Cancer Biome and Targeted Therapy
ISSN: 3070-9989
www.cancerbiometherapy.com

REVIEW · OPEN ACCESS

Reprogramming CAR-T Cell Therapy for Solid Tumors: Combination Strategies to Overcome Resistance

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Received April 12, 2026
Accepted June 11, 2026
Published August 12, 2026
DOI: 10.66505/cbtt.v1i3.47

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Abstract

Chimeric antigen receptor T (CAR-T) cell therapy has achieved unprecedented clinical success in hematologic malignancies, yet translating these outcomes to solid tumors has proven substantially more challenging. Unlike blood cancers, solid tumors present multiple interconnected barriers, including antigen heterogeneity, inefficient trafficking and infiltration, physical stromal constraints, metabolic competition, and profoundly immunosuppressive tumor microenvironments that collectively limit CAR-T cell persistence and function. Increasing evidence indicates that no single engineering modification is sufficient to overcome these obstacles, driving the development of combination strategies that integrate CAR-T cells with immune checkpoint blockade, cytokine and chemokine modulation, targeted therapies, radiotherapy, oncolytic viruses, cancer vaccines, biomaterial-based delivery platforms, and next-generation synthetic biology approaches. Concurrent advances in armored CAR-T cells, logic-gated circuits, multi-antigen targeting systems, and non-viral gene engineering are enabling more precise, adaptable, and controllable cellular therapies. In this review, we examine the biological mechanisms underlying resistance to CAR-T therapy in solid tumors and critically evaluate emerging combination strategies designed to enhance tumor recognition, trafficking, persistence, and antitumor activity. We further discuss translational challenges, including manufacturing complexity, toxicity management, and patient selection, and propose a framework for developing precision combination CAR-T therapies tailored to the unique biology of individual tumors. Collectively, these advances are reshaping CAR-T cell therapy from a single-agent cellular intervention into a programmable, increasingly integrated platform for solid-tumor immunotherapy.

Keywords: Chimeric antigen receptor T cells (CAR-T); tumor microenvironment; immunotherapy; immune checkpoint blockade; multi-antigen targeting; cellular immunotherapy; synthetic biology

1. Introduction

Chimeric antigen receptors (CARs) are modular synthetic receptors composed of four principal components: (1) an extracellular antigen-binding domain, (2) a hinge region, (3) a transmembrane domain, and (4) one or more intracellular signaling domains. CAR-T cells recognize tumor-associated antigens in a major histocompatibility complex (MHC)-independent manner, thereby triggering downstream signaling cascades that drive T-cell proliferation, cytotoxic activity, and sustained antitumor responses (5).

Over the past decade, CAR-T cell therapy has emerged as a transformative modality in oncology, demonstrating remarkable clinical efficacy and durable responses, particularly in hematological malignancies. Several FDA-approved products, including axicabtagene ciloleucel (Yescarta) and tisagenlecleucel (Kymriah), have achieved substantial success in treating CD19-positive leukemias and lymphomas, establishing CAR-T cells as a paradigm of engineered cell therapy (1, 2). However, despite encouraging preclinical data and early clinical investigations, translation to solid tumors has been considerably less effective. This limitation largely reflects the intrinsic complexity of solid tumors, including antigen heterogeneity, inadequate T-cell trafficking and infiltration, a highly immunosuppressive tumor microenvironment (TME), metabolic constraints, and progressive T-cell exhaustion (5, 6), all of which collectively impair CAR-T cell function and persistence. To address these challenges, combinatorial strategies that integrate CAR-T cells with complementary therapeutic modalities have emerged as a promising approach to enhance efficacy in solid tumors (7-9).

This review focuses on combination strategies to overcome the major barriers limiting CAR-T cell efficacy in solid tumors and discusses how emerging technologies may support the rational development of next-generation therapeutic platforms (Figure 1). Here, we distinguish this review from previous CAR-T solid tumor reviews by adopting a barrier-oriented framework for combination therapy. Rather than simply cataloging individual combinatorial modalities, we organize recent advances around the key biological obstacles that limit CAR-T efficacy in solid tumors, including antigen heterogeneity, impaired trafficking and infiltration, immunosuppressive TME, metabolic dysfunction, T-cell exhaustion, and limited persistence (5, 6).

This framework emphasizes that effective combination strategies should not be viewed as isolated adjuncts but as coordinated interventions designed to restore CAR-T cell function at multiple stages of the antitumor response. Importantly, the barriers encountered by CAR-T cells in solid tumors are highly interconnected rather than independent. Antigen heterogeneity promotes immune escape, inadequate infiltration limits target engagement, and persistent immunosuppressive and metabolic pressures accelerate T-cell dysfunction. Consequently, durable clinical responses will likely require multi-layered therapeutic interventions that simultaneously address several biological barriers rather than single-mechanism solutions. In doing so, this review provides a mechanistic and translational perspective for the rational development of next-generation CAR-T combination therapies for solid tumors.

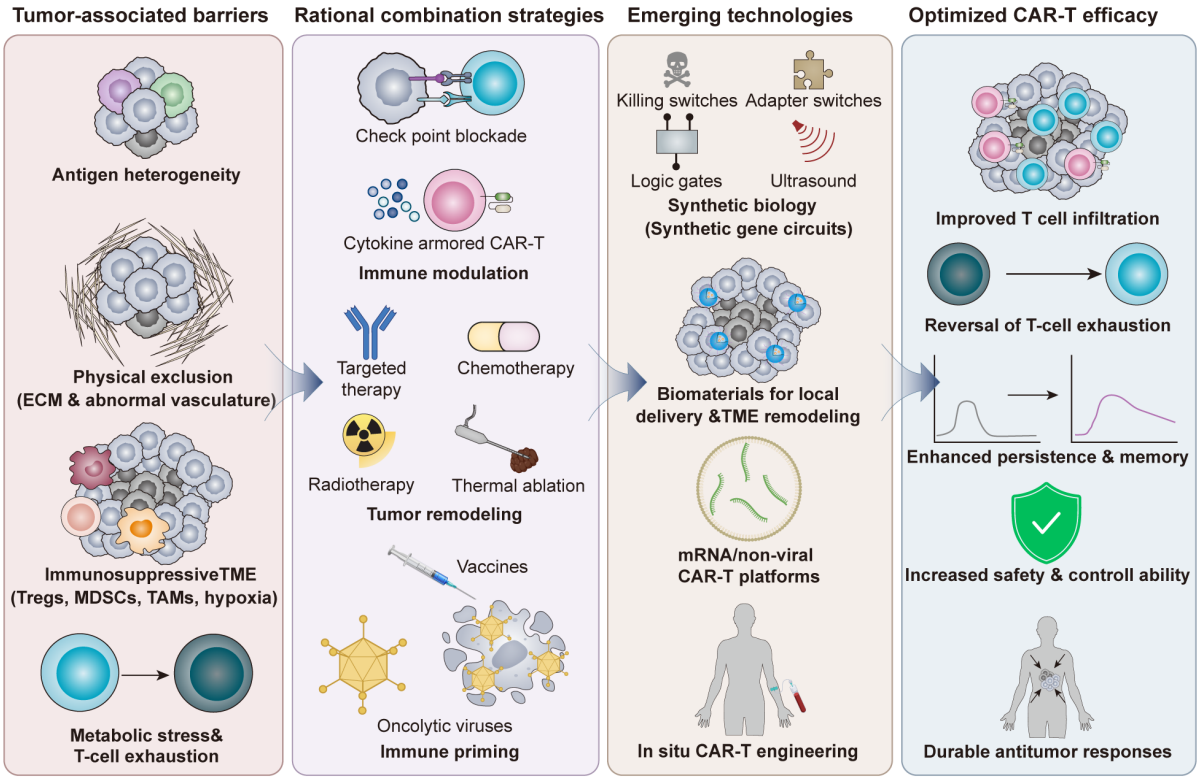


Figure 1. **Overcoming Barriers in Solid Tumors: Integrated Strategies Enhancing CAR-T Therapy.** *Major barriers to CAR-T cell therapy in solid tumors, including antigen heterogeneity, physical exclusion, an immunosuppressive tumor microenvironment (TME), and T-cell exhaustion, limit therapeutic efficacy. Rational combination strategies and emerging technologies are being developed to overcome these barriers and improve CAR-T cell infiltration, persistence, safety, and the durability of antitumor responses.*

