer	CLDN6				PD1/CD28	Chen et al. (2021) Simon et al. (2023)
Neuroblastoma	CAR-M-FcRγ				IL15 tTF-NGR-peptide	Xu et al. (2019) Liu et al. (2022) Altvater et al. (2023)
Ovarian cancer	TAG72 TAG72/CD47	IL12			Anti-CD40 antibodies bispecific protein of anti-PD-1 fused with TGF-β trap	Lee et al. (2023) Hombach et al. (2022) Zhang et al. (2021) Chen et al. (2021) Shu et al. (2021)
Melanoma			S1P		VHH-secreting	Chakraborty et al. (2019) Xie et al. (2020)
Osteosarcoma	CD276/FGFR4		Inosine	SCD1 inhibitor		Klysz et al. (2024) Sugi et al. (2023) Timpanaro et al. (2023)
Prostate cancer	PRDM1/NR4A3					Jung et al. (2022)
Colorectal cancer		Cbl-b			PD-1-TREM2	Chen et al. (2023) Zhao et al. (2023)
Lung cancer	LunX B7-H3 VEGFR2 EphA2	Foxo1		Sulforaphane		Hu et al. (2020) Marchais et al. (2023) Zhu et al. (2021) Zhong et al. (2023) Shen et al. (2021) Li et al. (2018)
Lymphoma and lymphocytic leukemia	CD19/CD22	NOTCH1 TET2 PIK3CD PIK3R3 GM-CSF	NAD+ PRODH2 CD38	Serpin B9 HDACi Qi Yin San Liang San (SLS) BCL-2 inhibitor	EVs CD47-SIRPα 4-1BB and optimized CD28 BB-ζ-C3aR CAR-Ts CD40L	Wilkens et al. (2022) Fraietta et al. (2018) Walsh et al. (2024) Garcia et al. (2024) Wang et al. (2021) Aharon et al. (2021) Kimman et al. (2023) Xu et al. (2018) Dong et al. (2024) Lee et al. (2022) Dacek et al. (2023) Roselli et al. (2021) Cox et al. (2022) Ye et al. (2022) Lai et al. (2022) Kuhn et al. (2019) Ma et al. (2023) Huang et al. (2024)

We have described and summarized recent research progress, novel CAR designs, and combination strategies aimed at enhancing the cytotoxic efficiency of CAR-T cell therapy in solid tumors (Table 2). Table 3 summarizes biomarkers and associated signaling pathways related to the activation, proliferation, cytotoxic and metastasis of CAR-T cells. However, the combined effects of metabolic modulators, molecular targeted drugs, and CAR-T cell therapy are complex and not yet fully understood, with current research being limited. Further studies are

Table 3 Key biomarkers associated with signaling pathways related to the activation, proliferation, cytotoxic and metastasis of CAR-T cells

Effects	Biomarker	Signaling pathways and metabolic mechanisms
CAR-T cell activation	1. SHP-1	1. TNF-α, IL-2, IFN-γ secretion
CAR-T cell proliferation	1. PD-L1 2. IL-18	1. PD1/PD-L1 pathway 2. IL-13, IFN-γ secretion
CAR-T cell cytotoxicity	1. B7-H3 2. tmTNF-α 3. CD40L 4. MSLN 5. TREM2 6. IL-4/15 7. IL-12 8. PIK3C 9. PIK3R3 10. NAD	1. B7-H3/ B7-H3 ligand 2. NTF-ERK-GST, NTF-NF-κB 3. CD40/CD40L 4. AKT, ERK1/2, JNK 5. RAS/MEK/ERK 6. AKT, JAK/STAT, IFN-γ secretion 7. IL-17, IFN-γ secretion 8. PI3K/AKT 9. PI3K/AKT 10. NAD + salvage pathway
CAR-T cell exhaustion and differentiation	1. PRDM1/NR4A3 2. FOXO1 3. NOTCH1 4. Cbl-b 5. TET2 6. PRODH2 7. SphK1	1. PRDM1/NR4A3/TCF7 2. AKT,MAPK 3. NOTCH-FOXM1 4. TCR/Cbl-b/STAT 5. c-MYC, mTORC1 6. Proline metabolism 7. Lipid metabolism
Tumor cell proliferation	1. LunX 2. CLDN6 3. EphA2 4. VEGFR-2 5. Glutamine 6. INO1	1. ERK, JNK pathway 2. STAT3 3. AKT, ERK1/2 4. VEGF/VEGFR 5. Glutamine/polyamine/hypusine 6. Glutamine/polyamine

needed to elucidate the molecular mechanisms underlying combination therapies, along with larger clinical trials to assess their efficacy and safety.

Balancing between toxicity and efficacy

Despite the remarkable efficacy of CAR-T cell therapies in treating various malignancies, safety concerns have emerged. Notably, Cytokine Release Syndrome (CRS) and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) are the most frequently reported adverse events in CAR-T cell therapies [179, 180].

Cytokine release syndrome

CRS typically begins with mild, generalized symptoms such as fever, hypotension, rigors, tachycardia, tachypnea, and hypoxemia [181]. In most cases, CRS is reversible without lasting neurological damage if symptoms are promptly recognized and treated [182]. However, delays in diagnosis and treatment can occur due to the current diagnostic criteria and severity grading system. Alex et al. developed a machine learning-based method to aid in the rapid diagnosis of CRS. They found that specific cytokines and chemokines were consistently associated with CRS and used machine learning algorithms to identify CRS based on cytokine peak concentrations, achieving over 90% accuracy [183].

CRS is associated with elevated levels of several cytokines, including IL-6, IL-8, IL-10, INF-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein-1β, and monocyte chemoattractant protein-1 [184]. IL-6 is a key mediator of CRS and is critically involved in life-threatening toxicity. IL-6 blockades, such as siltuximab (IL-6 antibody) and tocilizumab (IL-6 receptor antibody), are widely used to manage CRS [185]. Administering IL-6 receptor antibodies too early can disrupt normal IL-6 levels, causing side effects [186], while delayed administration can lead to severe toxicity [187, 188]. Thus, it is crucial to develop new therapies to prevent CRS before CAR-T cell infusion and determine the optimal timing for IL-6 blockade administration. Li et al. developed an IL-6 sponge (IL6S) that selectively absorbs abnormally elevated IL-6 during CRS without affecting the antitumor efficacy of CAR-T cells. Additionally, the sponge can be easily removed with a syringe, simplifying the removal of both the material and the absorbed IL-6 [189]. This offers a promising new solution for managing CRS in the clinic.

GM-CSF is another key cytokine associated with CAR-T cell-related adverse events [190]. Lenzilumab, a humanized monoclonal antibody that neutralizes GM-CSF, has been shown to enhance CAR-T cell cytotoxicity and prevent CRS [190]. However, safety assessments are still ongoing [191].

Immune effector cell-associated neurotoxicity syndrome

ICANS is a form of neurotoxicity that can occur after CAR-T therapy, manifesting as symptoms such as difficulty concentrating, confusion, delirium, aphasia, lethargy, tremors, and seizures [192]. Corticosteroid therapy and tocilizumab are the first-line treatments for ICANS [193]. However, these therapies may impact the long-term efficacy of CAR-T cell treatments [194]. Various preventive strategies, including early use of corticosteroids, tocilizumab, and the addition of ibrutinib, have shown success in reducing systemic CAR-T cell depletion [195]. When ICANS occurs, reducing the cumulative corticosteroid dose may improve OS and progression-free survival (PFS). Mikalai et al. reported a case where a patient with grade IV ICANS was treated with intrathecal chemotherapy, including methotrexate, cytarabine, and dexamethasone, which reduced the ICANS from grade IV to grade 0 within one day. This allowed the cessation of systemic corticosteroid use, preserving the efficacy of CAR-T cells. This suggests a promising method for managing high-grade ICANS while minimizing damage to CAR-T cell efficacy [196].

Optimization of CAR-T cell production

While CAR-T cell therapies have demonstrated efficacy in various malignancies, they are limited by high costs and lengthy production times. Various strategies are being explored to reduce costs, increase accessibility, and expand the applications of CAR-T therapies. In vivo CAR-T preparation and allogeneic CAR-T cell therapies have emerged as key areas of interest in "next-generation" CAR-T cell research. (Fig. 7).

The traditional in-vivo, Autologous CAR-T therapies compared to allogenic CAR-T therapies; The ex-vivo CAR-T therapies use vectors including LV, LNP, AAV, and is used directly to transduce patient T cells.

In vivo CAR-T cell therapies

The in vivo generation of CAR-T cells allows direct transduction of patient T cells, enabling new gene expression and CAR production within one to two days. This method may prevent GvHD, a life-threatening toxicity caused by recognizing healthy tissues. As gene therapies expand to more indications, improving vectors becomes essential. Michaels et al. outlined various vector-based technologies, such as lentiviral vectors (LV), adeno-associated viruses (AAV), synthetic polymer nanocarriers (NC), and lipid nanoparticles (LNP) [197].

Currently, LV vectors are widely used in clinical trials to deliver transgenes into primary cells ex vivo, such as T cells and hematopoietic stem cells, making them valuable tools for treating genetic and acquired diseases [198]. However, global LV vector manufacturing capacity is limited, and costs are high, making it essential to enhance cell lines for LV vector production [199]. Laffaldano et al. used CRISPR library screening to identify genetic perturbations, creating cell lines with higher LV vector titers and improved productivity [200].

AAVs are currently the most widely used gene delivery vectors for various disease models [201]. AAVs demonstrate strong ability to attach to and enter target

