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Review

Harnessing CAR T-Cells Against Breast Cancer: A Comprehensive Review

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Simple Summary

Breast cancer is an aggressive disease with limited treatment options and a high risk of recurrence. Unlike other breast cancer types, triple-negative breast cancer lacks well-defined molecular targets, making standard targeted therapies ineffective. Recent evidence shows that these tumors are highly immunogenic and contain cancer stem cells that survive treatment and drive relapse. Advances in immunotherapy, particularly CAR T-cell therapy, have shown strong success in blood cancers, but their use in solid tumors remains challenging due to immune suppression, physical barriers, and tumor diversity. This review highlights how newer generations of CAR T-cells are being engineered to improve tumor targeting, persistence, and safety, with a focus on eliminating cancer stem cells. By summarizing current strategies and challenges, the work provides a foundation for developing more effective immune-based therapies and supports future research aimed at improving outcomes in triple-negative breast cancer and other solid tumors.

Abstract

Breast cancer represents a highly heterogeneous malignancy encompassing multiple molecular subtypes, each with distinct therapeutic responses and clinical outcomes. Conventional treatment strategies—including surgery, chemotherapy, radiotherapy, endocrine, and targeted therapies—have improved survival rates but continue to face major limitations due to tumor relapse, metastasis, and therapy-induced resistance. In recent years, chimeric antigen receptor (CAR) T-cell therapy has emerged as a transformative modality in cancer immunotherapy, offering targeted and durable antitumor activity. While its efficacy in hematological malignancies is well established, translation to solid tumors such as breast cancer remains hindered by a complex interplay of tumor-intrinsic and microenvironmental barriers. This review delineates the mechanistic underpinnings of CAR T-cell function and highlights the multifaceted challenges posed by solid tumors, including antigen heterogeneity, an immunosuppressive tumor microenvironment, inadequate trafficking and infiltration, limited T-cell persistence, and safety-related cytotoxicities. Finally, emerging strategies and innovations aimed at overcoming these barriers are discussed, highlighting the potential and future direction of CAR T-cell therapy in breast cancer management. Collectively, this review underscores the translational potential of CAR T-cell therapy for breast cancer and outlines the rational strategies required to enhance its clinical applicability and therapeutic efficacy in solid tumor settings.

Keywords: CAR T-cell therapy; tumor microenvironment; breast cancer; antigen heterogeneity

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1. Introduction

Breast cancer is the most prevalent form of cancer affecting women worldwide [1,2]. This disease is broadly categorized into three molecular subtypes based on the expression of specific hormone receptors: hormone receptor-positive (HR-positive), HER2-enriched, and triple-negative breast cancer (TNBC). Each subtype differs significantly in terms of prognosis, treatment options, and biological behavior. Among these, TNBC is characterized by the absence of estrogen receptors, progesterone receptors, and HER2 expression, which contributes to its aggressive clinical course and poorer outcomes.

TNBC patients generally exhibit lower survival rates and reduced responsiveness to conventional treatments such as hormone therapy and HER2-targeted agents, which are effective in other breast cancer subtypes [3,4]. A major challenge in managing TNBC is the lack of well-defined molecular targets, which limits the effectiveness of targeted therapies currently available for other forms of breast cancer. Furthermore, TNBC is distinguished by a higher prevalence of circulating immunogenic cells, indicating an elevated level of immune system activity within the tumor microenvironment (TME).

This heightened immunogenicity positions TNBC as the most immunogenic among the breast cancer subtypes, thereby making immunotherapy an especially promising therapeutic approach [4]. Recent advances in immunotherapeutic strategies, including immune checkpoint inhibitors, have shown encouraging results by leveraging the body's immune system to mount a robust anti-tumor response. Consequently, immunotherapy represents a hopeful avenue for improving treatment outcomes and survival rates in patients diagnosed with TNBC [5,6].

2. Existing Treatment Options

For early-stage invasive breast cancer, breast-conserving surgery (BCS) and mastectomy remain the primary treatment modalities [7]. Among these, BCS followed by adjuvant radiotherapy has demonstrated excellent long-term outcomes, with 10-year locoregional recurrence (LRR) rates reported to be approximately 2% for estrogen receptor-positive (ER-positive) and HER2-positive breast cancers, and around 5% for TNBC [8,9]. These findings underscore the efficacy of BCS combined with radiotherapy as the preferred treatment approach for early invasive breast cancers, offering the dual benefits of effective disease control and breast preservation.

However, certain patient populations may not be ideal candidates for radiotherapy following BCS. Contraindications to radiotherapy, such as prior radiation exposure or certain comorbidities, as well as the presence of suspicious micro-calcifications on imaging, increase the risk of treatment failure and recurrence, thereby necessitating consideration of alternative therapeutic strategies [10]. In instances where patients experience local recurrence after initial BCS, total mastectomy is generally regarded as the standard of care to achieve optimal disease control and reduce the risk of further recurrence.

Immunotherapy

Adoptive cell therapy (ACT) refers to a form of immunotherapy that involves the engineering and manipulation of the patient's immune system to enhance its ability to recognize and eliminate malignant cells. Among the various approaches within ACT, chimeric antigen receptor (CAR) T-cell therapy has emerged as a ground-breaking strategy. This technique involves the genetic modification of T-cells to express CARs—synthetic receptors that enable these immune cells to specifically identify and target tumor-associated antigens presented on the surface of cancer cells [11]. Leukapheresis represents the initial stage in the production of CAR T-cells, encompassing the collection of blood from the patient to isolate T-cells, followed by the removal of myeloid cells through elutriation. Subsequently, T-lymphocytes are enriched, transgenes are introduced, and ex vivo expansion occurs [12].

CAR T-cell therapy harnesses the specificity and cytotoxic potential of T-lymphocytes, allowing for the selective targeting and destruction of malignant cells while sparing normal tissues. This precision is achieved through the engineered T-cells' ability to recognize unique antigenic markers that are typically overexpressed on cancer cells. Figure 1 summarizes the various possible ways in which CAR T-cells act in building the immune response against cancer cells. The first clinical application of CAR T-cell therapy was reported in 2017, marking a significant milestone in the treatment of hematological malignancies such as certain leukemias and lymphomas [13].

