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Review

Review

Decoding signaling architectures: CAR versus TCR dynamics in solid tumor immunotherapy

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Abstract

The T cell receptor (TCR) initiates signaling by specifically recognizing peptide-MHC complexes, triggering the phosphorylation of CD3 chain immunoreceptor tyrosine-based activation motifs (ITAMs). This recruits kinases such as ZAP70, triggering a tightly regulated signaling cascade that governs T cell activation, differentiation, and effector functions. In contrast, the chimeric antigen receptor (CAR) is a synthetic construct that bypasses MHC restriction by fusing an antigen-binding domain with intracellular signaling modules (usually CD3ζ and co-stimulatory domains) from the TCR complex and other receptors. CAR-T cell therapy has revolutionized the treatment of hematologic malignancies, resulting in durable remission of B-cell leukemia, lymphoma, and multiple myeloma. However, its efficacy in solid tumors is limited by intrinsic barriers: poor CAR-T-cell trafficking/infiltration into tumors, the immunosuppressive tumor microenvironment (TME), intratumoral metabolic competition, and tumor antigen heterogeneity/loss. To improve CAR-T-cell function in solid tumors, numerous studies have explored multiple strategies: engineering CARs to boost immune synapse formation via optimized receptor clustering, increasing the ITAM number/strength to amplify downstream signaling, and incorporating novel/multiple co-stimulatory domains to sustain T-cell activation and persistence. Additionally, approaches include the use of CAR-T cells that secrete pro-inflammatory cytokines, epigenetic reprogramming to preserve T-cell stemness and functionality, and the use of synthetic biology tools for tunable/logic-gated CAR activation. Here, we summarize the current understanding of CAR signaling dynamics and highlight recent breakthrough strategies designed to overcome these challenges in solid tumors. These advances narrow the liquid-solid tumor efficacy gap, holding promise for better clinical outcomes in patients with solid malignancies and a new era of personalized immunotherapy.

Key words TCR, CAR-T, solid tumor, immunotherapy

Introduction

Immunotherapy has emerged as a transformative approach in oncology, with T cell-based therapies at the forefront of innovation. Among these, CAR-engineered T cells and TCR-based therapies represent two powerful strategies that harness the immune system to target malignancies. While CAR-T cells have demonstrated remarkable success in hematologic cancers by recognizing surface antigens independent of presentation, their efficacy in solid tumors remains limited due to challenges such as immunosuppressive microenvironments, poor trafficking, and antigen heterogeneity. In contrast, TCR-based therapies leverage the natural precision of MHC-restricted antigen recognition, offering potential against intracellular targets but facing hurdles related to MHC down-regulation and off-target toxicity.

Understanding the fundamental differences in signaling architecture between CAR and TCR is critical to optimizing T cell therapies for solid tumors. This review delves into the structural and functional divergence between these two systems, examines the role of various co-stimulatory domains in shaping CAR-T cell fate, and surveys current clinical trials and innovative strategies aimed at overcoming the barriers to effective solid tumor immunotherapy. By integrating insights from basic science and clinical advances, we aim to highlight pathways toward more durable and targeted immunotherapeutic interventions.

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CAR versus TCR Signaling: Structural and Functional Divergence

CAR signaling

Chimeric antigen receptor (CAR) is a type of artificial receptor designed to mimic the T-cell receptor (TCR) by incorporating intracellular signaling domains from the TCR complex. Their modular design comprises four modules: an antigen recognition domain, a hinge region, a transmembrane (TM) domain, and an intracellular signaling domain [1,2]. The antigen recognition domain is often derived from the variable regions of a monoclonal antibody, such as a single-chain variable fragment (scFv), variable heavy domain (VH), or light chain, which can recognize cell-surface and soluble antigens [3–5]. Non-antibody approaches, such as natural ligand/receptor pairs, are also used for the antigen recognition domain [6–9]. The hinge region provides flexibility and distance for synapse formation and influences receptor accessibility, stability, and signaling [10]. The TM domain anchors the CAR to the cell membrane and plays a critical role in modulating its surface expression, stability, and propensity for receptor aggregation [11]. This extracellular domain is followed by a transmembrane region and intracellular signaling sequences from one (for second generation CARs) or two (for third generation CARs) co-stimulatory receptors, followed by the intracellular region of CD3ζ [12]. This enables CAR-T cells to recognize and destroy cancer cells like how native T cells recognize and eliminate infected cells.

In contrast to major histocompatibility complex (MHC)-restricted conventional T cells, CARs are typically designed to recognize non-MHC cell surface proteins [13]. Upon antigen binding, CARs undergo oligomerization, leading to the phosphorylation of the intracellular CD3ζ immunoreceptor tyrosine-based activation motif (ITAM) by lymphocyte cell-specific protein-tyrosine kinase (LCK). Zeta-chain-associated protein kinase 70 (ZAP-70) is subsequently recruited and phosphorylates many known substrates, including LAT, SLP-76, and PLC-γ, upon CAR ligation [14,15]. Additionally, the CAR structure includes 1–2 co-stimulator domains. The most frequently used domains are derived from the CD28 family (like CD28) or the tumor necrosis factor receptor (TNFR) family (like 4-1BB). Most studies of CAR signaling have shown that CD28-containing CARs signal more rapidly and intensely than 4-1BB-containing CARs by facilitating CD3ζ phosphorylation by LCK [16]. 4-1BB-containing CARs recruit the THEMIS/SHP1 phosphatase complex to attenuate CD3ζ-chain phosphorylation [16]. In addition, they also induce different signaling pathways, which will be discussed in detail later (Figure 1).

However, this integrated and simplified architecture may result in less precise signal modulation than endogenous TCRs do. For example, in a study comparing GD2-targeted CAR variants in varicella zoster virus-specific T cells, the GD2-CAR.CD28ζ-modified cells showed robust proliferation upon TCR restimulation, whereas those lacking co-stimulatory domains or with 4-1BB signaling had impaired expansion and endogenous TCR function [17]. Additionally, tonic signaling, which causes spontaneous receptor activation and accelerates T-cell exhaustion, is a significant challenge in CAR-T-cell therapy. Positively charged patches (PCPs) on the CAR's antigen-binding domain drive this abnormal activation by inducing receptor clustering. Strategies such as reducing PCPs or increasing ionic strength during *ex vivo* expansion can suppress this signaling and reduce exhaustion [18].

TCR signaling

The core TCR complex consists of two TCR chains and six CD3 chains (two ε chains, one γ chain, one δ chain and two ζ chains) [19]. Additionally, several other components, including coreceptors, kinases, and ligands, play essential roles in the TCR signaling pathway [20]. More than 95% of mature T cells express TCRα and TCRβ isoforms [21]. In this review, we focus on αβ T-cell signaling.

The TCR binds to its specific antigenic peptide present in the groove of the MHC molecule, and this binding is strengthened by mechanical forces that facilitate conformational changes and enhance affinity [22]. Recent studies have shown that agonist peptides form catch bonds with TCRs, where force increases the bond lifetime, thereby increasing calcium signaling and T cell activation [23]. This mechanotransduction is essential for precise antigen discrimination and initiating immune responses [24]. Structurally, TCRs function as anisotropic mechanosensors: the applied force generates torque on CD3 subunits, triggering intracellular signaling through lipid bilayer deformation. This process depends on the TCR structure, lipid environment, and other biochemical factors [25]. TCR activation involves phase separation between CD3ε and LCK via ionic interactions, forming condensates that increase LCK-mediated CD3 phosphorylation in ITAMs and amplify signaling. The binding of the LCK SH3 domain to the exposed RK motif resulted in local augmentation of LCK activity and CD3 phosphorylation [26]. Interestingly, this process is tightly regulated, as phosphorylated CD3ε recruits Csk to dissolve the condensate and inhibits LCK [27–29]. ZAP70 kinase is subsequently recruited and activated through transphosphorylation, leading to LAT phosphorylation and the assembly of the LAT-SLP76 signalosome, which activates downstream pathways [30–32]. Additionally, the TCR signaling cascade is regulated, with phosphorylated LAT forming liquid-phase condensates enriched in PLCγ1 [33], SOS1 [34], GRB2 [35], and GADS [36], driving ERK/MAPK activation [37] and SLP76 phosphorylation within minutes of stimulation [38]. In combination with co-stimulation signaling, such as CD28-B7, T cell proliferation capacity and effective function, including cytotoxicity, are increased (Figure 1).

Mechanical forces actively modulate T cell activation through TCR-pMHC interactions, influencing the specificity and magnitude of the immune response. They facilitate the dynamic translocation of TCR and MHC, which is essential for the initiation and propagation of TCR signaling. This interplay enhances TCR-MHC interaction stability and modulates the subsequent intracellular signaling cascade. For instance, studies have demonstrated that TCRs can be activated by monovalent ligands only when these ligands are membrane associated, underscoring the importance of mechanical forces in TCR activation [39]. Recent studies have indicated that β2 integrin mechanical signaling can separate T cell proliferation from differentiation, resulting in the production of stem cell-like CAR-T cells. β2 integrin activation recruits 14-3-3ζ, causing YAP phosphorylation and inactivation. This inactivation of YAP releases the transcription factor MafG, which activates stemness genes, thus generating therapeutic stem cell-like CAR-T cells [40]. Additionally, T cells can sense the mechanical rigidity of their microenvironment, with stiffer matrices enhancing T cell activation and proliferation [41]. The TCR acts as a mechanoreceptor, with local membrane bending increasing TCR signaling domain accessibility to phosphorylation, facilitating selective agonist recognition [42]. DNA origami tension sensors reveal that

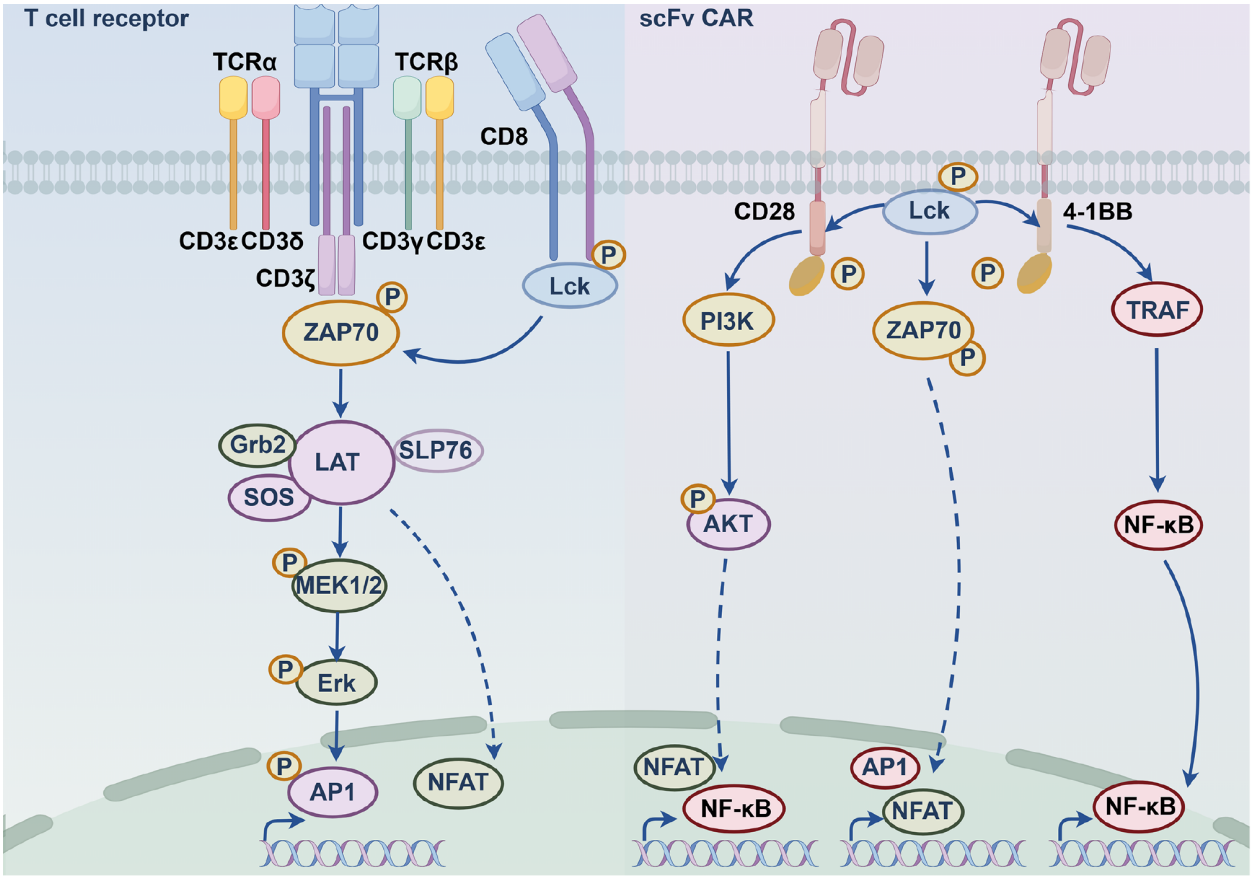


Figure 1. TCR versus CAR signaling: structural and functional divergence This figure contrasts the structures and downstream signaling cascades of the TCR and CAR. On the left, the TCR complex consists of α/β chains (for pMHC recognition) and CD3 subunits (with ITAMs). Upon pMHC binding, LCK phosphorylates CD3ζ ITAMs, recruits ZAP70, and activates pathways like Grb2-SOS-ERK and PI3K-AKT, with CD8 acting as a coreceptor. On the right, the modular CAR includes an extracellular antigen-binding domain (*e.g.*, scFv), a hinge, a transmembrane domain, and intracellular modules (CD3ζ plus co-stimulatory domains like CD28 or 4-1BB). Antigen binding induces oligomerization; CD28 accelerates effector function, while 4-1BB activates NF-κB via TRAF to enhance T cell persistence. The figure also highlights key differences: TCR relies on MHC restriction, while CAR is MHC-independent.

TCR-antigen bonds experience forces in the range of 5–10 pN, which are influenced by factors such as the cell state and F-actin activity [43]. By understanding these mechanical aspects, we can gain deeper insights into how T cells recognize and respond to antigens, which is fundamental to immune response initiation.

TCR-T-cell therapy involves engineering T cells to express a specific TCR that can recognize a tumor-associated antigen presented on MHC molecules. TCR-T therapy has shown remarkable efficacy in tumor models. For example, TBI-1301 achieved a 50% objective response rate in synovial sarcoma [44], and TCR-T cells targeting melanoma antigens such as MART-1 have demonstrated significant tumor regression [45]. However, the utility of TCR-T cells remains dependent on MHC-I expression and the tumor antigens they present. Some epithelial tumors often downregulate MHC-I expression, rendering TCR-T cells ineffective at recognizing and targeting these cells [46]. Additionally, identifying tumor-specific TCR remains challenging [47].

Co-stimulatory Domains in CAR-T Cells: CD28, 4-1BB, CD27, ICOS, OX40, and Others

While CAR-T immunotherapy has shown excellent potential for treating hematological malignancies, breakthroughs in the field of solid tumors are urgently needed [48]. A critical component of CAR design is the inclusion of co-stimulatory domains, which enhance T-cell activation, proliferation, and persistence. For example, incorporating specific signaling domains, such as CD28 and 4-1BB, can significantly increase CAR-T cell persistence and functionality [49]. Additionally, many different co-stimulatory domains lead to distinct signaling mechanisms and varying clinical efficacy.

CD28: potent effector responses to accelerated exhaustion

The CD28 co-stimulatory domain, which is widely used for its strong activation signals, greatly impacts downstream signaling pathways in CAR constructs, enhancing CAR-T-cell anti-tumor efficacy. It functions primarily by enhancing CD3ζ phosphorylation kinetics, leading to increased ERK activation via the MAPK pathway, which is essential for T cell activation and function [50]. CD28 co-stimulation also augments CAR signaling via the PI3K-AKT [51] pathway and promotes T-cell glycolysis [52]. CD28 co-stimulation also enhances CAR signaling through the LCK/CD3ζ/ZAP70 axis, increasing their anti-tumor efficacy and long-term cytotoxicity [53].

The incorporation of CD28 signaling into CAR designs has been

shown to significantly increase T-cell anti-tumor activity. CD28 co-stimulation has been shown to enhance trafficking, expansion, and tumor control in solid tumors such as neuroblastoma and ovarian carcinoma [54]. It was also found to be more effective than 4-1BB in promoting CAR-T-cell expansion at the tumor site and inducing tumor regression in preclinical models that target antigens such as L1CAM and HER2 [54]. Its ability to inhibit the CD73-mediated regulatory activity of $CD8^+$ T cells is crucial, as it prevents these cells from becoming suppressive in the tumor microenvironment and thus strengthens the overall immune response against tumors [55]. In addition to its role in CAR-T-cell therapy, CD28 plays a significant role in other immunotherapeutic approaches. CD28 co-stimulation is superior to 4-1BB co-stimulation in generating CAR-natural killer (CAR-NK) cells, resulting in better activation, cytotoxicity, and *in vivo* anti-tumor efficacy [56]. The development of bispecific antibodies such as NI-3201, which mediate PD-L1-dependent CD28 co-stimulation, provides a novel therapeutic option by enhancing T cell activity and anti-tumor function through dual mechanisms [57]. Moreover, CD28 plays a critical role in PD-1-targeted therapies, as the CD28-B7 co-stimulatory pathway is indispensable for rescuing exhausted $CD8^+$ T cells during PD-1 blockade [58]. Thus, the integration of CD28 signaling into immunotherapeutic strategies is highly promising for enhancing therapeutic outcomes [59,60].

A primary drawback is that, compared with those with co-stimulatory domains such as 4-1BB, CD28-based CAR-T cells often exhibit limited *in vivo* sustainability [61–64]. The rapid clearance of CD28 CAR-T cells is often attributed to T-cell exhaustion, where T cells lose their functional capabilities over time. Additionally, it is associated with increased regulatory T cell (Treg) infiltration, which can suppress antitumor immunity. Studies have shown the reduced efficacy of CD28-based CAR-T cells in solid tumors because Treg cells hinder T-cell-mediated attacks on cancer cells [65]. CD28 co-stimulation also increases the risk of cytokine release syndrome (CRS) [66,67]. Researchers have identified specific amino acid residues within the CD28 domain that contribute to this exhaustion, and modifying these residues has been shown to increase the durability of the anti-tumor response [68,69]. These findings suggest that while CD28 induces strong effector responses, strategies are needed to mitigate its associated accelerated exhaustion and overcome the inhibitory effects of Treg cells in solid tumor immunotherapy.