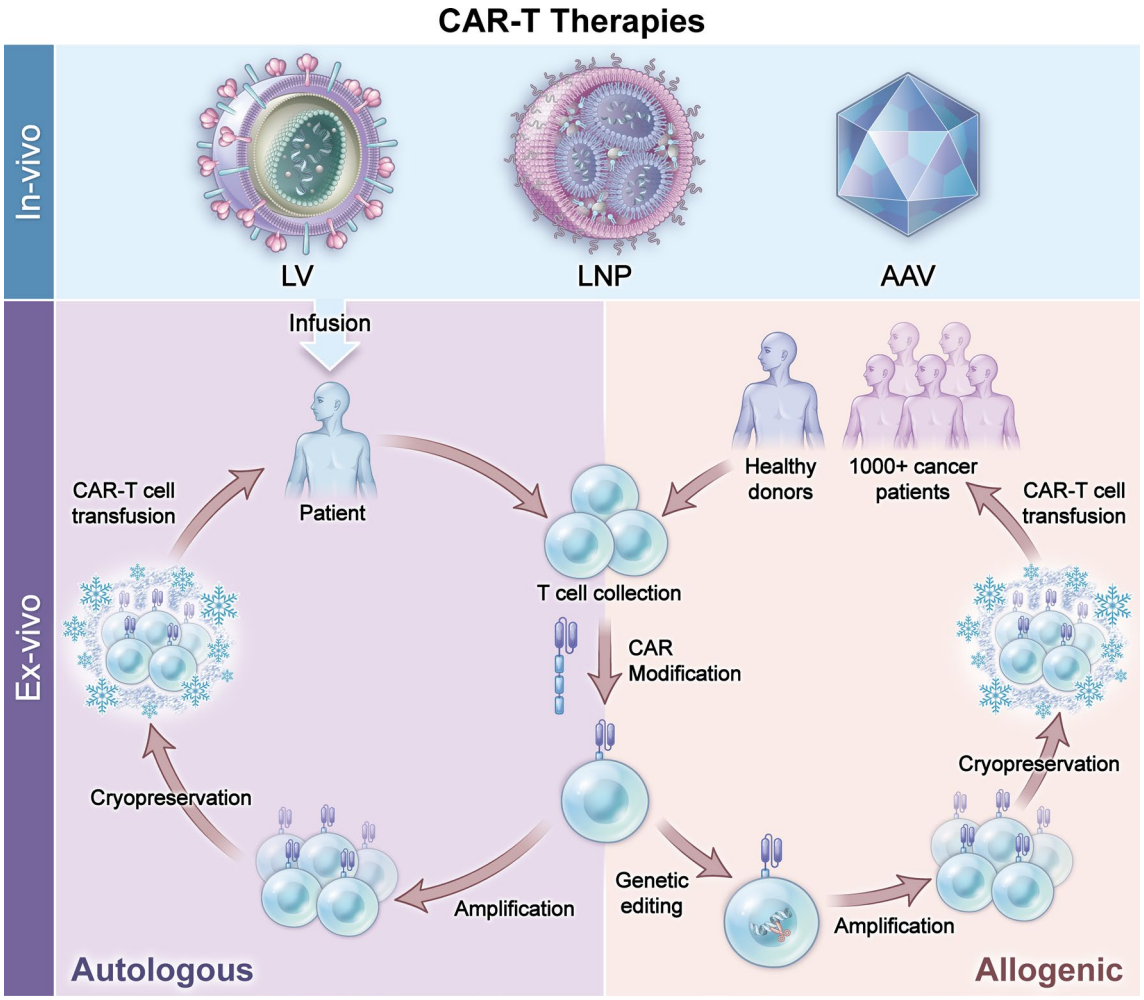

Fig. 7 Different CAR-T cell productions

cells, exhibiting cytotoxicity. Cao et al. used AAVs to deliver Vaccinia Virus (VV) B8R to tumor cells in pre-immunized mice and observed that AAV gene therapy slowed tumor growth. Moço et al. used AAV serotype 6 to integrate an anti-CD19 CAR into human T cells, achieving stable CAR expression. Importantly, this led to the knockout of the endogenous T cell receptor, enabling the use of allogenic donors, supporting an "off-the-shelf" universal CAR-T therapy [202]. However, transduction efficiency in primary cells, particularly T lymphocytes, remains relatively low. Wang et al. developed a method using OKT3 and genistein to culture AAVs, increasing gene expression by sevenfold [203].

Developing lentiviral vectors from wild-type viruses requires evaluating the viral genome and selecting components for safe, efficient gene transfer [204]. Lipid-based nanoparticles (LNPs) have emerged as revolutionary carriers due to their favorable physicochemical properties, making them especially useful for immunotherapeutic delivery [205]. The LNP-mRNA delivery system offers advantages such as no host genome integration, lower cost, reduced toxicity, and modifiability compared to viral vectors [206]. LNPs generally accumulate in the liver after systemic administration due to serum protein adsorption. Billingsley MM et al. developed an antibody-conjugated LNP (Ab-LNP) platform with extrahepatic tropism for CAR-T cell therapies [207]. This platform enables extrahepatic transfection, enhances delivery to the spleen, and generates functional CAR-T cells in vivo [208].

Zhou et al. developed an in vivo LNP system with CD3 antibody modification for targeted transfection, delivering a combinatorial gene encoding interleukin-6 short hairpin RNA (IL-6 shRNA) and CD19 CAR [209]. They successfully transformed T-cells into IL-6-down-regulated CAR-T cells, which targeted CD19-expressing leukemia while reducing CRS caused by IL-6. This demonstrated that the LNP system exhibited comparable anti-tumor effects to traditional ex vivo CAR-T cells. Furthermore, LNPs coated with antibodies targeting specific cells, such as T cells, can release mRNA and have been proven effective in producing vaccines against SARS-CoV-2 [210]. The LNP-mRNA system has also been shown to be effective in treating various cardiac diseases, including myocarditis, cardiac fibrosis, and myocardial infarction [211]. Hamilton et al. developed an mRNA/siRNA co-delivery platform for simultaneous gene expression and targeted gene disruption in T cells [212]. This system enhances CAR expression and PD-1 knockdown without affecting overall T cell activation, showing great promise for advancing cancer immunotherapies.

Allogenic CAR-T therapies

Although CAR-T cell therapies have proven effective, they face limitations, particularly the time-consuming and highly personalized manufacturing of autologous CAR-T cells. Universal "off-the-shelf" allogeneic CAR-T therapies with low alloreactivity are currently under development [213, 214]. The most used allogeneic cell source for CAR-T manufacturing is peripheral blood mononuclear cells (PBMCs) from healthy donors, or stem cells derived from PBMCs [215]. This enables a rapid and standardized manufacturing protocol.

Induced pluripotent stem cells (iPSCs) are a renewable cell source undergoing rapid development [216]. Since iPSCs can be derived from various somatic cells, they offer the potential for tailor-made regenerative medicine, eliminating the need for harmful immunosuppressive drugs used in allogeneic transplantation [213]. iPSCs derived from T cells inherit rearranged TCR genes, allowing the regeneration of various T cell types. These cytotoxic cells can serve as the base for CAR introduction, enabling the creation of a large cell bank for broad application [217]. Wang et al. demonstrated that iPSCs reprogrammed from $CD62L^+$ naive and memory T cells, followed by CD19-CAR engineering and 3D-organoid differentiation, produced $CD8\alpha\beta^+$ CAR-T cells, providing a feasible method to generate potent, allogeneic "off-the-shelf" CAR-T cells [218]. Jing et al. efficiently induced iPSC-derived $CD8\alpha\beta^+$ T cells by knocking down EZH1, demonstrating superior antitumor efficacy in vivo through memory-like T cells producing effector T cells [219]. Macrophages have gained attention in immunotherapy for their immunomodulatory capacity, ability to infiltrate solid tumors, and tumor cell phagocytosis [220, 221]. Lei et al. engineered induced pluripotent stem cell-derived macrophages (iMACs) with toll-like receptor 4 intracellular toll/IL-1R (TIR) domain-containing CARs. The TIR-containing CAR enables macrophages to achieve antigen-dependent M1 polarization, providing an advantage in cancer immunotherapy [222].

Despite the promise of allogeneic CAR-T therapies, two key challenges remain: life-threatening GvHD and host versus graft rejection (HvGR) [223]. To enhance CAR-T cell expansion and prevent host immune rejection of allogeneic CAR-T cells, a host preconditioning regimen is needed [224]. Derippe et al. developed a population-based pharmacokinetic-pharmacodynamic model to analyze the interplay between lymphodepletion, the host immune system, homeostatic cytokines, and universal CAR-T cell pharmacokinetics [225]. This model can optimize preconditioning regimens for future clinical applications.

One strategy to prevent GvHD involves using gene editing techniques like CRISPR/Cas9 or zinc fingers to

knock out the TCR [226]. However, research indicates that TCR knockout in allogeneic CAR-T cells may reduce long-term persistence compared to autologous CAR-T cells [227]. Interestingly, another study found that HLA-II deficiency improved CAR-T persistence and developed TCR, HLA-I/II triple-edited CAR-T cells that effectively resisted allogeneic NK rejection [228]. This provides novel insights for improving the persistence of allogeneic CAR-T cells and guiding future developments. Another approach is to use T cells from compatible donors. Buflao et al. reported treating relapsed/refractory B-cell precursor acute lymphoblastic leukemia with donor-derived CAR-T cells [229]. Only one of 13 patients developed GvHD, which was rapidly controlled with steroids and ruxolitinib. This demonstrated no increased toxicity compared to autologous CAR-T cells.

Application of novel technologies (Fig. 8)

Artificial Intelligence can identify abnormal CAR-T cells and predict adverse events like CRS; Organoids can be analyzed to discover new targets and to assess patient response to therapies; Gut microbiome can serve as non-invasive prognostic markers to predict and improve therapy outcome.

Artificial intelligence

Due to individual cellular differences, patients undergoing CAR-T therapy may experience side effects that can

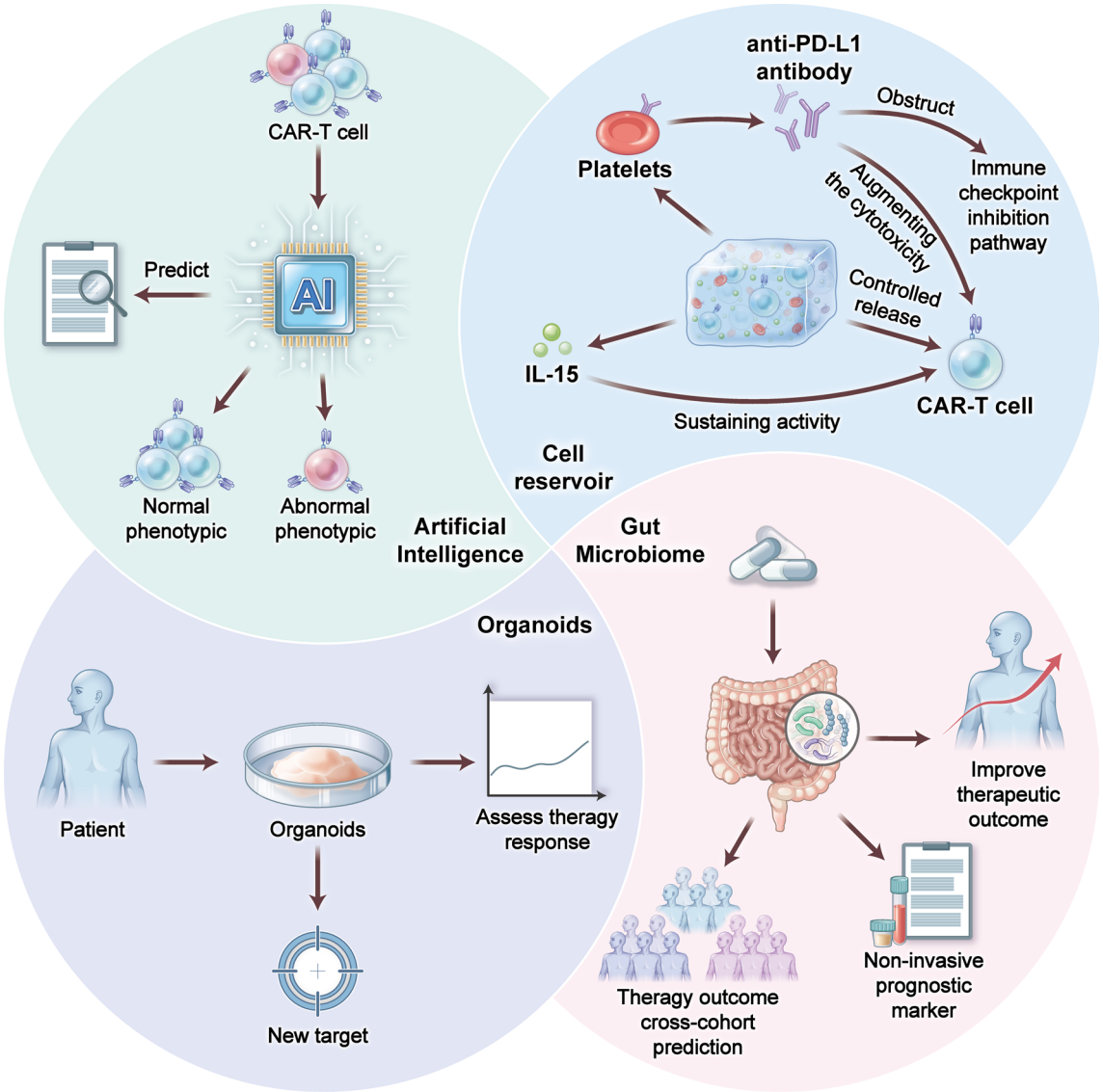

Fig. 8 Application of novel technologies

be debilitating or even fatal. However, because CAR-T cells exhibit heterogeneity and share similar phenotypes with other cells, only a limited number of blood morphologists can rapidly and accurately identify abnormal phenotypic cells. Additionally, biochemical analysis and training novice blood morphologists to recognize CAR-T cells are time-consuming and costly. Deep learning (DL), a state-of-the-art computer vision technique, has achieved significant success in medical cell image classification [230]. Zhang et al. developed a deep learning model, RCMNet, to identify CAR-T cells, achieving 99.63% top-1 accuracy on a public dataset [18]. DL has also demonstrated its ability to predict patient responses to therapy based on individual characteristics, aiding in the development of personalized treatment strategies. Wei et al. found that AI can predict and assess adverse events like CRS [181]. A key challenge for AI is identifying potential targets for CAR-T cell therapies. Daniels et al. developed a CAR library with over 2300 synthetic costimulatory domains that influence T cell outcomes. They used neural networks to filter, cluster, and interpret data to identify the best combinations for assessing neoantigens or designing TCRs [231].

