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Review

MicroRNA-Orchestrated Modulation of CAR-T Cells: Overcoming Functional Barriers Toward Next-Generation Immunotherapy

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Abstract

Chimeric antigen receptor (CAR) T-cell therapy has revolutionized treatment outcomes in hematologic cancers, yet its impact on solid tumors remains limited due to barriers such as antigen heterogeneity, functional exhaustion, immunosuppressive microenvironments, and inefficient tumor infiltration. Recent studies identify microRNAs (miRNAs) as central regulators of T-cell activation, persistence, differentiation, and metabolic adaptation, functioning as post-transcriptional rheostats rather than simple on/off switches. By modulating checkpoint pathways, chemokine responsiveness, and resistance to hypoxic stress, specific miRNAs-including miR-17~92, miR-155, miR-210, and miR-21-have demonstrated potential to enhance CAR-T performance across multiple functional axes. Here, we synthesize evidence from mechanistic research, preclinical studies, and early clinical observations on emerging delivery strategies such as synthetic oligonucleotide inhibitors/mimics, vectorized circuits, circular-RNA systems, and exosome-based carriers. Together, these findings suggest that miRNA engineering offers a versatile platform for next-generation CAR-T design, enabling multi-layered functional reprogramming while underscoring the need for rigorous clinical validation in solid tumor settings.

1. Introduction

One of the most inventive developments in cancer immunotherapy is the use of CAR T cells, which have shown impressive results in the treatment of hematologic cancers [1]. With CAR T technology, tumor-associated antigens can be precisely targeted, and malignant cells can be effectively eliminated by fusing the cytotoxic capability of T cells with the antigen-recognition ability of antibodies [2]. Even in patients who are resistant to traditional treatments, CAR T therapy has consistently produced long-lasting remissions in blood malignancies, making it a revolutionary therapeutic approach.

Since its conceptualization in the late 1980s, CAR-T cell technology has undergone several generational refinements that have profoundly shaped its clinical efficacy. The first-generation CARs incorporated only the CD3ζ signaling domain, providing antigen specificity but limited proliferation and persistence. The addition of co-stimulatory motifs such as CD28 or 4-1BB in second-generation CARs markedly enhanced T-cell survival and cytokine production [3]. Third-generation constructs integrated dual co-stimulatory domains (e.g., CD28 and 4-1BB) for improved activation thresholds and enhanced persistence [4]. More recent fourth-generation "armored" CAR-Ts include cytokine secretion modules or ligand expression systems, such as IL-12 or CD40L, to counteract immunosuppressive tumor microenvironments [5]. Experimental studies further demonstrate the superior cytotoxic potential of these fourth-generation CARs through incorporation of multiple costimulatory motifs and cytokine secretion capabilities [6]. Finally, fifth-generation CAR-T constructs-often referred to as TRUCKs (T cells Redirected for Universal Cytokine Killing)-are equipped with inducible signaling modules such as STAT3/5 activation cascades, allowing them to sustain activity and metabolic fitness within immunosuppressive milieus [7]. It has been much harder to translate these achievements into solid tumors [2,8]. The effectiveness of CAR T in solid tumors and hematologic malignancies is severely hampered by fundamental characteristics, such as an immunosuppressive tumor microenvironment (TME), extensive extracellular matrix, antigen heterogeneity, and limited immune cell penetration [9]. Reduced durability and less than ideal long-term tumor control result from CAR T cells' frequent functional fatigue

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and decreased proliferative potential, even when they are able to penetrate tumor tissue. CAR T cell growth and long-term persistence in vivo are acknowledged as key factors influencing the effectiveness of treatment [10]. Memory-like T cells typically have longer lifespans, more capacity for proliferation, and enhanced resilience to fatigue. Terminally differentiated or fatigued cells, on the other hand, not only lack prolonged antitumor immunity but also have diminished cytotoxic capability. Therefore, one of the main goals in maximizing the therapeutic performance of CAR T cells is to direct them toward memory phenotypes [11].

To better evaluate these barriers and improve translational relevance, preclinical validation using patient-derived xenograft (PDX) models has become increasingly essential. Such systems preserve tumor heterogeneity and microenvironmental complexity, providing a more faithful bridge between in vitro discovery and clinical application [12,13].