4-1BB: metabolic resilience and long-term persistence

The 4-1BB co-stimulatory domain, a member of the TNFR superfamily and known as CD137, plays a pivotal role in enhancing CAR-T-cell efficacy and persistence [70,71]. When CAR binds to an antigen, 4-1BB binds to the TNF receptor-associated factor (TRAF) family to modulate the activation of the canonical and noncanonical nuclear factor κB (NF-κB) pathways [51,72]. The NF-κB pathway protects T cells from apoptosis by inducing anti-apoptotic genes (*e.g.*, Bcl-2 and cIAPs) [73,74]. It also modulates the Fas-mediated apoptosis pathway, sometimes increasing tumor cell sensitivity to apoptosis [75,76]. Moreover, the interaction between 4-1BB and TRAF not only enhances NF-κB signaling but also contributes to the formation of protein complexes that are distinct from those formed during TCR signaling [77]. It provides signals that increase T-cell survival, proliferation, and anti-tumor activity [70,78,79].

The inclusion of the 4-1BB co-stimulatory domain in CAR-T cells has been shown to increase their persistence and anti-tumor efficacy. Studies have demonstrated that, compared with CAR-T cells with CD28 co-stimulation, 4-1BB-based CAR-T cells exhibit a less differentiated memory status, which is associated with improved perseverance and proliferation [80]. In addition, research comparing CD19 CAR-T cells with CD28 co-stimulated and 4-1BB co-stimulated (BBζ) domains revealed that BBζ CAR-T cells exhibited greater *ex vivo* survival and expansion [72]. This was attributed to the activation of noncanonical nuclear factor κB (ncNF-κB) signaling in BBζ CAR-T cells [81]. This domain also reduces T-cell exhaustion, a common issue in CAR-T cell therapy for solid tumors, by ameliorating tonic signaling, thereby improving overall anti-tumor efficacy [62,81–83]. Furthermore, the 4-1BB co-stimulatory domain positively influences the metabolic state of T cells. Compared with predominant oxidative metabolism, CD28-mediated costimulation results in pronounced glycolytic metabolism and increased susceptibility to exhaustion and lower susceptibility to exhaustion of 4-1BB-mediated co-stimulation [52]. Additionally, compared with CD28-based CARs, 4-1BB co-stimulated CAR-T cells enhance immune synapse formation and proinflammatory gene activation more effectively. This is due to its autonomous signaling and receptor homodimerization capability, which boosts anti-tumor responses and persistence [84].

In solid tumors, the tumor microenvironment (TME) presents challenges such as immunosuppressive factors and physical barriers, limiting T-cell infiltration and function. While 4-1BB signaling in CAR-T cells supports T-cell survival and proliferation, it may not overcome the hostile TME, leading to suboptimal anti-tumor responses. Compared with CD28 co-stimulation, which offers stronger initial T-cell activation, 4-1BB-based CAR-T cells often show weaker antitumor activity in solid tumors, where a more aggressive immune response is needed [80]. Additionally, the persistence of 4-1BB co-stimulated CAR-T cells can result in prolonged exposure to the immunosuppressive TME, causing T-cell exhaustion, characterized by reduced cytokine production and cytotoxic activity, which further diminishes therapeutic potential [62]. In addition, 4-1BB-containing CARs recruit the THEMIS/SHP1 phosphatase complex to attenuate CD3ζ-chain phosphorylation [16], which slows CAR-T-cell function, leading to BBζ CAR-T cells having limited efficacy against low-antigen-expressing targets compared with CD28 co-stimulated CAR-T cells [85]. 4-1BB in CARs causes cell aggregation and death without CAR tonic signaling. Studies have shown that 4-1BB sequesters A20 at the cell membrane in a TRAF-dependent manner, resulting in A20 deficiency. This leads to NF-κB hyperactivity, ICAM-1-driven cell aggregation, and cell death via RIPK1/RIPK3/MLKL. Genetic modifications such as overexpressing A20 or deleting the TRAF-binding motif of 4-1BB can prevent cell clustering and death, enhancing the anti-tumor ability of 4-1BB co-stimulated CAR-T cells [51].

CD27: bridging innate and adaptive immunity

CD27, a member of the tumor necrosis factor receptor superfamily, plays a key role in T cell activation by providing a co-stimulatory signal crucial for effective immune responses [86]. Its interaction with CD70 enhances T cell proliferation and differentiation into effector and memory T cells, making it a promising target in cancer immunotherapy [87,88]. CD27 could recruit the signaling adaptor TRAF2 and the phosphatase SHP-1, thereby modulating TCR and

CD28 signals. The modulation of TCR signals promotes transcription factor circuits that induce memory-associated rather than effector-associated gene programs [89]. In addition, CD27 signaling can also activate *de novo* nucleotide and protein synthesis [90] and Pim kinase [91] signaling pathways, leading to the regulation of T cell expansion, differentiation, and effector functions. Overall, the CD27 co-stimulatory domain significantly enhances the survival and anti-tumor activity of redirected human T cells [92,93].

CD27 as a co-stimulatory domain in CAR-T cells, enhances anti-tumor efficacy *in vivo*, improving human cancer regression in xenogeneic allograft models. [87]. This is due to increased antigen-stimulated effector functions (*e.g.*, cytokine secretion, cytotoxicity), proliferation, and resistance to apoptosis. *In vivo*, CD27-bearing CAR-T cells have increased persistence post-infusion, leading to better cancer regression [87]. Additionally, CD27-costimulated CAR-T cells achieve better tumor control than CD28-costimulated CAR-T cells do by promoting memory-relevant properties [89].

Further research has demonstrated that CD27 signaling can be effectively combined with other co-stimulatory signals to improve CAR-T-cell efficacy. For example, combining CD27 with CD28 and 4-1BB signaling in CAR-T cells targeting colorectal tumors resulted in enhanced proliferation, reduced expression of immune checkpoint receptors, and increased generation of memory stem T cells, thereby improving anti-tumor capacity in xenograft models [88,92]. Another example is the use of a bispecific antibody targeting both CD27 and EGFR, which induces cancer cell-localized crosslinking and activation of CD27, thereby enhancing T-cell cytotoxicity and proliferation in EGFR-expressing cancers [94]. These approaches highlight the potential of CD27 co-stimulation in conjunction with targeted therapies to overcome the immunosuppressive barriers of solid tumors.

CD27 activates pathways vital for T-cell responses but faces challenges in the TME, especially in solid tumors. Its interaction with CD70 can increase the number of Tregs, thus suppressing tumor-specific T-cell responses. This may promote tumor growth by increasing angiogenesis and reducing Treg apoptosis, creating an immunosuppressive environment [95]. Thus, CD27 might enhance immune responses but also increase the number of Tregs and support tumor growth, posing a challenge for solid tumor therapies. This highlights the need for caution and strategies to counteract these effects when CD27 co-stimulatory domains are used in CAR-T cells.

ICOS: tailoring helper T-cell responses

ICOS, a member of the TNF receptor superfamily, plays a crucial role in tailoring helper T-cell responses, particularly in CAR-T-cell therapies [96]. It is expressed on activated T cells and interacts with ICOS-L, which is found on antigen-presenting cells and tumor cells within the TME [97,98]. This interaction induces the recruitment of PI3K, leading to the production of membrane-bound phosphatidylinositol 3,4,5-trisphosphate (PIP_3), culminating in the activation of Akt to promote T cell proliferation and survival [99,100]. This signaling pathway has a dual role, both in promoting anti-tumor immunity and potentially facilitating tumor progression through Treg activity [101]. Recent studies have highlighted the potential of ICOS as a biomarker for predicting response to immunotherapy [102]. ICOS expression on T cells is correlated with better outcomes in patients receiving immune checkpoint blockade therapies targeting PD-1 and CTLA-4 [103,104]. The presence of $ICOS^+$ T cells in the TME indicates increased immune activity and better clinical responses, suggesting that ICOS could be an indicator of effective T cell-mediated immune responses in cancer patients [105–107]. In CAR-T-cell therapy, integrating ICOS into constructs enhances cytotoxicity and promotes T-cell persistence, which is crucial for sustained anti-tumor activity [108,109].

When combined with other co-stimulatory domains, such as OX40, ICOS has been shown to enhance CAR-T-cell cytotoxicity and promote a T-cell persistence phenotype, improving anti-tumor activity in both *in vitro* and *in vivo* models. These findings suggest that ICOS can significantly contribute to the long-term efficacy of CAR-T cells in the solid TME [110]. The dual co-stimulation approach improves CAR-T cell anti-tumor efficacy and persistence, as shown in studies with dual anti-CD19/CD20 CAR constructs [111]. The unique signaling pathways of ICOS contribute to a more robust immune response, potentially addressing the limitations of some CAR-T cells in solid tumors [112]. The molecular mechanisms by which co-stimulatory domains such as ICOS enhance CAR-T-cell function are being elucidated, offering insights into optimizing these domains for better therapeutic outcomes [78]. Additionally, ICOS can influence the tumor microenvironment; Tregs expressing CARs with ICOS domains have shown potential in controlling allograft rejection [113], indicating that ICOS can modulate immune responses in complex tissue environments. This ability to integrate native and CAR-mediated co-stimulatory signals underscores the versatility of ICOS in enhancing CAR-T-cell therapies for solid tumors [114].

In addition to CAR-T cells, ICOS plays a role in the immune system, such as in anti-atherosclerotic processes, by downregulating vascular smooth muscle phagocytosis and proliferation [115]. These findings suggest that ICOS can modulate immune responses in a broader context, which could benefit comprehensive cancer immunotherapies. ICOS expression also serves as an indicator of T cell-mediated responses to cancer immunotherapy. A single ICOS co-stimulator can enhance T cell proliferation and survival but may not provide optimal co-stimulatory signals for effective anti-tumor activity in solid tumors. These tumors often have a hostile microenvironment with immunosuppressive factors, physical barriers, and a lack of suitable antigens. Although ICOS promotes T-cell activation, it may not adequately overcome these barriers, leading to suboptimal CAR-T-cell performance. Moreover, ICOS expression can be downregulated in certain TMEs, further limiting its effectiveness [115]. Additionally, the use of ICOS as a co-stimulatory domain in CAR-T cells may inadvertently promote regulatory Treg activity, which can suppress the immune response against tumors. This is particularly concerning in solid tumors, where Tregs are often linked to a poor prognosis. The potential for ICOS to enhance Treg function could counteract the therapeutic effects of CAR-T cells, making it a less favorable choice for solid tumor applications [116]. Further research is needed to optimize co-stimulatory domains for CAR-T-cell therapy in solid tumors, possibly by combining ICOS with other domains or modifying ICOS signaling pathways to better suit the complex landscape of solid malignancies.

OX40

OX40, a TNFR superfamily member, plays a critical role in T cell activation, proliferation, and survival, promoting the expansion and effector differentiation of both $CD4^+$ and $CD8^+$ T cells [117,118].

When included in CAR constructs, OX40 can increase T-cell proliferation and survival through pathways such as the NF-κB, MAPK, and PI3K-AKT [119]. Research has shown that OX40 co-stimulation enhances CAR-T cell persistence when CAR-T cells are repeatedly stimulated by target cells. This is critical for reducing relapse rates after CAR-T-cell therapy. Both *in vitro* and *in vivo* studies have shown that OX40 CAR-T cells have greater proliferation and enhanced immune memory than other co-stimulatory domains, such as 4-1BB, making them promising options for improving the durability of CAR-T-cell responses [120–122]. Additionally, OX40 co-stimulation can reverse tumor-induced T-cell anergy, a state that limits anti-tumor immunity. By engaging OX40, CAR-T cells can restore the proliferative capacity of tumor-reactive T cells, reduce tumor growth, and improve the survival of tumor-bearing hosts [118]. Moreover, OX40 signaling can increase $CD4^+$ and $CD8^+$ T-cell clonal expansion and effector differentiation, which are crucial for effective tumor elimination [117]. Furthermore, OX40 co-stimulation can suppress the secretion of inhibitory cytokines such as IL-10, which otherwise weakens T-cell-mediated immunity while preserving the production of pro-inflammatory cytokines [123]. In multiple myeloma, a hematologic cancer, OX40 integration into CAR-T cells has yielded promising results. BCMA-BBZ-OX40 CAR-T-cell therapy, which was generated via a rapid manufacturing platform, demonstrated enhanced cytotoxicity and reduced exhaustion in preclinical studies. It also improved proliferation and T-cell stemness, leading to strong responses in patients with relapsed/refractory multiple myeloma, with a high overall response rate, and many patients achieved complete responses [124]. These findings highlight the potential of OX40 in overcoming the immunosuppressive tumor microenvironment in solid tumors.

Emerging co-stimulators: CD2, GITR, and HVEM

CD2

CD2, expressed on T and NK cells, is a co-stimulatory protein crucial for cell adhesion and recognition. Its interaction with CD58 is essential for T cell activation and the formation of immunological synapses between T cells and antigen-presenting cells, playing a key role in effective immune responses against tumors. However, in some cancers, such as diffuse large B-cell lymphoma, down-regulation of CD58 on tumor cells can reduce T-cell activation and function, and low CD58 expression is linked to shorter progression-free survival. Additionally, TILs with reduced CD2 expression are associated with defective cytotoxicity and T cell exhaustion, complicating the immune response in non-hematological neoplasms [125]. CD2 and CD28 co-stimulation trigger overlapping yet distinct signaling pathways. CD28 co-stimulation is particularly important for proper NF-κB activation, whereas CD2 signals more strongly to the S6-ribosomal protein [126].

CD2 co-stimulation enhances T cell activation, proliferation, and survival. CD2 can compensate for the absence of other co-stimulatory domains and enhance CAR-T-cell functional activity, offering advantages in solid tumors where strong T-cell activation is needed to overcome the immunosuppressive TME [127]. However, the use of CD2 as a co-stimulatory domain in CAR-T cells presents challenges. One issue is the risk of on-target off-tumor effects due to CD2 ligand expression on normal tissues [128]. Additionally, the efficacy of CD2-mediated co-stimulation in solid tumors is still under investigation, and more clinical data are needed to fully understand its benefits and limitations. Current research focuses on balancing effective tumor targeting with minimizing potential toxicity. Preclinical studies have shown that CAR-T cells incorporating CD2 co-stimulatory domains can produce high levels of IL-2 and exhibit increased proliferation and persistence [127]. In parallel, the development of an allogeneic CD2-deleted UCART2 for T-cell malignancies revealed that CD2 deletion affects CAR-T-cell function. However, this effect can be compensated for by rhIL-7-hyFc, thereby improving efficacy and survival [129].

GITR

Glucocorticoid-induced TNFR-related protein (GITR) is a co-stimulatory receptor that plays a significant role in modulating immune responses and cancer immunotherapy. Its activation enhances effector T cell function while inhibiting Tregs, promoting anti-tumor immunity. This dual role makes GITR a promising target for solid tumor immunotherapy. GITR activates T cells and inhibits Treg-mediated suppression. When activated by its ligand or agonistic antibodies, GITR increases $CD8^+$ and $CD4^+$ T cell proliferation and activation and depletes or impairs Tregs in the tumor microenvironment, further enhancing tumor targeting [130,131].

Studies have shown that incorporating GITR into CAR-T cells can increase anti-tumor efficacy by promoting T-cell proliferation and survival and reducing T-cell exhaustion, which is common in the tumor microenvironment [81]. The unique signaling pathways of GITRs can lead to a more robust and sustained immune response against tumors. However, the use of GITR in CAR-T cells has potential disadvantages. There is a risk of on-target off-tumor cytotoxicity, where CAR-T cells might attack normal tissues with low-level target antigen expression. Additionally, the complex interplay between GITR signaling and other immune pathways can lead to unpredictable outcomes, requiring careful CAR construct design and testing [113,132]. Preclinical studies have shown promising results, with GITR-enhanced CAR-T cells demonstrating improved persistence and anti-tumor activity in various solid tumor models [133]. However, translating these findings into clinical success needs further research to balance efficacy and safety.

HVEM

The herpes virus entry mediator (HVEM) is a significant player in the immune response and has been identified as a potential target in cancer immunotherapy, particularly in solid tumors. As a co-stimulatory molecule that mediates both stimulatory and inhibitory signals, HVEM is an attractive therapeutic target. Its role in tumor biology, particularly in NSCLC, reveals that HVEM can suppress metastasis by inhibiting glycolysis and modulating macrophage polarization [134]. Interaction with B and T lymphocyte attenuators (BTLAs) could enhance anti-tumor immunity. BTLA is involved in immune escape mechanisms, where upregulated BTLA can weaken T cell responses against cancer. Targeting this axis could reverse tumor-induced T cell dysfunction, providing a new avenue for cancer immunotherapy [135].

In CAR-T-cell therapy, high BTLA expression is correlated with poor clinical response. However, deleting BTLA in CAR-T cells has improved tumor control, indicating that the BTLA/HVEM axis is a crucial immune checkpoint in CAR-T-cell immunotherapy [136]. HVEM co-stimulation enhances CAR-T-cell function through a novel downstream signaling pathway that increases metabolic activity. This metabolic reprogramming is crucial, as it endows CAR-T cells with improved function and persistence within the

hostile TME. Studies have shown that HVEM CAR-T cells exhibit more robust cytokine release and cytotoxicity than traditional co-stimulatory domains (CSDs), such as 4-1BB or CD28 [112]. This enhanced functionality is attributed to the ability of HVEM to attenuate T-cell exhaustion, a common challenge in the treatment of solid tumors [137]. HVEM CAR-T cells maintain low levels of exhaustion even after prolonged antigen-dependent proliferation and efficiently differentiate into central and effector memory subsets, which is associated with elevated energy metabolism and reduced exhaustion [138]. Despite these advantages, the use of HVEM in CAR-T cells has potential disadvantages and challenges. The novel signaling pathways activated by HVEM may cause unforeseen off-target effects or toxicities, which requires thorough preclinical and clinical investigations. Additionally, the long-term persistence and safety of HVEM CAR-T cells in patients need evaluation to ensure that no adverse effects or autoimmune responses occur.

Common logic versus distinctive traits

All co-stimulatory domains enhance T cell activation and anti-tumor efficacy by increasing CD3ζ ITAM phosphorylation and converging on PI3K or TRAF/NF-κB signaling, influencing therapeutic outcomes through signal strength, duration, and metabolic coupling. CD28 offers high-intensity/short-lived signals for potent cytotoxicity but risks rapid exhaustion, whereas 4-1BB provides modest-intensity/long-lived signals, supporting metabolic resilience and memory formation. Intermediate modules such as CD27 and OX40 balance these traits. ICOS selectively activates Akt through PIP3 production, fine-tuning helper T cell responses. GITR activates the NF-κB and MAPK pathways with varying intensities and durations. HVEM uniquely balances stimulatory and inhibitory signals, offering potential for metabolic reprogramming in the TME. CD28-based CAR-T cells show superior initial anti-tumor activity in hematological cancers but limited persistence in solid tumors due to rapid exhaustion. 4-1BB CAR-T cells demonstrate long-term persistence but may require antigen density thresholds for effective activation. CD27 and ICOS CAR-T cells show promise in preclinical models but require careful ligand expression analysis to avoid unintended immune modulation. OX40 and GITR CAR-T cells offer novel mechanisms for overcoming T-cell anergy and exhaustion but require validation in diverse solid tumor contexts. HVEM CAR-T cells present unique metabolic advantages but need thorough evaluation of off-target effects and long-term safety profiles.