Although AI-based CAR-T therapy approaches are still limited, AI holds promise for uncovering strategies to enhance CAR-T cell therapy effectiveness.

Organoids

Organoids, which are in vitro 3D culture systems derived from self-organizing stem cells that resemble tissues or organs, offer an ideal platform for developing new materials and targets for CAR-T therapies. Yu et al. established bladder cancer organoid lines representing basal and luminal subtypes and developed second-generation CAR-T cells targeting MUC1 [232]. They subsequently developed a sensitive in vitro platform for evaluating CAR-T cell-mediated cytotoxicity. This demonstrated the potential of organoids in assessing personalized responses to CAR-T therapy.

Gut microbiome

The gut microbiota plays a crucial role in maintaining intestinal barrier integrity and homeostasis [233]. Increasing evidence suggests that the gut microbiome may influence the efficacy of cancer immunotherapy [234]. Stein-Thoeringer et al. recently reported that broad-spectrum antibiotic treatment prior to CD19-targeted CAR-T cell therapy is linked to adverse outcomes [235]. Smith et al. found that antibiotic exposure before CD19 CAR-T cell infusion was associated with significantly reduced survival and increased immune effector cell-associated neurotoxicity syndrome (ICANS) [236]. This suggests that gut microbiota could serve as a biomarker for predicting outcomes in patients undergoing CAR-T therapy. Additionally, Hu et al. found that in patients with hematologic malignancies (B-ALL, B-NHL, MM) treated with BCMA or CD19 CAR-T cells, dynamic changes in the gut microbiome were significantly correlated with therapeutic response and CRS severity [237]. This may allow for cross-cohort microbiome-based predictions of CAR-T immunotherapy outcomes. The gut microbiota may also contribute to immune-related adverse events (irAEs). More studies are needed to develop novel interventions targeting commensal bacteria to minimize irAEs and improve immunotherapy safety [233]. In conclusion, while research on the role of the gut microbiota in CAR-T therapy is still in its early stages, current findings suggest its potential as a non-invasive prognostic marker. Modulating the gut microbiota could help mitigate CAR-T-induced adverse effects and improve therapeutic outcomes.

Cell reservoir

Previous studies have shown that directly injecting CAR-T cells into the tumor site leads to rapid loss of activity and cell death. Additionally, tumor cells evade T cell recognition and inhibit their cytotoxicity through immune checkpoint inhibition and the immunosuppressive tumor microenvironment.

In response to these challenges, researchers developed a "cell reservoir" technology for localized, sustained, and controlled release of cells. This approach involves loading CAR-T cells, anti-PD-L1 antibody-modified platelets, and IL-15 cytokine-loaded nanoparticles into a biocompatible implantable hydrogel. The hydrogel's slow-release capability controls the release of CAR-T cells, preventing rapid inactivation and sustaining activity through IL-15. In the postoperative inflammatory environment, PD-L1-engineered platelets may activate, secreting anti-PD-L1 antibodies that block immune checkpoint inhibition and enhance CAR-T cell cytotoxicity, suppressing cancer recurrence. This novel "cell reservoir" approach serves as a localized, sustained-release system for cells [238].

The integration of CRISPR gene editing with CAR-T therapy offers new opportunities for cancer treatment and facilitates the translation of CAR-T research into clinical applications [239]. However, significant challenges remain regarding the safety, efficacy, and scalability of Cas9-based gene editing platforms. Specifically, Cas9-mediated editing can induce permanent alterations in T cell DNA, posing risks of irreversible outcomes. To address this issue, scientists are refining and enhancing gene editing methods. Researchers at Stanford University developed the MEGA RNA CRISPR editing system, capable of modifying cell RNA to regulate immune cell metabolism without permanently altering genes. Initial

Table 4 Ongoing and completed clinical trials of optimizing CAR-T cell therapy

Study name	NCT number	Indications	CAR-T cell type	Phase
CD4CAR for CD4 + leukemia and lymphoma	NCT03829540	Relapsed or refractory T-cell leukemia and lymphoma	CD4 + CART	1
Study of CD4-targeted chimeric antigen receptor T-cells (CD4-CAR-T) in subjects with relapsed or refractory T-cell lymphoma	NCT04712864	Relapsed or refractory T-cell lymphoma	CD4 + CART	1
CD4-specific CAR T cells (CD4 CAR T cells) for relapsed/refractory T cell malignancies	NCT04162340	Relapsed and/or refractory T cell lymphoma	CD4 + CART	1
Chimeric antigen receptor T cell therapy redirected to CD4 (CD4CAR)as a second line treatment for chronic myelomonocytic leukemia, CMML	NCT06071624	Relapsed or refractory CML	CD4 + CART	1
Chimeric antigen receptor T cell redirected to target CD4 positive relapsed refractory acute myeloid leukemia (AML) as a bridge to allogeneic stem cell transplant	NCT06197672	Relapsed or refractory AML	CD4 + CART	1
A clinical study of CD19 universal CAR-γδT cells in active systemic lupus erythematosus	NCT06106893	Active severe systemic lupus erythematosus	CD19 Universal CAR-γδT	1 and 2
Long-term follow-up study of allogeneic gamma delta (γδ) CAR T cells	NCT04911478	Subjects who received an adicet bio allogeneic γδ CAR T cell product that has been genetically engineered to express the anti-CD20 CAR by transduction with a self-inactivating, replication incompetent gamma retroviral vector	ADI-001 (CD19 Universal CAR-γδT)	NA
CAR-γ δ T cells in the treatment of relapsed and refractory CD7 positive T cell-derived malignant tumors	NCT04702841	Relapsed and refractory CD7 Positive T cell-derived malignancies	CAR-γ δ T cells	1
A safety and efficacy study of allogeneic CAR gamma-delta T cells in subjects with relapsed/refractory solid tumors (CAR001)	NCT06150885	Relapsed/refractory NSCLC, TNBC, CRC or GBM	HLA-G-CAR.BiTE allogeneic γδ T cells	1 and 2
CD19.CAR allogeneic NKT for patients with relapsed or refractory B-cell malignancies (ANCHOR)	NCT03774654	Relapsed or refractory B-cell malignancies	CD19.CAR-aNKT cells	1
CAR T cell receptor immunotherapy targeting VEGFR2 for patients with metastatic cancer	NCT01218867	Patients with metastatic cancer	Anti-VEGFR2 CAR CD8 plus PBL	1 and 2
Genetically engineered cells (EGFRt/19-28z/IL-12 CAR T cells) for the treatment of relapsed or refractory CD19 + hematologic malignancies	NCT06343376	Relapsed or refractory CD19 + hematologic malignancies	EGFRt/19-28z/IL-12 CAR T	1
NKG2D CAR-T(KD-025) in the treatment of advanced NKG2DL + solid tumors	NCT05382377	Advanced NKG2DL + solid tumors	NKG2D CAR-T(KD-025)	1
CD123/CLL1 CAR-T cells for R/R AML (STPHI_0001)	NCT03631576	Relapse/refractory B-cell acute myeloid leukemia	CD123/CLL1 CAR-T Cells	2 and 3
Safety and efficacy of CD19 and CD22 targeted CAR-T therapy for relapsed/refractory B cell leukemia and lymphoma	NCT04648475	Relapsed/refractory B cell leukemia and lymphoma	CD19 and CD22 targeted CAR-T cells	1 and 2
CD19/CD22 bispecific CAR-T cell therapy for relapsed/refractory B-cell lymphoma or ALL	NCT06081478	Relapsed/refractory B-cell lymphoma or ALL	CD19/CD22-bispecific CAR-T	2
Clinical study on the efficacy and safety of c-Met/PD-L1 CAR-T cell injection in the treatment of HCC	NCT03672305	Rrimary hepatocellular carcinoma	c-Met/PD-L1 CAR-T	1
CAR T and PD-1 knockout engineered T cells for esophageal cancer	NCT03706326	Advanced esophageal cancer	Anti-MUC1 CAR-T cells, PD-1 knockout Engineered T cells	1 and 2
Exploratory study of MSLN-CAR T cells secreting PD1/CTLA-4 nanoantibody for the treatment of advanced solid tumors	NCT06248697	Advanced solid tumors	αPD1/CTLA4-MSLN-CAR T cells	1

Table 4 (continued)

Study name	NCT number	Indications	CAR-T cell type	Phase
Chidamide bridging for CAR-T therapy	NCT05370547	Recurrent or refractory (r/r) B-cell non-Hodgkin's lymphoma	Anti-CD19 CAR-T cells	1 and 2
Study to evaluate the role of siltuximab in treatment of CRS and ICANS related to CAR-T cell therapy	NCT04975555	CRS and ICANS in patients who will receive CAR T-cell therapy for the treatment of hematological malignancies	Siltuximab	2

NSCLC, non-small cell lung cancer; CML, non-small cell lung cancer (NSCLC); TNBC, triple negative breast cancer; CRC, colorectal cancer; GBM, glioblastoma multiforme; ALL, acute lymphoblastic leukemia; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurological syndrome

studies of the MEGA platform's T cell editing showed no significant cancer inhibition advantage compared to traditional CAR-T or normal T cells. However, over time, these edited T cells exhibited sustained antitumor activity, with up to a tenfold increase in efficacy and proliferation, significantly slowing cancer growth [240].

Ongoing and completed clinical trials of optimizing CAR-T cell therapy

Additionally, we emphasize the complexities of these strategies and their potential impact on clinical outcomes. Representative clinical trials of optimized CAR-T therapies are listed in Table 4. The most common strategy is the use of bispecific CAR-T therapies, particularly CD19/22 bispecific CAR-T. As of this review, 205 studies on clinicaltrials.gov are investigating allogenic/universal CAR-T therapies, demonstrating the promising potential of off-the-shelf treatments. Although allogenic CAR-T therapies still carry risks of CRS and ICANS, various strategies have been explored to optimize administration and production, aiming to reduce toxicity and enhance cytotoxic efficacy.

Conclusion

The phenotypic diversity of solid tumors necessitates more precise CAR structural designs to enhance the specificity and cytotoxicity of CAR-T cells, thereby improving their ability to target and kill tumor cells. Identifying novel, precise targets holds significant research value. For example, targeting LunX and EphA2 in lung cancer, TAG72 in ovarian cancer and tmTNF-α in breast cancer has been successfully applied in vitro and in vivo. Moreover, dual-targeted CAR-T cells can be precisely localized at the tumor site and can exert high cytotoxicity against tumor cells while the adjacent tissues are not damaged, enabling accurate application of CAR-T cell therapy.

Optimizing the costimulatory domains and enhancing CSR can also be promising strategies for CAR design. The immunosuppressive nature of the TME poses a major obstacle to CAR-T cell infiltration into solid tumors. Targeting immunosuppressive factors in the TME, such as blocking the PD-1/PD-L1 checkpoint and targeting immunosuppressive cells, could remodel the TME and enhance CAR-T cell efficacy. However, the complex interactions between CAR-T cells, tumor cells, the TME, and immune factors remain poorly understood, requiring further research. Targeting CAR-T cell metabolism can promote a more scalable, less depleting, and more cytotoxic phenotype. This strategy can also modify the unfavorable TME to make it more conducive to CAR-T cell function. CAR-T cell metabolism is influenced by CAR structure, including costimulatory domains and key molecule expression, which requires further study.