2. Barriers to CAR-T efficacy in solid tumors

2.1 Tumor antigen heterogeneity and on-target/off-tumor toxicity

The immune system can identify and eliminate abnormal cells, maintain cellular homeostasis, and protect the organism from malignant transformation. However, cancer cells employ sophisticated mechanisms to evade immune surveillance (10), allowing uncontrolled proliferation and ultimately leading to life-threatening tumors. In tumor immune evasion, both intra-tumor heterogeneity (ITH) and spatial and temporal heterogeneity significantly limit CAR-T cell recognition and eradication of solid tumor cells (11). ITH refers to the genetic, phenotypic, and functional diversity within an individual tumor (12). For instance, immune cells may effectively target and eliminate subpopulations that express specific neoantigens, while failing to recognize other subclones that lack these antigens. Under selective pressure, immune-resistant clones expand, contributing to tumor progression and therapeutic resistance (13). In addition, spatial and temporal heterogeneity play critical roles in shaping tumor immune evasion, enabling tumor cells to adapt to heterogeneous microenvironmental and systemic conditions (11, 14).

Table 1: Clinical trials of CAR-T cell therapy (ClinicalTrials.gov)

Target Antigen	National Clinical Trials Identifier	Cancer Type	Trial Phase
HER2	NCT00924287	Metastatic cancers	I/II

CEACAM5	NCT01212887	CEACAM5-positive malignancy	I
CAIX	NCT04969354	Renal cancer	I
CLDN18.2	NCT03874897	Gastrointestinal cancers	I
ROR1	NCT05694364	CLL, MCL, ALL, DLBCL, TNBC	I
MSLN	NCT06885697	Mesothelioma	I
GD2	NCT02107963	GD2-positive solid tumors	I
B7-H3, (CD276)	NCT07231081	B7-H3/CD276-positive advanced solid tumors	I
CD70	NCT05947487	CD70-positive advanced/metastatic solid tumors	I/II
GPC3	NCT05003895	Hepatocellular carcinoma or other solid tumor malignancy	I

Abbreviations: HER2, human epidermal growth factor receptor 2; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; CAIX, carbonic anhydrase IX; CLDN18.2, claudin 18.2; ROR1, receptor tyrosine kinase-like orphan receptor 1; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; ALL, acute lymphoblastic leukemia; DLBCL, diffuse large B-cell lymphoma; TNBC, triple-negative breast cancer; MSLN, mesothelin; GD2, disialoganglioside 2; B7-H3, B7 homolog 3 (CD276); CD70, cluster of differentiation 70; GPC3, glypican-3.

This can lead to severe adverse effects known as on-target/off-tumor toxicity, as reported in several CAR-T clinical trials against solid tumors (15–17). For example, targeting HER2 has been associated with substantial toxicity, and a clinical trial involving HER2-specific CAR-T cells for colorectal cancer was terminated due to fatal pulmonary and multi-organ failure (NCT00924287) (18). Therefore, these factors collectively make CAR-T therapy more challenging to apply to solid tumors.

2.2 Poor trafficking and tumor infiltration

Effective CAR-T therapy requires efficient migration and infiltration into solid tumors. However, solid tumors impede CAR-T cell trafficking through abnormal tumor vasculature, a dense extracellular matrix (ECM), and mismatches between CAR-T cell chemokine receptors and tumor-secreted chemokines (6).

During migration, CAR-T cells initially rely on selectin-ligand interactions to mediate margination and adhesion to the vessel wall. Subsequently, chemokines activate their receptors on CAR-T cells, inducing integrin expression that enables firm adhesion to endothelial cells. Finally, intercellular adhesion molecules (ICAMs), vascular cell adhesion molecules (VCAMs), and chemokines coordinate to facilitate trans-endothelial migration (19). However, tumor vasculature poses major obstacles. Compared with normal vasculature, tumor vessels exhibit structural abnormalities and altered expression of adhesion molecules, including VCAM1 and ICAM1 (20, 21). These abnormalities cause vascular distortion and disorganized blood flow, thereby impairing CAR-T cell adhesion and extravasation (22, 23).

Additionally, the ECM further restricts infiltration. Cancer-associated fibroblasts (CAFs), abundant within the ECM, secrete large amounts of matrix components, including collagen, fibronectin, hyaluronan, glycoproteins, proteoglycans, and tenascin C (24). These components accumulate and become extensively cross-linked by lysyl oxidases (LOXs) and LOX-like proteins (LOXLs), forming a dense fibrotic barrier (25). These physical and biochemical barriers significantly hinder CAR-T cell trafficking and infiltration.

Another key limitation is chemokine-receptor mismatch. Chemokines are essential mediators of T-cell trafficking, with receptors such as CXCR3, CCR5, CCR2, and CCR6 playing critical roles (26, 27). However, solid tumors often secrete insufficient levels of T-cell-recruiting chemokines such as CCL5 (28, 29). Moreover, CAR-T cells derived from peripheral blood frequently lack expression of the corresponding receptors (30), including CCR2 and CXCR4. This mismatch between tumor-derived chemokines and CAR-T cell receptor profiles represents a major barrier to effective tumor homing (31). Furthermore, variability in chemokine expression across tumor types limits the universal adaptability of CAR-T cells.

2.3 Immunosuppressive tumor microenvironment

Beyond physical barriers, the TME exerts strong immunosuppressive effects. It contains multiple immunosuppressive immune cell populations and a range of cytokines and chemokines that drive immune cells toward an immunosuppressive phenotype. Regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) are the principal immunosuppressive components within the TME (22).

Previous studies have shown that FOXP3-expressing Tregs constitute a major immunosuppressive population in tumors (32, 33). Compared with Tregs in normal tissues, tumor-associated Tregs display enhanced proliferation and activation, expressing high levels of CTLA4, TIGIT, CD25, CCR4, and CCR8 (34, 35). Highly suppressive Treg subsets are further characterized by transcription factors such as BATF and IRF4 (36, 37), as well as co-stimulatory receptors including OX40 and GITR (38). Tregs suppress CAR-T cell function through multiple mechanisms. First, they secrete inhibitory cytokines such as IL-10, TGF-β, and IL-35, which suppress effector T-cell activity (39-41). They also express inhibitory receptors, including TIGIT, LAG-3, and PD-1, thereby reinforcing immunosuppression (42–45). Second, Tregs engage in metabolic competition by consuming essential nutrients. For example, CD39/CD73 expression contributes to an immunosuppressive metabolic environment (46), while high CD25 expression reduces IL-2 levels, a key cytokine for T-cell activation and proliferation (47, 48). Additionally, Tregs promote tryptophan metabolism via IDO, producing immunosuppressive kynurenine (49). They can also suppress effector T cells through CTLA-4-mediated ligand competition (50) and direct cytotoxicity via perforin and granzyme B (51).

TAMs and MDSCs further reinforce immunosuppression. They promote Treg recruitment via chemokines such as CCL17, CCL22, and CCL8 (52) and secrete inhibitory cytokines (e.g., TGF-β, IL-10) while expressing immune checkpoint ligands such as PD-L1, B7-H4, and VISTA (53, 54). Moreover, TAMs contribute to vascular abnormalities (55), whereas MDSCs induce metabolic stress through arginine depletion and oxidative stress (54, 56). Tumor-associated hypoxia and metabolic byproducts further exacerbate immunosuppression and impair T-cell function (57).

2.4 CAR-T cell exhaustion and metabolic dysregulation

CAR-T cell exhaustion is a major factor limiting therapeutic efficacy in solid tumors. It is characterized by reduced proliferation, cytokine production, and cytotoxicity, driven by chronic antigen exposure and immunosuppressive signaling (58).