The selection of co-stimulatory domains in CAR-T-cell design for solid tumor immunotherapy should balance the activation intensity, metabolic support, and counteraction of immunosuppressive mechanisms. CD28 or CD2 is suitable for the rapid reduction of large tumor burdens. For long-term tumor surveillance, domains supporting T-cell persistence, such as 4-1BB and HVEM, are preferred. In tumors with high Treg infiltration, combining GITR with CD28 enhances CAR-T-cell efficacy. For glucose-deprived TMEs, dual 4-1BB plus HVEM signaling provides metabolic advantages. These evidence-based design principles can guide the development of more effective, context-specific CAR-T-cell therapies for solid tumors. Please refer to Table 1 for a summary of the key features and limitations of each co-stimulatory domain.

Current Clinical Trials of CAR-T Cell Therapies in Solid Tumors

Understanding CAR signaling is crucial for determining the outcomes of solid tumor immunotherapy. TCRs function through MHC-restricted, mechanosensitive activation, whereas CARs, with their synthetic design, bypass the limitations of antigen presentation. These distinct mechanisms influence T cell activation, durability, and tumor infiltration, which in turn influence the efficacy of immunotherapy. Different modifications of CAR signaling have been extensively studied in clinical trials focused on T-cell therapy for solid tumors. Here, we summarize the current clinical trials on CAR-T cells in solid tumors in Table 2 and provide an in-depth analysis of the key trends and insights derived from these trials.

The clinical trials detailed in Table 2 highlight several key trends in the development of CAR-T-cell therapies for solid tumors. First, there is a growing emphasis on dual-antigen targeting strategies, such as those targeting GD2/PSMA and GD2/CD70, as seen in trials. These approaches aim to address the issue of antigen escape and enhance therapeutic efficacy by simultaneously targeting multiple tumor-associated antigens. Second, the integration of additional CARs that secrete cytokines or other effector molecules is becoming more prevalent. For example, trials incorporating HER2/TRAIL-R2 tandem CARs aimed to induce bystander cytotoxicity in antigen-negative tumor cells. Third, the use of gene editing, such as the disruption of endogenous checkpoints such as PD-1, to enhance CAR-T-cell function has been explored in several trials. This strategy aims to improve the persistence and anti-tumor activity of CAR-T cells in the immunosuppressive TME.

Moreover, the trials reflect the diversity of antigen targets being investigated, ranging from well-established targets such as EGFR and CEA to emerging targets such as CLDN18.2, B7H3, and CD70. The geographical distribution of these trials also indicates a global

Table 1. Side-by-side functional comparison of different co-stimulatory domains

Domain	Key adaptor/pathway	Exhaustion level	Cytokine profile	Persistence	Main limitation
CD28	PI3K-Akt, LCK/ZAP70	Rapid	Th1 + Th2	Short	CRS risk; Treg recruitment
4-1BB	TRAF, NF-κB (ncNF-κB)	Pronounced	Th1	Long	Slow kinetics; poor low-antigen killing
CD27	TRAF2–SHP1 memory circuit	NA	Th1 + Th17	Medium to Long	CD70-mediated Treg expansion
ICOS	PI3K-Akt-Pim	NA	Th1 + Tfh	Long	May empower Treg
OX40	TRAF-NF-κB	NA	Th1 + Th9	Long	Systemic T-cell activation
CD2	CD58-S6 ribosomal	NA	Th1	NA	Broad normal-tissue ligand
GITR	TRAF-NF-κB	NA	Th1	Long	Treg depletion linked to systemic inflammation
HVEM	BTLA checkpoint release	Low	Th1 + CTL	Long	Unknown off-target toxicities

Table 2. Current clinical trials of CAR-T cell therapies in solid tumors

Antigen	Tumor target	Sponsor	Phase	NCT number	Study start
IL13Ra2, EGFR	Glioblastoma	University of Pennsylvania	Phase 1	NCT05168423	2023-02-24
EGFR/B7-H3	Advanced solid tumors	Second Affiliated Hospital of Guangzhou Medical University	Early Phase 1	NCT05341492	2022-05-01
EGFR/TGFβR-KO	Advanced solid tumors	Chinese PLA General Hospital	Phase 1	NCT04976218	2022-03-15
EGFRvIII	Glioblastoma	Marcela V. Maus, MD, PhD	Phase 1	NCT05660369	2023-03-22
EGFRvIII	Glioblastoma	Hideho Okada, MD, PhD	Phase 1	NA	2024-04-30
CEA	Colorectal carcinoma	Chongqing Precision Biotech Co., Ltd	Phase 1 Phase 2	NCT04348643	2020-02-20
CEA	Colorectal carcinoma	Ruijin Hospital	Early Phase 1	NCT04513431	2020-08-30
CEA	Advanced solid tumors	Weijia Fang, MD	Phase 1	NCT05396300	2022-05-25
CEA	Advanced malignant tumors	Chongqing Precision Biotech Co., Ltd	Phase 1	NCT05415475	2021-09-10
CEA	Advanced malignant tumors	Chongqing Precision Biotech Co., Ltd	Phase 1 Phase 2	NCT05538195	2022-06-07
GPC3	Hepatocellular carcinoma	Zhejiang University	Phase 1	NCT05155189	2021-12-09
GPC3	Hepatocellular carcinoma	Zhejiang University	Phase 1	NCT04756648	2021-03-10
GPC3/MSLN	Advanced malignant tumors	Second Affiliated Hospital of Guangzhou Medical University	Phase 1	NCT06196294	2024-05-10
ROR1	NSCLC, TNBC, ovarian carcinoma	Lyell Immunopharma, Inc.	Phase 1	NCT05274451	2022-03-29
GD2	Diffuse intrinsic pontine gliomas (DIPG), spinal diffuse midline glioma (DMG)	Crystal Mackall, MD, Stanford University	Phase 1	NCT04196413	2020-06-04
GD2	Diffuse midline glioma (DMG), refractory CNS tumors	Baylor College of Medicine	Phase 1	NCT04099797	2020-02-03
GD2/PSMA	Recurrent or refractory solid tumors	Shenzhen Geno-Immune Medical Institute	Phase 1 Phase 2	NCT05437315	2022-06-30
GD2/CD70	Advanced malignant tumors	Shenzhen Geno-Immune Medical Institute	Phase 1 Phase 2	NCT05438368	2022-06-30
GD2/CD56	Advanced malignant tumors	Shenzhen Geno-Immune Medical Institute	Phase 1 Phase 2	NCT05437328	2022-06-30
CLDN18.2	Gastric/esophagogastric conjugate adenocarcinoma, pancreatic cancer	CARsgen Therapeutics Co., Ltd	Phase 1 Phase 2	NCT04581473	2020-10-23
CLDN18.2	Gastric cancer, gastroesophageal junction cancer, pancreatic cancer	Peking University	Phase 1	NCT03874897	2019-03-26
CLDN18.2	Gastric/gastroesophageal junction (GC/GEJ), pancreatic cancer (PC)	CARsgen Therapeutics Co., Ltd	Phase 1 Phase 2	NCT04404595	2020-10-23
CLDN18.2	Gastric cancer (GC)	Suzhou Immunofoco Biotechnology Co., Ltd	Phase 1	NCT05472857	2022-08-08
CLDN18.2	Advanced solid tumors	Shanghai East Hospital	Phase 1	NCT04467853	2020-09-21
CLDN18.2	Advanced solid tumors	Innovent Biologics (Suzhou) Co., Ltd	Phase 1	NCT05199519	2021-12-13
CLDN18.2	Pancreatic cancer	CARsgen Therapeutics Co., Ltd	Phase 1	NCT05911217	2023-07-11
CLDN18.2	Advanced solid tumors	Shenzhen University General Hospital	Not Applicable	NCT05620732	2022-10-01
CLDN18.2/PD-L1	Advanced solid tumors	Sichuan University	Phase 1	NCT06084286	2024-01-01
CLDN6	Recurrent or refractory solid tumors	BioNTech Cell & Gene Therapies GmbH	Phase 1	NCT04503278	2020-09-16
PSMA	Prostate cancer, salivary gland cancers	Poseida Therapeutics, Inc.	Phase 1	NCT04249947	2020-02-28
PSMA	Prostate cancer	Bioray Laboratories	Phase 1	NCT04053062	2020-07-16
PSMA/CD70	Advanced solid tumors	Shenzhen Geno-Immune Medical Institute	Phase 1 Phase 2	NCT05437341	2022-06-30
MSLN	Advanced refractory solid tumors	Shanghai Pudong Hospital	Phase 1	NCT05531708	2021-04-02
MSLN	Solid tumors	Shanghai Mengchao Cancer Hospital	Early Phase 1	NCT05373147	2020-10-30
MSLN	Solid tumors	Wuhan Union Hospital	Early Phase 1	NCT04489862	2020-05-13
MSLN	Recurrent or refractory solid tumors	Tongji Hospital	Early Phase 1	NCT05141253	2022-01-12

(*Continued*)

Antigen	Tumor target	Sponsor	Phase	NCT number	Study start
MSLN	Relapsed or refractory solid tumors	The First Affiliated Hospital with Nanjing Medical University	Early Phase 1	NCT05166070	2022-01-01
MSLN	Solid tumors	Shanghai Cell Therapy Group Co., Ltd	Early Phase 1	NCT04503980	2020-03-26
B7-H3	Advanced solid tumors	Shanghai Hansoh Biomedical Co., Ltd	Phase 1	NCT05276609	2021-11-28
B7-H3	Glioblastoma multiforme	Beijing Tiantan Hospital	Phase 1	NCT05241392	2022-01-27
B7-H3	Pediatric solid tumor	Seattle Children's Hospital	Phase 1	NCT04483778	2020-07-13
B7-H3	Solid tumors	St. Jude Children's Research Hospital	Phase 1	NCT04897321	2022-07-06
B7-H3	Advanced solid tumor	PersonGen BioTherapeutics (Suzhou) Co., Ltd	Phase 1	NCT05190185	2021-06-01
HER2	Advanced solid tumors	Bellicum Pharmaceuticals	Phase 1	NCT04650451	2020-12-07
HER2	Solid tumors	Shanghai PerHum Therapeutics Co., Ltd	Phase 1	NCT04511871	2020-07-09
HER2	Solid tumors	Baylor College of Medicine	Phase 1	NCT03740256	2020-12-14
HER2	Solid tumors	Sichuan University	Early Phase 1	NCT04684459	2021-03-12
HER2/TRAIL-R2	Metastatic breast cancer	Baylor College of Medicine	Phase 1	NCT06251544	2025-06
CD70	Relapsed or refractory renal cell carcinoma	CRISPR Therapeutics AG	Phase 1	NCT04438083	2020-06-16
CD70	Advanced/metastatic solid tumors	Chongqing Precision Biotech Co., Ltd	Phase 1	NCT05420545	2021-12-31
NKG2D/DR5	Solid tumors	Shenzhen Hospital, University of Chinese Academy of Sciences	Phase 1	MR-44-23-010294	2023-06-01
NKG2DL	Solid tumors	The Affiliated Hospital of the Chinese Academy of Military Medical Sciences	Early Phase 1	NCT05382377	2022-05-17
NKG2DL/CLDN18.2	Solid tumors	The Affiliated Hospital of the Chinese Academy of Military Medical Sciences	Phase 1	NCT05583201	2022-10-13
NKG2DL/CLDN18.2	Solid tumors	Peking University	Phase 1	NCT06134960	2023-11-16
OX40	Advanced solid tumors	Second Affiliated Hospital of Guangzhou Medical University	Phase 1	NCT04952272	2021-03-01
VEGFR1/PD-L1	Serosal cavity metastases of malignant tumor	Sichuan University	Phase 1	NCT05477927	2023-11-28
CD44/CD133/IL7Ra	Recurrent glioblastoma	Beijing Tiantan Hospital	Phase 1	NCT05577091	2023-09-30
GUCY2C	Advanced digestive system neoplasms	Beijing Immunochina Medical Science & Technology Co., Ltd	Early Phase 1	NCT05287165	2022-03-10

collaborative effort, with institutions in the United States, China, and Europe actively contributing to the advancement of CAR-T-cell therapy for solid tumors. Overall, these clinical trials represent critical steps toward optimizing CAR-T-cell therapies and overcoming the challenges associated with solid tumor immunotherapy. By learning from the signaling dynamics of TCRs and refining CAR designs through iterative clinical testing, researchers are paving the way for more effective and durable treatments for patients with solid malignancies.

Challenges and Strategies of CAR-T Cell Therapies in Solid Tumors

While these trials are still ongoing, several challenges in solid tumors remain that hinder their clinical translation (Figure 2).

Low infiltration and suppressive microenvironments

After being introduced into cancer patients, CAR-T cells encounter numerous challenges imposed by the TME. The evasion of antitumor immune responses can begin even before the transferred T cells engage tumor cells, partly through mechanisms such as the CD47 "do not eat me" signal, which is overexpressed on tumors [139]. Unlike disseminated or circulating tumors, solid tumors establish physical barriers that impede T cell infiltration through a complex network of molecular and cellular mechanisms. These include the production of chemokines and other chemical cues that selectively attract immunosuppressive cells [140]; cancer-associated fibroblasts (CAFs) foster a dense, fibrotic milieu by irregularly depositing extracellular matrix (ECM) components [141], thus hampering T cell movement, and abnormal blood vessels with reduced expression of adhesion molecules crucial for T cell infiltration [142,143]. To increase CAR-T-cell efficacy, strategies such as targeting the tumor vasculature to improve trafficking and infiltration have been proposed [144]. Additionally, genetic modifications of cytokine signaling pathways have been shown to increase CAR-T-cell infiltration and functionality within the TME [145,146]. Additionally, targeting the tumor stroma, which often serves as a barrier to immune cell infiltration, has been shown to enhance CAR-T-cell accessibility and efficacy, offering potential for improving outcomes in solid tumor treatment [147–149]. Moreover, combining CAR-T cells with oncolytic viruses has been demon-

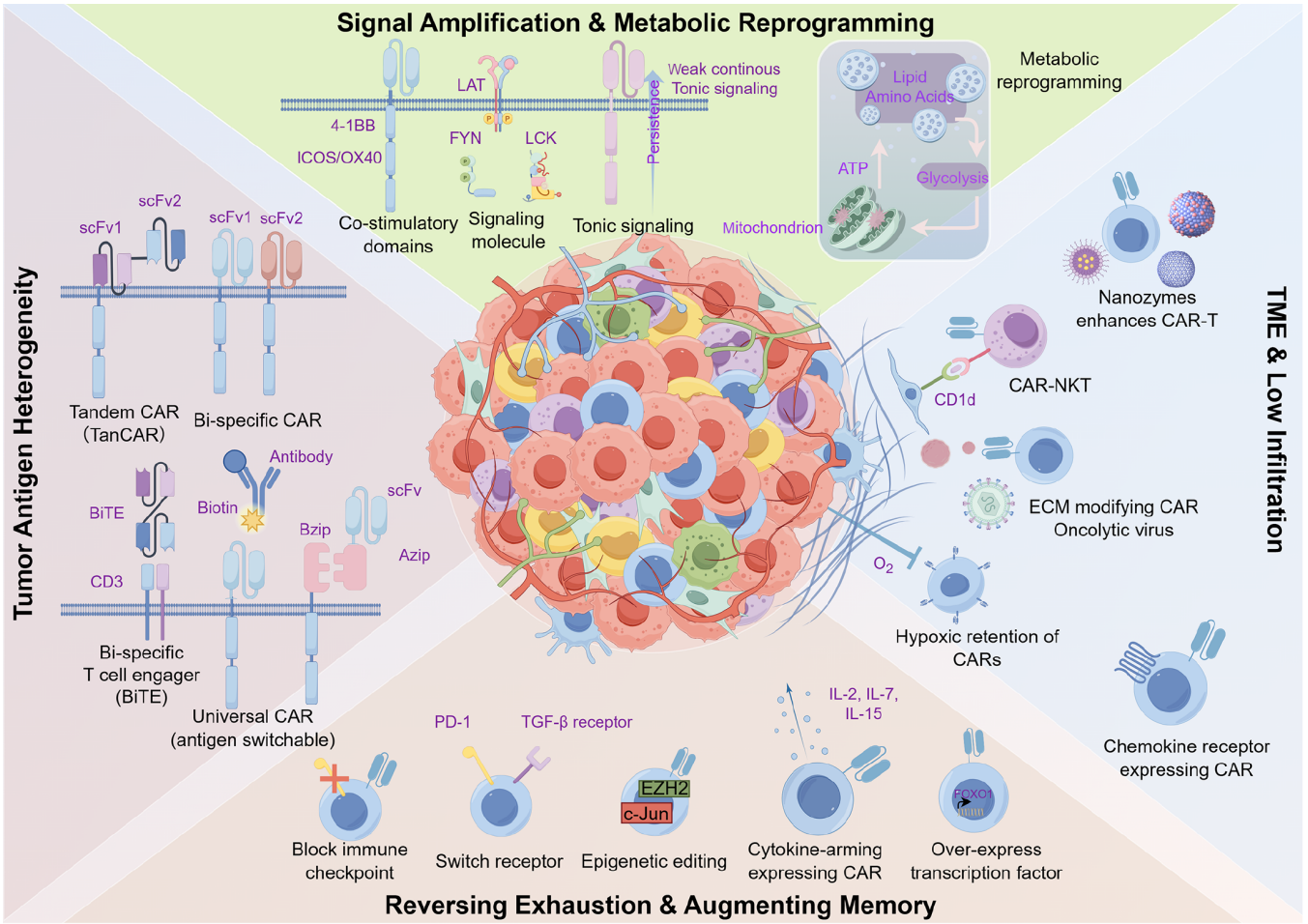


Figure 2. Challenges of CAR-T-cell therapies in solid tumors and strategies to overcome these challenges This figure outlines the main challenges of CAR-T cell therapy in solid tumors and corresponding solutions. Core challenges include poor CAR-T infiltration (due to tumor physical barriers), immunosuppressive TME (suppressive cells/cytokines, checkpoints), metabolic competition (tumor nutrient sequestration), tumor antigen heterogeneity/loss, and CAR-T exhaustion. Strategies to address these include engineering CAR-T cells to express chemokine receptors (for infiltration), armoring CAR-T cells to secrete pro-inflammatory cytokines (to reverse TME suppression), metabolic reprogramming (to enhance nutrient utilization), developing dual-target CARs (to counter antigen issues), and combining with checkpoint inhibitors (to reduce exhaustion).

strated to increase their anti-tumor activity [150]. Through trafficking into solid tumors, CAR-T cells still face an immunosuppressive TME characterized by the infiltration of multiple immune cells, including myeloid-derived suppressive cells [151], tumor-associated macrophages [152], tumor-associated neutrophils [153], and Tregs [154,155]. These suppressive cells can suppress CAR-T-cell function in several ways, including by producing immunosuppressive cytokines, upregulating receptors that inhibit T-cell activation, and creating an environment that is metabolically unfavorable for T cells [156–158].