Enhancing effector molecules can boost CAR-T cell efficacy, but IFN-γ is closely linked to CAR-T-induced CRS. CAR-T-derived EVs offers a promising approach to improve CAR-T efficacy while reducing CRS incidence. The selection of T cell subsets for CAR-T production still requires further study. Current studies suggest that T cell subtype selection and in vivo expansion are closely linked to tumor progression and recurrence. More research is needed to understand the relationship between therapeutic outcomes and the molecular characteristics of T cell subtypes. Combining CAR-T therapy with VHH (nanobodies) is an emerging trend. VHHs have low immunogenicity, stability, and high affinity, and can serve as alternatives to scFv, reducing CAR aggregation and depletion [241]. Currently, several CAR-T therapies incorporating nanobodies have entered clinical trials or reached the market. Gene modification strategies require a deep understanding of relevant signaling pathways and mechanisms. Additionally, artificial intelligence can help create precise models to predict optimal T cell stimulation levels for each patient, enabling flexible manufacturing of more consistent and potent CAR-T cells [242].

Abbreviations

AAV	Adenovirus-related vector
AI	Artificial intelligence
ALL	Acute lymphoblastic leukemia
APCs	Antigen-presenting cells
CAR-T	Chimeric antigen receptor T cell
CRS	Cytokine release syndrome
CSR	Chimeric-switch receptor
CTLs	Cytotoxic T lymphocytes
DCs	Dendritic cells
DLBCL	Diffuse large B-cell lymphoma
EVs	Extracellular vesicles
GM-CSF	Granulocyte–macrophage colony stimulating factor
GvHD	Graft-versus-host disease
HDACi	Histone deacetylase inhibitor
HvGR	The host versus graft rejection
IAPs	Inhibitors of apoptosis proteins
ICANS	Immune effector cell-associated neurotoxicity syndrome
IDO1	Indoleamine 2,3-dioxygenase 1
ILs	Interleukins
iMACs	Induced pluripotent stem cell-derived macrophages
INO	Inosine
iPSCs	Induced pluripotent stem cells
KDR	Kinase insert domain receptor
liso-cel	Lisocabtagene maraleucel
LNP	Lipid nanoparticles
LV	Lentiviral vector
MDSCs	Myeloid-derived suppressor cells
MHC	Major histocompatibility complex
MM	Multiple myeloma
NAD+	Nicotinamide adenine dinucleotide
NC	Synthetic polymer nanocarriers
NHL	Non-Hodgkin's lymphoma
NKT	Natural killer T cell
NSCLC	Non-small cell lung cancer
PBMC	Peripheral blood mononuclear cells
RTK	Receptor tyrosine kinase
S1P	Sphingosine-1-phosphate

SCD1	Stearoyl-CoA desaturase 1
scFv	Single-chain variable fragment
SFN	Sulforaphane
SLS	Qi Yin San Liang San
SphK1	Sphingosine kinase 1
TAMs	Tumor-associated macrophages
TIL	Tumor infiltrating lymphocytes
TIR	Toll-like receptor 4 intracellular toll/IL-1R
TME	Tumor microenvironment
tmTNF-α	Transmembrane tumor necrosis factor-alpha
TN/SCM	Naive/stem memory T cell
TNF	Tumor necrosis factor receptor
Treg	Regulatory T cell
TREM2	Triggering the receptor expressed on myeloid cells 2
VEGFR-2	Vascular endothelial growth factor 2
VHH	Variable heavy domain of heavy chain antibodies
ZOL	Zoledronate

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Kexin Ai and Bowen Liu analyzed the data and writed the manuscript, Xiaomei Chen, Chunxin Huang and Jingping Yang supervised the writing of the article, Weiya Zhang, Xin Du and Jianyu Weng collected the clinic data; Peilong Lai and Kongming Wu designed the research and provided critical reviews.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Chen R, Chen L, Wang C, Zhu H, Gu L, Li Y, et al. CAR-T treatment for cancer: prospects and challenges. Front Oncol. 2023;13:1288383.
2. Mazinani M, Rahbarizadeh F. CAR-T cell potency: from structural elements to vector backbone components. Biomark Res. 2022;10(1):70.
3. Liu Z, Lei W, Wang H, Liu X, Fu R. Challenges and strategies associated with CAR-T cell therapy in blood malignancies. Exp Hematol Oncol. 2024;13(1):22.
4. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439–48.
5. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. Blood Rev. 2019;34:45–55.
6. Zeng W, Zhang P. Resistance and recurrence of malignancies after CAR-T cell therapy. Exp Cell Res. 2022;410(2): 112971.
7. Qu C, Zhang H, Cao H, Tang L, Mo H, Liu F, et al. Tumor buster—where will the CAR-T cell therapy 'missile' go? Mol Cancer. 2022;21(1):201.
8. Tang L, Pan S, Wei X, Xu X, Wei Q. Arming CAR-T cells with cytokines and more: innovations in the fourth-generation CAR-T development. Mol Ther. 2023;31(11):3146–62.
9. Freen-van Heeren JJ. Post-transcriptional control of T-cell cytokine production: implications for cancer therapy. Immunology. 2021;164(1):57–72.
10. Maalej KM, Merhi M, Inchakalody VP, Mestiri S, Alam M, Maccalli C, et al. CAR-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. Mol Cancer. 2023;22(1):20.
11. Albelda SM. CAR T cell therapy for patients with solid tumours: key lessons to learn and unlearn. Nat Rev Clin Oncol. 2024;21(1):47–66.
12. Lemoine J, Ruella M, Houot R. Overcoming intrinsic resistance of cancer cells to CAR T-cell killing. Clin Cancer Res. 2021;27(23):6298–306.
13. Benmebarek MR, Karches CH, Cadilha BL, Lesch S, Endres S, Kobold S. Killing mechanisms of chimeric antigen receptor (CAR) T cells. Int J Mol Sci. 2019;20(6):1.
14. Dimitri A, Herbst F, Fraietta JA. Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. Mol Cancer. 2022;21(1):78.
15. Wang L, Zhang L, Dunmall LC, Wang YY, Fan Z, Cheng Z, et al. The dilemmas and possible solutions for CAR-T cell therapy application in solid tumors. Cancer Lett. 2024;591: 216871.
16. Liu H, Pan C, Song W, Liu D, Li Z, Zheng L. Novel strategies for immuno-oncology breakthroughs with cell therapy. Biomark Res. 2021;9(1):62.
17. Zhao G, Zhang Y, Xu C-F, Wang J. In vivo production of CAR-T cells using virus-mimetic fusogenic nanovesicles. Sci Bull. 2023;69:354.
18. Zhang R, Han X, Lei Z, Jiang C, Gul I, Hu Q, et al. RCMNet: a deep learning model assists CAR-T therapy for leukemia. Comput Biol Med. 2022;150: 106084.
19. Mazinani M, Rahbarizadeh F. New cell sources for CAR-based immunotherapy. Biomark Res. 2023;11(1):49.
20. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive phenotyping of T cells using flow cytometry. Cytom Part A J Int Soc Anal Cytol. 2019;95(6):647–54.
21. Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell. 2013;153(6):1239–51.
22. Boulch M, Cazaux M, Cuffel A, Guerin MV, Garcia Z, Alonso R, et al. Tumor-intrinsic sensitivity to the pro-apoptotic effects of IFN-γ is a major determinant of $CD4^+$ CAR T-cell antitumor activity. Nat Cancer. 2023;4(7):968–83.
23. Yang Y, Kohler ME, Chien CD, Sauter CT, Jacoby E, Yan C, et al. TCR engagement negatively affects CD8 but not CD4 CAR T cell expansion and leukemic clearance. Sci Transl Med. 2017;9(417):1.
24. Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ, et al. Chimeric antigen receptor-modified T cells derived from defined $CD8^+$ and $CD4^+$ subsets confer superior antitumor reactivity in vivo. Leukemia. 2016;30(2):492–500.
25. Bulliard Y, Andersson BS, Baysal MA, Damiano J, Tsimberidou AM. Reprogramming T cell differentiation and exhaustion in CAR-T cell therapy. J Hematol Oncol. 2023;16(1):108.
26. Turtle CJ, Berger C, Sommermeyer D, Hanafi L-A, Pender B, Robinson EM, et al. Anti-CD19 chimeric antigen receptor-modified T cell therapy for B cell non-hodgkin lymphoma and chronic lymphocytic leukemia: fludarabine and cyclophosphamide lymphodepletion improves in vivo expansion and persistence of CAR-T cells and clinical outcomes. Blood. 2015;126(23):184.
27. Teoh J, Brown LF. Developing lisocabtagene maraleucel chimeric antigen receptor T-cell manufacturing for improved process, product quality and consistency across $CD19^+$ hematologic indications. Cytotherapy. 2022;24(9):962–73.
28. Fergusson JR, Fleming VM, Klenerman P. CD161-expressing human T cells. Front Immunol. 2011;2:36.
29. Konduri V, Joseph SK, Byrd TT, Nawas Z, Vazquez-Perez J, Hofferek CJ, et al. A subset of cytotoxic effector memory T cells enhances CAR T cell efficacy in a model of pancreatic ductal adenocarcinoma. Sci Transl Med. 2021;13(592):1.