MiRNAs are a family of short non-coding RNAs that range in size from 18 to 25 nucleotides. They have become important regulators of T cell function in recent years [14]. miRNAs can alter the pathways controlling T cell differentiation, proliferation, apoptosis, metabolism, and resistance to exhaustion by post-transcriptionally suppressing gene expression. While targeted modification of miRNA networks offers great promise to rewire CAR T cells toward more robust, long-lasting phenotypes, dysregulation of particular miRNAs has been associated with compromised immune function [15]. In light of these factors, the current review only discusses how miRNAs improve the functionality and persistence of CAR T cells in solid tumors. This work aims to identify important miRNA-mediated pathways, assess their therapeutic potential, and suggest strategic directions for the development of next-generation CAR T therapies that can overcome the particular challenges presented by solid tumor environments by combining knowledge from hematologic and solid tumor research.

2. CAR-T Cell Structure, Signaling Domains, and Generational Progression

The three primary components of chimeric antigen receptors (CARs) are an intracellular signaling endodomain, a transmembrane domain, and an external antigen-recognition ectodomain [16]. A hinge region is fused to a ligand-specific single-chain variable fragment (scFv) in the ectodomain. The variable domains of immunoglobulin heavy and light chains are joined by a flexible peptide linker to create the scFv29. The hinge offers structural flexibility for the best antigen binding and is frequently derived from immunoglobulin-like domains [17].

The CD3ζ signaling motif alone or in combination with one or more co-stimulatory domains can make up the endodomain. Co-stimulatory domains are necessary for complete T cell activation, even if the scFv controls antigen specificity. CAR-T cells are divided into five generations according to their endodomain architecture [18]. Only CD3ζ was present in first-generation CARs, which had poor persistence and needed outside cytokine assistance [19]. The introduction of a single co-stimulatory domain, generally referred to as CD28 or 4-1BB, by second-generation CARs enhanced survival, cytotoxicity, and proliferation [20]. Two co-stimulatory domains, such as CD28 with 4-1BB, ICOS, or OX40, were integrated into third-generation CARs [21]. To modify the tumor microenvironment, fourth-generation CARs, also known as TRUCKs, were designed to express extra functional molecules, such as switch receptors and chemokine receptors, or release cytokines [22]. Fifth-generation CARs then introduced IL-2 receptor-based motifs to support memory formation and sustain activity, enabling antigen-dependent JAK/STAT signaling (Figure 1) [23].

In order to eradicate endogenous TCR expression and improve CAR stability, more recent approaches use targeted genome editing, such as CRISPR-mediated insertion into the TRAC locus, whereas earlier generations depended on viral vector transduction with semi-random genomic integration [24]. Similar strategies improve anticancer activity and decrease tiredness by targeting the PDCD1 locus [25]. These developments show how CAR-T cells are increasingly being precision engineered to maximize their effectiveness.

Key issues, including functional exhaustion, limited durability, and the suppressive tumor microenvironment in solid tumors, have not, however, been adequately addressed by structural advances alone. Non-coding RNAs (ncRNAs) have a crucial role in regulating T cell differentiation, memory programming, and resistance to exhaustion at the transcriptional and post-transcriptional levels, where many of these restrictions are regulated [26]. The next stage in creating CAR-T treatments with long-lasting effectiveness against solid tumors may involve combining ncRNA regulation with enhanced CAR construction, as covered in the sections that follow.

Direct cytotoxicity, activation of death receptor pathways, and cytokine-mediated modification of the tumor microenvironment are the three primary ways whereby CAR T cells destroy tumor cells [27]. CAR T cells establish a lasting immunological synapse with target cells upon antigen identification through their modified receptor, which activates CD3ζ and co-stimulatory domains to drive effector responses. CARs have affinities that are frequently 100-1,000 times higher than natural TCR-peptide-MHC interactions, in contrast to conventional TCRs, and they can identify antigens regardless of MHC presentation [28].