Elevated collagen in the ECM contributes to T-cell exhaustion via LAIR1 signaling, which is activated by CD18-collagen interactions and mediates inhibitory signaling via SHP-1 (59). Persistent antigen stimulation also upregulates inhibitory receptors such as PD-1, CTLA-4, TIM-3, and LAG-3. Both intrinsic and extrinsic signals contribute to this process. Extrinsic suppression arises from inhibitory cytokines produced by Tregs and MDSCs, including IL-10 and TGF-β (60). Furthermore, the

metabolically hostile TME impairs T-cell bioenergetics. Abnormal tumor vasculature disrupts oxygen delivery, and rapid tumor growth leads to hypoxia (61). Stabilization of HIF-1α enhances VEGF and adenosine production, suppressing T-cell proliferation and cytotoxicity (62, 63).

Tumor cells also compete with T cells for essential nutrients, including glucose, glutamine, and arginine, while MDSCs and TAMs exacerbate nutrient depletion through arginase-1 (64). Additionally, tumor-derived lactate accumulation acidifies the TME, disrupts oxidative phosphorylation, reduces IFN-γ secretion, and promotes the recruitment of immunosuppressive cells (65). Together, these factors drive metabolic dysfunction and accelerate CAR-T cell exhaustion.

2.5 Limited persistence and memory formation

Limited CAR-T cell persistence is a major contributor to weak or transient therapeutic responses in solid tumors (66). In current applications, short-lived effector CAR-T cells predominate, whereas long-term persistence and memory formation remain insufficient.

Co-stimulatory domains shape differentiation outcomes. Second-generation CARs containing CD28 favor effector memory T cells, whereas those with 4-1BB promote central memory T cells with enhanced persistence (67–69). Mitochondrial fitness is a key determinant of T-cell persistence. Effector memory T cells rely on glycolysis, whereas central memory T cells depend on oxidative phosphorylation, which supports long-term survival and reactivation (70). Within the TME, tumor cells exhibit high rates of glycolysis, consuming large amounts of glucose and producing lactate, thereby inducing metabolic stress in CAR-T cells.

This altered metabolic environment, together with cytokine signaling, disrupts mitochondrial oxidative phosphorylation by inhibiting PDH activity and impairing CAR-T cell proliferation and survival. Signaling pathways, such as HIF-1α, further regulate the balance between mTORC1 and AMPK, thereby affecting cellular metabolism, growth, and autophagy (65, 71). Additionally, elevated cytokine levels, including IL-2, can drive CAR-T cells toward terminal effector differentiation, thereby reducing long-term persistence (72). Overall, metabolic dysregulation and cytokine signaling favor effector phenotypes over memory differentiation, limiting sustained antitumor immunity.

Collectively, these barriers form an integrated network of resistance rather than discrete obstacles. For example, poor infiltration limits tumor access, prolongs ineffective antigen exposure, and contributes to exhaustion, while immunosuppressive and metabolically hostile microenvironments further impair persistence and memory formation. This biological interdependence helps explain why single-agent CAR-T strategies have generally shown limited efficacy in solid tumors and supports combination approaches that can simultaneously target multiple resistance mechanisms.

3. Strategies of combination therapy

3.1 CAR-T plus immune checkpoint blockade

Because the major barriers limiting CAR-T activity in solid tumors stem from multiple interconnected biological processes, combinatorial approaches have emerged as a rational strategy to simultaneously enhance trafficking, overcome immunosuppression, prevent exhaustion, and improve long-term persistence (73).

Representative ongoing and completed clinical trials evaluating CAR-T combination strategies in solid tumors are summarized in Table 2.

Table 2: Clinical trials of CAR-T combination therapies in solid tumors (ClinicalTrials.gov)

Target Antigen	National Clinical Trials Identifier	Combination Therapy	Combination Type	Cancer Type
Mesothelin	NCT02414269	Pembrolizumab; cyclophosphamide	PD-1 antibody; lymphodepletion	Malignant pleural mesothelioma, lung cancer, breast cancer
EGFRvIII	NCT03726515	Pembrolizumab	PD-1 antibody	EGFRvIII-positive, MGMT-unmethylated glioblastoma (GBM)
IL13Rα2	NCT04003649	Nivolumab ± ipilimumab	Immune checkpoint blockade (PD-1 and CTLA-4 antibodies)	Recurrent/refractory glioblastoma (GBM)
PSMA	NCT03089203	Dominant-negative TGF-β receptor (TGFβRDN)	Armored CAR-T cells resistant to TGF-β signaling	Metastatic castration-resistant prostate cancer (mCRPC)
GPC3	NCT05103631; NCT04377932	IL-15	IL-15-armored GPC3-targeted CAR-T cells	Hepatocellular carcinoma (HCC) and pediatric solid tumors
EGFR	NCT03182816	CTLA-4 and PD-1 antibodies	Checkpoint inhibitor-secreting CAR-T cells	EGFR-positive advanced solid tumors
EGFRvIII	NCT05660369	T-cell-engaging antibody molecules (TEAMs)	TEAM-secreting CAR-T cells	Glioblastoma (GBM)
GD2	NCT01822652	Pembrolizumab	PD-1 blockade in iCasp9-engineered GD2 CAR-T cells	Neuroblastoma
HER2	NCT00902044	Cyclophosphamide, fludarabine	Lymphodepletion	Advanced sarcoma
Mesothelin	NCT02159716	Cyclophosphamide	Lymphodepletion	Epithelial ovarian cancer, malignant pleural mesothelioma, metastatic pancreatic ductal adenocarcinoma
PSCA	NCT05805371	Radiotherapy	Radiotherapy	Prostate cancer
CLDN6	NCT04503278	CARVac	CAR-T cell-amplifying RNA vaccine	CLDN6-positive solid tumors
Mesothelin	NCT01897415	In situ CAR-T	SS1 CAR-transfected autologous T cells	Metastatic pancreatic ductal adenocarcinoma (mPDAC)

Abbreviations: EGFRvIII, epidermal growth factor receptor variant III; MGMT, O6-methylguanine-DNA methyltransferase; GBM, glioblastoma; IL13Rα2, interleukin-13 receptor alpha 2; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PSMA, prostate-specific membrane antigen; TGF-β, transforming growth factor-beta; mCRPC, metastatic castration-resistant prostate cancer; GPC3, glypican-3; IL-15, interleukin-15; HCC, hepatocellular carcinoma; TEAMs, T-cell-engaging antibody molecules; GD2, disialoganglioside 2; PSCA, prostate stem cell antigen; CARVac, CAR-T cell-amplifying RNA vaccine; CLDN6, claudin 6; mPDAC, metastatic pancreatic ductal adenocarcinoma.

As discussed above, inhibitory receptors such as PD-1, CTLA-4, TIM-3, and LAG-3 play critical roles in T-cell dysfunction (74–77). Accordingly, blockade of the PD-1/PD-L1 and CTLA-4/B7 axes has become a well-established approach and is widely used in clinical practice (78, 79). Compared with CTLA-4 blockade, inhibition of the PD-1/PD-L1 axis is generally associated with a more favorable safety profile (80).

Accumulating evidence indicates that combining ICIs with CAR-T cell therapy yields synergistic antitumor effects (81, 82). In a phase II clinical trial of 44 patients with relapsed/refractory diffuse large B-cell lymphoma (R/R DLBCL), those receiving PD-1 inhibitor maintenance therapy after CAR-T infusion had significantly improved progression-free survival (PFS) (47.26% vs. 26.67%, $P = 0.0348$) and overall survival (OS) (65.06% vs. 39.29%, $P = 0.0227$) compared with CAR-T monotherapy. Importantly, no increase in cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) was observed (83).