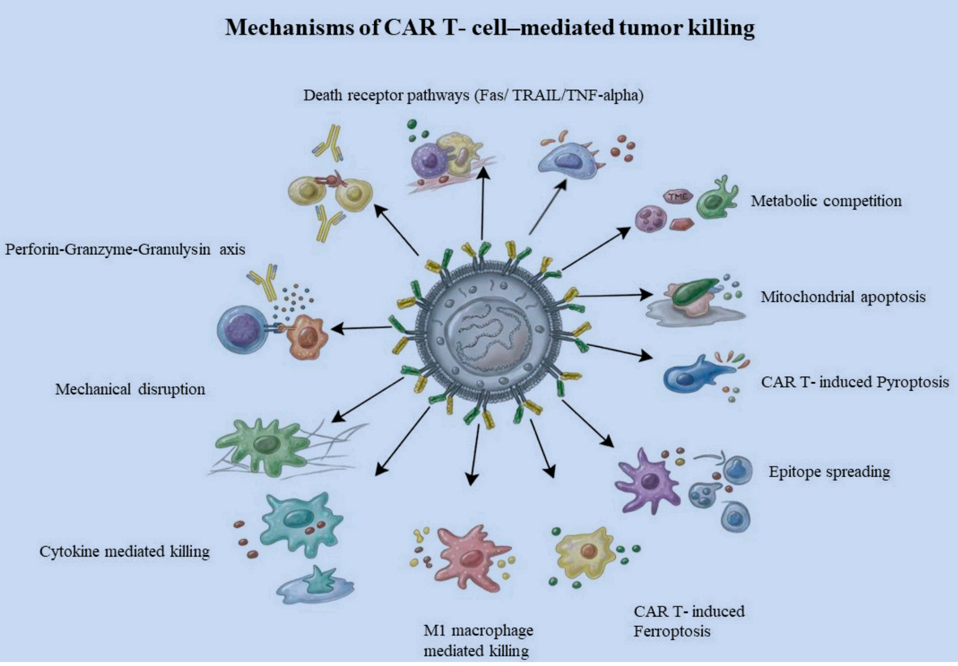

Figure 1. Killing mechanisms of CAR T-cells: CAR T- cells eliminate tumor cells through multiple complementary cytotoxic mechanisms within the tumor microenvironment. CAR T-cells induce direct cytolysis via perforin–granzyme–grnulysin axis, mediating pore formation and caspase-dependent apoptosis. Death-receptor signaling through Fas–FasL, TRAIL–DR4/DR5, and TNF-alpha activates extrinsic apoptotic cascades in antigen-expressing tumor cells. TNF-α–mediated apoptosis and cytokine-driven bystander killing extend cytotoxicity beyond direct cell–cell contact. Mitochondrial apoptosis via BID/BAX activation and cytochrome-c release amplifies intrinsic death signaling. Granulysin secretion contributes to membrane damage and mitochondrial dysfunction in susceptible tumor cells. Pyroptosis mediated by granzyme-B–dependent GSDME cleavage in susceptible tumor cells. Ferroptosis can be induced through IFN-driven lipid peroxidation pathways, sensitizing tumor cells to oxidative death. Metabolic competition within the TME restricts tumor growth while metabolically optimized CAR T- cells can preserve functional fitness. Mechanical disruption at the immunological synapse causes membrane rupture and cytoskeletal collapse. CAR T–cell derived cytokines activate macrophages, promoting phagocytosis and ROS/NO-dependent tumor killing. Epitope spreading recruits endogenous T-cells, NK cells, and dendritic cells, enabling multi-antigen immune surveillance and control of antigen-escape variants [14–21].

Since its initial application, CAR T-cell therapy has revolutionized the field of cancer immunotherapy by demonstrating remarkable efficacy in refractory and relapsed blood cancers, offering new hope to patients who had limited treatment options. Ongoing research continues to expand the potential of this approach to a wider range of malignancies and to improve its safety and effectiveness.

3. The Evolution of CAR T-Cell Therapy

CAR T-cells are broadly classified into five generations, distinguished by the arrangement of their intracellular signaling domains, These domains are central to orchestrating a range of T-cell functions, including lineage differentiation, induction of cytotoxic activity, cytokine production, and recruitment of additional immune effectors [22]. Together, these mechanisms contribute not only to efficient tumor clearance but also to the capacity for tumor targeting in a manner that bypasses MHC restriction.

The conceptual foundation of CAR technology was first demonstrated by Kuwana and colleagues in 1987, who provided proof-of-principle that antibody-derived antigen recognition could be coupled with T-cell signalling. This was achieved by fusing the constant region of the T-cell receptor (TCR) to the variable regions of an antibody directed against a bacterial antigen. Building on this seminal work, single-chain variable fragments (scFvs)—consisting of the variable heavy (VH) and light (VL) domains of a monoclonal antibody linked by a short, flexible peptide—have since become the standard extracellular antigen-recognition modules employed in CAR design [23].

The first generation of CAR T-cells was designed with a relatively simple architecture, consisting of a single intracellular signaling domain—either the CD3ζ chain or the FcεRIγ motif (Figure 2)—and lacking additional costimulatory elements. Early reports in the 1990s established proof-of-concept for this design, along with demonstrating that fusing a tumor antigen–specific single-chain variable fragment (scFv), such as one recognizing human epidermal growth factor receptor 2 (HER2) to the CD3ζ domain, could confer antigen-specific cytotoxicity [24–26].

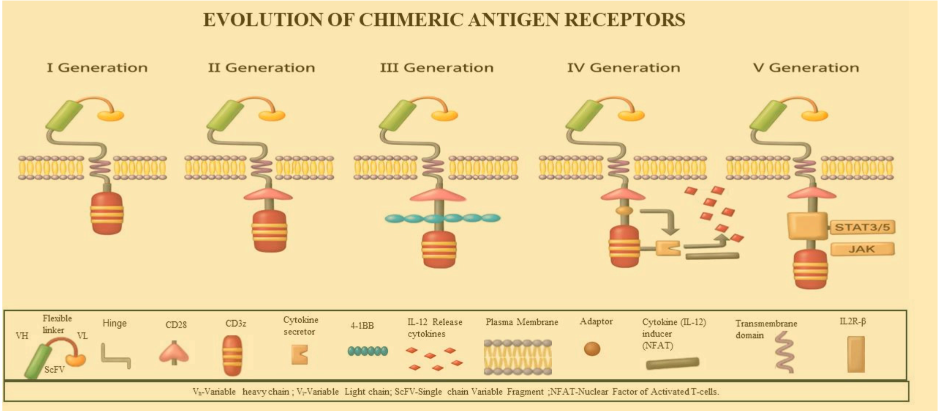

Figure 2. Evolution of chimeric antigen receptor (CAR) T-cell generations and signaling architecture. First-generation CARs comprise an extracellular single-chain variable fragment (scFv) linked solely to the intracellular CD3ζ activation domain, resulting in limited T-cell activation due to the absence of co-stimulation. Second-generation CARs incorporate an additional costimulatory domain (e.g., CD28 or 4-1BB) alongside CD3ζ, enabling dual signaling and improved effector function. Third-generation CARs further augment signaling by including two costimulatory domains in tandem with CD3ζ to enhance T-cell activation following antigen engagement. There are several different iterations categorized as Fourth-generation CAR T-cells. One well-described subtype is referred to as TRUCKs (T-cells Redirected for Universal Cytokine-mediated Killing), where CARs are based on second-generation constructs and incorporate an NFAT-responsive gene cassette that is activated following CAR engagement with its target antigen, resulting in localized cytokine release that acts as an additional downstream signaling and immune-modulatory mechanism following CAR activation. Fifth-generation CARs build on the second-generation framework by integrating a cytokine receptor signaling domain, typically IL-2Rβ (Interleukin-2 receptor beta chain) This additional intracellular module enables activation of the JAK–STAT pathway, leading to STAT3 and STAT5 signaling upon antigen engagement,

resulting in synergistic triple signaling through CD3ζ activation, co-stimulation, and intracellular cytokine receptor-mediated pathways [14,27–30].