Several innovative strategies have been developed to overcome these barriers. For example, owing to their photothermal and nanocatalytic properties, nanozymes can remodel the TME, helping to overcome immunosuppressive barriers. Notably, studies using B7-H3-targeted CAR-T cells in non-small cell lung cancer models have demonstrated that nanozyme treatment improves CAR-T cell infiltration and activation, resulting in enhanced anti-tumor activity and the transformation of the immune-hostile environment to support greater therapeutic efficacy [159–164]. Additionally, emerging studies from others and our group have demonstrated that CAR-T-cell therapy based on natural killer T (NKT) cells results in superior anti-tumor activity *in vivo* via CD1d-dependent immune responses in the TME. CAR-NKT cells eliminate CD1d-expressing M2-like macrophages and myeloid-derived suppressor cells (MDSCs). In addition, CAR-NKT cells promote epitope spreading and the activation of endogenous T cell responses against tumor-associated neoantigens [150,165].

In addition, the hypoxic feature of the tumor microenvironment due to rapid cell proliferation and poor vascularization significantly impacts cellular metabolism and function. In T cells, hypoxia triggers a metabolic shift from oxidative phosphorylation to glycolysis to maintain ATP production under low-oxygen conditions. However, this adaptation can lead to T-cell exhaustion, thereby reducing their anti-tumor efficacy [166,167]. Targeting metabolic pathways, such as modulating HIFs or inhibiting autophagy, presents a promising strategy to increase T-cell fitness and anti-tumor activity [168]. Recent studies have explored the engineering of T cells with hypoxia-inducible elements, such as hypoxia-inducible chimeric antigen receptors (HiCARs). HiCAR-T cells, which are activated under hypoxic conditions, show selective

tumor cell targeting and reduced off-target effects, improving both the safety and efficacy of CAR-T-cell therapies in solid tumors [169]. Moreover, strategies to improve CAR-T-cell metabolism via the use of drugs that activate AMPK and inhibit mTOR, such as metformin and rapamycin, have also been shown to help overcome hypoxic conditions [170].

Signal amplification to avoid dysfunction

The signal transduction domains (STDs) significantly impact CAR signal input efficiency. The incorporation of specific STDs from molecules such as CD3ζ and CD28 enhances cytokine secretion and cytotoxicity in CAR-T cells, highlighting the importance of the STD structure and signaling in modulating CAR activity [171]. However, inefficient CAR proximal signaling remains a bottleneck, reducing antigen sensitivity. Studies indicate that the inefficient recruitment and activation of key signaling proteins, such as ZAP-70, are significantly reduced in current CAR designs, limiting the efficacy of CAR-T-cell therapies, especially those that target tumors with low antigen expression [172].

To address these challenges, strategies such as integrating binding affinity and inputting signaling molecules have been proposed for rational CAR design. By adjusting the affinity of CARs and utilizing tonic signaling, researchers can increase T-cell functionality and sustain long-term tumor control, underscoring the need to balance CAR binding affinity and tonic signaling to optimize T-cell responses [173]. Another promising approach involves adjusting the charge density of CARs to optimize tonic signaling and CAR-T-cell fitness. Modifying the positively charged patches on the CAR antigen-binding domain can regulate CAR clustering and tonic signaling, potentially improving the *in vivo* persistence and anti-tumor function of CAR-T cells [18].

Moreover, compensating CAR-T cells with signaling molecules such as LCK, LAT, or FYN was demonstrated to efficiently overcome these barriers. LCK enhances T-cell activation and anti-tumor responses by phosphorylating signaling proteins and helps manage CAR-T-cell exhaustion and cytokine release [16]. LAT enhances CAR-T-cell sensitivity and persistence, particularly in antigen-low leukemias, through the ALA-CAR-T-cell platform, which enhances signaling and cytotoxicity [174]. FYN influences T-cell responses, and its modulation can promote regulatory T-cell differentiation, potentially reducing CAR-T-cell exhaustion and improving therapeutic outcomes [175].

In addition, the combination of two different co-stimulatory domains has been explored to enhance CAR-T cell function by balancing initial activation and long-term persistence. For example, the combination of CD28 with other co-stimulatory domains, such as 4-1BB, to enhance CAR-T-cell function by balancing initial activation and long-term persistence has been explored [176,177]. The combination of 4-1BB with ICOS can improve the persistence and anti-tumor efficacy of CAR-T cells, providing a synergistic effect that enhances their therapeutic potential in solid tumors [108]. In solid tumors, combining OX40 with ICOS has been studied to further improve CAR-T-cell efficacy. This combination can promote a T-cell persistence phenotype and increase cytotoxicity against tumor cells, thereby increasing overall survival and long-term CAR-T-cell persistence *in vivo* [110]. These strategies show that combining two other co-stimulatory signals has the potential to overcome the limitations of CAR-T cell therapy in solid tumors [78,81].

Metabolic reprogramming for nutrient competition

The clinical implications of TCR versus CAR signaling in solid tumors are profoundly influenced by metabolic reprogramming, which is a critical factor determining the efficacy of T cell-based therapies. In solid tumors, the metabolic landscape is often hostile to immune cells and is characterized by nutrient deprivation and the production of immunosuppressive metabolites. This hostile environment necessitates robust metabolic adaptation by T cells to maintain their anti-tumor activity [178,179]. Recent studies have shown that targeting metabolic pathways such as the AMPK and PPAR pathways can overcome the restrictions of the tumor microenvironment [180]. Some transcription factors, such as Foxp3 and Foxo1, when introduced into advanced CAR-T cells, have been shown to reprogram cellular metabolism, reduce exhaustion, and enhance anti-tumor efficacy [181,182]. These findings underscore the importance of metabolic reprogramming in overcoming the metabolic deficiencies induced by the TME, thereby enhancing the therapeutic potential of TCR/CAR-based therapies.

Mitochondrial remodeling

Mitochondrial remodeling is essential for effective immune responses. The dynamic processes of mitochondrial fusion and fission, supported by proteins such as Opa1, maintain the metabolic efficiency of memory T cells by promoting oxidative phosphorylation and fatty acid oxidation [183]. Moreover, overexpressing PGC-1α, a key regulator of mitochondrial biogenesis, enhances $CD8^+$ T cell memory and anti-tumor immunity by increasing mitochondrial activity and metabolic fitness [184,185]. These findings highlight the importance of mitochondrial dynamics in establishing and maintaining immunological memory, which is vital for effective vaccine responses and protective immunity against infections [186–189].

Restricted glycolysis

Inhibiting glycolysis in T cells also offers a promising strategy to increase their fitness and function in cancer immunotherapy. Glycolysis is essential for T cell activation and effector functions but can cause metabolic exhaustion. Targeting this pathway helps reprogram T cell metabolism, providing an edge in the nutrient-deprived tumor microenvironment where cancer cells often outcompete immune cells [190–192]. Research has shown that inhibiting glycolysis can increase memory $CD8^+$ T cell formation, which is crucial for sustained anti-tumor immunity. For example, the use of 2-deoxyglucose has been shown to promote memory T cell generation, thereby enhancing the immunotherapeutic potential of T cells against cancer [193,194]. Furthermore, targeting tumor cell glycolysis can enhance checkpoint therapy efficacy, as observed in highly glycolytic tumors, where genetic glycolysis suppression improves outcomes [191,192]. Overall, inhibiting glycolysis and reprogramming T-cell metabolism constitute a viable approach to enhance the efficacy of TCR and CAR therapy in solid tumors [195].

Lipid supplementation

Furthermore, lipid metabolism is critical for T cell function, especially in the TME, where T cells compete with malignant cells for nutrients [178,196,197]. Lipid metabolic remodeling has also been explored to delay T-cell senescence and enhance anti-tumor potency [198], with preclinical results showing improved T-cell receptor clustering and sustained activation for better anti-tumor efficacy [199]. Studies indicate that enhancing fatty acid catabolism in T cells can improve their effector functions and persistence in the tumor microenvironment, thereby increasing the efficacy of

immunotherapies such as CAR-T cells [200,201]. Additionally, targeting lipid metabolism in Tregs can reduce their immunosuppressive function and strengthen the anti-tumor immune response [202]. Nanoparticle-mediated lipid metabolic reprogramming shows promise for improving T cell function in the TME. For example, nanoparticles can deliver lipid-modulating agents to T cells, promoting fatty acid oxidation over glycolysis and supporting T cell survival in glucose-deficient environments while enhancing their cytotoxicity against tumors [203]. Liposomal avasimibe, which modulates cholesterol in T cells, has improved T cell receptor clustering and activation, increasing anti-tumor efficacy in melanoma and glioblastoma mouse models [199,204].

Overdrive of glutaminolysis metabolism

Additionally, glutaminolysis metabolic reprogramming is essential for enhancing T cell fitness and function in the TME. Glutaminolysis provides energy and biosynthetic precursors for T cell proliferation and function, but it also fuels cancer cells, leading to nutrient competition [205,206]. Targeting this pathway can reduce glutamine availability for cancer cells, enhancing T cell metabolic fitness and anti-tumor efficacy [207,208]. Overactive glutaminolysis can support the energy and biosynthetic needs of activated T cells. This metabolic shift is essential for maintaining T cell function and preventing exhaustion in the hostile TME of solid tumors. Increasing glutaminolysis allows T cells to compete better for nutrients in the TME, potentially overcoming metabolic barriers and improving TCR and CAR therapy efficacy. These findings align with recent findings highlighting the importance of metabolic reprogramming in T cell-based immunotherapies and suggest that targeting metabolic pathways could enhance these treatments [184].

Augmenting memory

Enhancing T cell fitness, particularly in terms of memory and adaptability, is crucial for cancer immunotherapy [209,210]. Low CAR-T cell expansion post-infusion is associated with poor survival in patients with relapsed/refractory large B-cell non-Hodgkin lymphoma [211]. Research has shown that stem-like or memory-phenotype T cells have improved persistence and tumor control compared with conventional CAR-T cells in preclinical models [212]. Manipulating T cell differentiation pathways can also increase the degree of memory differentiation of CAR-T cells. One strategy is to target co-stimulatory domain signaling pathways to increase T cell function. The incorporation of specific co-stimulatory domains into CAR-T cells also significantly enhances their anti-tumor activity. For example, CD19 CAR-T cells with B-cell co-stimulatory molecules, such as CD79A/CD40, exhibit superior anti-tumor functions compared with those with conventional domains, such as CD28 or 4-1BB. CD79A/CD40-armed CAR-T cells upregulate genes involved in T-cell activation, proliferation, and NF-κB signaling while downregulating genes linked to exhaustion and apoptosis [213]. Additionally, kinase screening and rest of the strategies are reported to promote CAR-T-cell persistence by increasing CAR-T-cell memory [214,215]. MEK inhibition also improves CAR-T-cell memory by downregulating c-Fos and JunB [216]. Moreover, the overexpression of transcription factors such as Foxo1 in CAR-T cells enhances stem-like traits, mitochondrial fitness, and persistence, increasing solid tumor control [182,217]. It drives memory-associated gene expression, which is crucial for long-term efficacy. Foxo1 also promotes memory precursor T cell generation and central-memory T cell differentiation [218]. CAR-T cells engineered to overexpress the canonical AP-1 factor c-Jun have increased expansion potential, increased functional capacity, diminished terminal differentiation, and improved anti-tumor potency *in vivo* [219].

Moreover, cytokine priming is vital for enhancing T cell memory and adaptability. Key cytokines such as IL-7, IL-21, and IL-15 play crucial roles in this process. IL-7 exposure during priming increases memory $CD4^+$ T-cell generation by promoting survival [220]. Co-expressing cytokines such as IL-15 and IL-21 can prevent functional exhaustion and enhance the resilience of engineered T cells against solid tumors [221–223]. In addition, emerging studies have shown that several traditional negative cytokine signaling pathways, such as the IL-10, IL-4, and TGF-beta pathways, are also reported to help CAR-T cells resist dysfunction and mediate durable clearance of solid tumors and metastases [224–226]. These findings suggest that targeting cytokines could improve T cell fitness and immune responses.

Reverse exhaustion

T-cell exhaustion refers to a dysfunctional state of T cells characterized by reduced proliferative capacity [227], diminished cytokine production [227], and impaired cytotoxicity, often observed in chronic infections and tumors [228,229]. CAR-T-cell exhaustion is a major limitation in CAR-T-cell therapy for solid tumors.

Reversing exhaustion through epigenetic editing

Epigenetic and genetic editing offers potential solutions to rescue exhaustion. Epigenetic changes such as DNA methylation and histone modifications are crucial for gene expression and T cell function. For instance, the epigenetic regulator EZH2 can help CAR-T cells resist exhaustion, and EZH2 inhibition enhances CAR-T-cell resistance against multiple cancers [230–232]. In addition, disruption of the DNA methylcytosine dioxygenase TET2 reportedly enhances CAR-T-cell expansion, persistence, and anti-tumor efficacy, promoting a central memory phenotype and tumor remission [233]. Targeting TET2 has also been shown to restrain terminal exhaustion and improve anti-tumor responses in CAR-T cells [234,235]. TET2 loss can cause antigen-independent CAR-T-cell expansion, leading to tissue infiltration and genomic instability [236]. Inhibiting histone deacetylase (HDAC) has been shown to enhance memory maintenance and resistance to exhaustion in CAR-T cells [237,238]. Entinostat, an HDAC inhibitor, sustains CAR expression in CAR-NK cells, increasing their cytotoxic activity against myeloma cells [239]. HDAC inhibitors also sensitize cancer cells to CAR-T-cell clearance by upregulating NOXA [240]. These studies highlight the potential of epigenetic reprogramming in improving CAR-T-cell persistence and functionality.

Reversing exhaustion through transcription modification

Certain transcription factors play crucial roles in T-cell function and exhaustion [241]. For example, TOX and TOX2 are key regulators, especially in chronic infections and tumors, where they induce and maintain the exhausted state [242–244]. Nuclear factor of activated T cells (NFAT) is a key transcription factor that controls the T-cell exhaustion program, influencing the expression of inhibitory receptors and impairing T-cell function [245,246]. Other important factors, including IRF4, BATF, and NR4A, also act downstream of TCR signaling, contributing to the expression of inhibitory receptors and impaired T-cell function [247,248]. Strategies such as genetic editing of these transcription factors on CAR-T cells have also been

demonstrated to result in enhanced efficacy [249–251].

Reversing exhaustion through checkpoint blockade

Therapies such as anti-PD-1 and anti-PD-L1 can alter epigenetic changes [252], reverse T-cell exhaustion [253], and enhance anti-tumor activity [254], particularly in solid tumors with immunosuppressive microenvironments [252,255,256]. Strategies such as CAR-T-cell therapy and dual inhibition of the PD-1 and LAG-3 pathways have shown promise in restoring T-cell function [253,257]. Combination therapies that integrate checkpoint blockades with CAR-T-cell therapy are being explored to enhance the efficacy against solid tumors [258,259]. Additionally, knocking out immune checkpoint-related genes such as PD-1 promotes longer survival in tumor models by reducing exhaustion phenotypes [260]. Additionally, genetic editing to engineer CAR-T cells that express a PD-1 dominant negative receptor has improved anti-tumor efficacy in solid tumors [261–263].

Heterogeneity or loss of tumor antigen

Treatment failure and/or disease recurrence after CAR-T-cell therapy can be caused by epitope or antigen loss [264,265]. In particular, the inherently heterogeneous expression pattern of antigens in solid tumors can easily cause tumor escape after targeted immunotherapy [266,267]. Therefore, targeting multiple tumor-associated antigens (TAAs) is generally expected to improve the outcome of CAR-T-cell therapy in solid tumors. When constructing dual-targeting CAR-T cells, the different co-stimulation domain choices also need to be considered [177]. We propose an approach based on dual targeting, split co-stimulatory signaling, and a shared CD3ζ chain tailored to target that allows rapid and sustained anti-tumor effects to be achieved [177]. In addition, we and others reported that, compared with traditional scFv-based CARs, bispecific-human antibody VH domain-based CARs exhibited superior tumor-killing ability [4,268].

Other strategies to enhance CAR-T-cell therapies for solid tumors

The future of TCR and CAR therapies in solid tumors lies in continuous innovation and the integration of advanced technologies and combination strategies. By addressing the unique challenges of solid tumors, these therapies have the potential to revolutionize cancer treatment and offer new hope for patients with hard-to-treat malignancies [269–272].

Hybrid CAR designs and dynamic control systems

Hybrid CAR designs and dynamic control systems present significant potential for improving CAR-T-cell therapy in solid tumors. Hybrid CAR designs seek to merge the strengths of different receptor components to enhance antigen recognition and T-cell activation. For example, a study introduced a hybrid CAR in which the antigen-binding domain of a first-generation CAR was combined under the control of the CD3ε promoter, forming a CAR-CD3ε fusion protein [273]. This design harnesses the endogenous TCR signaling cascade, enabling robust CAR activity while preserving the TCR's primary antigen recognition function, potentially increasing CAR-T-cell specificity and functionality in solid tumors. In addition, dynamic control systems also offer promising avenues for enhancing CAR-T-cell therapy. These systems enable precise and adaptable regulation of CAR-T-cell activity by responding to changes in the tumor microenvironment. They can adjust CAR-T-cell activation and function on the basis of specific signals, such as alterations in antigen density or immunosuppressive factors, thereby optimizing therapeutic effects and reducing off-target effects and T-cell exhaustion [274].

Optogenetic and sonogenetic CARs

Optogenetic and sonogenetic CARs, which use light-sensitive proteins or ultrasensitive heat for precise T cell activity modulation, are promising platforms. They allow dynamic regulation of CAR-T-cell activation, potentially improving their fitness and persistence in the tumor microenvironment. Optogenetic and sonogenetic CARs offer advantages such as real-time control to reduce off-target effects and cytokine release syndrome. In addition, controlled CAR expression also triggers long-lasting CAR-T-cell activation and continuous destruction of cancer cells. They also enhance CAR-T-cell adaptability to the heterogeneous and immunosuppressive solid TME through condition control [275,276].