Similarly, emerging studies in solid tumors have highlighted the potential of this combination strategy. In a clinical study of malignant pleural mesothelioma, CAR-T monotherapy achieved a median survival of 17.7 months and a 1-year survival rate of 74%. Notably, patients receiving regional delivery of mesothelin-targeted CAR-T cells in combination with pembrolizumab showed improved outcomes, with a median survival of 23.9 months and a 1-year survival rate of 83% (84).

Mechanistically, ICIs enhance CAR-T cell persistence and function within the TME by alleviating inhibitory signaling, particularly via the PD-1/PD-L1 pathway, thereby reducing T-cell exhaustion and improving durability (85). Beyond restoring effector function, checkpoint blockade may also reshape CAR-T cell differentiation by preserving progenitor-like populations with greater proliferative capacity and long-term persistence. Emerging evidence suggests that maintaining these stem-like states may be particularly important for durable responses in solid tumors, where chronic antigen exposure continuously drives exhaustion programs.

However, therapeutic efficacy depends on several factors. The TME varies substantially across tumor types, and most solid tumors are considered “cold,” which may limit ICIs’ ability to promote CAR-T cell homing and accumulation at tumor sites. In addition, tumor heterogeneity may compromise the efficacy of combination therapy. For instance, PD-1 inhibitor maintenance therapy may have limited benefit when the PD-1/PD-L1 axis is not the dominant inhibitory pathway. Furthermore, a major limitation of early-generation CARs is irreversible CAR-T cell exhaustion. Because ICIs transiently block receptor-ligand interactions, they may not fully reverse an established exhausted state. Lastly, the overall impact of combining CAR-T cells with ICIs on tumor progression or regression is strongly influenced by the timing and route of administration.

3.2 CAR-T plus cytokine or costimulatory modulation

Fourth-generation CAR-T cells, also known as armored CAR-T cells, enhance antitumor activity by engineering the expression of cytokines or costimulatory molecules (86). By encoding cytokines, chemokines, or pro-inflammatory ligands (87), armored CAR-T cells can actively remodel the TME and recruit endogenous immune responses. Unlike systemic combination therapies that rely on external administration of immunomodulatory agents, armored CAR-T cells represent a cell-intrinsic combination strategy that delivers therapeutic payloads directly to tumor sites. This localized approach may improve the therapeutic index by concentrating immune activation within the tumor while minimizing systemic exposure.

Armored CAR-T cells are broadly classified into three categories: redirected universal cytokine killer (TRUCK) CAR-T cells, cytokine-regulatory CAR-T cells, and antibody-secreting CAR-T cells. TRUCK CAR-T cells secrete cytokines such as IL-12, IL-18, or IL-15 to enhance proliferation, persistence, and antitumor activity (88, 89). Cytokine-regulatory CAR-T cells are engineered to improve tumor trafficking and infiltration (90). Antibody-secreting CAR-T cells further enhance efficacy by releasing monoclonal antibodies, scFvs, or bispecific T-cell engagers (BiTEs) (91).

Several studies have demonstrated the therapeutic potential of armored CAR-T cells in solid tumors. In a phase I clinical trial (NCT03089203), PSMA-targeted, TGF-β-insensitive armored CAR-T cells showed an acceptable safety profile in patients with metastatic castration-resistant prostate cancer (mCRPC). CRS was observed, and PSA levels decreased by ≥30% in several patients (92). Similarly, IL-15-secreting CAR-T cells have demonstrated enhanced expansion and cytotoxicity. In multiple phase I trials (NCT02905188, NCT02932956, NCT05103631, NCT04377932), IL-15 co-expression significantly improved *the in vivo* efficacy of GPC3 CAR-T cells, achieving a disease control rate of 66% and an objective response rate of 33% (93). Furthermore, EGFRvIII-targeted CAR-T cells engineered to secrete T-cell-engaging antibody molecules (TEAMs) have shown promising early results, with sustained remissions in some patients (94).

Despite these advances, the clinical application of armored CAR-T cells is limited by increased toxicity, particularly a higher incidence of CRS. Compared with the remarkable achievements in hematologic malignancies, most studies of armored CAR-T cells for solid tumors remain in phase I/II clinical trials. A major challenge is the trade-off between antitumor potency and systemic toxicity. For example, an ongoing phase I clinical trial is evaluating the safety of different dose levels of IL-12-armored CAR-T cells in patients with recurrent MUC16-positive solid tumors. The study reported CRS at all dose levels, requiring clinical management, highlighting the potential risk of severe or even life-threatening toxicity (NCT02498912). Taken together, current strategies should focus on restricting transgene expression to tumor sites using synthetic promoters or inducible gene circuits, which will be essential for successful clinical translation.

3.3 CAR-T plus targeted therapy or chemotherapy

Combining CAR-T cell therapy with targeted agents can enhance tumor cell apoptosis through synergistic mechanisms. In addition to immune checkpoint blockade (75, 77, 84, 95, 96), kinase inhibitors (KIs) are another promising class of agents that modulate T-cell activation and reduce inhibitory signaling (97). Many KIs target aberrantly activated pathways, including the MAPK pathway (e.g., avutometinib and mirdametinib) and receptor tyrosine kinase (RTK) signaling (98). Notably, MEK1/2 inhibitors have been shown to attenuate CAR-T cell exhaustion and terminal differentiation induced by tonic signaling and antigen stimulation, thereby enhancing antitumor efficacy (99). Furthermore, specific KIs (e.g., UNC10225387B, UNC10225263A, UNC10112761A) have been reported to enrich for $CD45RA^{+}CCR7^{+}TCF1^{hi}$ T memory stem cells (T_{SCM})-like CAR-T cells and promote T-cell expansion (100). These findings suggest that kinase inhibition can improve CAR-T cell persistence and functionality.

Importantly, targeted therapies may serve functions beyond direct tumor cytotoxicity. Increasing evidence indicates that many targeted agents can remodel the tumor ecosystem by altering cytokine production, antigen presentation, stromal interactions, and immune cell recruitment. Consequently, their greatest value in CAR-T combinations may arise from their ability to condition tumors into more permissive immune environments rather than from direct antitumor effects alone.

Lymphodepleting chemotherapy remains a widely adopted strategy to facilitate CAR-T cell engraftment, expansion, and persistence (101). Standard regimens, typically including fludarabine and cyclophosphamide, deplete suppressive immune populations such as Tregs and MDSCs, disrupt tumor-associated barriers, and modulate chemokine signaling to enhance CAR-T cell trafficking (102). Clinical studies have demonstrated improved CAR T-cell expansion and tumor infiltration following lymphodepletion (103, 104). Beyond conventional chemotherapy, recent studies have highlighted the potential of epigenetic modulation to enhance CAR-T cell function. Wang et al. showed that CAR-T cells treated with low-dose decitabine (5-aza-2-deoxycytidine, DAC) exhibit enhanced antitumor activity, proliferation, and cytokine secretion.

Mechanistically, DAC induces DNA hypomethylation-mediated reprogramming, leading to higher expression of memory-associated genes and lower expression of exhaustion-associated genes under both *in vitro* and *in vivo* tumor stimulation conditions (105). These findings demonstrate that methylation inhibition can alleviate CAR-T cell exhaustion while promoting maintenance of the memory phenotype and effector function, thereby improving therapeutic durability.

However, chemotherapy may also have detrimental effects on CAR-T cells, including direct cytotoxicity, alterations in T-cell subsets, and increased expression of immune checkpoint molecules (106). Therefore, optimizing treatment regimens and integrating strategies such as targeted signaling modulation or epigenetic reprogramming will be critical to maximize therapeutic efficacy while minimizing adverse effects.