Despite these encouraging findings, the limitations of first-generation CARs quickly became evident. Although structurally analogous to endogenous TCRs, they failed to produce sufficient levels of interleukin-2 (IL-2), making them dependent on exogenous IL-2 supplementation to sustain activity. Moreover, these CAR T-cells exhibited poor proliferative capacity and limited in vivo persistence, both of which hindered their therapeutic efficacy in clinical settings [31–33]. Consequently, while first-generation CARs established the foundation for CAR-based immunotherapy, their restricted signaling capacity highlighted the need for further refinement, ultimately paving the way for second-generation designs incorporating costimulatory domains.

To overcome the limitations of first-generation CARs, researchers introduced intracellular costimulatory domains such as CD28, 4-1BB, and OX40 into CAR designs (Figure 2).

This modification significantly enhanced T-cell activation by providing a secondary signal upon antigen engagement, leading to improved proliferation, persistence, and cytotoxic function. Early comparative studies revealed that the addition of CD28 co-stimulation increased expansion and survival relative to CD3ζ-only constructs, while 4-1BB conferred superior persistence in the circulation, albeit with distinct effects on exhaustion and tonic signaling [34]. These insights laid the groundwork for the development of second- and third-generation CARs, which incorporated one or two costimulatory domains, respectively, reflecting the biological principle that endogenous T-cell receptor activation requires accessory signaling molecules [35]. Incorporation of CD28 or 4-1BB domains in particular was shown to enhance cytokine production, promote memory formation, and modulate metabolic pathways [36–40]. Subsequent iterations explored novel combinations of costimulatory modules to further optimize function: for instance, CARs engineered with both CD28 and OX40 suppressed CD28-driven interleukin-10 (IL-10) secretion, thereby countering immunosuppressive signaling [41], while ICOS paired with CD28 or 4-1BB improved in vivo persistence, and synthetic MyD88/CD40 signaling enhanced proliferation and antitumor activity [42,43]. Collectively, these advances not only established the clinical superiority of co-stimulation-enhanced CARs but also highlighted the importance of tailoring signaling domains to balance effector potency, persistence, and resistance to exhaustion. Refinements to third-generation CAR designs led to the emergence of fourth-generation CAR T-cells, which incorporated additional functional modules to enhance both efficacy and safety. These constructs often include transgenic elements—most commonly cytokines—that may be expressed either constitutively or inducibly upon antigen recognition. Some variants also integrate regulatory "safety switches," such as inducible Caspase-9, to enable controlled elimination of CAR T-cells in the event of severe toxicity [44–46]. Further advances introduced protease-based CARs engineered for dose-dependent regulation, thereby allowing external control over CAR activity to improve safety and therapeutic precision [47]. A notable subset of fourth-generation constructs, termed "T-cells redirected for universal cytokine-mediated killing" (TRUCKs), harness a nuclear factor of activated T-cells (NFAT)-responsive promoter to drive inducible cytokine expression—such as interleukin-12 (IL-12)—directly within the TME. This design allows CAR T-cells to deliver immunomodulatory cytokines in situ, thereby enhancing antitumor efficacy while minimizing systemic toxicity [48,49]. Expanding on this principle, so-called "armored" CAR T-cells have been developed to secrete additional soluble factors, including IL-12, IL-15, and IL-18, which strengthen effector responses, counteract immunosuppressive cell populations such as regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and promote sustained T-cell proliferation [44,48]. Other combinations, such as CCL19 with IL-7, have been explored to recruit endogenous immune subsets and support durable memory formation [50–52]. Collectively, these "armored" fourth-generation platforms represent a pivotal evolution in CAR design, equipping engineered T-cells not only with enhanced cytotoxic potential but also with the ability to actively remodel the tumor microenvironment.

Fifth-generation (5G) CAR T-cells represent a sophisticated evolution of cellular immunotherapy, building upon the foundational designs of second-generation (2G) CARs. While earlier iterations focused on enhancing T-cell activation through co-stimulatory domains, 5G CARs incorporate an additional dimension of signaling that closely mimics physiological T-cell activation. These next-generation constructs are specifically engineered to address critical limitations observed in earlier CAR generations—namely, T-cell exhaustion, poor persistence, and limited efficacy against solid tumors [53].

The central innovation of 5G CARs lies in their ability to engage the JAK-STAT signaling cascade in an antigen-dependent manner. This is achieved by incorporating a truncated cytoplasmic domain of the IL-2 receptor β-chain fused with a STAT3/STAT5-binding motif into a conventional 2G CAR backbone. Consequently, these cells simultaneously activate three key intracellular pathways: TCR signaling via the CD3ζ domain, co-stimulation via CD28, and cytokine signaling via JAK-STAT3/5. This design enables autonomous and sustained cytokine signaling without the need for exogenous cytokine support, thereby promoting T-cell persistence and functionality over time.

Preclinical studies have provided compelling evidence supporting the enhanced functionality of 5G CARs. Notably, Kagoya et al. demonstrated that JAK-STAT-augmented CAR T-cells outperformed earlier-generation counterparts in CD19+ leukemia xenograft models, exhibiting superior tumor control, increased expansion, and prolonged persistence [54]. These cells displayed a less differentiated, stem cell-like memory phenotype, reduced terminal differentiation, and lower rates of apoptosis. Enhanced secretion of effector cytokines such as IL-2, IFN-γ, and TNF-α contributed to improved cytotoxicity and offered a means of counteracting the immunosuppressive TME. In models of A375-CD19 melanoma, these CAR T-cells also showed improved tumor infiltration, underscoring their potential in addressing challenges associated with solid tumors [54].