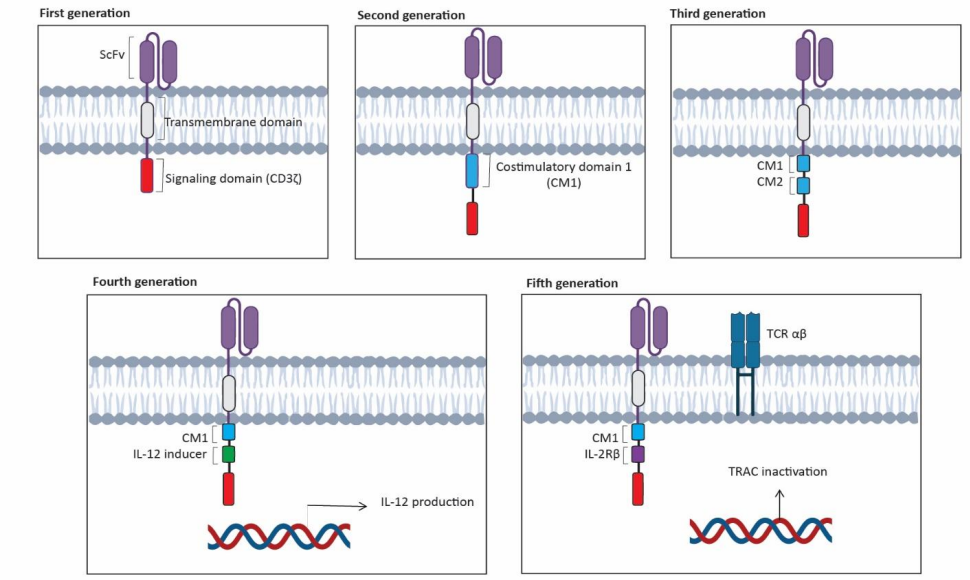

Figure 1. Structural evolution of CARs. The first generation of CARs includes a single-chain variable fragment (scFv) or ligand-based extracellular domain linked to the CD3ζ intracellular signaling region. The second generation adds one co-stimulatory domain, typically 4-1BB or CD28, to enhance T-cell activation. The third generation combines two co-stimulatory modules, most commonly 4-1BB and CD28, to further improve persistence and potency. Fourth-generation CARs, also called TRUCKs, incorporate genetic elements enabling cytokine release or additional signaling control. The fifth generation integrates three synergistic co-stimulatory and cytokine-linked signaling pathways for optimized antitumor response.

The main cytotoxic pathway is the perforin-granzyme axis, which causes apoptosis by secreting lytic granules that include perforin and granzymes, particularly GZMB and GZMA, at the synapse [29]. Fas-FasL-mediated apoptosis is a secondary route that, through bystander effects, can also target nearby tumor cells that do not have antigens [30]. Moreover, the release of cytokines like TNF-α and IFN-γ changes the tumor microenvironment and increases the tumor's susceptibility to immune attack (Figure 2) [31]. This is especially important in solid tumors, because CAR T infiltration and function are decreased by physical and immunosuppressive barriers [10].

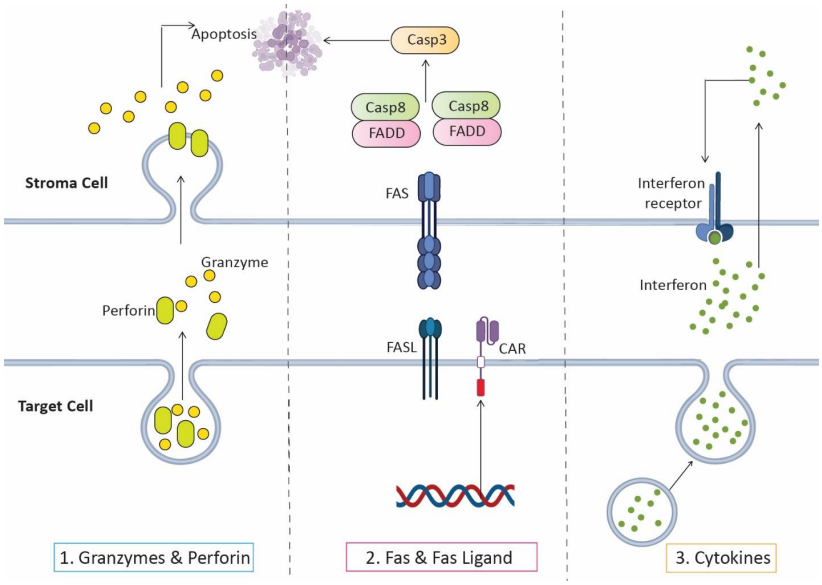

Figure 2. Antitumor mechanisms of CAR-T cells. (1) Perforin molecules form pores in tumor cell membranes, allowing granzymes to enter and trigger apoptosis. (2) CAR-T cells express high levels of Fas ligands, which bind to Fas receptors on tumor cells and induce programmed cell death. (3) Cytokines secreted by CAR-T cells remodel the tumor microenvironment by upregulating interferon signaling in stromal cells, enhancing immune activation, and amplifying antitumor effects.