3.4 CAR-T plus radiotherapy or thermal ablation

Radiotherapy, a widely used cancer treatment, exerts its antitumor effects primarily by inducing DNA damage, thereby impairing tumor cell proliferation and survival (107). Beyond direct cytotoxicity, radiotherapy can enhance antitumor immunity by recruiting and activating pro-inflammatory immune cells within the irradiated tumor microenvironment, thereby potentiating the efficacy of CAR-T cell therapy (108, 109). Clinical evidence supports combining radiotherapy with CAR-T therapy. A comparative study in patients with relapsed/refractory diffuse large B-cell lymphoma found that bridging radiotherapy (BRT) significantly improved progression-free survival (PFS) and overall survival (OS) compared with CAR-T monotherapy ($P = 0.001$, $P = 0.043$), without increasing the incidence of CAR-T-related toxicities during a median follow-up of 35.27 months (110). Similarly, accumulating preclinical and clinical studies indicate that radiotherapy can improve therapeutic outcomes in solid tumors when combined with CAR-T cells.

Mechanistically, radiotherapy enhances CAR-T efficacy through multiple pathways. Radiotherapy may be particularly attractive because it addresses several resistance mechanisms simultaneously. In addition to promoting antigen release and inflammatory signaling, irradiation can transiently normalize tumor vasculature, increase chemokine production, enhance antigen presentation, and facilitate immune cell trafficking. Such pleiotropic effects position radiotherapy as a potentially powerful platform for converting immunologically cold tumors into CAR-T-permissive environments. Ultra-high-dose-rate FLASH radiotherapy has been shown to reprogram macrophage lipid metabolism, thereby reversing tumor-associated immunosuppression (111). Additionally, low-dose irradiation can sensitize antigen-negative tumor cells to TRAIL-mediated apoptosis induced by CAR-T cells, effectively overcoming antigen heterogeneity and reducing tumor relapse (112). Consistently, Quach et al. (113) demonstrated that tumor-targeted radiation administered prior to CAR-T infusion significantly enhances therapeutic efficacy in solid tumors.

These findings collectively support the potential of radiotherapy to overcome key barriers, including antigen heterogeneity and an immunosuppressive TME. Radiotherapy is often used as a bridging therapy while awaiting CAR-T cell manufacturing and infusion. However, despite these promising findings, most current evidence on the combination of CAR-T cells and radiotherapy in solid tumors is limited to case reports, and no standardized dosing protocols have been established. Furthermore, patient-specific factors, irradiation fields, and treatment timing can significantly influence therapeutic outcomes. These limitations highlight the need for further investigation into the interplay between radiotherapy dose and timing, potential toxicities, and clinical outcomes.

In parallel, minimally invasive ablative approaches, including radiofrequency (RF) or microwave ablation (MWA) and cryoablation, have emerged as effective strategies for solid tumor management. Among these, MWA has been widely used for its ability to induce rapid tumor necrosis via localized thermal effects (114, 115). Notably, emerging evidence suggests that MWA can also modulate the tumor microenvironment to enhance CAR-T cell efficacy. In our work, we demonstrated that MWA promoted the polarization of recruited macrophages toward an M1 phenotype and enhanced their phagocytic function. When combined with CV1-secreting sCAR-T cells, which block the CD47-SIRPα "don't eat me" signal, this strategy further strengthened macrophage-mediated tumor clearance.

Together, these effects amplified antitumor immunity and induced a macrophage-dependent abscopal response against distant tumors with antigenic heterogeneity (8). In addition, combining MWA with AXL-targeted CAR-T cells in non-small cell lung cancer (NSCLC) improved CAR-T cell efficacy by remodeling the TME, alleviating hypoxia, enhancing mitochondrial oxidative metabolism, and promoting differentiation toward a central memory phenotype (9).

Overall, these combination strategies facilitate tumor antigen release, enhance local inflammation, and remodel the TME, thereby promoting antigen spreading, T-cell priming, and improved infiltration into tumor sites (116, 117). Collectively, radiotherapy and ablative therapies are promising approaches to overcome multiple barriers to CAR-T therapy in solid tumors.

3.5 CAR-T plus vaccines or oncolytic viruses

Vaccine-boosted CAR-T therapy has emerged as a promising strategy to enhance CAR-T cell function, particularly to address key limitations in solid tumors, including antigen heterogeneity, insufficient activation, and limited persistence. Tumor vaccines can be broadly categorized as whole-cell, genetically engineered, peptide/protein-based, or dendritic cell (DC) vaccines (118). Conceptually, vaccine-enhanced CAR-T therapy can be divided into two main approaches: those that leverage the host immune system to convert an immunosuppressive TME into an immunostimulatory one (119) and those that directly stimulate CAR-T cells through antigen-specific engagement (120).

Recent studies have demonstrated the potential of vaccine-mediated CAR-T activation. Ma et al. developed amphiphile CAR-T ligands (amph-ligands) that traffic to lymph nodes and decorate antigen-presenting cells, thereby priming CAR-T cells within the native lymphoid microenvironment (121). In a phase I/II clinical trial, CLDN6-targeted CAR-T cells, combined with CARVac-mediated *in vivo* expansion, showed a favorable safety profile and encouraging clinical activity in patients with $CLDN6^+$ advanced solid tumors (122). Peptide-based vaccines offer an additional strategy to overcome tumor antigen heterogeneity by enhancing CAR-specific immune activation and sustaining CAR-T cytotoxicity. Preclinical studies have shown that peptide vaccination (e.g., CMVpp65) significantly promotes CAR-T cell proliferation and augments antitumor responses *in vivo* (123). Similarly, dendritic cell-based vaccines further enhance CAR-T efficacy by presenting tumor antigens and facilitating

efficient T-cell priming and activation (124). Despite these encouraging findings, the clinical translation of vaccine-enhanced CAR-T therapy requires careful optimization of antigen selection, vaccine platforms, dosing regimens, and safety considerations.

Oncolytic virotherapy (OV) is another complementary strategy that exerts antitumor effects through selective tumor cell lysis and the induction of systemic antitumor immunity (125, 126). To date, several OVs have been approved for clinical use, including Rigvir (SND005), Oncorine (H101), Imlygic (Talimogene laherparepvec, T-VEC), and Delytact (teserpaturev/G47Δ) (127–130). Recent advances in OV engineering have further strengthened the synergy between OV and CAR-T therapy. For instance, Chen et al. developed a tumor-specific vaccinia virus (TTVΔTK-IL21) expressing IL-21, which demonstrated superior antitumor efficacy when combined with CAR-T therapy compared with monotherapy (131). In addition, Caroline et al. engineered a multifunctional OV (CAdTrio) that simultaneously delivers a CD44v6-targeting BiTE, IL-12, and a PD-L1-blocking antibody, thereby enhancing HER2 CAR-T cytotoxicity *in vitro* (132).

From a clinical maturity perspective, studies combining CAR-T cells with cancer vaccines remain largely confined in phase I/II clinical trials. To date, no CAR-T plus cancer vaccine combination regimen has been approved for commercial use, partly due to challenges posed by tumor heterogeneity. Similarly, studies combining CAR-T cells with oncolytic viruses (OVs) are mainly limited to preclinical animal models and early-phase clinical trials. Several barriers remain, including the dense extracellular matrix, which impedes intratumoral OV penetration, and the difficulty of in situ delivery to multiple metastatic lesions.

In terms of safety, CAR-T cells combined with cancer vaccines may cause local inflammation at the injection site and low-grade fever. In contrast, CAR-T cells combined with OVs may carry a higher risk of severe CRS and systemic inflammation due to their potent immune-activating mechanisms. Overall, cancer vaccines and OVs can enhance CAR-T therapy by promoting antigen presentation, amplifying immune activation, and remodeling the tumor microenvironment.

These strategies may help overcome key barriers, including antigen heterogeneity, insufficient immune priming, and immunosuppression, thereby improving CAR-T cell persistence and therapeutic efficacy. From a mechanistic perspective, vaccines and oncolytic viruses address a limitation that extends beyond CAR-T cells themselves: the absence of robust endogenous antitumor immunity. By promoting antigen spreading and broadening immune recognition beyond the CAR-targeted antigen, these approaches may reduce the risk of antigen-loss escape and generate more durable systemic immune surveillance. Nevertheless, future studies should focus on optimizing vector design, delivery strategies, and tumor-specific targeting to maximize therapeutic benefit while minimizing off-target effects.