In parallel to the integration of cytokine signaling, researchers have explored novel synthetic regulatory strategies to improve safety and functional control of CAR T-cells. One promising avenue involves the inclusion of antigen-inducible membrane receptors—such as IL-2 receptor components—to conditionally activate the JAK/STAT pathway, thereby refining cytokine-driven responses while minimizing off-target effects [54].

Furthermore, the development of switchable CAR systems offers a significant leap toward safer and more controllable therapies. These include both ON-switch and OFF-switch mechanisms that allow for drug-mediated modulation of CAR activity. For instance, lenalidomide-gated CAR constructs exemplify the concept of external pharmacologic control, albeit with some trade-offs in in vitro potency [55]. Despite this, their improved safety profile renders them attractive candidates for further research.

Expanding on this concept, Li et al., introduced the VIPER (VersatIle ProtEase Regulatable) CAR platform, which utilizes viral protease domains to enable small molecule-inducible ON and OFF switches [55]. This modular system permits multi-antigen targeting and the design of sophisticated logic-gated CAR circuits. Importantly, VIPER CARs demonstrated limited systemic toxicity in a cytokine storm model, highlighting their translational potential for clinical use [56].

While these advancements mark significant progress in the field, several challenges remain. Ongoing research is essential to optimize dosing strategies, mitigate risks such as cytokine release syndrome (CRS), and broaden the applicability of 5G CAR T-cell therapies across diverse hematological and solid malignancies.

4. CAR T-Therapy and Cancer Stem Cells

Tumors consist of a heterogeneous array of cell types, among which cancer stem cells (CSCs) represent a small yet critically important subpopulation of undifferentiated cells capable of giving rise to the differentiated progeny that constitute the tumor bulk. According to the CSC hypothesis, tumor formation parallels normal tissue development in that a subset of "cancer-initiating cells" maintains itself through asymmetric division while simultaneously generating multiple differentiated lineages [57]. These tumorigenic CSCs exhibit distinct surface marker profiles—

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including CD29, CD34$^+$, CD38$^-$, CD166, CD133$^+$/$^-$, Lin, Sca-1, and EpCAM—many of which are associated with stem-like traits and have enabled their isolation via fluorescence-activated cell sorting or other immunoselection strategies [58–60]. However, the application of these markers requires caution, as several are also expressed by non-CSC populations [61,62].

Within breast cancer, a discrete CSC subset—termed breast cancer stem cells (BCSCs)—has been identified and is now recognized as a key driver of therapeutic resistance and disease recurrence. Despite the rapid expansion of breast cancer treatment modalities, persistent chemo resistance and adverse effects continue to underscore the need for strategies capable of targeting this resilient population. BCSCs have been repeatedly implicated in treatment survival and post-therapy tumor re-emergence, making them a major determinant of relapse [63] and high BCSC fractions correlate strongly with poor clinical outcomes [64].

Because CAR T-cells are designed to recognize tumor-associated surface antigens with high specificity and inhibit tumor proliferation, CSC-restricted or CSC-enriched markers represent promising targets for immunotherapeutic intervention. Owing to their capacity to recognize and eliminate tumor cells via tumor-associated antigen binding, CAR T-cells represent strong candidates for targeting CSCs. For example, Chen et al. showed that GD2-specific CAR T-cells effectively depleted side-population cells and eradicated established tumors in a neuroblastoma mouse model [65]. Multiple studies employing CAR T-cells engineered against established CSC markers have further demonstrated that these cells can efficiently eliminate CSC subsets while sparing normal stem cells or exhibiting only minimal cytotoxic effects on them [66–71]. A range of CSC-associated markers are therefore considered potentially targetable by CAR T-cell approaches. For instance, extensive studies have demonstrated that markers such as CD133, CD90, ALDH, and EpCAM are expressed across CSCs from multiple tumor types, providing molecular entry points for CAR-T–mediated elimination of CSCs and for the suppression of tumor recurrence and metastasis [72].

Current CSC-directed CAR T-cell strategies can be broadly divided into two categories. The first involves engineering CAR T-cells against CSC-specific antigens—such as CD133, EpCAM, or ALDH—and assessing their cytotoxic potential through in vitro assays and in vivo animal models. The second approach targets “general” antigens shared by both CSCs and bulk tumor cells. Although these CAR T-cell constructs are not originally designed for CSC specificity, the presence of the same markers on CSC surfaces enables collateral killing of CSCs during co-culture or in vivo treatment [73]. However, this broadened targeting specificity introduces risks: notable “on-target, off-tumor” toxicities have been reported, particularly within the hematopoietic compartment, an effect likely attributable to CD133 expression on CD34$^+$ progenitor cells.

Identifying antigens appropriate for CSC targeting presents unique challenges distinct from those encountered when identifying conventional tumor antigens for platforms such as TCR-based therapies. Current hurdles include: (i) the requirement for strict tumor specificity to avoid on-tumor, off-target toxicity, which is generally more severe with CAR T-cells compared with monoclonal antibody-based therapies [74]; (ii) the necessity for antigens to be exclusively expressed on the surface of CSCs—rather than intracellularly—because CAR T-cells recognize only surface-exposed antigens in an HLA-independent manner; (iii) the frequent reliance on functional properties such as self-renewal rather than stable phenotypic markers to define CSCs; and (iv) the absence of surface antigen expression in certain CSC populations. Consequently, next-generation CAR T-cell designs (e.g., iCAR-expressing CAR T-cells) and combination strategies (e.g., differentiation therapy followed by CAR T-cell treatment) may help mitigate or overcome these limitations [75].

One of the most effective strategies for identifying CSCs within tumors is the use of CSC-specific biomarkers. Depending on their cellular localization, these markers can be categorized as intracellular or cell-surface markers (Table 1).

Table 1. Some identified BCSC markers.

BCSC Marker	Targeting Mechanism	Preclinical Safety	Therapeutic Rationale	References
MUC1-C / MUC1	Induces tumor cell death through disruption of oncogenic MUC1-C signaling.	Built-in tumor specificity reduces damage to normal tissues.	Enhanced efficacy and limited off-tumor activation.	[76]
EpCAM	Eliminates epithelial tumor bulk through surface adhesion molecule targeting	Antigen-density–dependent targeting due to EpCAM expression on normal epithelium.	Marked reduction in epithelial tumor burden.	[77]
GD2	Eradicates stem-like metastatic initiators to block tumor spread	Favorable preclinical safety through selective targeting of stem-like metastatic cells.	Disrupts metastatic seeding in TNBC models.	[78]