SynNotch receptors

SynNotch receptors offer a key advantage in enhancing CAR-T-cell specificity and persistence by integrating multiple signals before activation, potentially reducing on-target, off-tumor toxicity, which is a major concern in solid tumor therapies due to the scarcity of highly tumor-specific antigens [277]. By programming T cells to respond only to a specific antigen combination, SynNotch circuits improve therapeutic precision [278].

Recent studies have shown that SynNotch CAR circuits can enhance solid tumor recognition and promote persistent anti-tumor activity in mouse models. For example, the identification of alkaline phosphatase placental-like 2 as a tumor-specific antigen in various solid tumors, such as mesothelioma and ovarian cancer, suggests that SynNotch CAR-T cells could effectively target a broad range of solid tumors. Additionally, the use of SynNotch receptors, such as IL-12, to control local cytokine expression increases CAR-T-cell anti-tumor activity without causing systemic toxicity [279,280]. Moreover, engineering T cells with SynNotch receptors to recognize and respond to angiogenic signals in the tumor microenvironment provides another way to increase CAR-T-cell efficacy [281].

Combinatorial strategies

To increase the effectiveness of TCR and CAR therapy in solid tumors, combination approaches such as chemotherapy, radiation, or immune checkpoint inhibitors are being explored.

Radiotherapy synergy Combining radiotherapy with CAR-T-cell therapy is a promising strategy for improving CAR-T-cell efficacy in solid tumors. Radiotherapy can modulate the TME to increase CAR-T-cell infiltration and activity while inducing immunogenic cell death to increase the anti-tumor immune response [282,283]. It also promotes a chemokine gradient for better CAR-T-cell tumor infiltration and increases tumor-associated antigen expression, improving CAR-T-cell activation [284]. Additionally, radiotherapy can induce systemic immune responses through abscopal effects, which may further potentiate the efficacy of CAR-T-cell therapy [285]. These synergistic effects suggest that combining radiotherapy with CAR-T-cell therapy could help overcome barriers to CAR-T-cell effectiveness in solid tumors. Future directions for CAR-T-cell therapy in solid tumors, especially when CAR-T-cell therapy is combined with radiotherapy, should focus on tackling antigenic heterogeneity and the immunosuppressive TME [286].

Combining therapies with immune checkpoint blockade Combining CAR-T-cell therapy with immune checkpoint blockade is a promising approach, as checkpoint inhibitors can counteract immunosuppressive signals within the TME, enhancing T-cell

persistence and function. Preclinical and clinical studies have demonstrated a synergistic effect, suggesting that this combination could lead to more durable responses in solid tumors [258,287]. As discussed in section "Reversing exhaustion through checkpoint blockade".

Other combination strategies The integration of oncolytic virotherapy with CAR-T-cell therapy is being explored. Oncolytic viruses can selectively infect and kill tumor cells while modulating the TME to increase immune cell infiltration and activation. This approach has shown promise in preclinical models, where oncolytic viruses have been engineered to express tumor antigens or immunomodulatory molecules, thereby increasing CAR-T-cell efficacy [288]. Additionally, approaches such as vaccines [289] and biomaterials [290,291] are being investigated to increase CAR-T-cell efficiency and overcome solid tumor limitations [292,293].

Conclusion and Future Direction

The contrast between TCR precision and CAR adaptability highlights the necessity for integrative strategies in solid tumor immunotherapy. Advances in co-stimulatory engineering, metabolic reprogramming, and epigenetic modulation offer transformative potential. The future of CAR engineering is moving toward innovative approaches such as logic-gated CARs and CRISPR-enhanced signaling. Logic-gated CARs, which utilize multiple receptors to distinguish between tumor and normal cells, offer enhanced precision and reduced off-target effects. CRISPR-enhanced signaling enables precise genomic modifications, improving the functionality and persistence of CAR-T cells. These advancements hold promise for overcoming current limitations in CAR-T-cell therapies, particularly in solid tumors.

Synthetic CARs can further learn from or integrate TCR features to improve their effectiveness. TCRs exhibit high antigen recognition precision and can present endogenous peptides, expanding the antigen repertoire. By incorporating these features, synthetic CARs could achieve more nuanced antigen recognition and sustained T-cell responses. For example, integrating TCR MHC restriction for specificity with CAR flexibility in targeting non-peptide antigens may increase therapeutic precision.

TCR-CAR hybrid approaches also present significant potential. These hybrid systems combine the strengths of both receptors, enabling T cells to respond to a broader array of tumor-associated antigens. For example, leveraging the ability of the TCR to recognize a wide range of antigens processed and presented by tumor cells while utilizing the capacity of the CAR to engage non-peptide antigens and provide co-stimulatory signals could enhance therapeutic efficacy. However, strategies for optimizing pairing and signaling integration between TCR and CAR components, as well as strategies to mitigate potential drawbacks such as off-target effects or T-cell exhaustion, must be developed.

In conclusion, the future of CAR-T-cell therapy in solid tumors hinges on continuous innovations in CAR engineering, strategic integration of TCR features, and careful development of TCR-CAR hybrid approaches. Collaborative efforts between basic researchers and clinicians remain essential for translating these strategies into durable clinical responses, providing hope for patients with refractory solid tumors.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Roybal KT, Lim WA. Synthetic immunology: hacking immune cells to expand their therapeutic capabilities. *Annu Rev Immunol* 2017, 35: 229–253
2. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol* 2019, 17: 147–167
3. Chang ZNL, Lorenzini MH, Chen X, Tran U, Bangayan NJ, Chen YY. Rewiring T-cell responses to soluble factors with chimeric antigen receptors. *Nat Chem Biol* 2018, 14: 317–324
4. Wang G, Zhou X, Fucà G, Dukhovlinova E, Shou P, Li H, Johnston C, *et al.* Fully human antibody V_H domains to generate mono and bispecific CAR to target solid tumors. *J Immunother Cancer* 2021, 9: e002173
5. Ranganathan R, Shou P, Ahn S, Sun C, West J, Savoldo B, Dotti G. CAR T cells targeting human immunoglobulin light chains eradicate mature B-cell malignancies while sparing a subset of normal B cells. *Clin Cancer Res* 2021, 27: 5951–5960
6. Ellebrecht CT, Bhoj VG, Nace A, Choi EJ, Mao X, Cho MJ, Di Zenzo G, *et al.* Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science* 2016, 353: 179–184
7. Schmidts A, Ormhøj M, Choi BD, Taylor AO, Bouffard AA, Scarfò I, Larson RC, *et al.* Rational design of a trimeric APRIL-based CAR-binding domain enables efficient targeting of multiple myeloma. *Blood Adv* 2019, 3: 3248–3260
8. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, Ostberg JR, *et al.* Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N Engl J Med* 2016, 375: 2561–2569
9. Bagley SJ, Desai AS, Fraietta JA, Silverbush D, Chafamo D, Freeburg NF, Gopikrishna GK, *et al.* Intracerebroventricular bivalent CAR T cells targeting EGFR and IL-13Rα2 in recurrent glioblastoma: a phase 1 trial. *Nat Med* 2025, 31: 2778–2787
10. Ying Z, Huang XF, Xiang X, Liu Y, Kang X, Song Y, Guo X, *et al.* A safe and potent anti-CD19 CAR T cell therapy. *Nat Med* 2019, 25: 947–953
11. Flemming A. Trogocytosis of CARs depends on transmembrane domains. *Nat Rev Immunol* 2025, 25: 156
12. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov* 2013, 3: 388–398
13. Guedan S, Calderon H, Posey Jr. AD, Maus MV. Engineering and design of chimeric antigen receptors. *Mol Ther-Methods Clin Dev* 2019, 12: 145–156
14. Salter AI, Ivey RG, Kennedy JJ, Voillet V, Rajan A, Alderman EJ, Voytovich UJ, *et al.* Phosphoproteomic analysis of chimeric antigen receptor signaling reveals kinetic and quantitative differences that affect cell function. *Sci Signal* 2018, 11: eaat6753
15. Ramello MC, Benzaïd I, Kuenzi BM, Lienlaf-Moreno M, Kandell WM, Santiago DN, Pabón-Saldaña M, *et al.* An immunoproteomic approach to characterize the CAR interactome and signalosome. *Sci Signal* 2019, 12: eaap9777
16. Sun C, Shou P, Du H, Hirabayashi K, Chen Y, Herring LE, Ahn S, *et al.* THEMIS-SHP1 recruitment by 4-1BB tunes LCK-mediated priming of chimeric antigen receptor-redirected T cells. *Cancer Cell* 2020, 37: 216–225.e6

17. Omer B, Castillo PA, Tashiro H, Shum T, Huynh MTA, Cardenas M, Tanaka M, *et al.* Chimeric antigen receptor signaling domains differentially regulate proliferation and native T cell receptor function in virus-specific T cells. *Front Med* 2018, 5: 343
18. Chen J, Qiu S, Li W, Wang K, Zhang Y, Yang H, Liu B, *et al.* Tuning charge density of chimeric antigen receptor optimizes tonic signaling and CAR-T cell fitness. *Cell Res* 2023, 33: 341–354
19. Zhao X, Shao S, Hu L. The recent advancement of TCR-T cell therapies for cancer treatment. *Acta Biochim Biophys Sin* 2024, 56: 663–674
20. Kuhns MS, Badgandi HB. Piecing together the family portrait of TCR-CD3 complexes. *Immunol Rev* 2012, 250: 120–143
21. Shah K, Al-Haidari A, Sun J, Kazi JU. T cell receptor (TCR) signaling in health and disease. *Sig Transduct Target Ther* 2021, 6: 412
22. Hwang W, Mallis RJ, Lang MJ, Reinherz EL. The *αβ* TCR mechanosensor exploits dynamic ectodomain allostery to optimize its ligand recognition site. *Proc Natl Acad Sci USA* 2020, 117: 21336–21345
23. Liu B, Chen W, Evavold BD, Zhu C. Accumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling. *Cell* 2014, 157: 357–368
24. Jeffreys N, Brockman JM, Zhai Y, Ingber DE, Mooney DJ. Mechanical forces amplify TCR mechanotransduction in T cell activation and function. *Appl Phys Rev* 2024, 11: 011304
25. Kim ST, Shin Y, Brazin K, Mallis RJ, Sun ZYJ, Wagner G, Lang MJ, *et al.* TCR mechanobiology: torques and tunable structures linked to early T cell signaling. *Front Immun* 2012, 3: 76
26. Hartl FA, Beck-Garcìa E, Woessner NM, Flachsmann LJ, Cárdenas RMHV, Brandl SM, Taromi S, *et al.* Noncanonical binding of Lck to CD3ε promotes TCR signaling and CAR function. *Nat Immunol* 2020, 21: 902–913
27. Dong D, Zheng L, Lin J, Zhang B, Zhu Y, Li N, Xie S, *et al.* Structural basis of assembly of the human T cell receptor–CD3 complex. *Nature* 2019, 573: 546–552
28. Pandey PR, Różycki B, Lipowsky R, Weikl TR. Structural variability and concerted motions of the T cell receptor – CD3 complex. *eLife* 2021, 10: e67195
29. Chen H, Xu X, Hu W, Wu S, Xiao J, Wu P, Wang X, *et al.* Self-programmed dynamics of T cell receptor condensation. *Proc Natl Acad Sci USA* 2023, 120: e2217301120
30. Au-Yeung BB, Shah NH, Shen L, Weiss A. ZAP-70 in signaling, biology, and disease. *Annu Rev Immunol* 2018, 36: 127–156
31. Voisinne G, Locard-Paulet M, Froment C, Maturin E, Menoita MG, Girard L, Mellado V, *et al.* Kinetic proofreading through the multi-step activation of the ZAP70 kinase underlies early T cell ligand discrimination. *Nat Immunol* 2022, 23: 1355–1364
32. Anto NP, Arya AK, Muraleedharan A, Shaik J, Nath PR, Livneh E, Sun Z, *et al.* Cyclophilin A associates with and regulates the activity of ZAP70 in TCR/CD3-stimulated T cells. *Cell Mol Life Sci* 2023, 80: 7
33. Zeng L, Palaia I, Šarić A, Su X. PLCγ1 promotes phase separation of T cell signaling components. *J Cell Biol* 2021, 220: e202009154
34. Kim MJ, Chang JS, Park SK, Hwang JI, Ryu SH, Suh PG. Direct interaction of SOS1 ras exchange protein with the SH3 domain of phospholipase C-γ1. *Biochemistry* 2000, 39: 8674–8682
35. Houtman JCD, Yamaguchi H, Barda-Saad M, Braiman A, Bowden B, Appella E, Schuck P, *et al.* Oligomerization of signaling complexes by the multipoint binding of GRB2 to both LAT and SOS1. *Nat Struct Mol Biol* 2006, 13: 798–805
36. Sommers CL, Menon RK, Grinberg A, Zhang W, Samelson LE, Love PE. Knock-in mutation of the distal four tyrosines of linker for activation of T cells blocks murine T cell development. *J Exp Med* 2001, 194: 135–142
37. Su X, Ditlev JA, Hui E, Xing W, Banjade S, Okrut J, King DS, *et al.* Phase separation of signaling molecules promotes T cell receptor signal transduction. *Science* 2016, 352: 595–599
38. Tousley AM, Rotiroti MC, Labanieh L, Rysavy LW, Kim WJ, Lareau C, Sotillo E, *et al.* Co-opting signalling molecules enables logic-gated control of CAR T cells. *Nature* 2023, 615: 507–516
39. Lin JJ, O'Donoghue GP, Wilhelm KB, Coyle MP, Low-Nam ST, Fay NC, Alfieri KN, *et al.* Membrane association transforms an inert anti-TCRβ Fab′ ligand into a potent T cell receptor agonist. *Biophys J* 2020, 118: 2879–2893
40. Lv J, Si T, Wang D, Zhang C, Zhou Y, Yu J, Jiang Y, *et al.* Mechanical signaling via β2 integrin decouples T cell proliferation and differentiation for generating stem cell-like CAR T cells. *Immunity* 2025, 58: 2289–2304. e10
41. Majedi FS, Hasani-Sadrabadi MM, Thauland TJ, Li S, Bouchard LS, Butte MJ. T-cell activation is modulated by the 3D mechanical microenvironment. *Biomaterials* 2020, 252: 120058
42. Al-Aghbar MA, Jainarayanan AK, Dustin ML, Roffler SR. The interplay between membrane topology and mechanical forces in regulating T cell receptor activity. *Commun Biol* 2022, 5: 40
43. Hu Y, Duan Y, Velusamy A, Narum S, Rogers J, Salaita K. DNA Origami Tension Sensors (DOTS) to study T cell receptor mechanics at membrane junctions. *bioRxiv* 2023
44. Kawai A, Ishihara M, Nakamura T, Kitano S, Iwata S, Takada K, Emori M, *et al.* Results from phase I/II study of NY-ESO-1-specific TCR gene-transduced T cell therapy (TBI-1301, mipetresgene autoleucel) in patients with advanced synovial sarcoma. *J Clin Oncol* 2023, 41: 11558
45. Chodon T, Comin-Anduix B, Chmielowski B, Koya RC, Wu Z, Auerbach M, Ng C, *et al.* Adoptive transfer of MART-1 T-cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. *Clin Cancer Res* 2014, 20: 2457–2465
46. Kong S, Zhang J, Wang L, Li W, Guo H, Weng Q, He Q, *et al.* Mechanisms of low MHC I expression and strategies for targeting MHC I with small molecules in cancer immunotherapy. *Cancer Lett* 2025, 611: 217432
47. Zhang H, Liu L, Zhang J, Chen J, Ye J, Shukla S, Qiao J, *et al.* Investigation of antigen-specific T-cell receptor clusters in human cancers. *Clin Cancer Res* 2020, 26: 1359–1371
48. Mu X, Chen C, Dong L, Kang Z, Sun Z, Chen X, Zheng J, *et al.* Immunotherapy in leukaemia. *Acta Biochim Biophys Sin* 2023, 55: 974–987
49. Zhao Z, Condomines M, van der Stegen SJC, Perna F, Kloss CC, Gunset G, Plotkin J, *et al.* Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. *Cancer Cell* 2015, 28: 415–428
50. Rohrs JA, Siegler EL, Wang P, Finley SD. ERK activation in CAR T cells is amplified by CD28-Mediated increase in CD3ζ phosphorylation. *iScience* 2020, 23: 101023
51. Dou Z, Bonacci TR, Shou P, Landoni E, Woodcock MG, Sun C, Savoldo B, *et al.* 4-1BB-encoding CAR causes cell death via sequestration of the ubiquitin-modifying enzyme A20. *Cell Mol Immunol* 2024, 21: 905–917
52. Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey Jr. AD, Patel PR, *et al.* Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity* 2016, 44: 380–390
53. Acharya S, Basar R, Daher M, Rafei H, Li P, Uprety N, Ensley E, *et al.* CD28 costimulation augments CAR signaling in NK cells via the LCK/CD3ζ/ZAP70 signaling axis. *Cancer Discov* 2024, 14: 1879–1900
54. Textor A, Grunewald L, Anders K, Klaus A, Schwiebert S, Winkler A, Stecklum M, *et al.* CD28 co-stimulus achieves superior CAR T cell