3.6 Multi-antigen targeting strategies

Antigen heterogeneity remains a major barrier to CAR-T cell efficacy in solid tumors. Because many solid tumor antigens are heterogeneously expressed and may also be shared with normal tissues, single-antigen CAR-T strategies can promote antigen-negative immune escape or increase the risk of on-target/off-tumor toxicity (5, 6, 11, 14). Accordingly, multi-antigen targeting has emerged as a rational strategy to broaden tumor recognition while improving therapeutic specificity.

Several engineering approaches have been developed to address this challenge. Tandem CARs incorporate two antigen-binding domains within a single CAR construct, allowing a CAR-T cell to recognize either of two tumor antigens. Bicistronic CAR-T cells express two independent CAR molecules from the same vector, enabling parallel recognition of distinct tumor-associated antigens.

More recently, trivalent CAR designs have extended this principle by targeting three antigens simultaneously, aiming to reduce antigen-loss relapse in highly heterogeneous tumors. These multi-targeted formats may improve tumor coverage, but their clinical translation requires careful optimization of antigen selection, receptor affinity, signaling strength, and safety.

Logic-gated CAR-T systems provide an additional layer of specificity. “AND-gate” designs require recognition of two antigens before full T-cell activation, thereby reducing the likelihood of attacking normal tissues that express only one target antigen. One representative approach integrates synNotch receptors with CAR constructs, in which engagement of a priming antigen induces CAR expression against a second antigen. This design enables spatially restricted CAR-T activation within tumors expressing the appropriate antigen combination and may reduce on-target/off-tumor toxicity (133, 134).

Conversely, “OR-gate” strategies permit activation through either of two antigens and may be particularly useful for tumors with heterogeneous or unstable antigen expression. “NOT-gate” systems, although less clinically mature, are designed to suppress CAR-T activation in the presence of antigens expressed by normal tissues, thereby improving discrimination between malignant and healthy cells.

Adapter and universal CAR platforms offer another strategy for flexible antigen recognition. Rather than permanently encoding specificity for a single tumor antigen, these systems pair a universal T-cell receptor with externally administered adaptor molecules that bridge CAR-T cells to tumor antigens. By changing the adaptor, the same CAR-T cell product can, in theory, be redirected to different targets. This modular design may enable titratable control of CAR-T activity, sequential targeting of multiple antigens, and rapid interruption of treatment in the event of toxicity. Small-molecule–regulated ON-switch systems further support this concept by enabling reversible, dose-dependent control of CAR-T activity.

Overall, multi-antigen and adaptive recognition platforms shift CAR-T therapy from static, single-target recognition to programmable tumor sensing. These strategies directly address antigen heterogeneity, antigen-loss escape, and on-target/off-tumor toxicity, making them highly relevant to solid tumors. Future studies should determine which multi-antigen architectures provide the optimal balance among breadth of tumor recognition, functional persistence, manufacturing feasibility, and clinical safety. Collectively, these combination approaches reveal several emerging design principles for successful CAR-T therapy in solid tumors.

First, interventions that simultaneously address multiple resistance mechanisms generally outperform strategies targeting a single barrier.

Second, localized or inducible therapeutic activity appears superior to systemic immune activation because it may improve efficacy while limiting toxicity.

Third, durable responses increasingly depend on preserving stem-like and memory-associated CAR-T states rather than maximizing short-term effector activity. These principles suggest that future CAR-T platforms will likely integrate multiple complementary functions within a single programmable therapeutic system (Figure 2).

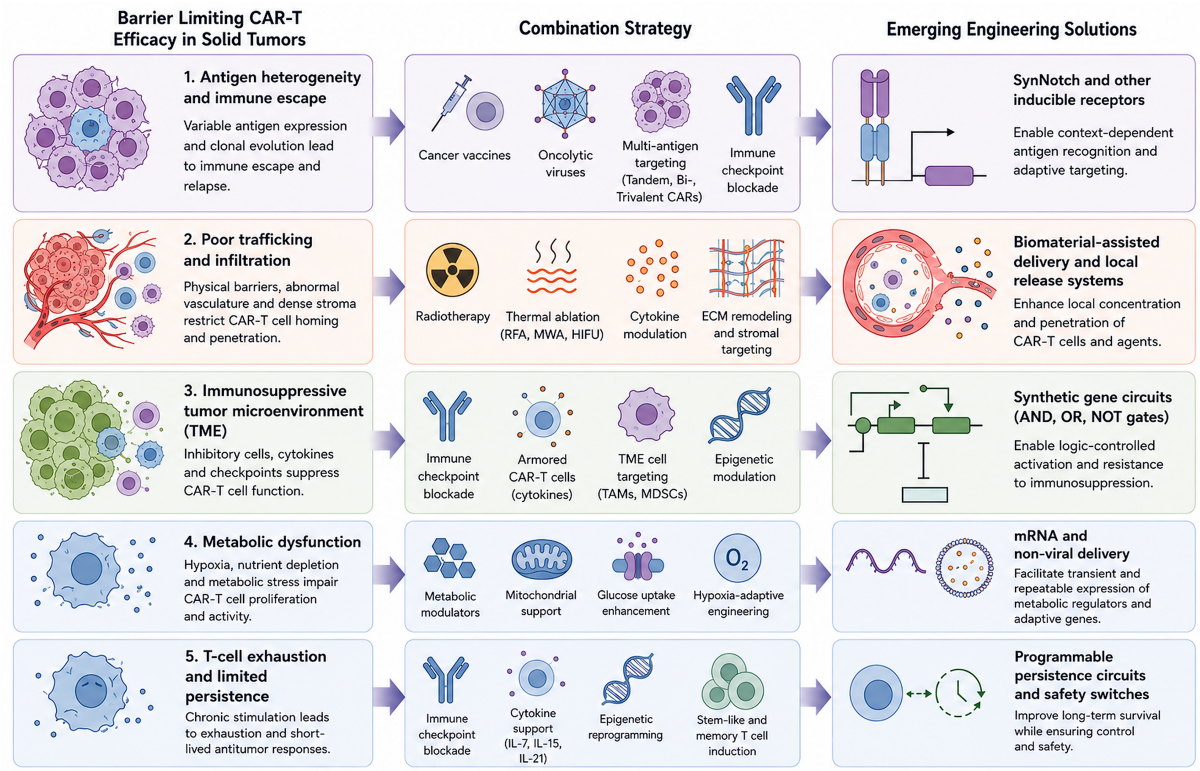


Figure 2. Barrier-to-solution framework for overcoming resistance to CAR-T cell therapy in solid tumors. *Major biological barriers to CAR-T cell efficacy in solid tumors are linked to rational combination strategies and emerging engineering technologies designed to overcome them. Antigen heterogeneity may be addressed through vaccines, oncolytic viruses, and multi-antigen targeting platforms, supported by adaptive recognition systems such as synNotch receptors. Poor trafficking and infiltration can be improved through radiotherapy, thermal ablation, cytokine modulation, and extracellular matrix remodeling, with biomaterial-assisted delivery systems enhancing local accumulation and penetration. Immunosuppressive tumor microenvironments may be overcome through immune checkpoint blockade, armored CAR-T cells, stromal-targeting approaches, and epigenetic modulation, supported by synthetic gene circuits that enable context-dependent activation. Metabolic dysfunction can be mitigated through metabolic reprogramming and hypoxia-adaptive engineering, whereas T-cell exhaustion and limited persistence may be addressed through checkpoint inhibition, cytokine support, epigenetic reprogramming, and programmable persistence circuits. Together, these integrated strategies illustrate the transition from conventional CAR-T therapy toward programmable and adaptive cellular immunotherapies capable of overcoming multiple resistance mechanisms simultaneously.*

4. Emerging technologies enhancing CAR-T combination therapy

4.1 Synthetic biology and gene circuit designs

To address the complexity of the TME and reduce on-target/off-tumor toxicity, synthetic biology has emerged as a powerful approach to enhance CAR-T cell function by integrating programmable genetic circuits (133, 134). Recent advances have led to the development of engineered CAR-T systems, including hypoxia-, reactive oxygen species (ROS)-, and pH-responsive CARs, as well as cytokine-sensing designs and immune checkpoint knockout strategies (135, 136).