CD133, also referred to as prominin-1, is a pentaspan transmembrane glycoprotein encoded by the PROM1 gene [79]. High levels of CD133 expression have been reported across diverse CSC populations, including those derived from leukemia, brain, liver, breast, pancreatic, and ovarian tumors [80–83]. Owing to its broad involvement in CSC biology, CD133 has emerged as a promising therapeutic target, and in recent years multiple CD133-directed agents—most notably monoclonal antibodies—have been explored [84]. Preclinical investigations evaluating CD133-targeted CAR T-cells have demonstrated significant antitumor activity. In an orthotopic glioma mouse model, Hu et al. showed that CD133 CAR T-cells markedly suppressed tumor progression and improved survival [85]. Similarly, Zhu et al. reported that CD133-specific CAR T-cells effectively eliminated glioblastoma CSCs in both in vitro and in vivo settings [86]. Beyond T-cells, CD133-targeted CAR natural killer-cells have also been tested; one study demonstrated that such CAR-NK cells efficiently recognized and eradicated CD133$^+$ primary ovarian cancer cells as well as established ovarian cancer cell lines [87]. Collectively, these data suggest that CD133-directed CAR-based therapies hold substantial promise for CSC eradication. However, clinical investigation remains limited; to date, only one study has evaluated CD133-targeted CAR T-cells against patient-derived glioblastoma stem cells [88]. Given that CD133 is expressed not only on malignant neural stem cells but also on normal neural stem cells, concerns regarding potential off-tumor toxicity remain. Approaches such as intratumoral delivery of CD133-targeted CAR T-cells have been proposed to mitigate these risks [89]. Mechanistically, CD133 (PROM1) can activate intracellular pathways including PI3K/AKT, Src, and β-catenin, thereby contributing to tumor progression [79]. Moreover, combinations of CSC markers may enhance specificity: for instance, CD44 and CD133 have been co-utilized to identify CSC populations in gallbladder cancer [90] ,while in colorectal cancer both CD44$^+$/CD133$^-$ and CD44$^-$/CD133$^+$ subpopulations have been shown to possess CSC properties [91,92].

EpCAM (CD326) is a type I transmembrane glycoprotein traditionally associated with epithelial cell–cell adhesion [93]. Beyond its structural role, EpCAM promotes epithelial–mesenchymal transition by suppressing E-cadherin expression, thereby enhancing migratory and invasive potential. Consistent with these functions, EpCAM contributes to cell signaling, differentiation, proliferation, and migration, and is recognized as a prominent CSC marker in hepatocellular carcinoma (HCC) and colorectal cancer [94,95]. Indeed, EpCAM$^+$ HCC cell lines such as HuH1 and HuH7 display robust tumor-initiating capacity and generate sizeable tumors in SCID mouse models [94]. These biological properties have positioned EpCAM as an attractive target for adoptive cell therapies. Over the past decade, multiple investigations have shown that EpCAM-directed CAR T-cells exert potent antitumor activity.

For example, Zhang et al. observed that EpCAM CAR T-cells secrete cytotoxic mediators, including TNF-α and IFN-γ, enabling efficient elimination of EpCAM$^+$ cancer cells in vitro and significant inhibition of tumor growth in colorectal xenograft models [96]. Parallel findings by Wu et al. demonstrated that these CAR T-cells eradicate PC3M prostate cancer cells in vitro and markedly suppress tumor progression in NOD/SCID mice [97]. Further supporting the therapeutic value of this antigen, Deng et al. reported that EpCAM-specific CAR T-cells not only eliminate PC3M prostate cells that overexpress EpCAM but also prolong survival in models using PC3 cells with comparatively low EpCAM expression [98]. Optimizing EpCAM-targeted CAR T-cells—particularly through tuning CAR affinity—may help achieve effective tumor control while minimizing off-tumor toxicity associated with physiological EpCAM expression [89].

5. Challenges Faced in the Case of Solid Tumors

Despite the transformative success of CAR T-cell therapies in hematologic malignancies, their translation to solid tumors has been met with substantial challenges. These limitations are primarily rooted in the complex and immunosuppressive architecture of the solid TME, which poses a multifactorial barrier to effective immunotherapy. Unlike blood cancers, where CAR T-cells have relatively unimpeded access to malignant targets, solid tumors exhibit a constellation of physiological and biochemical obstacles that restrict T-cell function and persistence [99–101].

The TME is a highly complex and dynamic ecosystem composed not only of malignant cells but also a diverse repertoire of non-neoplastic constituents, including fibroblasts, adipocytes, pericytes, immune cells, and other stromal elements [102]. This multifaceted milieu is further shaped by a dense stromal architecture, aberrant vasculature, immunosuppressive cellular networks, and a profoundly dysregulated chemokine landscape, which together impose formidable barriers to effective immune cell infiltration and antitumor immunity [103]. Upregulation of immune checkpoint ligands, secretion of pro-tumorigenic and anti-inflammatory cytokines, and physical exclusion of lymphocytes from tumor islets collectively limit CAR T-cell infiltration and cytotoxicity [104]. Moreover, antigenic heterogeneity and the scarcity of truly tumor-specific targets further compound the difficulty in achieving selective and sustained CAR T-cell activation [99].

These barriers do not operate in isolation but rather form a highly interconnected system of resistance. For example, the adaptive remodeling of the tumor microenvironment may simultaneously reduce antigen expression and modulate chemokine gradients, thereby hindering T-cell recruitment and activation. Such interactions underscore the need for a systems-level understanding of tumor-immune dynamics rather than piecemeal solutions targeting single mechanisms [105].

5.1. Immunosuppressive Tumor Microenvironment

Beyond its cellular makeup, the TME also incorporates extracellular matrix (ECM) components, abnormal vasculature, and a complex milieu of chemokines, cytokines, and other soluble factors that together orchestrate the pathophysiology of tumor progression [106,107].

Rather than serving as a passive backdrop, the TME functions as an active participant in tumor evolution, exerting a profound influence on the malignant phenotype of neoplastic cells. Reciprocal interactions between cancer cells and their microenvironment drive processes such as immune evasion, angiogenesis, metastasis, and resistance to therapy, thereby reinforcing the TME's role as a dynamic enabler of carcinogenesis and disease progression [108].

A critical barrier to the success of CAR T-cell therapy in solid tumors lies in the immunosuppressive nature of this microenvironment. Studies have revealed that only 1–2% of infused CAR T-cells are capable of infiltrating the tumor core, severely limiting their cytotoxic activity against solid tumor cells [109]. This poor infiltration is partly due to the structural and biochemical obstacles embedded within the TME, including dense fibrotic stroma, ECM components such as glycoproteins, fibrous proteins, proteoglycans, and polysaccharides, all of which contribute to the formation of physical and immunological barriers [107,110].