30. Brandes M, Willimann K, Bioley G, Lévy N, Eberl M, Luo M, et al. Cross-presenting human γδ T cells induce robust $CD8^+$αβ T cell responses. Proc Nat Acad Sci. 2009;106(7):2307–12.
31. Rozenbaum M, Meir A, Aharony Y, Itzhaki O, Schachter J, Bank I, et al. Gamma-delta CAR-T cells show CAR-directed and independent activity against leukemia. Front Immunol. 2020;11:1347.
32. Frieling JS, Tordesillas L, Bustos XE, Ramello MC, Bishop RT, Cianne JE, et al. γδ-Enriched CAR-T cell therapy for bone metastatic castrate-resistant prostate cancer. Sci Adv. 2023;9(18):eadf108.
33. Zhai X, You F, Xiang S, Jiang L, Chen D, Li Y, et al. MUC1-Tn-targeting chimeric antigen receptor-modified Vγ9Vδ2 T cells with enhanced antigen-specific anti-tumor activity. Am J Cancer Res. 2021;11(1):79–91.
34. Makkouk A, Yang XC, Barca T, Lucas A, Turkoz M, Wong JTS, et al. Off-the-shelf Vdelta1 gamma delta T cells engineered with glypican-3 (GPC-3)-specific chimeric antigen receptor (CAR) and soluble IL-15 display robust antitumor efficacy against hepatocellular carcinoma. J Immunother Cancer. 2021;9(12): e003441.
35. Zhang P, Zhang G, Wan X. Challenges and new technologies in adoptive cell therapy. J Hematol Oncol. 2023;16(1):97.
36. Shao R, Li Z, Xin H, Jiang S, Zhu Y, Liu J, et al. Biomarkers as targets for CAR-T/NK cell therapy in AML. Biomark Res. 2023;11(1):65.
37. Jiang W, Gu G, Zhang Y, Song Y, Shi M, Wang G, et al. Novel mesothelin-targeted chimeric antigen receptor-modified UNKT cells are highly effective in inhibiting tumor progression. Pharmacol Res. 2023;197: 106942.
38. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Kaer LVJNRI. NKT cells: what's in a name? Nat Rev Immunol. 2004;4(3):231–7.
39. Alizadeh D, Wong RA, Yang X, Wang D, Pecoraro JR, Kuo C-F, et al. IL15 enhances CAR-T cell antitumor activity by reducing mTORC1 activity and preserving their stem cell memory phenotype. Cancer Immunol Res. 2019;7(5):759–72.
40. Liu D, Song L, Wei J, Courtney AN, Gao X, Marinova E, et al. IL-15 protects NKT cells from inhibition by tumor-associated macrophages and enhances antimetastatic activity. J Clin Invest. 2012;122(6):2221–33.
41. Xu X, Huang W, Heczey A, Liu D, Guo L, Wood M, et al. NKT cells coexpressing a GD2-specific chimeric antigen receptor and IL15 show enhanced in vivo persistence and antitumor activity against neuroblastoma. Clin Cancer Res. 2019;25(23):7126–38.
42. Arcangeli S, Bove C, Mezzanotte C, Camisa B, Falcone L, Manfredi F, et al. CAR T cell manufacturing from naive/stem memory T lymphocytes enhances antitumor responses while curtailing cytokine release syndrome. J Clin Invest. 2022;132(12):1.
43. Larson SM, Walthers CM, Ji B, Ghafouri SN, Naparstek J, Trent J, et al. CD19/CD20 bispecific chimeric antigen receptor (CAR) in naive/memory T cells for the treatment of relapsed or refractory non-hodgkin lymphoma. Cancer Discov. 2023;13(3):580–97.
44. El Marabti E, Abdel-Wahab O. Enhancing CD19 chimeric antigen receptor T cells through memory-enriched T cells. Clin Cancer Res. 2023;29(4):694–6.
45. Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, et al. Recent advances in CAR-T cell engineering. J Hematol Oncol. 2020;13(1):86.
46. Zheng X, Cheng M, Fu B, Fan X, Wang Q, Yu X, et al. Targeting LUNX inhibits non-small cell lung cancer growth and metastasis. Cancer Res. 2015;75(6):1080–90.
47. Hu Z, Zheng X, Jiao D, Zhou Y, Sun R, Wang B, et al. LunX-CAR T cells as a targeted therapy for non-small cell lung cancer. Mol Ther Oncolytics. 2020;17:361–70.
48. Simon AG, Lyu SI, Laible M, Woll S, Tureci O, Sahin U, et al. The tight junction protein claudin 6 is a potential target for patient-individualized treatment in esophageal and gastric adenocarcinoma and is associated with poor prognosis. J Transl Med. 2023;21(1):552.
49. Mackensen A, Haanen J, Koenecke C, Alsdorf W, Wagner-Drouet E, Borchmann P, et al. CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. Nat Med. 2023;29(11):2844–53.
50. Zhao P, Jiang D, Huang Y, Chen C. EphA2: a promising therapeutic target in breast cancer. J Genet Genom. 2021;48(4):261–7.
51. Li N, Liu S, Sun M, Chen W, Xu X, Zeng Z, et al. Chimeric antigen receptor-modified T cells redirected to EphA2 for the immunotherapy of non-small cell lung cancer. Transl Oncol. 2018;11(1):11–7.
52. Zhong M, Chalbatani GM, Deng M, Li Q, Gharagouzloo E, Hamblin MR, et al. Functional characterization and development of novel human kinase insert domain receptor chimeric antigen receptor T-cells for immunotherapy of non-small cell lung cancer. Eur J Pharm Sci. 2023;180: 106331.
53. Liu J, Yang S, Cao B, Zhou G, Zhang F, Wang Y, et al. Targeting B7–H3 via chimeric antigen receptor T cells and bispecific killer cell engagers augments antitumor response of cytotoxic lymphocytes. J Hematol Oncol. 2021;14(1):21.
54. Nishimura CD, Corrigan D, Zheng XY, Galbo PM Jr, Wang S, Liu Y, et al. TOP CAR with TMIGD2 as a safe and effective costimulatory domain in CAR cells treating human solid tumors. Sci Adv. 2024;10(19):eadk1857.
55. Zhang Z, Lin G, Yan Y, Li X, Hu Y, Wang J, et al. Transmembrane TNF-alpha promotes chemoresistance in breast cancer cells. Oncogene. 2018;37(25):3456–70.
56. Yang P, Zeng Y, Yang F, Peng X, Hu Y, Tan X, et al. Transmembrane TNF-alpha as a novel biomarker for the diagnosis of cytokine storms in a mouse model of multiple organ failure. Inflammation. 2023;46(1):359–69.
57. Ba H, Dai Z, Zhang Z, Zhang P, Yin B, Wang J, et al. Antitumor effect of CAR-T cells targeting transmembrane tumor necrosis factor alpha combined with PD-1 mAb on breast cancers. J Immunother Cancer. 2023;11(1):1.
58. Jun Lee EH, Cullen C, Murad JP, Gumber D, Park AK, Yang J, et al. Antigen-dependent IL-12 signaling in CAR T cells promotes regional to systemic disease targeting. Nat Commun. 2023;14(1):4737.
59. Wang XY, Bian MR, Lin GQ, Yu L, Zhang YM, Wu DP. Tandem bispecific CD123/CLL-1 CAR-T cells exhibit specific cytolytic effector functions against human acute myeloid leukaemia. Eur J Haematol. 2024;112(1):83–93.
60. Yan T, Zhu L, Chen J. Current advances and challenges in CAR T-Cell therapy for solid tumors: tumor-associated antigens and the tumor microenvironment. Exp Hematol Oncol. 2023;12(1):14.
61. Ma Y, Dai H, Cui Q, Liu S, Kang L, Lian X, et al. Decitabine in combination with fludarabine and cyclophosphamide as a lymphodepletion regimen followed by CD19/CD22 bispecific targeted CAR T-cell therapy significantly improves survival in relapsed/refractory B-ALL patients. Exp Hematol Oncol. 2023;12(1):36.
62. Ma R, You F, Tian S, Zhang T, Tian X, Xiang S, et al. Enhanced efficacy of CD19/CD22 bispecific CAR-T cells with EAAAK linker on B-cell malignancies. Eur J Haematol. 2024;112(1):64–74.
63. Liu S, Zhang X, Dai H, Cui W, Yin J, Li Z, et al. Which one is better for refractory/relapsed acute B-cell lymphoblastic leukemia: single-target (CD19) or dual-target (tandem or sequential CD19/CD22) CAR T-cell therapy? Blood Cancer J. 2023;13(1):1.
64. Jung IY, Narayan V, McDonald S, Rech AJ, Bartoszek R, Hong G, et al. BLIMP1 and NR4A3 transcription factors reciprocally regulate antitumor CAR T cell stemness and exhaustion. Sci Transl Med. 2022;14(670):eabn7336.
65. Shu R, Evtimov VJ, Hammett MV, Nguyen NN, Zhuang J, Hudson PJ, et al. Engineered CAR-T cells targeting TAG-72 and CD47 in ovarian cancer. Mol Ther Oncolytics. 2021;20:325–41.
66. Timpanaro A, Piccand C, Dzhumashev D, Anton-Joseph S, Robbi A, Moser J, et al. CD276-CAR T cells and dual-CAR T cells targeting CD276/FGFR4 promote rhabdomyosarcoma clearance in orthotopic mouse models. J Exp Clin Cancer Res. 2023;42(1):293.
67. He Y, Vlaming M, van Meerten T, Bremer E. The implementation of TNFRSF co-stimulatory domains in CAR-T cells for optimal functional activity. Cancers. 2022;14(2):1.
68. Zhang H, Snyder KM, Suhoski MM, Maus MV, Kapoor V, June CH, et al. 4–1BB is superior to CD28 costimulation for generating $CD8^+$ cytotoxic lymphocytes for adoptive immunotherapy. J Immunol. 2007;179(7):4910–8.
69. Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, et al. 4–1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med. 2015;21(6):581–90.