To improve specificity and minimize off-target effects, Kole T. Roybal et al. designed a combinatorial antigen-sensing circuit that integrates synNotch receptors with CAR constructs, creating a dual-receptor "AND-gate" system. In this design, synNotch activation by a primary antigen induces CAR expression, enabling T-cell activation only when both antigens are present on tumor cells (137).

In addition, synthetic gene circuits have been engineered to reprogram CAR-T cells in response to specific TME features, such as dense ECM, abnormal vasculature, hypoxia, oxidative stress, and

elevated protease activity. For example, fusion receptors that combine the extracellular domains of TGF-β receptors with the intracellular signaling domains of IL-2/IL-7 receptors convert inhibitory signals into activating signals, thereby enhancing CAR-T cell survival and function in suppressive environments (138).

To address tumor hypoxia, hypoxia-responsive CAR-T systems have been developed to selectively express CARs under low-oxygen conditions, thereby confining activity to tumor tissues and improving safety (139). Similarly, ON-switch CAR systems enable small-molecule-dependent, titratable, and reversible control of CAR-T cell activity (140). Furthermore, thermal gene switches incorporating heat-shock elements (HSEs) enable spatial and temporal control of CAR-T activation during photothermal stimulation (141).

Collectively, these advances in synthetic biology enable precise, context-dependent control of CAR-T cell activity, improving specificity, safety, and adaptability within complex tumor environments.

4.2 CAR-T plus biomaterials

To enhance the therapeutic efficacy of CAR-T cells in solid tumors, advances in biomaterials have provided promising strategies to overcome key barriers, including physical obstruction, the immunosuppressive TME, and treatment-related toxicity (142, 143). Increasing efforts have focused on developing drug delivery systems that enable precise, controlled co-delivery of CAR-T cells and immunostimulatory molecules, thereby improving therapeutic outcomes (144-146). To date, a variety of biomaterial-based platforms have been explored to support CAR-T therapy in solid tumors (147).

To prevent systemic CAR-T cell diffusion and excessive cytokine release, Wang et al. generated GD2-specific CAR-T cells (GD2.CAR-Ts) and delivered them locally to a retinoblastoma (RB) model via an injectable hydrogel system. The study showed that combining GD2.CAR-T cells with sustained interleukin-15 release effectively eliminated RB tumor cells without impairing visual function (148). To overcome the immunosuppressive TME, hydrogel systems integrated with nanoparticles can protect CAR-T cells from immunosuppressive factors while enabling sustained release of cytokines (e.g., IL-2, IL-15) and CAR-T cells, thereby prolonging local activity and enhancing persistence. Similarly, implantable biomaterials facilitate localized administration and create a transient inflammatory niche that supports CAR-T cell expansion and function *in vivo*. Abigail K. Grosskopf et al. developed a self-assembled injectable biomaterial platform based on polymer-nanoparticle (PNP) hydrogels for CAR-T cell delivery (149). This system functions as a selective barrier that restricts cytokine diffusion while permitting T-cell migration, thereby establishing an optimized microenvironment that supports prolonged CAR-T cell retention and antitumor activity. In addition, abundant fibrotic components, including collagen and hyaluronic acid, contribute to the formation of a dense extracellular matrix (ECM) in solid tumors, which restricts CAR-T cell infiltration into the tumor core. To overcome this physical barrier, biomaterial-based strategies have been developed to deliver or induce the local production of ECM-degrading enzymes. For instance, hyaluronic acid (HA)-based hydrogels have been shown to remodel the ECM, thereby enhancing CAR-T cell infiltration into tumor tissues (150). In addition, biomaterial-based strategies have been explored to simplify CAR-T cell therapy and reduce manufacturing complexity by enabling in vivo CAR-T cell generation. An implantable Multifunctional Alginate Scaffold for T Cell Engineering and Release (MASTER) has been developed, enabling *in situ* generation and release of functional CAR-T cells *in vivo* (151).

Moreover, biomaterial platforms have been extended to three-dimensional (3D) *in vitro* systems, which allow investigation of how the mechanical properties of the TME influence CAR-T cell behavior and serve as preconditioning platforms prior to clinical administration (152, 153).

Despite these advances, most biomaterial-based CAR-T strategies remain in preclinical development. Significant challenges remain, including large-scale manufacturing, batch-to-batch consistency, and long-term safety evaluation. Therefore, further optimization and translational studies are needed to fully realize the clinical potential of biomaterial-assisted CAR-T therapy.

4.3 Nonviral or mRNA-based CAR-T delivery

Conventional viral vectors are associated with risks such as CRS, ICANS, insertional mutagenesis, and secondary malignancies (154-156). In contrast, non-viral and mRNA-based CAR-T delivery strategies have emerged as promising alternatives, offering improved safety, flexibility, and cost-effectiveness (157, 158). These strategies make a significant contribution to both *ex vivo* engineering and *in vivo* programming.

In *ex vivo* engineering, T cells are purified from patients, activated, and then transduced with a viral vector. After expansion, cryopreservation, and extensive quality testing (159), the CAR-T cells are infused back into the original patient. However, retroviral and lentiviral vectors are difficult to control and may inadvertently disrupt critical genes or transcriptional regulatory elements. Therefore, non-viral delivery, including transposon systems, mRNA delivery, and genome-editing technologies, plays a pivotal role in *ex vivo* engineering of CAR-T. Platforms such as PiggyBac and Sleeping Beauty enable stable genomic integration of CAR constructs (160, 161). Early clinical evidence demonstrates detectable CAR-T persistence and clinical responses in NSCLC patients treated with piggyBac-engineered EGFR-CAR T cells (162). Moreover, mRNA-based delivery offers a transient, controllable alternative that avoids genomic integration (163). CAR-encoding mRNA can be introduced into isolated T cells via electroporation and then infused back into the original patient (164).

In the realm of *in vivo* programming, mRNA is being delivered using nanocarriers, including polymer-based nanoparticles and lipid nanoparticles (LNPs) (165, 166). Notably, targeted LNP technology is the core tool for efficient *in vivo* CAR-T reprogramming. Building on these advances, *in situ* CAR-T reprogramming has emerged as a next-generation strategy that enables direct genetic engineering of immune cells *in vivo*, thereby bypassing *ex vivo* manufacturing (159, 167, 168). For instance, targeted LNPs enable the *in vivo* generation of anti-FAP CAR-T cells, thereby reducing cardiac fibrosis and demonstrating potential to remodel the desmoplastic stroma in solid tumors (169). Recent developments in targeted delivery systems, such as CD8-specific LNPs (e.g., L829-tLNP) and myeloid-targeted LNP-based CAR therapies (e.g., MT-302/MT-303), further enable *in vivo* CAR expression and effective tumor control (170, 171), highlighting their potential to expand CAR-T accessibility and scalability.

Proof-of-concept studies have demonstrated the feasibility of generating functional CAR-T cells *in vivo* with CD8-targeted vectors (172), and multiple platforms are advancing toward clinical translation (173). Furthermore, mRNA-based approaches have shown favorable safety profiles in early clinical studies, including mesothelin-targeted CAR-T therapy for patients with pancreatic cancer (174).

However, mRNA-based CAR-T strategies remain limited by transient expression and stability, largely determined by structural elements such as the $5'$ cap, poly(A) tail, coding sequence, and untranslated regions (UTRs) (175). Consequently, repeated administration may be required, raising concerns about cumulative toxicity. To address these limitations, optimized mRNA engineering strategies, including modified cap structures (e.g., CleanCap®), extended poly(A) tails, and UTR optimization, have been

developed (176). Additionally, RNA circularization technologies such as high-yield scarless PIE (Hi-Scarless-PIE) have demonstrated improved CAR expression and antitumor efficacy compared with conventional linear mRNA constructs (177).