Furthermore, the TME houses a heterogeneous population of immune and stromal cells that actively suppress T-cell function. These include tumor-associated macrophages, myeloid-derived suppressor cells, regulatory T-cells, dendritic cells, and natural killer cells, as well as myeloid progenitor and effector T-cells [111,112]. These cells release immunosuppressive mediators such as

TGF-β and other cytokines that blunt CAR T-cell activity, while molecules such as TRIF-related adaptor molecule interfere with downstream signaling cascades crucial for T-cell function [112].

Compounding these cellular and molecular barriers are additional physiological constraints such as hypoxia, nutrient deprivation, and the buildup of metabolic waste products, all of which create a metabolically hostile environment that further compromises CAR T-cell efficacy [113]. Moreover, abnormal tumor vasculature and increased interstitial pressure hinder the effective trafficking and delivery of CAR T-cells or therapeutic agents to deeper tumor regions [110].

To address these formidable barriers, combinatorial strategies are being explored. Co-treatment with immune checkpoint inhibitors, for instance, offers a promising avenue to alleviate T-cell exhaustion and overcome the suppressive effects of the TME [114]. Engineering CAR T-cells with enhanced resistance to TME-derived inhibitory signals or improved capacity to degrade ECM components may also represent critical steps toward improving their performance in solid tumors.

5.2. Physical Barriers

The physical architecture of the tumor stroma presents a formidable barrier to the successful infiltration of CAR T-cells into solid tumors. Comprised of a dense ECM, blood and lymphatic vessels, fibroblasts, immune cells, and mesenchymal components, this structural network forms a compact and highly organized mesh that can significantly limit immune cell access [115]. In some instances, the degree of stromal compaction renders the tumor virtually inaccessible to therapeutic agents, including CAR T-cells—a major obstacle given the well-established correlation between T-cell infiltration and improved clinical outcomes [116].

This physical inaccessibility is further exacerbated by the dynamic interactions between stromal and malignant cells, which drive tumor evolution. These interactions induce a spectrum of metabolic, genetic, and morphological changes, transforming stromal elements into pro-tumorigenic agents that support cancer progression and metastasis. Key modulators such as proteoglycans and glycopeptides embedded in the ECM actively remodel immune responses during tumor development, thereby reinforcing immune evasion mechanisms [117].

Among the most influential stromal players are cancer-associated fibroblasts (CAFs), which arise through a phenotypic transition of normal fibroblasts. Once activated, CAFs express molecules such as fibroblast-activating protein and stromal-derived factor 1α (SDF1A), both of which contribute to ECM remodeling. By inducing collagen cross-linking and enzymatic degradation of matrix components, CAFs not only support tumor expansion but also fortify physical barriers that impede CAR T-cell infiltration [118].

In addition to local stromal barriers, anatomical structures such as the blood-brain barrier (BBB) present further challenges for CAR T-cell therapy, particularly in the treatment of central nervous system (CNS) tumors. Traditional intravenous delivery methods are often insufficient to enable CAR T-cells to cross the BBB, prompting exploration of alternative strategies including laser thermotherapy, electroporation, transcranial ultrasound, and other modalities designed to temporarily disrupt the barrier [119,120]. Direct delivery routes—such as intraventricular or intrathecal administration—are currently under investigation as targeted approaches capable of bypassing the BBB and delivering CAR T-cells directly into the CNS parenchyma [121].

However, efforts to modify or bypass the BBB are not without risk. Disruption of this critical interface can trigger serious CNS-related adverse effects, including cerebral edema and neuroinflammation [122]. Furthermore, even beyond the BBB, solid tumors harbor additional physical obstructions. Activated CAFs contribute to the generation of thicker, mechanically stressed collagen fibers that create a more rigid and supportive ECM structure conducive to tumor growth. Simultaneously, tumor basement membranes frequently exhibit breaches caused by both proteolytic degradation and mechanical realignment of ECM molecules, which complicates the orderly infiltration of therapeutic immune cells [123].

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Altogether, these multilayered structural and physiological barriers necessitate innovative engineering of CAR T-cells and novel delivery strategies to optimize their efficacy in the context of solid tumors.

5.3. Antigen Escape

One of the foremost challenges limiting the efficacy of CAR T-cell therapy in solid tumors is the significant antigenic heterogeneity displayed by these malignancies. This heterogeneity often facilitates a rapid phenomenon known as antigen escape, whereby tumor cells evade immune targeting by either mutating the antigen or expanding from a subpopulation of antigen-negative cells already present prior to treatment [124,125]. Such escape mechanisms critically undermine both the persistence and the therapeutic effectiveness of CAR T-cells.

To address this limitation, multi-antigen targeting strategies have been developed. By engineering CAR T-cells to recognize two distinct tumor-associated antigens simultaneously—a double-antigen-targeted approach—researchers aim to mitigate antigen escape while improving specificity and reducing off-target effects [126]. These dual-targeted therapies can take various forms, including the administration of two separate CAR T-cell populations, bicistronic CAR T-cell constructs, or tandem bispecific CAR T-cells, each designed to enhance anti-tumor efficacy through diversified antigen recognition [127], (Figure 3)