Tumor type, antigen density, CAR affinity, and co-stimulatory signals all affect how these pathways are balanced [32]. Crucially, new data indicates that transcriptional and post-transcriptional processes also influence these effector systems. For instance, long non-coding RNAs can affect immunological synapse integrity and death receptor signaling, whereas certain microRNAs control the synthesis of cytokines, granzymes, and perforin. It may therefore be possible to improve CAR T cytotoxicity, persistence, and adaptability-particularly in the harsh microenvironment of solid tumors-by comprehending and modifying these ncRNA-mediated regulatory layers.

3. Integrated Overview of CAR-T Cell Therapy Challenges and the Regulatory Role of microRNAs

The treatment paradigm for hematologic malignancies has been completely transformed by CAR T cell therapy, which has produced individuals with relapsed or refractory disease with previously unheard-of response rates and long-lasting remissions [33]. However, there are currently no FDA- or EMA-approved CAR-T therapies in this scenario, indicating that its clinical translation to solid tumors has remained minimal. This discrepancy highlights the significant anatomical and molecular distinctions between solid and hematologic malignancies.

Four interconnected domains can be used to broadly classify the obstacles limiting CAR-T efficacy. First, antigen loss or heterogeneity, which is most noticeable in solid tumors, impairs antigen engagement and recognition [34]. Second, poor persistence after injection and manufacturing-related tiredness pose a threat to intrinsic T cell fitness [35]. Third, through regulatory immune cells, inhibitory cytokines, nutritional depletion, hypoxia, and extracellular matrix density, the TME exerts significant immunosuppressive pressure [36]. Lastly, systemic toxicities, including immune effector cell-associated neurotoxicity (ICANS) and cytokine release syndrome (CRS), continue to be significant treatment challenges [37].

An increasing amount of data suggests that these various issues are linked by transcriptional and post-transcriptional regulatory layers rather than being in isolation. The capacity to fine-tune antigen expression, adhesion molecule stability, checkpoint receptor signaling, metabolic adaptability, cytokine secretion, and chemokine receptor balance has made miRNAs a key modulator among these regulators [38]. MiRNAs are positioned as prospective molecular levers for creating next-generation CAR-T cells because of their capacity to coordinate intricate gene networks [39].

With a focus on improving CAR-T efficacy in solid tumors, we break down each of the four key difficulty categories in the sections that follow and investigate how focused alteration of miRNA networks could lessen these barriers (Figure 3).

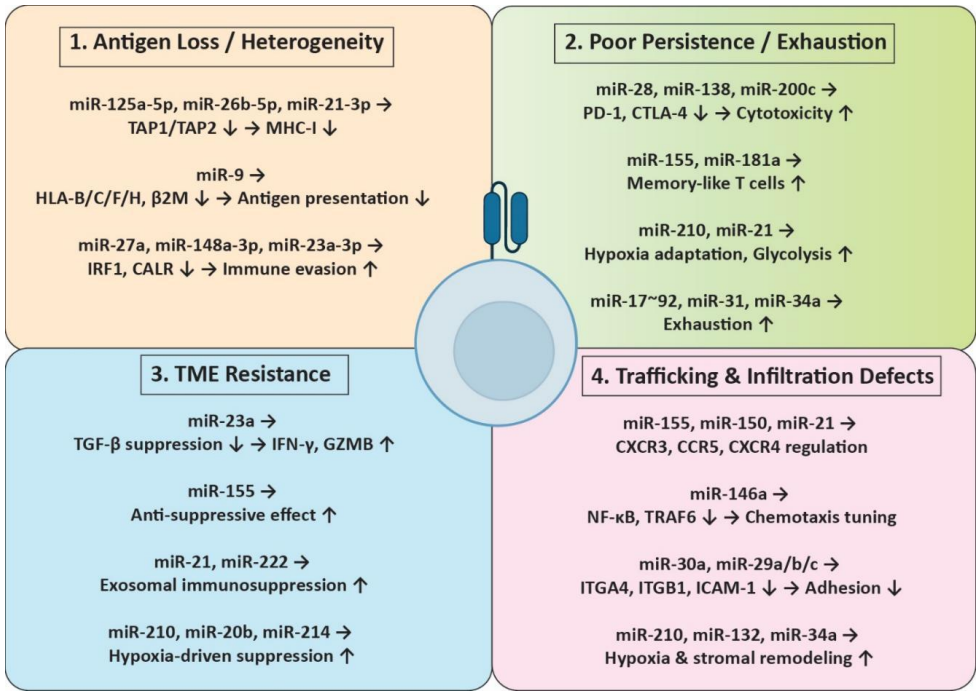

Figure 3. Integrated overview of CAR-T cell therapy challenges and regulatory roles of microRNAs**.** Schematic representation of four major domains limiting CAR-T cell efficacy in solid tumors: (1) antigen loss/heterogeneity, (2) poor persistence/exhaustion, (3) tumor microenvironment (TME) resistance, and (4) trafficking/infiltration defects. Key miRNAs involved in each domain are illustrated, highlighting their molecular targets and functional impact on antigen presentation, metabolic adaptation, immune checkpoint regulation, and migratory competence.