70. Roselli E, Boucher JC, Li G, Kotani H, Spitler K, Reid K, et al. 4–1BB and optimized CD28 co-stimulation enhances function of human mono-specific and bi-specific third-generation CAR T cells. J Immunother Cancer. 2021;9(10): e003354.
71. Chen Y, Zhu X, Liu H, Wang C, Chen Y, Wang H, et al. The application of HER2 and CD47 CAR-macrophage in ovarian cancer. J Transl Med. 2023;21(1):654.
72. Lai P, Chen X, Wang Y, Wang J, Zhang Y, Geng S, et al. C3aR costimulation enhances the antitumor efficacy of CAR-T cell therapy through Th17 expansion and memory T cell induction. J Hematol Oncol. 2022;15(1):68.
73. Zhang H, Li F, Cao J, Wang X, Cheng H, Qi K, et al. A chimeric antigen receptor with antigen-independent OX40 signaling mediates potent antitumor activity. Sci Transl Med. 2021;13(578):1.
74. Ramakrishna V, Sundarapandiyan K, Zhao B, Bylesjo M, Marsh HC, Keler T. Characterization of the human T cell response to in vitro CD27 costimulation with varlilumab. J Immunother Cancer. 2015;3:37.
75. Julamanee J, Terakura S, Umemura K, Adachi Y, Miyao K, Okuno S, et al. Composite CD79A/CD40 co-stimulatory endodomain enhances CD19CAR-T cell proliferation and survival. Mol Ther. 2021;29(9):2677–90.
76. Nunoya JI, Masuda M, Ye C, Su L. Chimeric antigen receptor T cell bearing herpes virus entry mediator co-stimulatory signal domain exhibits high functional potency. Mol Ther Oncolytics. 2019;14:27–37.
77. Golubovskaya VM, Berahovich R, Xu Q, Zhou H, Xu S, Guan J, et al. GITR domain inside CAR co-stimulates activity of CAR-T cells against cancer. Front Biosci. 2018;23(12):2245–54.
78. Liu G, Rui W, Zhao X, Lin X. Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment. Cell Mol Immunol. 2021;18(5):1085–95.
79. Chen C, Gu YM, Zhang F, Zhang ZC, Zhang YT, He YD, et al. Construction of PD1/CD28 chimeric-switch receptor enhances anti-tumor ability of c-Met CAR-T in gastric cancer. Oncoimmunology. 2021;10(1):1901434.
80. Zhang A, Wang S, Sun Y, Zhang Y, Zhao L, Yang Y, et al. Targeting and cytotoxicity of chimeric antigen receptor T cells grafted with PD1 extramembrane domain. Exp Hematol Oncol. 2023;12(1):85.
81. Prosser ME, Brown CE, Shami AF, Forman SJ, Jensen MC. Tumor PD-L1 co-stimulates primary human $CD8^+$ cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. Mol Immunol. 2012;51(3–4):263–72.
82. Liang Y, Liu H, Lu Z, Lei W, Zhang C, Li P, et al. CD19 CAR-T expressing PD-1/CD28 chimeric switch receptor as a salvage therapy for DLBCL patients treated with different CD19-directed CAR T-cell therapies. J Hematol Oncol. 2021;14(1):26.
83. Liaskou E, Patel SR, Webb G, Bagkou Dimakou D, Akiror S, Krishna M, et al. Increased sensitivity of Treg cells from patients with PBC to low dose IL-12 drives their differentiation into IFN-gamma secreting cells. J Autoimmun. 2018;94:143–55.
84. Hombach A, Barden M, Hannappel L, Chmielewski M, Rappl G, Sachinidis A, et al. IL12 integrated into the CAR exodomain converts $CD8^+$ T cells to poly-functional NK-like cells with superior killing of antigen-loss tumors. Mol Ther. 2022;30(2):593–605.
85. Gong W, Hoffmann JM, Stock S, Wang L, Liu Y, Schubert ML, et al. Comparison of IL-2 versus IL-7/IL-15 for the generation of NY-ESO-1-specific T cells. Cancer Immunol Immunother. 2019;68(7):1195–209.
86. Dabiri H, Safarzadeh Kozani P, Habibi Anbouhi M, Mirzaee Godarzee M, Haddadi MH, Basiri M, et al. Site-specific transgene integration in chimeric antigen receptor (CAR) T cell therapies. Biomark Res. 2023;11(1):67.
87. Chen Z, Hu Y, Mei H. Advances in CAR-engineered immune cell generation: engineering approaches and sourcing strategies. Adv Sci. 2023;10(35): e2303215.
88. Doan AE, Mueller KP, Chen AY, Rouin GT, Chen Y, Daniel B, et al. FOXO1 is a master regulator of memory programming in CAR T cells. Nature. 2024;629(8010):211–8.
89. Chan JD, Scheffler CM, Munoz I, Sek K, Lee JN, Huang YK, et al. FOXO1 enhances CAR T cell stemness, metabolic fitness and efficacy. Nature. 2024;629(8010):201–10.
90. Marchais M, Simula L, Phayanouvong M, Mami-Chouaib F, Bismuth G, Decroocq J, et al. FOXO1 inhibition generates potent nonactivated CAR T cells against solid tumors. Cancer Immunol Res. 2023;11(11):1508–23.
91. Reardon S. How to supercharge cancer-fighting cells: give them stem-cell skills. Nature. 2024;628(8008):486.
92. Wilkens AB, Fulton EC, Pont MJ, Cole GO, Leung I, Stull SM, et al. NOTCH1 signaling during $CD4^+$ T-cell activation alters transcription factor networks and enhances antigen responsiveness. Blood. 2022;140(21):2261–75.
93. Kondo T, Ando M, Nagai N, Tomisato W, Srirat T, Liu B, et al. The NOTCH-FOXM1 axis plays a key role in mitochondrial biogenesis in the induction of human stem cell memory-like CAR-T cells. Cancer Res. 2020;80(3):471–83.
94. Venuprasad K. Cbl-b and itch: key regulators of peripheral T-cell tolerance. Cancer Res. 2010;70(8):3009–12.
95. Kumar J, Kumar R, Kumar Singh A, Tsakem EL, Kathania M, Riese MJ, et al. Deletion of Cbl-b inhibits $CD8^+$ T-cell exhaustion and promotes CAR T-cell function. J Immunother Cancer. 2021;9(1):1.
96. Bhat AA, Nisar S, Mukherjee S, Saha N, Yarravarapu N, Lone SN, et al. Integration of CRISPR/Cas9 with artificial intelligence for improved cancer therapeutics. J Transl Med. 2022;20(1):534.
97. Mei H, Hari P, Hu Y. Exercise, "CALM" and make CAR-T therapy work better. Sci Bull. 2022;67(19):1925–8.
98. Liu M, Zhang L, Zhong M, Long Y, Yang W, Liu T, et al. CRISPR/Cas9-mediated knockout of intracellular molecule SHP-1 enhances tumor-killing ability of CD133-targeted CAR T cells in vitro. Exp Hematol Oncol. 2023;12(1):88.
99. Walsh ZH, Shah P, Kothapalli N, Shah SB, Nikolenyi G, Brodtman DZ, et al. Mapping variant effects on anti-tumor hallmarks of primary human T cells with base-editing screens. Nat Biotechnol. 2024. https://doi.org/10.1038/s41587-024-02235-x.
100. Garcia J, Daniels J, Lee Y, Zhu I, Cheng K, Liu Q, et al. Naturally occurring T cell mutations enhance engineered T cell therapies. Nature. 2024;626(7999):626–34.
101. Muyldermans S, Baral TN, Retamozzo VC, De Baetselier P, De Genst E, Kinne J, et al. Camelid immunoglobulins and nanobody technology. Vet Immunol Immunopathol. 2009;128(1–3):178–83.
102. Xie YJ, Dougan M, Ingram JR, Pishesha N, Fang T, Momin N, et al. Improved antitumor efficacy of chimeric antigen receptor T cells that secrete single-domain antibody fragments. Cancer Immunol Res. 2020;8(4):518–29.
103. Nasiri F, Safarzadeh Kozani P, Rahbarizadeh F. T-cells engineered with a novel VHH-based chimeric antigen receptor against CD19 exhibit comparable tumoricidal efficacy to their FMC63-based counterparts. Front Immunol. 2023;14:1063838.
104. Revets H, De Baetselier P, Muyldermans S. Nanobodies as novel agents for cancer therapy. Expert Opin Biol Ther. 2005;5(1):111–24.
105. De Meyer T, Muyldermans S, Depicker A. Nanobody-based products as research and diagnostic tools. Trends Biotechnol. 2014;32(5):263–70.
106. Vincke C, Loris R, Saerens D, Martinez-Rodriguez S, Muyldermans S, Conrath K. General strategy to humanize a camelid single-domain antibody and identification of a universal humanized nanobody scaffold. J Biol Chem. 2009;284(5):3273–84.
107. De Pauw T, De Mey L, Debacker JM, Raes G, Van Ginderachter JA, De Groof TWM, et al. Current status and future expectations of nanobodies in oncology trials. Expert Opin Investig Drugs. 2023;32(8):705–21.
108. Leslie M. Cilta-cel OK'd for multiple myeloma. Cancer Discov. 2022;12(5):1176.
109. Tredan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst. 2007;99(19):1441–54.
110. Toledo B, Zhu Chen L, Paniagua-Sancho M, Marchal JA, Peran M, Giovannetti E. Deciphering the performance of macrophages in tumour microenvironment: a call for precision immunotherapy. J Hematol Oncol. 2024;17(1):44.
111. Hoekstra ME, Bornes L, Dijkgraaf FE, Philips D, Pardieck IN, Toebes M, et al. Publisher correction: long-distance modulation of bystander tumor cells by $CD8^+$ T-cell-secreted IFN-γ. Nat Cancer. 2020;1(7):749.
112. Hong M, Clubb JD, Chen YY. Engineering CAR-T cells for next-generation cancer therapy. Cancer Cell. 2020;38(4):473–88.
113. McGowan E, Lin Q, Ma G, Yin H, Chen S, Lin Y. PD-1 disrupted CAR-T cells in the treatment of solid tumors: promises and challenges. Biomed Pharmacother. 2020;121: 109625.

114. Fan J, Yu Y, Yan L, Yuan Y, Sun B, Yang D, et al. GAS6-based CAR-T cells exhibit potent antitumor activity against pancreatic cancer. J Hematol Oncol. 2023;16(1):77.
115. Wang J, Wang Y, Pan H, Zhao L, Yang X, Liang Z, et al. Chemokine receptors CCR6 and PD1 blocking scFv E27 enhances anti-EGFR CAR-T therapeutic efficacy in a preclinical model of human non-small cell lung carcinoma. Int J Mol Sci. 2023;24(6):1.
116. Ganesh K, Massague J. TGF-beta inhibition and immunotherapy: checkmate. Immunity. 2018;48(4):626–8.
117. Chen X, Yang S, Li S, Qu Y, Wang HY, Liu J, et al. Secretion of bispecific protein of anti-PD-1 fused with TGF-beta trap enhances antitumor efficacy of CAR-T cell therapy. Mol Ther Oncolytics. 2021;21:144–57.
118. Xu J, Liu W, Fan F, Zhang B, Sun C, Hu Y. Advances in nano-immunotherapy for hematological malignancies. Exp Hematol Oncol. 2024;13(1):57.
119. Altvater B, Kailayangiri S, Spurny C, Flugge M, Meltzer J, Greune L, et al. CAR T cells as micropharmacies against solid cancers: combining effector T-cell mediated cell death with vascular targeting in a one-step engineering process. Cancer Gene Ther. 2023;30(10):1355–68.
120. French RR, Chan HT, Tutt AL, Glennie MJ. CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. Nat Med. 1999;5(5):548–53.
121. Curran KJ, Seinstra BA, Nikhamin Y, Yeh R, Usachenko Y, van Leeuwen DG, et al. Enhancing antitumor efficacy of chimeric antigen receptor T cells through constitutive CD40L expression. Mol Ther. 2015;23(4):769–78.
122. Kuhn NF, Purdon TJ, van Leeuwen DG, Lopez AV, Curran KJ, Daniyan AF, et al. CD40 ligand-modified chimeric antigen receptor T cells enhance antitumor function by eliciting an endogenous antitumor response. Cancer Cell. 2019;35(3):473–88.
123. Zhang Y, Wang P, Wang T, Fang Y, Ding Y, Qian Q. Chimeric antigen receptor T cells engineered to secrete CD40 agonist antibodies enhance antitumor efficacy. J Transl Med. 2021;19(1):82.
124. Li Y, Lu S, Xu Y, Qiu C, Jin C, Wang Y, et al. Overexpression of CD47 predicts poor prognosis and promotes cancer cell invasion in high-grade serous ovarian carcinoma. Am J Transl Res. 2017;9(6):2901–10.
125. Matlung HL, Szilagyi K, Barclay NA, van den Berg TK. The CD47-SIRPalpha signaling axis as an innate immune checkpoint in cancer. Immunol Rev. 2017;276(1):145–64.
126. Mathias MD, Sockolosky JT, Chang AY, Tan KS, Liu C, Garcia KC, et al. CD47 blockade enhances therapeutic activity of TCR mimic antibodies to ultra-low density cancer epitopes. Leukemia. 2017;31(10):2254–7.
127. Dacek MM, Kurtz KG, Wallisch P, Pierre SA, Khayat S, Bourne CM, et al. Potentiating antibody-dependent killing of cancers with CAR T cells secreting CD47-SIRPalpha checkpoint blocker. Blood. 2023;141(16):2003–15.
128. Nakamura K, Smyth MJ. TREM2 marks tumor-associated macrophages. Signal Transduct Target Ther. 2020;5(1):233.
129. Chen J, Zhu T, Jiang G, Zeng Q, Li Z, Huang X. Target delivery of a PD-1-TREM2 scFv by CAR-T cells enhances anti-tumor efficacy in colorectal cancer. Mol Cancer. 2023;22(1):131.
130. Avanzi MP, Yeku O, Li X, Wijewarnasuriya DP, van Leeuwen DG, Cheung K, et al. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell Rep. 2018;23(7):2130–41.
131. Glienke W, Dragon AC, Zimmermann K, Martyniszyn-Eiben A, Mertens M, Abken H, et al. GMP-compliant manufacturing of TRUCKs: CAR T cells targeting GD(2) and releasing inducible IL-18. Front Immunol. 2022;13:839783.
132. Harrer DC, Schenkel C, Bezler V, Kaljanac M, Hartley J, Barden M, et al. CAR triggered release of type-1 interferon limits CAR T-cell activities by an artificial negative autocrine loop. Cells. 2022;11(23):1.
133. Zeng R, Spolski R, Finkelstein SE, Oh S, Kovanen PE, Hinrichs CS, et al. Synergy of IL-21 and IL-15 in regulating $CD8^+$ T cell expansion and function. J Exp Med. 2005;201(1):139–48.
134. Zhou Y, Farooq MA, Ajmal I, He C, Gao Y, Guo D, et al. Co-expression of IL-4/IL-15-based inverted cytokine receptor in CAR-T cells overcomes IL-4 signaling in immunosuppressive pancreatic tumor microenvironment. Biomed Pharmacother. 2023;168:1.
135. Edeline J, Houot R, Marabelle A, Alcantara M. CAR-T cells and BiTEs in solid tumors: challenges and perspectives. J Hematol Oncol. 2021;14(1):65.
136. Yin Y, Rodriguez JL, Li N, Thokala R, Nasrallah MP, Hu L, et al. Locally secreted BiTEs complement CAR T cells by enhancing killing of antigen heterogeneous solid tumors. Mol Ther. 2022;30(7):2537–53.
137. Marofi F, Motavalli R, Safonov VA, Thangavelu L, Yumashev AV, Alexander M, et al. CAR T cells in solid tumors: challenges and opportunities. Stem Cell Res Ther. 2021;12(1):81.
138. Avisar A, Cohen M, Brenner B, Bronshtein T, Machluf M, Bar-Sela G, et al. Extracellular vesicles reflect the efficacy of wheatgrass juice supplement in colon cancer patients during adjuvant chemotherapy. Front Oncol. 2020;10:1659.
139. Aharon A, Horn G, Bar-Lev TH, Zagagi Yohay E, Waks T, Levin M, et al. Extracellular vesicles derived from chimeric antigen receptor-T cells: a potential therapy for cancer. Hum Gene Ther. 2021;32(19–20):1224–41.
140. Zhu S, Zhang T, Zheng L, Liu H, Song W, Liu D, et al. Combination strategies to maximize the benefits of cancer immunotherapy. J Hematol Oncol. 2021;14(1):156.
141. Peng JJ, Wang L, Li Z, Ku CL, Ho PC. Metabolic challenges and interventions in CAR T cell therapy. Sci Immunol. 2023;8(82):eabq3016.
142. Shen L, Xiao Y, Tian J, Lu Z. Remodeling metabolic fitness: strategies for improving the efficacy of chimeric antigen receptor T cell therapy. Cancer Lett. 2022;529:139–52.
143. Ye L, Park JJ, Peng L, Yang Q, Chow RD, Dong MB, et al. A genome-scale gain-of-function CRISPR screen in CD8 T cells identifies proline metabolism as a means to enhance CAR-T therapy. Cell Metab. 2022;34(4):595–614.
144. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. Cell. 2016;167(3):829-42.e13.
145. Summitt CB, Johnson LC, Jönsson TJ, Parsonage D, Holmes RP, Lowther WT. Proline dehydrogenase 2 (PRODH2) is a hydroxyproline dehydrogenase (HYPDH) and molecular target for treating primary hyperoxaluria. Biochem J. 2015;466(2):273–81.
146. PRODH2-mediated proline metabolism boosts CAR T-cell effector function. Cancer Discov. 2022;12(6):1405.
147. Liu Y, Adu-Berchie K, Brockman JM, Pezone M, Zhang DKY, Zhou J, et al. Cytokine conjugation to enhance T cell therapy. Proc Natl Acad Sci USA. 2023;120(1): e2213222120.
148. Yang Q, Hao J, Chi M, Wang Y, Xin B, Huang J, et al. Superior anti-tumor immunotherapy efficacy of kynureninase modified CAR-T cells through targeting kynurenine metabolism. Oncoimmunology. 2022;11(1):2055703.
149. Shao J, Hou L, Liu J, Liu Y, Ning J, Zhao Q, et al. Indoleamine 2,3-dioxygenase 1 inhibitor-loaded nanosheets enhance CAR-T cell function in esophageal squamous cell carcinoma. Front Immunol. 2021;12: 661357.
150. Li F, Zhang R, Li S, Liu J. IDO1: an important immunotherapy target in cancer treatment. Int Immunopharmacol. 2017;47:70–7.
151. Huang Q, Xia J, Wang L, Wang X, Ma X, Deng Q, et al. miR-153 suppresses IDO1 expression and enhances CAR T cell immunotherapy. J Hematol Oncol. 2018;11(1):58.
152. Caforio M, Sorino C, Caruana I, Weber G, Camera A, Cifaldi L, et al. GD2 redirected CAR T and activated NK-cell-mediated secretion of IFNγ overcomes MYCN-dependent IDO1 inhibition, contributing to neuroblastoma cell immune escape. J Immunother Cancer. 2021;9(3):1.
153. Patra T, Cunningham DM, Meyer K, Toth K, Ray RB, Heczey A, et al. Targeting Lin28 axis enhances glypican-3-CAR T cell efficacy against hepatic tumor initiating cell population. Mol Ther. 2023;31(3):715–28.
154. Huang Y, Shao M, Teng X, Si X, Wu L, Jiang P, et al. Inhibition of CD38 enzymatic activity enhances CAR-T cell immune-therapeutic efficacy by repressing glycolytic metabolism. Cell Rep Med. 2024;5(2): 101400.
155. Siracusa F, Durek P, McGrath MA, Sercan-Alp Ö, Rao A, Du W, et al. $CD69^+$ memory T lymphocytes of the bone marrow and spleen express the signature transcripts of tissue-resident memory T lymphocytes. Eur J Immunol. 2019;49(6):966–8.
156. Shinoda K, Tokoyoda K, Hanazawa A, Hayashizaki K, Zehentmeier S, Hosokawa H, et al. Type II membrane protein CD69 regulates the formation of resting T-helper memory. Proc Natl Acad Sci USA. 2012;109(19):7409–14.
157. Elmarsafawi AG, Hesterberg RS, Fernandez MR, Yang C, Darville LN, Liu M, et al. Modulating the polyamine/hypusine axis controls generation of $CD8^+$ tissue-resident memory T cells. JCI Insight. 2023;8(18):1.