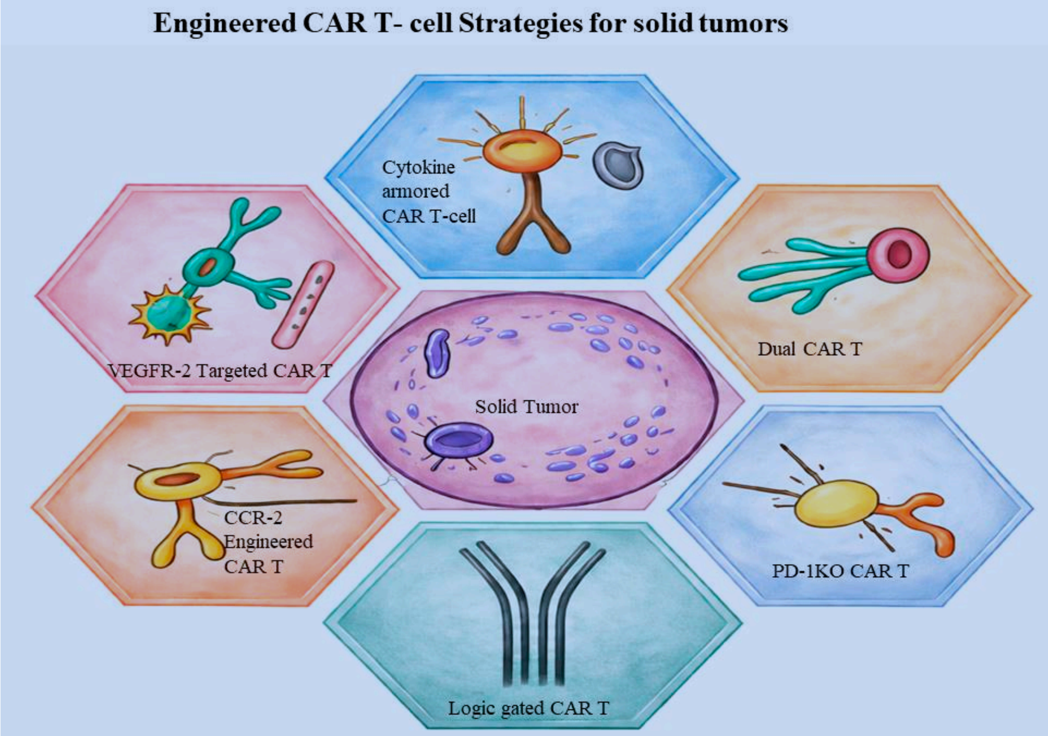

Figure 3. Schematic representation of advanced CAR T-cell engineering strategies designed to overcome major biological barriers in solid tumors. Cytokine-armored CAR T-cells are engineered to secrete immunomodulatory cytokines, enhancing local immune activation and antitumor efficacy in hostile tumor niches. VEGFR-2–targeted CAR T-cells exploit tumor vasculature targeting to disrupt tumor angiogenesis and enable indirect eradication of vascularized solid tumors. Dual CAR T-cells incorporate multiple antigen-recognition domains to address antigen heterogeneity and reduce tumor escape. CCR-2(Chemokine Receptor Type 2) engineered CAR T-cells express chemokine receptors that enhance trafficking and infiltration into chemokine-rich solid tumors. PD-1 knockout CAR T-cells, generated using genome-editing approaches, attenuate immune checkpoint–mediated suppression and restore T-cell effector function. Logic-gated CAR T-cells employ synthetic antigen-sensing circuits to refine tumor recognition, improve specificity, and limit off-tumor toxicity. Collectively, these engineered strategies aim to enhance CAR T-cell persistence, tumor infiltration, and antitumor activity in solid tumors [128–132].

Although single antigen-targeting CAR T-cells may initially elicit high response rates, tumor resistance often emerges because of partial or complete loss of the targeted antigen on malignant cells. This adaptive resistance, termed antigen escape, remains a significant barrier to durable responses and highlights the necessity for advanced CAR designs that can circumvent or pre-empt this mechanism.

5.4. Tumor Trafficking and Infiltration

CAR T-cell therapy for solid tumors faces considerable limitations compared to its application in hematological malignancies, primarily due to challenges in trafficking and infiltration. As previously mentioned, while CAR T-cells in hematologic cancers have direct access to tumor cells circulating in the bloodstream or residing in bone marrow, solid tumors present a more complex physical and immunosuppressive landscape that restricts CAR T-cell mobility and penetration [133,134]. The TME, combined with physical barriers such as the dense tumor stroma, impede effective CAR T-cell entry and function within solid tumor sites [135].

One key factor affecting CAR T-cell infiltration is the abnormal vasculature characteristic of solid tumors, which often displays reduced expression of endothelial adhesion molecules such as ICAM-1 and VCAM-1 that are crucial for lymphocyte extravasation [136,137]. This aberrant endothelium acts as a selective barrier, actively inhibiting immune cell entry by suppressing adhesion molecules and releasing factors such as ALCAM (activated leukocyte cell adhesion molecule), which modulate integrin-mediated arrest and impede T-cell trafficking. Additionally, the tumor-associated endothelial cells regulate cytokine production, further controlling immune cell migration and creating a hostile environment for CAR T-cell homing [136].

Despite occasional successful accumulation of CAR T-cells in the neoplastic stroma, their penetration into the tumor parenchyma remains notably limited. Vascular structures like high endothelial venules have been identified as facilitators of immune cell recruitment in certain cancers, including melanoma and breast cancer, highlighting potential routes for enhancing CAR T-cell infiltration [138,139]. The process of lymphocyte migration itself is tightly regulated by adhesion molecules—selectins, integrins, and chemokine receptors—that interact with chemokines to initiate lymphocyte rolling, firm adhesion, and eventual transendothelial migration toward the tumor [140].

However, the immunosuppressive TME often disrupts this coordinated trafficking by downregulating cytokine secretion and altering chemokine profiles, which diminishes T-cell recruitment and recognition [135,141,142]. Tumor and stromal cells in cancers such as pancreatic, ovarian, and breast carcinomas produce inhibitory chemokines likesuch as CXCL12 that restrict CAR T-cell proliferation and migration, effectively limiting their therapeutic delivery to tumor sites [143,144].

Moreover, manufacturing processes for CAR T-cells can inadvertently impair their homing capabilities. Extended ex vivo expansion may alter the expression of chemokine receptors necessary for effective trafficking, while loss of key enzymes such as heparanase—which degrades heparan sulfate in the tumor ECM—can reduce the ability of CAR T-cells to penetrate the dense stromal matrix and infiltrate tumor parenchyma [145].

Together, these physiological and technical hurdles underscore the complexity of achieving effective CAR T-cell localization and infiltration in solid tumors, necessitating innovative strategies to enhance trafficking, overcome stromal barriers, and modulate the tumor vasculature.

5.5. Target Antigen Selection

A major contributing factor to this difficulty is the inherent heterogeneity of tumor antigens, which varies markedly not only between different patients with the same tumor type but also among distinct cell populations within a single tumor. This variability complicates the process of antigen screening and selection, impeding the development of universally effective CAR T-cell therapies.

An additional critical challenge lies in ensuring target specificity to prevent “on-target off-tumor” toxicity. Many tumor-associated antigens are also expressed, albeit at varying levels, on normal healthy tissues. This nonspecific expression can inadvertently activate CAR T-cells against normal cells, causing collateral damage to essential tissues and posing potentially life-threatening risks to patients. The cytotoxic mechanisms underlying this adverse effect primarily involve the secretion of perforin and granzymes by CAR T-cells, which induce apoptosis in target cells, alongside the upregulation of T-cell surface molecules and the release of pro-inflammatory cytokines [146].

To maximize both the safety and efficacy of CAR T-cell therapies, it is imperative to identify novel antigens that exhibit exclusive expression on malignant cells while being absent from non-malignant tissues [146]. Approaches to mitigate off-tumor toxicity include fine-tuning CAR structural domains, designing logic-gated CAR T-cells that require multiple antigen signals for activation, incorporating suicide switches for controlled CAR T-cell elimination, regulating cytotoxicity levels and CAR expression, and utilizing localized delivery methods.

Table 2. Next-Generation CAR T- Circuit Design and Function.