3.1 Antigen Loss/Heterogeneity

A critical obstacle undermining CAR-T cell efficacy in solid tumors is the spatial and temporal heterogeneity of tumor-associated antigens (TAAs), which can vary across tumor subregions or emerge during therapy, enabling immune escape. Unlike hematologic malignancies with largely uniform antigen expression, solid tumors present uneven antigen landscapes that challenge consistent CAR-T targeting [40]. Emerging evidence implicates tumor-derived miRNAs in orchestrating antigen loss and downregulating antigen processing pathways, further diminishing CAR-T cell recognition and contributing to immune evasion.

3.1.1 Antigen Recognition and Adhesion Stability

Modified lymphocytes can identify tumor-associated antigens (TAAs) displayed on major histocompatibility complex (MHC) molecules and establish a stable immunological synapse with target cells, making this capacity one of the first checkpoints in CAR-T cell–mediated tumor clearance [39,41]. Even though CAR designs avoid the traditional MHC constraint, intact adhesion molecules and antigen processing machinery (APM) are nevertheless essential for the increased immune visibility of tumor cells and the potency of CAR-tumor engagement [42]. Tumors use miRNA networks to disrupt these systems, thereby decreasing immune visibility at the earliest stage of cytotoxicity.

3.1.2 Antigen Presentation

Since several nodes of the system are controlled in unison, MHC-I antigen presentation is especially susceptible to miRNA-mediated suppression. While miR-125a-5p targets TAP2, which exacerbates the loss of peptide supply, miR-26b-5p and miR-21-3p inhibit TAP1, a transporter necessary for peptide translocation into the ER [43,44]. Integrating stress responses with immunological escape, the UPR factor XBP1 triggers miR-346 under ER stress, which downregulates TAP1 and HLA heavy chains [44]. Surprisingly, miR-9 collapses the presenting scaffold and the peptide processing machinery in one step by reducing surface expression of HLA-B, -C, -F, and -H in addition to repressing TAP1, PSMB8/9/10, and β2M [45-47].

Through supplementary targets, additional miRNAs strengthen this degradation. According to Colangelo et al. (2016), miR-27a decreases calreticulin (CALR), which affects peptide loading and is associated with poor $CD8^+$ infiltration in colorectal cancer [48]. HLA-A, -B, and -C transcripts are directly bound by miR-148a-3p [49], but IRF1, the transcriptional driver of HLA-I, is suppressed by miR-23a-3p, especially when TGF-β signaling is present [50]. MHC-II is also altered: let-7i, miR-146b-5p, miR-185-5p, miR-145-5p, and miR-198 converge on CIITA, and miR-212-3p suppresses RFXAP, all of which work together to reduce the expression of HLA-II generated by IFN-γ [51-54]. These combined effects demonstrate how cancers use overlapping miRNAs to undermine several antigens presentations checkpoints, guaranteeing immune evasion redundancy.

3.1.3 Non-classical MHC Regulation

Tumors utilize molecules other than traditional MHC-I. Loss of HLA-G-targeting miRNAs (miR-148/152 family and miR-133a) permits unrestricted expression and engagement of inhibitory receptors on effector cells where HLA-G, a tolerogenic factor in pregnancy, is aberrantly increased in several malignancies [55-57]. Dense miRNA networks also commonly suppress NKG2D ligands, including MICA/B and ULBPs, which are typically triggered by cellular stress to mark malignancies for elimination [58-60]. While MICA/ULBP2 is suppressed by miR-20a, miR-519a-3p, and miR-93/106b [59,61-63], MICA/B is generally silenced by clusters comprising miR-373, miR-29 (a/b/c), miR-15b, miR-195, miR-16, miR-424, miR-106a, miR-107, and miR-17 [63]. This axis is further subverted by hypoxia: the circ_0000977/miR-153 imbalance raises soluble MICA (sMICA), which saturates NKG2D without offering killable targets, thereby converting a stress alarm into a decoy [64]. Taken together, these data show how tumors actively skew immune signals to misdirect CAR-T engagement in addition to suppressing understanding.