effector function against solid tumors than 4-1BB co-stimulus. *Cancers* 2021, 13: 1050
55. Lai YP, Kuo LC, Lin BR, Lin HJ, Lin CY, Chen YT, Hsiao PW, *et al.* CD28 engagement inhibits CD73-mediated regulatory activity of CD8+ T cells. *Commun Biol* 2021, 4: 595
56. Zhang P, Feng X, Niu X, Liu Z, Li M, Liu M, Yan D, *et al.* CD28 is superior to 4-1BB costimulation in generating CAR-NK cells for tumor immunotherapy. *Exp Hematol Oncol* 2025, 14: 28
57. Majocchi S, Lloveras P, Nouveau L, Legrand M, Viandier A, Malinge P, Charreton M, *et al.* NI-3201 is a bispecific antibody mediating PD-L1–dependent CD28 co-stimulation on T cells for enhanced tumor control. *Cancer Immunol Res* 2025, 13: 365–383
58. Kamphorst AO, Wieland A, Nasti T, Yang S, Zhang R, Barber DL, Konieczny BT, *et al.* Rescue of exhausted CD8 T cells by PD-1–targeted therapies is CD28-dependent. *Science* 2017, 355: 1423–1427
59. Lotze MT, Olejniczak SH, Skokos D. CD28 co-stimulation: novel insights and applications in cancer immunotherapy. *Nat Rev Immunol* 2024, 24: 878–895
60. Klebanoff CA, Rosenberg SA, Restifo NP. Prospects for gene-engineered T cell immunotherapy for solid cancers. *Nat Med* 2016, 22: 26–36
61. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014, 371: 1507–1517
62. Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, Smith JP, *et al.* 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat Med* 2015, 21: 581–590
63. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, *et al.* Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med* 2018, 378: 439–448
64. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Jäger U, *et al.* Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med* 2019, 380: 45–56
65. Kofler DM, Chmielewski M, Rappl G, Hombach A, Riet T, Schmidt A, Hombach AA, *et al.* CD28 costimulation impairs the efficacy of a redirected T-cell antitumor attack in the presence of regulatory T cells which can be overcome by preventing lck activation. *Mol Ther* 2011, 19: 760–767
66. Römer PS, Berr S, Avota E, Na SY, Battaglia M, ten Berge I, Einsele H, *et al.* Preculture of PBMCs at high cell density increases sensitivity of T-cell responses, revealing cytokine release by CD28 superagonist TGN1412. *Blood* 2011, 118: 6772–6782
67. Langenhorst D, Haack S, Göb S, Uri A, Lühder F, Vanhove B, Hünig T, *et al.* CD28 costimulation of T helper 1 cells enhances cytokine release in vivo. *Front Immunol* 2018, 9: 1060
68. Guedan S, Madar A, Casado-Medrano V, Shaw C, Wing A, Liu F, Young RM, *et al.* Single residue in CD28-costimulated CAR-T cells limits long-term persistence and antitumor durability. *J Clin Invest* 2020, 130: 3087–3097
69. Wei C, Huang X, Xu T, Fang Y, Wang F, He Q, Zhang P, *et al.* NECTIN-4-redirected T cell antigen coupler T cells bearing CD28 show superior antitumor responses against solid tumors. *Front Immunol* 2024, 15: 1456443
70. Song DG, Ye Q, Carpenito C, Poussin M, Wang LP, Ji C, Figini M, *et al.* *In vivo* persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). *Cancer Res* 2011, 71: 4617–4627
71. Kotanides H, Sattler RM, Lebron MB, Carpenito C, Shen J, Li J, Surguladze D, *et al.* Characterization of 7A5: a human CD137 (4-1BB) receptor binding monoclonal antibody with differential agonist properties that promotes antitumor immunity. *Mol Cancer Ther* 2020, 19: 988–998
72. Philipson BI, O'Connor RS, May MJ, June CH, Albelda SM, Milone MC. 4-1BB costimulation promotes CAR T cell survival through noncanonical NF-κB signaling. *Sci Signal* 2020, 13: eaay8248
73. Ho JQ, Asagiri M, Hoffmann A, Ghosh G, Agarwal S. NF-κB potentiates caspase independent hydrogen peroxide induced cell death. *PLoS ONE* 2011, 6: e16815
74. Abhari BA, McCarthy N, Agostinis P, Fulda S. NF-κB contributes to Smac mimetic-conferred protection from tunicamycin-induced apoptosis. *Apoptosis* 2019, 24: 269–277
75. Zhang L, Blackwell K, Altaeva A, Shi Z, Habelhah H, Luo K. TRAF2 phosphorylation promotes NF-κB–dependent gene expression and inhibits oxidative stress-induced cell death. *Mol Biol Cell* 2011, 22: 128–140
76. Liu F, Bardhan K, Yang D, Thangaraju M, Ganapathy V, Waller JL, Liles GB, *et al.* NF-κB directly regulates fas transcription to modulate fas-mediated apoptosis and tumor suppression. *J Biol Chem* 2012, 287: 25530–25540
77. Ritmeester-Loy SA, Draper IH, Bueter EC, Lautz JD, Zhang-Wong Y, Gustafson JA, Wilson AL, *et al.* Differential protein-protein interactions underlie signaling mediated by the TCR and a 4-1BB domain–containing CAR. *Sci Signal* 2024, 17: eadd4671
78. Zhao W, Yao Y, Li Q, Xue Y, Gao X, Liu X, Zhang Q, *et al.* Molecular mechanism of co-stimulatory domains in promoting CAR-T cell anti-tumor efficacy. *Biochem Pharmacol* 2024, 227: 116439
79. Zhong Q, Zhu Y, Zheng L, Shen H, Ou R, Liu Z, She Y, *et al.* Chimeric antigen receptor-T cells with 4-1BB co-stimulatory domain present a superior treatment outcome than those with CD28 domain based on bioinformatics. *Acta Haematol* 2018, 140: 131–140
80. Dunn ZS, Qu Y, MacMullan M, Chen X, Cinay G, Wang P. Secretion of 4-1BB ligand crosslinked to PD-1 checkpoint inhibitor potentiates chimeric antigen receptor T cell solid tumor efficacy. *Hum Gene Ther* 2023: hum.2022.068
81. 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: 299
82. Schoutrop E, El-Serafi I, Poiret T, Zhao Y, Gultekin O, He R, Moyano-Galceran L, *et al.* Mesothelin-specific CAR T cells target ovarian cancer. *Cancer Res* 2021, 81: 3022–3035
83. Ahn J, Xie P, Chen S, Shi G, Fan J, Zhang M, Tang H, *et al.* 4-1BB stimulation with concomitant inactivation of adenosine A2B receptors enhances CD8+ T cell antitumor response. *J Clin Invest* 2025, 135: e190841
84. Singh N, Frey NV, Engels B, Barrett DM, Shestova O, Ravikumar P, Cummins KD, *et al.* Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. *Nat Med* 2021, 27: 842–850
85. Xiong Y, Taleb M, Misawa K, Hou Z, Banerjee S, Amador-Molina A, Jones DR, *et al.* c-Kit signaling potentiates CAR T cell efficacy in solid tumors by CD28- and IL-2-independent co-stimulation. *Nat Cancer* 2023, 4: 1001–1015
86. Starzer AM, Berghoff AS. New emerging targets in cancer immunotherapy: CD27 (TNFRSF7). *ESMO Open* 2020, 4: e000629
87. Song DG, Ye Q, Poussin M, Harms GM, Figini M, Powell Jr DJ. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. *Blood* 2012, 119: 696–706
88. van de Ven K, Borst J. Targeting the T-cell co-stimulatory CD27/CD70

pathway in cancer immunotherapy: rationale and potential. *Immunotherapy* 2015, 7: 655–667
89. Jaeger-Ruckstuhl CA, Lo Y, Fulton E, Waltner OG, Shabaneh TB, Simon S, Muthuraman PV, *et al.* Signaling via a CD27-TRAF2-SHP-1 axis during naive T cell activation promotes memory-associated gene regulatory networks. *Immunity* 2024, 57: 287–302.e12
90. Tran Ngoc Minh T, Verleng LJ, Schrama E, Busselaar J, Staal MD, de Vries E, Anholts JDH, *et al.* CD27 costimulation supports metabolic fitness of CD4+ T cells by enhancing *de novo* nucleotide and protein synthesis. *J Immunol* 2025, 214: 1566–1581
91. Peperzak V, Veraar EAM, Keller AM, Xiao Y, Borst J. The pim kinase pathway contributes to survival signaling in primed CD8+ T Cells upon CD27 costimulation. *J Immunol* 2010, 185: 6670–6678
92. Zhang C, Jia J, Heng G, Li Y, Wang M, Chen J, Wang L, *et al.* CD27 agonism coordinates with CD28 and 4-1BB signal to augment the efficacy of CAR-T cells in colorectal tumor. *Med Oncol* 2023, 40: 123
93. Thomas LJ, He LZ, Marsh H, Keler T. Targeting human CD27 with an agonist antibody stimulates T-cell activation and antitumor immunity. *OncoImmunology* 2014, 3: e27255
94. Melo V, Nelemans LC, Vlaming M, Lourens HJ, Wiersma VR, Bilemjian V, Huls G, *et al.* EGFR-selective activation of CD27 co-stimulatory signaling by a bispecific antibody enhances anti-tumor activity of T cells. *Front Immunol* 2023, 14: 1191866
95. Claus C, Riether C, Schürch C, Matter MS, Hilmenyuk T, Ochsenbein AF. CD27 signaling increases the frequency of regulatory T cells and promotes tumor growth. *Cancer Res* 2012, 72: 3664–3676
96. Ward-Kavanagh LK, Lin WW, Šedý JR, Ware CF. The TNF receptor superfamily in co-stimulating and co-inhibitory responses. *Immunity* 2016, 44: 1005–1019
97. Khayyamian S, Hutloff A, Büchner K, Gräfe M, Henn V, Kroczek RA, Mages HW. ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory CD4+ T cells. *Proc Natl Acad Sci USA* 2002, 99: 6198–6203
98. Martin-Orozco N, Li Y, Wang Y, Liu S, Hwu P, Liu YJ, Dong C, *et al.* Melanoma cells express ICOS ligand to promote the activation and expansion of T-regulatory cells. *Cancer Res* 2010, 70: 9581–9590
99. Fos C, Salles A, Lang V, Carrette F, Audebert S, Pastor S, Ghiotto M, *et al.* ICOS ligation recruits the p50α PI3K regulatory subunit to the immunological synapse. *J Immunol* 2008, 181: 1969–1977
100. Chen H, Fu T, Suh WK, Tsavachidou D, Wen S, Gao J, Ng Tang D, *et al.* CD4 T cells require ICOS-mediated PI3K signaling to increase T-bet expression in the setting of anti-CTLA-4 therapy. *Cancer Immunol Res* 2014, 2: 167–176
101. Solinas C, Gu-Trantien C, Willard-Gallo K. The rationale behind targeting the ICOS-ICOS ligand costimulatory pathway in cancer immunotherapy. *ESMO Open* 2020, 5: e000544
102. Zhao X, Wang Y, Jiang X, Mo B, Wang C, Tang M, Rong Y, *et al.* Comprehensive analysis of the role of ICOS (CD278) in pan-cancer prognosis and immunotherapy. *BMC Cancer* 2023, 23: 194
103. Fan X, Quezada SA, Sepulveda MA, Sharma P, Allison JP. Engagement of the ICOS pathway markedly enhances efficacy of CTLA-4 blockade in cancer immunotherapy. *J Exp Med* 2014, 211: 715–725
104. Zhang M, Xia L, Yang Y, Liu S, Ji P, Wang S, Chen Y, *et al.* PD-1 blockade augments humoral immunity through ICOS-mediated CD4+ T cell instruction. *Int Immunopharmacol* 2019, 66: 127–138
105. Xiao Z, Mayer AT, Nobashi TW, Gambhir SS. ICOS is an indicator of T-cell–mediated response to cancer immunotherapy. *Cancer Res* 2020, 80: 3023–3032
106. Soldevilla MM, Villanueva H, Meraviglia-Crivelli D, Menon AP, Ruiz M, Cebollero J, Villalba M, *et al.* ICOS costimulation at the tumor site in combination with CTLA-4 blockade therapy elicits strong tumor immunity. *Mol Ther* 2019, 27: 1878–1891
107. Luo Y, Li H, Fang H, Gong T, Zhao Y, Cao W, Yu M, *et al.* ICOS+ CD4+ T cells promote antitumor immunity through Akt/STAT1/T-bet axis in MSS/pMMR colorectal cancer. *J Immunol* 2025, 214: 1422–1433
108. Guedan S, Posey Jr. AD, Shaw C, Wing A, Da T, Patel PR, McGettigan SE, *et al.* Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI Insight* 2018, 3: e96976
109. Herbrich S, Chaib M, Sharma P. ICOS-expressing CAR-T cells mediate durable eradication of triple-negative breast cancer and metastasis. *J Immunother Cancer* 2025, 13: e011564
110. Moreno-Cortes E, Franco-Fuquen P, Garcia-Robledo JE, Forero J, Booth N, Castro JE. ICOS and OX40 tandem co-stimulation enhances CAR T-cell cytotoxicity and promotes T-cell persistence phenotype. *Front Oncol* 2023, 13: 1200914
111. Wang Y, Zhong K, Ke J, Chen X, Chen Y, Shu W, Chen C, *et al.* Combined 4-1BB and ICOS co-stimulation improves anti-tumor efficacy and persistence of dual anti-CD19/CD20 chimeric antigen receptor T cells. *Cytotherapy* 2021, 23: 715–723
112. Sun S, Huang C, Lu M, Xu H, Yuan Y, Zhao W, Hu X, *et al.* Herpes virus entry mediator costimulation signaling enhances CAR T-cell efficacy against solid tumors through metabolic reprogramming. *Cancer Immunol Res* 2023, 11: 515–529
113. Rosado-Sánchez I, Haque M, Salim K, Speck M, Fung VCW, Boardman DA, Mojibian M, *et al.* Tregs integrate native and CAR-mediated costimulatory signals for control of allograft rejection. *JCI Insight* 2023, 8: e167215
114. 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: 1085–1095
115. Zhong Z, Zhang Q, Tan L, Guo X, Gan C. T cell co-stimulator inducible co-stimulatory (ICOS) exerts potential anti-atherosclerotic roles through downregulation of vascular smooth muscle phagocytosis and proliferation. *Ann Transl Med* 2020, 8: 1597
116. Ralser DJ, Herr E, de Vos L, Kulcsár Z, Zarbl R, Klümper N, Gielen GH, *et al.* ICOS DNA methylation regulates melanoma cell-intrinsic ICOS expression, is associated with melanoma differentiation, prognosis, and predicts response to immune checkpoint blockade. *Biomark Res* 2023, 11: 56
117. Redmond WL, Ruby CE, Weinberg AD. The role of OX40-mediated co-stimulation in T-cell activation and survival. *Crit Rev Immunol* 2009, 29: 187–201
118. Redmond WL, Gough MJ, Weinberg AD. Ligation of the OX40 co-stimulatory receptor reverses self-Ag and tumor-induced CD8 T-cell anergy *in vivo*. *Eur J Immunol* 2009, 39: 2184–2194
119. Zhang H, Li F, Cao J, Wang X, Cheng H, Qi K, Wang G, *et al.* A chimeric antigen receptor with antigen-independent OX40 signaling mediates potent antitumor activity. *Sci Transl Med* 2021, 13: eaba7308
120. Tan J, Jia Y, Zhou M, Fu C, Tuhin IJ, Ye J, Monty MA, *et al.* Chimeric antigen receptors containing the OX40 signalling domain enhance the persistence of T cells even under repeated stimulation with multiple myeloma target cells. *J Hematol Oncol* 2022, 15: 39
121. Guercio M, Orlando D, Di Cecca S, Sinibaldi M, Boffa I, Caruso S, Abbaszadeh Z, *et al.* CD28.OX40 co-stimulatory combination is associated with long in vivo persistence and high activity of CAR.CD30 T-cells. *Haematologica* 2021, 106: 987–999
122. Hombach AA, Abken H. Costimulation by chimeric antigen receptors revisited the T cell antitumor response benefits from combined CD28-