158. Reinfeld BI, Madden MZ, Wolf MM, Chytil A, Bader JE, Patterson AR, et al. Cell-programmed nutrient partitioning in the tumour microenvironment. Nature. 2021;593(7858):282–8.
159. Wang Y, Wang F, Wang L, Qiu S, Yao Y, Yan C, et al. NAD^+ supplement potentiates tumor-killing function by rescuing defective TUB-mediated NAMPT transcription in tumor-infiltrated T cells. Cell Rep. 2021;36(6):109516.
160. Chakraborty P, Vaena SG, Thyagarajan K, Chatterjee S, Al-Khami A, Selvam SP, et al. Pro-survival lipid sphingosine-1-phosphate metabolically programs T cells to limit anti-tumor activity. Cell Rep. 2019;28(7):1879–93.
161. Lemoine J, Ruella M, Houot R. Born to survive: How cancer cells resist CAR T cell therapy. J Hematol Oncol. 2021;14(1):199.
162. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med. 2016;375(26):2561–9.
163. Song EZ, Wang X, Philipson BI, Zhang Q, Thokala R, Zhang L, et al. The IAP antagonist birinapant enhances chimeric antigen receptor T cell therapy for glioblastoma by overcoming antigen heterogeneity. Mol Ther Oncolytics. 2022;27:288–304.
164. Xu Y, Li S, Wang Y, Liu J, Mao X, Xing H, et al. Induced CD20 expression on B-cell malignant cells heightened the cytotoxic activity of chimeric antigen receptor engineered T cells. Hum Gene Ther. 2019;30(4):497–510.
165. DeAngelo DJ. The use of novel monoclonal antibodies in the treatment of acute lymphoblastic leukemia. Hematol Am Soc Hematol Educ Progr. 2015;2015:400–5.
166. Teeling JL, Mackus WJ, Wiegman LJ, van den Brakel JH, Beers SA, French RR, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol. 2006;177(1):362–71.
167. Shimizu R, Kikuchi J, Wada T, Ozawa K, Kano Y, Furukawa Y. HDAC inhibitors augment cytotoxic activity of rituximab by upregulating CD20 expression on lymphoma cells. Leukemia. 2010;24(10):1760–8.
168. Kaiserman D, Bird PI. Control of granzymes by serpins. Cell Death Differ. 2010;17(4):586–95.
169. van Houdt IS, Oudejans JJ, van den Eertwegh AJ, Baars A, Vos W, Bladergroen BA, et al. Expression of the apoptosis inhibitor protease inhibitor 9 predicts clinical outcome in vaccinated patients with stage III and IV melanoma. Clin Cancer Res. 2005;11(17):6400–7.
170. Kimman T, Slomp A, Martens A, Grabherr S, Li S, van Diest E, et al. Serpin B9 controls tumor cell killing by CAR T cells. J Immunother Cancer. 2023;11(3):1.
171. Sugi T, Katoh Y, Ikeda T, Seta D, Iwata T, Nishio H, et al. SCD1 inhibition enhances the effector functions of $CD8^+$ T cells via ACAT1-dependent reduction of esterified cholesterol. Cancer Sci. 2023;115:48.
172. Dong S, Wang P, Zhang L, Zhang X, Li X, Wang J, et al. The Qi Yin San Liang San decoction enhances anti-CD19 CAR-T cell function in the treatment of B-cell lymphomas. J Ethnopharmacol. 2024;319(Pt 1):117109.
173. Palliyaguru DL, Yuan JM, Kensler TW, Fahey JW. Isothiocyanates: translating the power of plants to people. Mol Nutr Food Res. 2018;62(18):e1700965.
174. Lenzi M, Fimognari C, Hrelia P. Sulforaphane as a promising molecule for fighting cancer. Cancer Treat Res. 2014;159:207–23.
175. Shih YL, Wu LY, Lee CH, Chen YL, Hsueh SC, Lu HF, et al. Sulforaphane promotes immune responses in a WEHI-3-induced leukemia mouse model through enhanced phagocytosis of macrophages and natural killer cell activities in vivo. Mol Med Rep. 2016;13(5):4023–9.
176. Geisel J, Bruck J, Glocova I, Dengler K, Sinnberg T, Rothfuss O, et al. Sulforaphane protects from T cell-mediated autoimmune disease by inhibition of IL-23 and IL-12 in dendritic cells. J Immunol. 2014;192(8):3530–9.
177. Shen C, Zhang Z, Tian Y, Li F, Zhou L, Jiang W, et al. Sulforaphane enhances the antitumor response of chimeric antigen receptor T cells by regulating PD-1/PD-L1 pathway. BMC Med. 2021;19(1):283.
178. Lee YG, Guruprasad P, Ghilardi G, Pajarillo R, Sauter CT, Patel R, et al. Modulation of BCL-2 in both T cells and tumor cells to enhance chimeric antigen receptor T-cell immunotherapy against cancer. Cancer Discov. 2022;12(10):2372–91.
179. Wang L, Lv Y, Zhou L, Wu S, Zhu Y, Fu S, et al. Cytokine-based models for efficient differentiation between infection and cytokine release syndrome in patients with hematological malignancies. Exp Hematol Oncol. 2024;13(1):28.
180. Frey N, Porter D. Cytokine release syndrome with chimeric antigen receptor T cell therapy. Biol Blood Marrow Transplant. 2019;25(4):e123–7.
181. Wei Z, Xu J, Zhao C, Zhang M, Xu N, Kang L, et al. Prediction of severe CRS and determination of biomarkers in B cell-acute lymphoblastic leukemia treated with CAR-T cells. Front Immunol. 2023;14:1273507.
182. Lei W, Xie M, Jiang Q, Xu N, Li P, Liang A, et al. Treatment-related adverse events of chimeric antigen receptor T-cell (CAR T) in clinical trials: a systematic review and meta-analysis. Cancers. 2021;13(15):3912.
183. Bogatu A, Wysocka M, Wysocki O, Butterworth H, Pillai M, Allison J, et al. Meta-analysis informed machine learning: supporting cytokine storm detection during CAR-T cell therapy. J Biomed Inform. 2023;142:104367.
184. Murthy H, Iqbal M, Chavez JC, Kharfan-Dabaja MA. Cytokine release syndrome: current perspectives. Immunotargets Ther. 2019;8:43–52.
185. Le RQ, Li L, Yuan W, Shord SS, Nie L, Habtemariam BA, et al. FDA approval summary: tocilizumab for treatment of chimeric antigen receptor T cell-induced severe or life-threatening cytokine release syndrome. Oncologist. 2018;23(8):943–7.
186. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. Nat Med. 2018;24(6):739–48.
187. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188–95.
188. Si S, Teachey DT. Spotlight on tocilizumab in the treatment of CAR-T-cell-induced cytokine release syndrome: clinical evidence to date. Ther Clin Risk Manag. 2020;16:705–14.
189. Li X, Gong N, Tian F, Zhang S, Zhang Y, Wang Y, et al. Suppression of cytokine release syndrome during CAR-T-cell therapy via a subcutaneously injected interleukin-6-adsorbing hydrogel. Nat Biomed Eng. 2023;7(9):1129–41.
190. Sterner RM, Sakemura R, Cox MJ, Yang N, Khadka RH, Forsman CL, et al. GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. Blood. 2019;133(7):697–709.
191. Jain MD, Miklos DB, Jacobson CA, Timmerman JM, Sun J, Nater J, et al. Axicabtagene ciloleucel in combination with the 4–1BB agonist utomilumab in patients with relapsed/refractory large B-cell lymphoma: phase 1 results from ZUMA-11. Clin Cancer Res. 2023;29(20):4118–27.
192. Santomasso BD, Park JH, Salloum D, Riviere I, Flynn J, Mead E, et al. Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute lymphoblastic leukemia. Cancer Discov. 2018;8(8):958–71.
193. Santomasso BD, Nastoupil LJ, Adkins S, Lacchetti C, Schneider BJ, Anadkat M, et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO guideline. J Clin Oncol. 2021;39(35):3978–92.
194. Strati P, Ahmed S, Furqan F, Fayad LE, Lee HJ, Iyer SP, et al. Prognostic impact of corticosteroids on efficacy of chimeric antigen receptor T-cell therapy in large B-cell lymphoma. Blood. 2021;137(23):3272–6.
195. Topp MS, van Meerten T, Houot R, Minnema MC, Bouabdallah K, Lugtenburg PJ, et al. Earlier corticosteroid use for adverse event management in patients receiving axicabtagene ciloleucel for large B-cell lymphoma. Br J Haematol. 2021;195(3):388–98.
196. Gauthier J, Hirayama AV, Purushe J, Hay KA, Lymp J, Li DH, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. Blood. 2020;135(19):1650–60.
197. Michels A, Ho N, Buchholz CJ. Precision medicine: in vivo CAR therapy as a showcase for receptor-targeted vector platforms. Mol Ther. 2022;30(7):2401–15.
198. Milone MC, O'Doherty U. Clinical use of lentiviral vectors. Leukemia. 2018;32(7):1529–41.
199. Perry C, Rayat A. Lentiviral vector bioprocessing. Viruses. 2021;13(2):1.
200. Iaffaldano BJ, Marino MP, Reiser J. CRISPR library screening to develop HEK293-derived cell lines with improved lentiviral vector titers. Front Genome Ed. 2023;5:1218328.

201. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. Clin Microbiol Rev. 2008;21(4):583–93.
202. Cao D, Song Q, Li J, Chard Dunmall LS, Jiang Y, Qin B, et al. Redirecting anti-Vaccinia virus T cell immunity for cancer treatment by AAV-mediated delivery of the VV B8R gene. Mol Ther Oncolytics. 2022;25:264–75.
203. Wang D, Zhou Q, Qiu X, Liu X, Zhang C. Optimizing rAAV6 transduction of primary T cells for the generation of anti-CD19 AAV-CAR-T cells. Biomed Pharmacother. 2022;150: 113027.
204. Perry C, Mujahid N, Takeuchi Y, Rayat A. Insights into product and process related challenges of lentiviral vector bioprocessing. Biotechnol Bioeng. 2023;121:2466.
205. Cheng Z, Fobian SF, Gurrieri E, Amin M, D'Agostino VG, Falahati M, et al. Lipid-based nanosystems: the next generation of cancer immune therapy. J Hematol Oncol. 2024;17(1):53.
206. Wang S, Liang B, Wang W, Li L, Feng N, Zhao Y, et al. Viral vectored vaccines: design, development, preventive and therapeutic applications in human diseases. Signal Transduct Target Ther. 2023;8(1):149.
207. Billingsley MM, Gong N, Mukalel AJ, Thatte AS, El-Mayta R, Patel SK, et al. In vivo mRNA CAR T cell engineering via targeted ionizable lipid nanoparticles with extrahepatic tropism. Small. 2023;20: e2304378.
208. Yu Y, Gao Y, He L, Fang B, Ge W, Yang P, et al. Biomaterial-based gene therapy. MedCommun. 2023;4(3): e259.
209. Zhou JE, Sun L, Jia Y, Wang Z, Luo T, Tan J, et al. Lipid nanoparticles produce chimeric antigen receptor T cells with interleukin-6 knockdown in vivo. J Control Release. 2022;350:298–307.
210. Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, et al. mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability. Int J Pharm. 2021;601: 120586.
211. Morfino P, Aimo A, Castiglione V, Galvez-Monton C, Emdin M, Bayes-Genis A. Treatment of cardiac fibrosis: from neuro-hormonal inhibitors to CAR-T cell therapy. Heart Fail Rev. 2023;28(2):555–69.
212. Hamilton AG, Swingle KL, Joseph RA, Mai D, Gong N, Billingsley MM, et al. Ionizable lipid nanoparticles with integrated immune checkpoint inhibition for mRNA CAR T cell engineering. Adv Healthc Mater. 2023;12(30): e2301515.
213. Iriguchi S, Kaneko S. Toward the development of true "off-the-shelf" synthetic T-cell immunotherapy. Cancer Sci. 2019;110(1):16–22.
214. Aparicio C, Acebal C, Gonzalez-Vallinas M. Current approaches to develop "off-the-shelf" chimeric antigen receptor (CAR)-T cells for cancer treatment: a systematic review. Exp Hematol Oncol. 2023;12(1):73.
215. Abraham-Miranda J, Menges M, Atkins R, Mattie M, Kanska J, Turner J, et al. CAR-T manufactured from frozen PBMC yield efficient function with prolonged in vitro production. Front Immunol. 2022;13:1007042.
216. Themeli M, Kloss CC, Ciriello G, Fedorov VD, Perna F, Gonen M, et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. Nat Biotechnol. 2013;31(10):928–33.
217. Kawamoto H, Masuda K, Nagano S. Regeneration of antigen-specific T cells by using induced pluripotent stem cell (iPSC) technology. Int Immunol. 2021;33(12):827–33.
218. Wang Z, McWilliams-Koeppen HP, Reza H, Ostberg JR, Chen W, Wang X, et al. 3D-organoid culture supports differentiation of human CAR$^+$ iPSCs into highly functional CAR T cells. Cell Stem Cell. 2022;29(4):651–3.
219. Jing R, Scarfo I, Najia MA, Lummertz da Rocha E, Han A, Sanborn M, et al. EZH1 repression generates mature iPSC-derived CAR T cells with enhanced antitumor activity. Cell Stem Cell. 2022;29(8):1181–96.
220. Hadiloo K, Taremi S, Heidari M, Esmaeilzadeh A. The CAR macrophage cells, a novel generation of chimeric antigen-based approach against solid tumors. Biomark Res. 2023;11(1):103.
221. Li M, Yang Y, Xiong L, Jiang P, Wang J, Li C. Metabolism, metabolites, and macrophages in cancer. J Hematol Oncol. 2023;16(1):80.
222. Lei A, Yu H, Lu S, Lu H, Ding X, Tan T, et al. A second-generation M1-polarized CAR macrophage with antitumor efficacy. Nat Immunol. 2024;25(1):102–16.
223. Zhao Q, Li F. Advances in allogeneic chimeric antigen receptor T cells. Acta Acad Med Sci. 2023;45(6):967–72.
224. Testa U, Sica S, Pelosi E, Castelli G, Leone G. CAR-T cell therapy in B-cell acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis. 2024;16(1): e2024010.
225. Derippe T, Fouliard S, Marchiq I, Dupouy S, Almena-Carrasco M, Geronimi J, et al. Mechanistic modeling of the interplay between host immune system, IL-7 and UCART19 allogeneic CAR-T cells in adult B-cell acute lymphoblastic leukemia. Cancer Res Commun. 2022;2(11):1532–44.
226. Zhao J, Lin Q, Song Y, Liu D. Universal CARs, universal T cells, and universal CAR T cells. J Hematol Oncol. 2018;11(1):132.
227. Stenger D, Stief TA, Kaeuferle T, Willier S, Rataj F, Schober K, et al. Endogenous TCR promotes in vivo persistence of CD19-CAR-T cells compared to a CRISPR/Cas9-mediated TCR knockout CAR. Blood. 2020;136(12):1407–18.
228. Li W, Zhu X, Xu Y, Chen J, Zhang H, Yang Z, et al. Simultaneous editing of TCR, HLA-I/II and HLA-E resulted in enhanced universal CAR-T resistance to allo-rejection. Front Immunol. 2022;13:1052717.
229. Del Bufalo F, Becilli M, Rosignoli C, De Angelis B, Algeri M, Hanssens L, et al. Allogeneic, donor-derived, second-generation, CD19-directed CAR-T cells for the treatment of pediatric relapsed/refractory BCP-ALL. Blood. 2023;142(2):146–57.
230. Oei RW, Hou G, Liu F, Zhong J, Zhang J, An Z, et al. Convolutional neural network for cell classification using microscope images of intracellular actin networks. PLoS ONE. 2019;14(3): e0213626.
231. Daniels KG, Wang S, Simic MS, Bhargava HK, Capponi S, Tonai Y, et al. Decoding CAR T cell phenotype using combinatorial signaling motif libraries and machine learning. Science. 2022;378(6625):1194–200.
232. Yu L, Li Z, Mei H, Li W, Chen D, Liu L, et al. Patient-derived organoids of bladder cancer recapitulate antigen expression profiles and serve as a personal evaluation model for CAR-T cells in vitro. Clin Transl Immunol. 2021;10(2): e1248.
233. Li Z, Xiong W, Liang Z, Wang J, Zeng Z, Kolat D, et al. Critical role of the gut microbiota in immune responses and cancer immunotherapy. J Hematol Oncol. 2024;17(1):33.
234. Abid MB, Shah NN, Maatman TC, Hari PN. Gut microbiome and CAR-T therapy. Exp Hematol Oncol. 2019;8:31.
235. Stein-Thoeringer CK, Saini NY, Zamir E, Blumenberg V, Schubert ML, Mor U, et al. A non-antibiotic-disrupted gut microbiome is associated with clinical responses to CD19-CAR-T cell cancer immunotherapy. Nat Med. 2023;29(4):906–16.
236. Smith M, Dai A, Ghilardi G, Amelsberg KV, Devlin SM, Pajarillo R, et al. Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. Nat Med. 2022;28(4):713–23.
237. Hu Y, Li J, Ni F, Yang Z, Gui X, Bao Z, et al. CAR-T cell therapy-related cytokine release syndrome and therapeutic response is modulated by the gut microbiome in hematologic malignancies. Nat Commun. 2022;13(1):5313.
238. Hu Q, Li H, Archibong E, Chen Q, Ruan H, Ahn S, et al. Inhibition of post-surgery tumour recurrence via a hydrogel releasing CAR-T cells and anti-PDL1-conjugated platelets. Nat Biomed Eng. 2021;5(9):1038–47.
239. Song P, Zhang Q, Xu Z, Shi Y, Jing R, Luo D. CRISPR/Cas-based CAR-T cells: production and application. Biomark Res. 2024;12(1):54.
240. Tieu V, Sotillo E, Bjelajac JR, Chen C, Malipatlolla M, Guerrero JA, et al. A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary human T cells. Cell. 2024;187(5):1278–95.
241. Safarzadeh Kozani P, Naseri A, Mirarefin SMJ, Salem F, Nikbakht M, Evazi Bakhshi S, et al. Nanobody-based CAR-T cells for cancer immunotherapy. Biomark Res. 2022;10(1):24.
242. Zhang DKY, Adu-Berchie K, Iyer S, Liu Y, Cieri N, Brockman JM, et al. Enhancing CAR-T cell functionality in a patient-specific manner. Nat Commun. 2023;14(1):506.

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