Circuit Type	**Core Logic**	**Biological Function**	**Solid Tumor Benefit**	**Reference**
Feedback-Controlled CAR	The CAR T-cell maintains internal activation balance.	Limits excessive CAR activation.	Minimizes activation toxicity, improving CAR T-cell safety	[147]
Hypoxia-responsive CAR	They detect hypoxia within tumor microenvironments.	Activates only within hypoxic tumor regions	Hypoxia-limited CAR T-cell activation reduces off-target effects	[148]
Kill-Switch Enhanced CAR	Kill-switch prevents damage to normal tissues	Allows emergency CAR T- shutdown	Prevents normal tissue toxicity via CAR T-cell shutdown	[149]
Metabolic CAR	CAR T-cell performance improves under metabolic stress	Enhances CAR T-cell survival in hostile TME.	Survives hostile TME.	[150]
Armored CAR T-cell (PD-1 scFv-secreting)	CAR T-cell delivers checkpoint-blocking PD-1 scFv.	Overcomes PD-L1 immunosuppression, enhancing CAR T-cell function	Enhances CSC targeting and improves CAR T-cell persistence.	[151]

Furthermore, a promising strategy to circumvent the limitations posed by antigen expression on normal tissues involves targeting tumor-restricted post-translational modifications. Notably, solid tumors often overexpress truncated O-glycans such as Tn (GalNAcα1-O-Ser/Thr) and sialyl-Tn (STn) (NeuAcα2–6-GalNAcα1-O-Ser/Thr), which are largely absent in healthy cells. These tumor-specific glycoforms offer a refined target profile that may enhance selectivity and reduce off-tumor effects, thereby advancing the precision of CAR T-cell therapies for solid malignancies [152].

5.6. Adverse Reactions

While CAR T-cell therapy has undeniably transformed the landscape of cancer treatment, its adoption as a frontline option remains limited due to the high incidence of toxicities, some of which can be fatal. The occurrence and severity of adverse effects such as CRS, hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), and immune effector cell-associated neurotoxicity syndrome (ICANS) are influenced by multiple factors including the CAR construct design, the specific antigen targeted, and the tumor type involved [153].

CAR T-cell therapy-related toxicities predominantly manifest as CRS and neurotoxicity, both of which can complicate treatment across hematological and solid tumors. CRS results from an exaggerated immune response characterized by excessive secretion of inflammatory cytokines, leading to a spectrum of clinical symptoms such as fever, fatigue, muscle pain, hypotension, hypoxia, coagulopathies, capillary leak syndrome, and even multi-organ failure, representing a significant risk of mortality [154]. Management of severe CRS often involves administration of immunosuppressive agents such as tocilizumab, either alone or in combination with corticosteroids [155]. ICANS represents a potentially life-threatening neurotoxic complication associated with CAR T-cell therapy. Patients may experience neurological symptoms including delirium, aphasia, encephalopathy, seizures, tremors, and in rare instances, rapid cerebral edema [156,157]. Proposed mechanisms underlying ICANS include disruption of the BBB and elevated production of cytokines such as IL-6, IL-8, IP-10, and MCP-1 [158].

The introduction of co-stimulatory domains in next-generation CAR designs, while enhancing efficacy, may also exacerbate the risk of severe toxicities. To address these challenges, innovative safety measures such as incorporating inducible suicide genes, such as iCaspase9, have been developed. These suicide switches enable timely termination of CAR T-cells, thereby mitigating cytotoxic damage and systemic adverse effects [159].

6. Challenges Faced with CAR T-Cell Targeting of Cancer Stem Cells

CSC-targeted CAR T-cell therapy holds considerable promise but faces substantial challenges across multiple functional domains. Toxicity remains a primary barrier, as many CSC markers—including CD133 and ALDH—are also expressed on normal progenitor cells, creating significant on-target/off-tumour risks [160–162]. Strategies to address this include the use of safer antigens, dual-targeted CAR constructs to enhance tumour specificity [163], intratumoral delivery to reduce systemic exposure [164], and incorporation of suicide genes or inhibitory receptors as built-in safety switches [165,166]. Additional adverse events, such as CRS and ICANS, remain clinically important and are managed with IL-6 blockade, corticosteroids, and supportive interventions [167–170].

Limited persistence and potency—particularly in solid tumours—further restrict therapeutic efficacy [171]. Enhancements have been achieved through optimized co-stimulatory domains such as: 4-1BB, ICOS, OX40, and CD27,71–74 integration of immune checkpoint blockade elements targeting PD-1, CTLA-4, TIM3, LAG3; and A2AR,74 and cytokine-armoured CAR T-cells engineered to express IL-12, IL-18, IL-7, IL-15, or IL-21 [172–175]. More recently, CARs incorporating JAK-STAT signalling domains demonstrated superior proliferative and anti-tumour capacity [54].

Overcoming poor trafficking into solid tumours remains essential, as CSCs are often shielded by dense stroma [176]. Approaches include local delivery [177],chemokine receptor engineering (CCR4, CCR2b, CXCR2) [178–180], and combining CAR T-cell therapy with cytoreductive treatments [181]. CAR T-cell infiltration is further hindered by ECM barriers, driving interest in FAP-targeted CAR T-cells [182], VEGFR2-targeted vascular disruption [183], and heparanase-expressing CAR T-cells [143].

Within the immunosuppressive TME, inhibitory cytokines, suppressive immune cells, and PD-L1 expression on CSCs reduce CAR T-cell functionality [184–190]. Supporting CAR T-cells with cytokine secretion [175], neutralizing TGF-β or IL-10 [187], in combination with PD-1/PD-L1 or CTLA-4 blockade offer promising strategies [114,188–191]. Finally, pronounced heterogeneity within CSC populations contributes to antigen escape [192–196]. Bispecific or multi-target CAR T-cells [197] as well as CAR T-cells engineered to secrete BiTEs that recruit bystander T-cells [198–200] represent key innovations to overcome this limitation.

7. Conclusions

In response to the challenges presented in the preceding sections, recent strategies have focused on engineering CAR T-cells to better navigate and resist the immunosuppressive cues of the TME. Approaches such as the incorporation of synthetic receptors responsive to tumor-derived signals, armored CAR constructs that secrete pro-inflammatory cytokines, and combination therapies targeting immune checkpoints have shown promise in enhancing T-cell infiltration, proliferation, and durability. These innovations aim not only to improve therapeutic efficacy but to fundamentally reshape the immunological landscape within solid tumors. Ultimately, overcoming the multifaceted resistance mechanisms of the TME holds the potential to catalyze a paradigm shift in the treatment of solid malignancies. A comprehensive and integrated approach—grounded in an in-depth understanding of tumor biology and immune evasion—will be essential for unlocking the full potential of CAR T-cell therapy in these complex settings.

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