OX40 signalling. *Intl J Cancer* 2011, 129: 2935–2944
123. Hombach AA, Heiders J, Foppe M, Chmielewski M, Abken H. OX40 costimulation by a chimeric antigen receptor abrogates CD28 and IL-2 induced IL-10 secretion by redirected CD4+ T cells. *OncoImmunology* 2012, 1: 458–466
124. Wang T, Yang Y, Ma L, Feng R, Li J, Zhang C, Bai J, ***et al.*** BCMA-BBZ-OX40 CAR-T therapy using an instant manufacturing platform in multiple myeloma. *J Immunother Cancer* 2024, 12: e009476
125. Ghosh A, Marques-Piubelli ML, Wang X, Sheu TG, Cheng J, Khan K, Lu W, ***et al.*** CD2-negative lymphoma-associated T-cells: a potential mechanism of immune-evasion in diffuse large B-cell lymphoma. *Virchows Arch* 2022, 481: 659–663
126. Kalland ME, Oberprieler NG, Vang T, Taskén K, Torgersen KM. T cell-signaling network analysis reveals distinct differences between CD28 and CD2 costimulation responses in various subsets and in the MAPK pathway between resting and activated regulatory T cells. *J Immunol* 2011, 187: 5233–5245
127. Cheadle EJ, Rothwell DG, Bridgeman JS, Sheard VE, Hawkins RE, Gilham DE. Ligation of the CD2 co-stimulatory receptor enhances IL-2 production from first-generation chimeric antigen receptor T cells. *Gene Ther* 2012, 19: 1114–1120
128. Lo DJ, Weaver TA, Stempora L, Mehta AK, Ford ML, Larsen CP, Kirk AD. Selective targeting of human alloresponsive CD8+ Effector memory T cells based on CD2 expression. *Am J Transplant* 2011, 11: 22–33
129. Xiang J, Devenport JM, Carter AJ, Staser KW, Kim MY, O' Neal J, Ritchey JK, ***et al.*** An "off-the-shelf" CD2 universal CAR-T therapy for T-cell malignancies. *Leukemia* 2023, 37: 2448–2456
130. Riccardi C, Ronchetti S, Nocentini G. Glucocorticoid-induced TNFR-related gene (GITR) as a therapeutic target for immunotherapy. *Expert Opin Ther Targets* 2018, 22: 783–797
131. Buzzatti G, Dellepiane C, Del Mastro L. New emerging targets in cancer immunotherapy: the role of GITR. *ESMO Open* 2020, 4: e000738
132. Honikel MM, Olejniczak SH. Co-stimulatory receptor signaling in CAR-T cells. *Biomolecules* 2022, 12: 1303
133. Liang X, Huang Y, Li D, Yang X, Jiang L, Zhou W, Su J, ***et al.*** Distinct functions of CAR-T cells possessing a dectin-1 intracellular signaling domain. *Gene Ther* 2023, 30: 411–420
134. Yao Y, Li B, Chen C, Wang J, Yao F, Li Z. HVEM as a tumor-intrinsic regulator in non-small cell lung cancer: suppression of metastasis via glycolysis inhibition and modulation of macrophage polarization. *Pharmacol Res* 2025, 213: 107604
135. Pasero C, Olive D. Interfering with coinhibitory molecules: BTLA/HVEM as new targets to enhance anti-tumor immunity. *Immunol Lett* 2013, 151: 71–75
136. Guruprasad P, Carturan A, Zhang Y, Cho JH, Kumashie KG, Patel RP, Kim KH, ***et al.*** The BTLA–HVEM axis restricts CAR T cell efficacy in cancer. *Nat Immunol* 2024, 25: 1020–1032
137. Nunoya J, Imuta N, Masuda M. Chimeric antigen receptor T cell bearing herpes virus entry mediator co-stimulatory signal domain exhibits exhaustion-resistant properties. *Int J Mol Sci* 2024, 25: 8662
138. Nunoya J, 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
139. Yuan H, Zhu L, Yang L, Yi Y, Lv T. CD47-mediated tumor microenvironment remodeling: a central mechanism in immune evasion. *Acta Biochim Biophys Sin* 2025, 57: 1557–1573
140. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol* 2017, 17: 559–572
141. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. *Nat Rev Clin Oncol* 2018, 15: 366–381
142. Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. *Curr Opin Immunol* 2015, 33: 55–63
143. Huang Y, Kim BYS, Chan CK, Hahn SM, Weissman IL, Jiang W. Improving immune–vascular crosstalk for cancer immunotherapy. *Nat Rev Immunol* 2018, 18: 195–203
144. Akbari P, Katsarou A, Daghighian R, van Mil LWHG, Huijbers EJM, Griffioen AW, van Beijnum JR. Directing CAR T cells towards the tumor vasculature for the treatment of solid tumors. *Biochim Biophys Acta Rev Cancer* 2022, 1877: 188701
145. Ghahri-Saremi N, Akbari B, Soltantoyeh T, Hadjati J, Ghassemi S, Mirzaei HR. Genetic modification of cytokine signaling to enhance efficacy of CAR T cell therapy in solid tumors. *Front Immunol* 2021, 12: 738456
146. Moon EK, Carpenito C, Sun J, Wang LCS, Kapoor V, Predina J, Powell Jr. DJ, ***et al.*** Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin Cancer Res* 2011, 17: 4719–4730
147. Mondino A, Vella G, Icardi L. Targeting the tumor and its associated stroma: one and one can make three in adoptive T cell therapy of solid tumors. *Cytokine Growth Factor Rev* 2017, 36: 57–65
148. McKenna MK, Englisch A, Brenner B, Smith T, Hoyos V, Suzuki M, Brenner MK. Mesenchymal stromal cell delivery of oncolytic immunotherapy improves CAR-T cell antitumor activity. *Mol Ther* 2021, 29: 1808–1820
149. Liu C, Wang Q, Li L, Gao F, Zhang Y, Zhu Y. The peptide-based bispecific CAR T cells target EGFR and tumor stroma for effective cancer therapy. *Int J Pharm* 2024, 663: 124558
150. Fu R, Qi R, Xiong H, Lei X, Jiang Y, He J, Chen F, ***et al.*** Combination therapy with oncolytic virus and T cells or mRNA vaccine amplifies antitumor effects. *Sig Transduct Target Ther* 2024, 9: 118
151. Wang Y, Jia A, Bi Y, Wang Y, Yang Q, Cao Y, Li Y, ***et al.*** Targeting myeloid-derived suppressor cells in cancer immunotherapy. *Cancers* 2020, 12: 2626
152. La Fleur L, Botling J, He F, Pelicano C, Zhou C, He C, Palano G, ***et al.*** Targeting MARCO and IL37R on immunosuppressive macrophages in lung cancer blocks regulatory T cells and supports cytotoxic lymphocyte function. *Cancer Res* 2021, 81: 956–967
153. Germann M, Zangger N, Sauvain MO, Sempoux C, Bowler AD, Wirapati P, Kandalaft LE, ***et al.*** Neutrophils suppress tumor-infiltrating T cells in colon cancer via matrix metalloproteinase-mediated activation of TGF β. *EMBO Mol Med* 2020, 12: e10681
154. Liu C, Chikina M, Deshpande R, Menk AV, Wang T, Tabib T, Brunazzi EA, ***et al.*** Treg cells promote the SREBP1-dependent metabolic fitness of tumor-promoting macrophages via repression of CD8+ T cell-derived interferon-γ. *Immunity* 2019, 51: 381–397.e6
155. Cheever A, Townsend M, O'Neill K. Tumor microenvironment immunosuppression: a roadblock to CAR T-cell advancement in solid tumors. *Cells* 2022, 11: 3626
156. Farooq MA, Ajmal I, Hui X, Chen Y, Ren Y, Jiang W. β2-Adrenergic receptor mediated inhibition of T cell function and its implications for CAR-T cell therapy. *Int J Mol Sci* 2023, 24: 12837
157. Zou F, Lu L, Liu J, Xia B, Zhang W, Hu Q, Liu W, ***et al.*** Engineered triple inhibitory receptor resistance improves anti-tumor CAR-T cell performance via CD56. *Nat Commun* 2019, 10: 4109
158. Zheng S, Che X, Zhang K, Bai Y, Deng H. Potentiating CAR-T cell function in the immunosuppressive tumor microenvironment by

inverting the TGF-β signal. *Mol Ther* 2025, 33: 688–702
159. Zhu L, Liu J, Zhou G, Liu TM, Dai Y, Nie G, Zhao Q. Remodeling of tumor microenvironment by tumor-targeting nanozymes enhances immune activation of CAR T cells for combination therapy. *Small* 2021, 17: e2102624
160. Xu X, Zhang Y, Meng C, Zheng W, Wang L, Zhao C, Luo F. Nanozymes in cancer immunotherapy: metabolic disruption and therapeutic synergy. *J Mater Chem B* 2024, 12: 9111–9143
161. Yang Q, Wu Q, Liu H, Wu J, Ma F, Tian X. Tumor microenvironment–responsive and modulatory manganese-based nanoenzyme for enhanced tumor immunotherapy. *Front Pharmacol* 2024, 15: 1518983
162. Wu W, Li H, Chen W, Hu Y, Wang Z, She W, Huang L, *et al.* CAR T cell membrane camouflaged nanocatalyst augments CAR T cell therapy efficacy against solid tumor. *Small* 2024, 20: e2401299
163. Murad JP, Tilakawardane D, Park AK, Lopez LS, Young CA, Gibson J, Yamaguchi Y, *et al.* Pre-conditioning modifies the TME to enhance solid tumor CAR T cell efficacy and endogenous protective immunity. *Mol Ther* 2021, 29: 2335–2349
164. Wang X, Fan R, Mu M, Zhou L, Zou B, Tong A, Guo G. Harnessing nanoengineered CAR-T cell strategies to advance solid tumor immunotherapy. *Trends Cell Biol* 2025, 35: 782–798
165. Delfanti G, Cortesi F, Perini A, Antonini G, Azzimonti L, de Lalla C, Garavaglia C, *et al.* TCR-engineered iNKT cells induce robust antitumor response by dual targeting cancer and suppressive myeloid cells. *Sci Immunol* 2022, 7: eabn6563
166. Scharping NE, Rivadeneira DB, Menk AV, Vignali PDA, Ford BR, Rittenhouse NL, Peralta R, *et al.* Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. *Nat Immunol* 2021, 22: 205–215
167. Liu YN, Yang JF, Huang DJ, Ni HH, Zhang CX, Zhang L, He J, *et al.* Hypoxia induces mitochondrial defect that promotes T cell exhaustion in tumor microenvironment through MYC-regulated pathways. *Front Immunol* 2020, 11: 1906
168. Frezza C, Zheng L, Tennant DA, Papkovsky DB, Hedley BA, Kalna G, Watson DG, *et al.* Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. *PLoS One* 2011, 6: e24411
169. Liao Q, He H, Mao Y, Ding X, Zhang X, Xu J. Engineering T cells with hypoxia-inducible chimeric antigen receptor (HiCAR) for selective tumor killing. *Biomark Res* 2020, 8: 56
170. Hatae R, Kyewalabye K, Yamamichi A, Chen T, Phyu S, Chuntova P, Nejo T, *et al.* Enhancing CAR-T cell metabolism to overcome hypoxic conditions in the brain tumor microenvironment. *JCI Insight* 2024, 9: e177141
171. Fujiwara K, Kitaura M, Tsunei A, Kusabuka H, Ogaki E, Okada N. Structure of the signal transduction domain in second-generation CAR regulates the input efficiency of CAR signals. *Int J Mol Sci* 2021, 22: 2476
172. Gudipati V, Rydzek J, Doel-Perez I, Gonçalves VDR, Scharf L, Königsberger S, Lobner E, *et al.* Inefficient CAR-proximal signaling blunts antigen sensitivity. *Nat Immunol* 2020, 21: 848–856
173. Barden M, Elsenbroich PR, Haas V, Ertelt M, Pervan P, Velas L, Gergely B, *et al.* Integrating binding affinity and tonic signaling enables a rational CAR design for augmented T cell function. *J Immunother Cancer* 2024, 12: e010208
174. Pham-Danis C, Novak AJ, Danis E, McClellan SM, Leach L, Yarnell MC, Ebmeier CC, *et al.* Restoration of LAT activity improves CAR T cell sensitivity and persistence in response to antigen-low acute lymphoblastic leukemia. *Cancer Cell* 2025, 43: 482–502.e9
175. Wu L, Brzostek J, Sakthi Vale PD, Wei Q, Koh CKT, Ong JXH, Wu L, *et al.* CD28-CAR-T cell activation through FYN kinase signaling rather than LCK enhances therapeutic performance. *Cell Rep Med* 2023, 4: 100917
176. Drent E, Poels R, Ruiter R, van de Donk NWCJ, Zweegman S, Yuan H, de Bruijn J, *et al.* Combined CD28 and 4-1BB costimulation potentiates affinity-tuned chimeric antigen receptor–engineered T cells. *Clin Cancer Res* 2019, 25: 4014–4025
177. Hirabayashi K, Du H, Xu Y, Shou P, Zhou X, Fucá G, Landoni E, *et al.* Dual-targeting CAR-T cells with optimal co-stimulation and metabolic fitness enhance antitumor activity and prevent escape in solid tumors. *Nat Cancer* 2021, 2: 904–918
178. Ecker C, Guo L, Voicu S, Gil-de-Gómez L, Medvec A, Cortina L, Pajda J, *et al.* Differential reliance on lipid metabolism as a salvage pathway underlies functional differences of T cell subsets in poor nutrient environments. *Cell Rep* 2018, 23: 741–755
179. Li W, Chen J, Guo Z. Targeting metabolic pathway enhance CAR-T potency for solid tumor. *Int Immunopharmacol* 2024, 143: 113412
180. Jalil AT, Al-Kazzaz HH, Hassan FA, Mohammed SH, Merza MS, Aslandook T, Elewadi A, *et al.* Metabolic reprogramming of anti-cancer T cells: targeting AMPK and PPAR to optimize cancer immunotherapy. *Ind J Clin Biochem* 2025, 40: 165–175
181. Niu C, Wei H, Pan X, Wang Y, Song H, Li C, Qie J, *et al.* Foxp3 confers long-term efficacy of chimeric antigen receptor-T cells via metabolic reprogramming. *Cell Metab* 2025, 37: 1426–1441.e7
182. Chan JD, Scheffler CM, Munoz I, Sek K, Lee JN, Huang YK, Yap KM, *et al.* FOXO1 enhances CAR T cell stemness, metabolic fitness and efficacy. *Nature* 2024, 629: 201–210
183. Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang CH, Sanin DE, Qiu J, *et al.* Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* 2016, 166: 63–76
184. Lontos K, Wang Y, Joshi SK, Frisch AT, Watson MLJ, Kumar A, Menk AV, *et al.* Metabolic reprogramming via an engineered PGC-1α improves human chimeric antigen receptor T-cell therapy against solid tumors. *J Immunother Cancer* 2023, 11: e006522
185. Dumauthioz N, Tschumi B, Wenes M, Marti B, Wang H, Franco F, Li W, *et al.* Enforced PGC-1α expression promotes CD8 T cell fitness, memory formation and antitumor immunity. *Cell Mol Immunol* 2021, 18: 1761–1771
186. Borges da Silva H, Beura LK, Wang H, Hanse EA, Gore R, Scott MC, Walsh DA, *et al.* The purinergic receptor P2RX7 directs metabolic fitness of long-lived memory CD8+ T cells. *Nature* 2018, 559: 264–268
187. Li W, Zhang L. Rewiring mitochondrial metabolism for CD8+ T cell memory formation and effective cancer immunotherapy. *Front Immunol* 2020, 11: 1834
188. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, Ferris RL, *et al.* The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity* 2016, 45: 701–703
189. Frisch AT, Wang Y, Xie B, Yang A, Ford BR, Joshi S, Kedziora KM, *et al.* Redirecting glucose flux during in vitro expansion generates epigenetically and metabolically superior T cells for cancer immunotherapy. *Cell Metab* 2025, 37: 870–885.e8
190. Xu K, Yin N, Peng M, Stamatiades EG, Chhangawala S, Shyu A, Li P, *et al.* Glycolytic ATP fuels phosphoinositide 3-kinase signaling to support effector T helper 17 cell responses. *Immunity* 2021, 54: 976–987.e7
191. Renner K, Bruss C, Schnell A, Koehl G, Becker HM, Fante M, Menevse AN, *et al.* Restricting glycolysis preserves T cell effector functions and augments checkpoint therapy. *Cell Rep* 2019, 29: 135–150.e9
192. Lei J, Yang Y, Lu Z, Pan H, Fang J, Jing B, Chen Y, *et al.* Taming metabolic competition via glycolysis inhibition for safe and potent tumor immunotherapy. *Biochem Pharmacol* 2022, 202: 115153

193. Sukumar M, Gattinoni L. The short and sweet of T-cell therapy. *OncoImmunology* 2014, 3: e27573
194. Martins CP, New LA, O'Connor EC, Previte DM, Cargill KR, Tse IL, Sims-Lucas S, *et al.* Glycolysis inhibition induces functional and metabolic exhaustion of CD4+ T cells in type 1 diabetes. *Front Immunol* 2021, 12: 669456
195. Yin Z, Bai L, Li W, Zeng T, Tian H, Cui J. Targeting T cell metabolism in the tumor microenvironment: an anti-cancer therapeutic strategy. *J Exp Clin Cancer Res* 2019, 38: 403
196. Liu X, Hartman CL, Li L, Albert CJ, Si F, Gao A, Huang L, *et al.* Reprogramming lipid metabolism prevents effector T cell senescence and enhances tumor immunotherapy. *Sci Transl Med* 2021, 13: eaaz6314
197. Rumiano L, Manzo T. Lipids guide T cell antitumor immunity by shaping their metabolic and functional fitness. *Trends Endocrinol Metab* 2025, 36: 802–814
198. Xu Y, Ding L, Wu M, Wang X, Wang L, Xu Z, Xia Y, *et al.* Lipid metabolic remodeling delays senescence of T cells to potentiate their immunity against solid tumors. *J Immunother Cancer* 2025, 13: e010403
199. Hao M, Hou S, Li W, Li K, Xue L, Hu Q, Zhu L, *et al.* Combination of metabolic intervention and T cell therapy enhances solid tumor immunotherapy. *Sci Transl Med* 2020, 12: eaaz6667
200. Zhang Y, Kurupati R, Liu L, Zhou XY, Zhang G, Hudaihed A, Filisio F, *et al.* Enhancing CD8+ T Cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. *Cancer Cell* 2017, 32: 377–391.e9
201. Lou W, Gong C, Ye Z, Hu Y, Zhu M, Fang Z, Xu H. Lipid metabolic features of T cells in the tumor microenvironment. *Lipids Health Dis* 2022, 21: 94
202. Yang L, Wang X, Wang S, Shen J, Li Y, Wan S, Xiao Z, *et al.* Targeting lipid metabolism in regulatory T cells for enhancing cancer immunotherapy. *Biochim Biophys Acta Rev Cancer* 2025, 1880: 189259
203. Kim D, Wu Y, Li Q, Oh YK. Nanoparticle-mediated lipid metabolic reprogramming of T cells in tumor microenvironments for immunometabolic therapy. *Nano-Micro Lett* 2021, 13: 31
204. Chen C, Wang Z, Ding Y, Qin Y. Manipulating T-cell metabolism to enhance immunotherapy in solid tumor. *Front Immunol* 2022, 13: 1090429
205. Matés JM, Di Paola FJ, Campos-Sandoval JA, Mazurek S, Márquez J. Therapeutic targeting of glutaminolysis as an essential strategy to combat cancer. *Semin Cell Dev Biol* 2020, 98: 34–43
206. Sharma S, Agnihotri N, Kumar S. Targeting fuel pocket of cancer cell metabolism: a focus on glutaminolysis. *Biochem Pharmacol* 2022, 198: 114943
207. Valvo V, Parietti E, Deans K, Ahn SW, Park NR, Ferland B, Thompson D, *et al.* High-throughput in situ perturbation of metabolite levels in the tumor micro-environment reveals favorable metabolic condition for increased fitness of infiltrated T-cells. *Front Cell Dev Biol* 2022, 10: 1032360
208. Dong C, Zhao Y, Han Y, Li M, Wang G. Targeting glutamine metabolism crosstalk with tumor immune response. *Biochim Biophys Acta Rev Cancer* 2024, 1880: 189257
209. Wang T, Shen Y, Luyten S, Yang Y, Jiang X. Tissue-resident memory CD8+ T cells in cancer immunology and immunotherapy. *Pharmacol Res* 2020, 159: 104876
210. Okła K, Farber DL, Zou W. Tissue-resident memory T cells in tumor immunity and immunotherapy. *J Exp Med* 2021, 218: e20201605
211. Abadir E, Wayte R, Li W, Gupta S, Yang S, Reaiche E, Debosz K, *et al.* Reduced CAR T expansion post infusion is associated with poor survival in patients with large B cell lymphoma after two or more therapies. *Transplant Cell Ther* 2025
212. Meyran D, Zhu JJ, Butler J, Tantalo D, MacDonald S, Nguyen TN, Wang M, *et al.* T_{STEM}-like CAR-T cells exhibit improved persistence and tumor control compared with conventional CAR-T cells in preclinical models. *Sci Transl Med* 2023, 15: eabk1900
213. Ung S, Choochuen P, Khopanlert W, Maneechai K, Sangkhathat S, Terakura S, Julamanee J. Enrichment of T-cell proliferation and memory gene signatures of CD79A/CD40 costimulatory domain potentiates CD19 CAR-T cell functions. *Front Immunol* 2022, 13: 1064339
214. Song F, Tsahouridis O, Stucchi S, Walhart T, Mendell S, Hardy PB, Axtman M, *et al.* A multi-kinase inhibitor screen identifies inhibitors preserving stem-cell-like chimeric antigen receptor T cells. *Nat Immunol* 2025, 26: 279–293
215. Gurusamy D, Henning AN, Yamamoto TN, Yu Z, Zacharakis N, Krishna S, Kishton RJ, *et al.* Multi-phenotype CRISPR-Cas9 screen identifies p38 kinase as a target for adoptive immunotherapies. *Cancer Cell* 2020, 37: 818–833.e9
216. Wang X, Tao X, Chen P, Jiang P, Li W, Chang H, Wei C, *et al.* MEK inhibition prevents CAR-T cell exhaustion and differentiation via downregulation of c-Fos and JunB. *Sig Transduct Target Ther* 2024, 9: 293
217. Doan AE, Mueller KP, Chen AY, Rouin GT, Chen Y, Daniel B, Lattin J, *et al.* FOXO1 is a master regulator of memory programming in CAR T cells. *Nature* 2024, 629: 211–218
218. Kim MV, Ouyang W, Liao W, Zhang MQ, Li MO. The transcription factor foxo1 controls central-memory CD8+ T cell responses to infection. *Immunity* 2013, 39: 286–297
219. Lynn RC, Weber EW, Sotillo E, Gennert D, Xu P, Good Z, Anbunathan H, *et al.* c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature* 2019, 576: 293–300
220. Guo Z, Wang G, Miyahara Y, Khattar M, Linkes SP, Wang C, Xia J, *et al.* IL-7, but not thymic stromal lymphopoietin (TSLP), during priming enhances the generation of memory CD4+ T cells. *Immunol Lett* 2010, 128: 116–123
221. Nguyen R, Doubrovina E, Mousset CM, Jin BY, Okada R, Zhang X, Clavel A, *et al.* Cooperative armoring of CAR and TCR T cells by T cell–restricted IL15 and IL21 universally enhances solid tumor efficacy. *Clin Cancer Res* 2024, 30: 1555–1566
222. Snell LM, MacLeod BL, Law JC, Osokine I, Elsaesser HJ, Hezaveh K, Dickson RJ, *et al.* CD8+ T cell priming in established chronic viral infection preferentially directs differentiation of memory-like cells for sustained immunity. *Immunity* 2018, 49: 678–694.e5
223. Steffin D, Ghatwai N, Montalbano A, Rathi P, Courtney AN, Arnett AB, Fleurence J, *et al.* Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. *Nature* 2025, 637: 940–946
224. Zhao Y, Chen J, Andreatta M, Feng B, Xie YQ, Wenes M, Wang Y, *et al.* IL-10-expressing CAR T cells resist dysfunction and mediate durable clearance of solid tumors and metastases. *Nat Biotechnol* 2024, 42: 1693–1704
225. Feng B, Bai Z, Zhou X, Zhao Y, Xie YQ, Huang X, Liu Y, *et al.* The type 2 cytokine Fc–IL-4 revitalizes exhausted CD8+ T cells against cancer. *Nature* 2024, 634: 712–720
226. Hou AJ, Chang ZNL, Lorenzini MH, Zah E, Chen YY. TGF-β–responsive CAR-T cells promote anti-tumor immune function. *Bioeng Transla Med* 2018, 3: 75–86
227. Lawton ML, Inge MM, Blum BC, Smith-Mahoney EL, Bolzan D, Lin W, McConney C, *et al.* Multiomic profiling of chronically activated CD4+ T cells identifies drivers of exhaustion and metabolic reprogramming. *PLoS Biol* 2024, 22: e3002943

228. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T cell exhaustion during chronic viral infection and cancer. *Annu Rev Immunol* 2019, 37: 457–495
229. Waibl Polania J, Hoyt-Miggelbrink A, Tomaszewski WH, Wachsmuth LP, Lorrey SJ, Wilkinson DS, Lerner E, *et al.* Antigen presentation by tumor-associated macrophages drives T cells from a progenitor exhaustion state to terminal exhaustion. *Immunity* 2025, 58: 232–246.e6
230. Porazzi P, Nason S, Yang Z, Carturan A, Ghilardi G, Guruprasad P, Patel RP, *et al.* EZH1/EZH2 inhibition enhances adoptive T cell immunotherapy against multiple cancer models. *Cancer Cell* 2025, 43: 537–551.e7
231. Kailayangiri S, Altvater B, Lesch S, Balbach S, Göttlich C, Kühnemundt J, Mikesch JH, *et al.* EZH2 inhibition in ewing sarcoma upregulates GD2 expression for targeting with gene-modified T cells. *Mol Ther* 2019, 27: 933–946
232. Isshiki Y, Chen X, Teater M, Karagiannidis I, Nam H, Cai W, Meydan C, *et al.* EZH2 inhibition enhances T cell immunotherapies by inducing lymphoma immunogenicity and improving T cell function. *Cancer Cell* 2025, 43: 49–68.e9
233. Fraietta JA, Nobles CL, Sammons MA, Lundh S, Carty SA, Reich TJ, Cogdill AP, *et al.* Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature* 2018, 558: 307–312
234. Dimitri AJ, Baxter AE, Chen GM, Hopkins CR, Rouin GT, Huang H, Kong W, *et al.* TET2 regulates early and late transitions in exhausted CD8$^+$ T cell differentiation and limits CAR T cell function. *Sci Adv* 2024, 10: eadp9371
235. Kim Y, Jeun M, Lee HK, Choi JU, Park S, Park CH. TET2 downregulation enhances the antitumor efficacy of CD19 CAR T cells in a preclinical model. *Exp Hematol Oncol* 2025, 14: 23
236. Jain N, Zhao Z, Feucht J, Koche R, Iyer A, Dobrin A, Mansilla-Soto J, *et al.* TET2 guards against unchecked BATF3-induced CAR T cell expansion. *Nature* 2023, 615: 315–322
237. Zhu M, Han Y, Gu T, Wang R, Si X, Kong D, Zhao P, *et al.* Class I HDAC inhibitors enhance antitumor efficacy and persistence of CAR-T cells by activation of the Wnt pathway. *Cell Rep* 2024, 43: 114065
238. McGuire CK, Meehan AS, Couser E, Bull L, Minor AC, Kuhlmann-Hogan A, Kaech SM, *et al.* Transcriptional repression by HDAC3 mediates T cell exclusion from *Kras* mutant lung tumors. *Proc Natl Acad Sci USA* 2024, 121: e2317694121
239. Jo DH, Kaczmarek S, Khan AUH, Pervin J, Clark DM, Gadde S, Wang L, *et al.* Entinostat, a histone deacetylase inhibitor, enhances CAR-NK cell anti-tumor activity by sustaining CAR expression. *Front Immunol* 2025, 16: 1533044
240. Yan X, Chen D, Wang Y, Guo Y, Tong C, Wei J, Zhang Y, *et al.* Identification of NOXA as a pivotal regulator of resistance to CAR T-cell therapy in B-cell malignancies. *Sig Transduct Target Ther* 2022, 7: 98
241. Berrien-Elliott MM, Yuan J, Swier LE, Jackson SR, Chen CL, Donlin MJ, Teague RM. Checkpoint blockade immunotherapy relies on T-bet but not eomes to induce effector function in tumor-infiltrating CD8+ T cells. *Cancer Immunol Res* 2015, 3: 116–124
242. Khan O, Giles JR, McDonald S, Manne S, Ngiow SF, Patel KP, Werner MT, *et al.* TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. *Nature* 2019, 571: 211–218
243. Scott AC, Dündar F, Zumbo P, Chandran SS, Klebanoff CA, Shakiba M, Trivedi P, *et al.* TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* 2019, 571: 270–274
244. Alfei F, Kanev K, Hofmann M, Wu M, Ghoneim HE, Roelli P, Utzschneider DT, *et al.* TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* 2019, 571: 265–269
245. Martinez GJ, Pereira RM, Äijö T, Kim EY, Marangoni F, Pipkin ME, Togher S, *et al.* The transcription factor NFAT promotes exhaustion of activated CD8+ T cells. *Immunity* 2015, 42: 265–278
246. Bengsch B, Wherry EJ. The importance of cooperation: partnerless NFAT induces T cell exhaustion. *Immunity* 2015, 42: 203–205
247. Grusdat M, McIlwain DR, Xu HC, Pozdeev VI, Knievel J, Crome SQ, Robert-Tissot C, *et al.* IRF4 and BATF are critical for CD8+ T-cell function following infection with LCMV. *Cell Death Differ* 2014, 21: 1050–1060
248. Man K, Gabriel SS, Liao Y, Gloury R, Preston S, Henstridge DC, Pellegrini M, *et al.* Transcription factor IRF4 promotes CD8+ T cell exhaustion and limits the development of memory-like T cells during chronic infection. *Immunity* 2017, 47: 1129–1141.e5
249. Seo H, González-Avalos E, Zhang W, Ramchandani P, Yang C, Lio CWJ, Rao A, *et al.* BATF and IRF4 cooperate to counter exhaustion in tumor-infiltrating CAR T cells. *Nat Immunol* 2021, 22: 983–995
250. Nakagawara K, Ando M, Srirat T, Mise-Omata S, Hayakawa T, Ito M, Fukunaga K, *et al.* NR4A ablation improves mitochondrial fitness for long persistence in human CAR-T cells against solid tumors. *J Immunother Cancer* 2024, 12: e008665
251. Chen J, López-Moyado IF, Seo H, Lio CWJ, Hempleman LJ, Sekiya T, Yoshimura A, *et al.* NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* 2019, 567: 530–534
252. Chen X, Pan X, Zhang W, Guo H, Cheng S, He Q, Yang B, *et al.* Epigenetic strategies synergize with PD-L1/PD-1 targeted cancer immunotherapies to enhance antitumor responses. *Acta Pharm Sin B* 2020, 10: 723–733
253. Zhou X, Zhou F, Zhang L. Dual inhibition of PD-1 and LAG-3: Uncovering mechanisms to reverse T cell exhaustion and enhance anti-tumor immunity. *Sci Bull* 2025, 70: 624–626
254. Van Damme H, Dombrecht B, Kiss M, Roose H, Allen E, Van Overmeire E, Kancheva D, *et al.* Therapeutic depletion of CCR8$^+$ tumor-infiltrating regulatory T cells elicits antitumor immunity and synergizes with anti-PD-1 therapy. *J Immunother Cancer* 2021, 9: e001749
255. Pei L, Liu Y, Liu L, Gao S, Gao X, Feng Y, Sun Z, *et al.* Roles of cancer-associated fibroblasts (CAFs) in anti-PD-1/PD-L1 immunotherapy for solid cancers. *Mol Cancer* 2023, 22: 29
256. Zhang H, Liu L, Liu J, Dang P, Hu S, Yuan W, Sun Z, *et al.* Roles of tumor-associated macrophages in anti-PD-1/PD-L1 immunotherapy for solid cancers. *Mol Cancer* 2023, 22: 58
257. Budimir N, Thomas GD, Dolina JS, Salek-Ardakani S. Reversing T-cell exhaustion in cancer: lessons learned from PD-1/PD-L1 immune checkpoint blockade. *Cancer Immunol Res* 2022, 10: 146–153
258. Grosser R, Cherkassky L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. *Cancer Cell* 2019, 36: 471–482
259. Yoon D, Osborn M, Tolar J, Kim C. Incorporation of immune checkpoint blockade into chimeric antigen receptor T cells (CAR-Ts): combination or built-in CAR-T. *Int J Mol Sci* 2018, 19: 340
260. Lau E, Kwong G, Fowler TW, Sun BC, Donohoue PD, Davis RT, Bryan M, *et al.* Allogeneic chimeric antigen receptor-T cells with CRISPR-disrupted programmed death-1 checkpoint exhibit enhanced functional fitness. *Cytotherapy* 2023, 25: 750–762
261. Chen N, Morello A, Tano Z, Adusumilli PS. CAR T-cell intrinsic PD-1 checkpoint blockade: a two-in-one approach for solid tumor immunotherapy. *OncoImmunology* 2017, 6: e1273302
262. Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, Sadelain M, *et al.* Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest* 2016, 126: 3130–3144
263. Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, Yates KB, *et al.* Subsets of exhausted CD8+ T cells differentially mediate tumor

control and respond to checkpoint blockade. *Nat Immunol* 2019, 20: 326–336
264. Lin H, Yang X, Ye S, Huang L, Mu W. Antigen escape in CAR-T cell therapy: mechanisms and overcoming strategies. *Biomed Pharmacother* 2024, 178: 117252
265. Kokalaki E, Ma B, Ferrari M, Grothier T, Hazelton W, Manzoor S, Costu E, *et al.* Dual targeting of CD19 and CD22 against B-ALL using a novel high-sensitivity aCD22 CAR. *Mol Ther* 2023, 31: 2089–2104
266. George JT, Levine H. Optimal cancer evasion in a dynamic immune microenvironment generates diverse post-escape tumor antigenicity profiles. *eLife* 2023, 12: e82786
267. Roerden M, Castro AB, Cui Y, Harake N, Kim B, Dye J, Maiorino L, *et al.* Neoantigen architectures define immunogenicity and drive immune evasion of tumors with heterogenous neoantigen expression. *J Immunother Cancer* 2024, 12: e010249
268. Dai Z, Mu W, Zhao Y, Cheng J, Lin H, Ouyang K, Jia X, *et al.* T cells expressing CD5/CD7 bispecific chimeric antigen receptors with fully human heavy-chain-only domains mitigate tumor antigen escape. *Sig Transduct Target Ther* 2022, 7: 85
269. Date V, Nair S. Emerging vistas in CAR T-cell therapy: challenges and opportunities in solid tumors. *Expert Opin Biol Ther* 2021, 21: 145–160
270. Abdalsalam NMF, Ibrahim A, Saliu MA, Liu TM, Wan X, Yan D. MDSC: a new potential breakthrough in CAR-T therapy for solid tumors. *Cell Commun Signal* 2024, 22: 612
271. He W, Cui K, Farooq MA, Huang N, Zhu S, Jiang D, Zhang X, *et al.* TCR-T cell therapy for solid tumors: challenges and emerging solutions. *Front Pharmacol* 2025, 16: 1493346
272. Tsimberidou AM, Van Morris K, Vo HH, Eck S, Lin YF, Rivas JM, Andersson BS. T-cell receptor-based therapy: an innovative therapeutic approach for solid tumors. *J Hematol Oncol* 2021, 14: 102
273. Manske K, Dreßler L, Fräßle SP, Effenberger M, Tschulik C, Cletiu V, Benke E, *et al.* Miniaturized CAR knocked onto CD3ε extends TCR function with CAR specificity under control of endogenous TCR signaling cascade. *J Immunol Methods* 2024, 526: 113617
274. Li C, Zhao X, Wu C, Liu L, Zhao N. Periodic event-triggered dynamic output feedback control for networked control systems subject to packet dropouts. *ISA Trans* 2023, 140: 97–108
275. Nguyen NT, Huang K, Zeng H, Jing J, Wang R, Fang S, Chen J, *et al.* Nano-optogenetic engineering of CAR T cells for precision immunotherapy with enhanced safety. *Nat Nanotechnol* 2021, 16: 1424–1434
276. Liu L, He P, Wang Y, Ma F, Li D, Bai Z, Qu Y, *et al.* Engineering sonogenetic EchoBack-CAR T cells. *Cell* 2025, 188: 2621–2636.e20
277. Hyrenius-Wittsten A, Su Y, Park M, Garcia JM, Alavi J, Perry N, Montgomery G, *et al.* SynNotch CAR circuits enhance solid tumor recognition and promote persistent antitumor activity in mouse models. *Sci Transl Med* 2021, 13: eabd8836
278. Choe JH, Watchmaker PB, Simic MS, Gilbert RD, Li AW, Krasnow NA, Downey KM, *et al.* SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. *Sci Transl Med* 2021, 13: eabe7378
279. Luo H, Wu X, Sun R, Su J, Wang Y, Dong Y, Shi B, *et al.* Target-dependent expression of IL12 by synNotch receptor-engineered NK92 cells increases the antitumor activities of CAR-T cells. *Front Oncol* 2019, 9: 1448
280. Khan SH, Choi Y, Veena M, Lee JK, Shin DS. Advances in CAR T cell therapy: antigen selection, modifications, and current trials for solid tumors. *Front Immunol* 2024, 15: 1489827
281. Wang Z, Wang F, Zhong J, Zhu T, Zheng Y, Zhao T, Xie Q, *et al.* Using apelin-based synthetic Notch receptors to detect angiogenesis and treat solid tumors. *Nat Commun* 2020, 11: 2163
282. Smith EL, Mailankody S, Staehr M, Wang X, Senechal B, Purdon TJ, Daniyan AF, *et al.* BCMA-targeted CAR T-cell therapy plus radiotherapy for the treatment of refractory myeloma reveals potential synergy. *Cancer Immunol Res* 2019, 7: 1047–1053
283. Fang PQ, Gunther JR, Wu SY, Dabaja BS, Nastoupil LJ, Ahmed S, Neelapu SS, *et al.* Radiation and CAR T-cell therapy in lymphoma: future frontiers and potential opportunities for synergy. *Front Oncol* 2021, 11: 648655
284. Misawa K, Bhat H, Adusumilli PS, Hou Z. Combinational CAR T-cell therapy for solid tumors: requisites, rationales, and trials. *Pharmacol Ther* 2025, 266: 108763
285. Holtermann A, Gislon M, Angele M, Subklewe M, von Bergwelt-Baildon M, Lauber K, Kobold S. Prospects of synergy: local interventions and CAR T cell therapy in solid tumors. *BioDrugs* 2024, 38: 611–637
286. Hull CM, Maher J. Approaches for refining and furthering the development of CAR-based T cell therapies for solid malignancies. *Expert Opin Drug Discov* 2021, 16: 1105–1117
287. Alcantara M, Du Rusquec P, Romano E. Current clinical evidence and potential solutions to increase benefit of CAR T-cell therapy for patients with solid tumors. *OncoImmunology* 2020, 9: 1777064
288. Liu Y, Zheng Y, Deng T, Huang Y, Liu Z, Zhan B, Zhou X, *et al.* Oncolytic herpes simplex virus delivery of dual CAR targets of CD19 and BCMA as well as immunomodulators to enhance therapeutic efficacy in solid tumors combined with CAR T cell therapy. *Front Oncol* 2022, 12: 1037934
289. Sun S, Ding Z, Gao L, Hammock BD, Huang X, Xu ZP, Wang X, *et al.* A dendritic/tumor fusion cell vaccine enhances efficacy of nanobody-based CAR-T cells against solid tumor. *Theranostics* 2023, 13: 5099–5113
290. Su FY, Mac QD, Sivakumar A, Kwong GA. Interfacing biomaterials with synthetic T cell immunity. *Adv Healthcare Mater* 2021, 10: e2100157
291. Pandit S, Agarwalla P, Song F, Jansson A, Dotti G, Brudno Y. Implantable CAR T cell factories enhance solid tumor treatment. *Biomaterials* 2024, 308: 122580
292. Huang M, Deng J, Gao L, Zhou J. Innovative strategies to advance CAR T cell therapy for solid tumors. *Am J Cancer Res* 2020, 10: 1979–1992
293. Shum T, Kruse RL, Rooney CM. Strategies for enhancing adoptive T-cell immunotherapy against solid tumors using engineered cytokine signaling and other modalities. *Expert Opin Biol Ther* 2018, 18: 653–664