Overall, non-viral or mRNA-based CAR-T delivery represents a complementary and evolving strategy in both *ex vivo* engineering and *in vivo* programming. These strategies enable the production of safer, more controllable, and scalable CAR-T cells, offering substantial potential for improving therapeutic outcomes in solid tumors.

5. Discussion

5.1 Biological Challenges

Despite substantial advances in CAR-T engineering and combination therapy, several biological barriers continue to constrain therapeutic efficacy in solid tumors.

When combined with ICIs, CAR-T cells may enhance CAR-T cell activity but also increase the incidence and severity of immune-related adverse events (irAEs). Importantly, different ICIs have distinct toxicity profiles, further complicating clinical management (178,179). For instance, a phase I trial (NCT03726515) evaluating EGFRvIII-targeted CAR-T cells in combination with pembrolizumab found no significant therapeutic benefit in glioblastoma (180). These findings underscore the need for more precise combinatorial strategies and alternative approaches, such as CRISPR/Cas9-mediated disruption of inhibitory receptors (e.g., PD-1), to enhance efficacy while minimizing toxicity (181).

Similarly, combination strategies that use exogenous cytokines, costimulatory modulation, or kinase inhibitors may exacerbate systemic toxicity because of their narrow therapeutic windows (154, 182). To address this limitation, approaches that enable spatiotemporally controlled activation, such as biomaterial-based delivery systems (183) and in situ expression strategies (e.g., armored CAR-T cells) (184), are actively being explored. These strategies aim to localize therapeutic activity within the TME, thereby reducing off-target effects.

In addition, combining CAR-T therapy with chemotherapy or radiotherapy introduces further complexity. These treatments can trigger innate immune responses by releasing damage-associated molecular patterns (DAMPs) from dying tumor cells (185). This may synergize with CAR-T activity but also increase the risk of systemic inflammation and toxicity. Moreover, higher radiation doses and larger treatment volumes are associated with greater damage to normal tissues (186). To mitigate these risks, engineered CAR-T cells with context-dependent activation, such as those incorporating synthetic gene circuits, have been proposed (187).

Importantly, these challenges are highly interconnected rather than independent. Antigen heterogeneity, inadequate trafficking, immunosuppressive signaling, metabolic dysfunction, and progressive exhaustion collectively drive treatment resistance in solid tumors. Consequently, future therapeutic success will likely depend on combination strategies that simultaneously overcome multiple resistance mechanisms, rather than on addressing individual barriers in isolation.

5.2 Translational and Manufacturing Challenges

Beyond biological barriers, the increasing complexity of next-generation CAR-T platforms introduces important translational, manufacturing, and regulatory challenges.

Despite these advances, several challenges remain for emerging platforms. For biomaterial-assisted CAR-T therapy, long-term safety concerns, including immunogenicity and toxicity from degradation byproducts, remain incompletely characterized (188). In parallel, most synthetic gene circuit-based approaches remain in preclinical development and lack standardized protocols for large-scale manufacturing and clinical translation.

Furthermore, critical translational challenges persist across CAR-T combination strategies, including optimizing dosing schedules, identifying predictive biomarkers, and stratifying patients. In situ CAR-T reprogramming also faces unique limitations, as the complexity of the in vivo TME cannot be fully recapitulated in current preclinical models. In addition, delivery-related challenges, including transient mRNA expression that requires repeated dosing, integration risks associated with transposon systems, and suboptimal genome-editing efficiency that may lead to genomic instability, remain to be addressed. The lack of long-term safety data and standardized manufacturing and quality-control frameworks further complicates clinical implementation.

Moreover, as CAR-T products become increasingly sophisticated through the incorporation of multi-antigen targeting, synthetic gene circuits, biomaterials, and programmable control systems, balancing therapeutic potency with manufacturing feasibility and economic sustainability will become a major consideration for widespread clinical adoption.

5.3 Toward Precision Combination CAR-T Therapy

An important emerging concept is that solid tumors differ substantially in the dominant mechanisms limiting CAR-T efficacy. While some tumors are primarily characterized by antigen heterogeneity, others are dominated by stromal exclusion, immune suppression, metabolic dysfunction, hypoxia, or inadequate persistence. Consequently, future CAR-T combination strategies will likely require biomarker-guided approaches that match specific therapeutic interventions to the underlying resistance mechanisms in individual tumors. Importantly, successful implementation of precision CAR-T therapy will require biomarkers that are not only prognostic but also actionable, enabling selection of the most appropriate combination strategy for each resistance landscape.

Advances in single-cell sequencing, spatial transcriptomics, multiplex imaging, and circulating biomarker analyses are providing unprecedented insights into tumor architecture and immune landscapes. These technologies may help identify dominant barriers within individual tumors and support the rational selection of combination therapies. In this context, precision-based frameworks that integrate molecular profiling with advanced CAR engineering may improve therapeutic efficacy while minimizing unnecessary toxicity.

Rather than applying uniform treatment strategies across all patients, future CAR-T therapy may increasingly rely on individualized combinations tailored to the specific biological characteristics of each tumor ecosystem.

5.4 Future Outlook

Overall, although CAR-T combination therapies have shown significant promise in overcoming key barriers in solid tumors, substantial challenges remain. Continued efforts to integrate advances in cellular engineering, immunology, biomaterials, and clinical oncology will be essential to developing safer, more effective, and more controllable next-generation CAR-T therapies for solid tumors.

Collectively, the field is transitioning from conventional CAR-T cell therapy toward increasingly programmable and adaptive cellular therapeutics that can sense, respond to, and remodel the tumor microenvironment. Combination strategies that integrate immune checkpoint blockade, cytokine modulation, targeted and epigenetic therapies, radiotherapy, thermal ablation, vaccines, oncolytic viruses, biomaterials, and emerging engineering technologies are beginning to address the multifaceted barriers that have historically limited success in solid tumors.

Importantly, advances in synthetic biology, multi-antigen targeting platforms, non-viral engineering technologies, and in situ CAR-T generation are expanding the therapeutic landscape, enabling more precise, controllable, and scalable cellular immunotherapies. The ultimate goal is to develop CAR-T platforms that simultaneously overcome antigen escape, improve tumor trafficking, resist immunosuppression, maintain metabolic fitness, and provide programmable safety mechanisms. Achieving this objective will require continued interdisciplinary collaboration across immunology, synthetic biology, bioengineering, biomaterials science, and clinical oncology. These efforts are expected to accelerate the translation of next-generation CAR-T therapies into durable, broadly applicable treatment options for patients with solid tumors.

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Declarations

Funding Statement: This research was supported by the National Natural Science Foundation of China (No. 82203380 to WC, No. 82402409 to BHC), the Guangdong Basic and Applied Basic Research Foundation (No. 2023A1515011416 to WC), and the Guangzhou Basic and Applied Basic Research Foundation (No. 2024A04J4419 to WC).

Competing Interest: The authors declare no financial or personal relationships with other individuals or organizations that could inappropriately influence or bias the content of this work. All authors have read the final version of the manuscript and confirm that there are no competing interests.

Consent for publication: All authors have approved the final version of the manuscript.

Use of Artificial Intelligence Disclosure: This article was written by human contributors. Artificial intelligence-based tools were used to improve grammar, language, and readability without affecting the article's scientific content, data interpretation, or conclusions. The authors reviewed and verified all content to ensure its accuracy and integrity. The authors prepared the figures with assistance from artificial intelligence, reviewed and edited them in PowerPoint.

Data Availability Statement: No datasets were generated or analyzed in the current study.

Ethics approval and consent to participate: Not applicable, as this study did not involve the conduct of research.

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CRediT authorship contribution statement: WC, MLL, BKC, YPW, CZ, and MTL: Writing - review & editing. JYC: Conceptualization, Funding acquisition, Writing – review & editing. BHC: Conceptualization, Funding acquisition, Writing – review & editing. All authors contributed to the work and approved the final version of the manuscript.

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