3.1.4 Adhesion and Immune Synapse Formation

Sustained immunological synapse development and recognition are both necessary for stable tumor elimination. Thus, adhesion molecules constitute a second regulatory layer. MiR-221, miR-222, and miR-339 limit ICAM-1, which stabilizes T cell–tumor interaction via LFA-1, resulting in decreased granzyme delivery and shorter synapse dwell periods [65]. Similar downregulation of CD44, which miR-34a targets, affects tumor cell adhesion and migratory dynamics and is associated with weakened immune engagement in bladder and prostate cancer [66]. Through the simultaneous degradation of antigen presentation and adherence, tumors create a multi-layered barrier that compels CAR-T cells to engage in brief, non-fatal interactions.

3.2 Poor Persistence/Exhaustion

In addition to their ability to eradicate tumor cells, CAR-T cells' long-term effectiveness is based on their ability to endure, withstand fatigue, and continue to function normally in the hostile TME. Relapses connect with terminally exhausted (Tex) populations, but sustained responses are consistently associated with central memory (Tcm) and stem-like memory (Tscm) fractions, according to clinical follow-up of CAR-T patients. There is growing

evidence that miRNAs rewire metabolic resilience, lineage commitment, and checkpoint pathways to orchestrate this equilibrium [67].

3.2.1 Checkpoint Pathways And Exhaustion Resistance

Persistent antigen stimulation triggers the chronic production of PD-1, CTLA-4, TIM-3, and LAG-3, which gradually reduces effector functions. Many miRNAs attenuate this maladaptive course. MiR-28 restores cytokine release in worn-out T cells by directly suppressing PD-1, TIM-3, and BTLA [68]. According to Li et al., miR-138 increases cytotoxicity by suppressing both PD-1 and CTLA-4 [69]. While miR-200c modifies PD-L1/PD-1 loops between T cells and malignancies [70].

In addition to these intrinsic pathways, tumor-intrinsic miRNAs can also shape PD-L1 expression and thereby influence the intensity of inhibitory signaling, as shown in several tumor profiling studies [71]. Collectively, these findings underscore that checkpoint-targeting miRNAs function as dynamic modulators rather than simple binary switches, suggesting that their therapeutic manipulation must balance activation and restraint to avoid immune overdrive.

3.2.2 Memory Versus Effector Lineage Control

miRNA plays a crucial role in controlling the destiny of CAR-T cells, determining whether they develop into long-lasting memory pools or transient effectors. Targeting SOCS1, miR-155 increases STAT activity and encourages the growth of memory-like CD8+ T cells [72]. By maintaining Blimp-1, on the other hand, miR-23a enforces terminal effector differentiation [73]. While miR-181a fine-tunes TCR thresholds, guaranteeing a robust yet controlled response [74], miR-146a's absence increases cytokine release and memory potential [75]

This paradigm is strengthened by CAR-T-specific findings: In preclinical lymphoma models, the induced expression of miR-155 boosted the metabolic fitness and persistence of CAR-T cells [76,77]. These direct CAR-T results support the use of miRNA engineering as a tool to influence lineage trajectories throughout the cell-making process.

3.2.3 Metabolic Resilience

Metabolic flexibility is necessary for survival in hypoxic, nutrient-deficient tumor environments. Exhausted T-cell subsets remain trapped in glycolysis, whereas memory-like cells rely predominantly on fatty acid oxidation and oxidative phosphorylation (OXPHOS). MiR-210 strengthens glycolytic dependence under hypoxic conditions and contributes to mitochondrial dysfunction [78]. Other regulators-such as miR-33, which modulates cholesterol metabolism for membrane integrity [79], and miR-21, which enhances glycolysis through PTEN suppression [80]-further shape the metabolic adaptability of CAR-T cells. Broader evidence from cancer models highlights similar axes, including miR-378a-3p, which represses Glut1 [81], and miR-133a-3p, which limits glutamine metabolism in gastric cancer [82]. Collectively, these findings indicate that miRNAs act as integral components of metabolic reprogramming networks that define CAR-T persistence and functionality within hostile tumor microenvironments.

3.2.4 Differentiation Brakes and Accelerators

Numerous miRNAs function as drivers of pro-exhaustion. The miR-17~92 cluster promotes proliferation at the expense of premature exhaustion [83], miR-31 disrupts IFN-γ regulation [84], and miR-34a imposes terminal differentiation by suppressing SIRT1/Notch1 [85].

3.3 TME Resistance and the Regulatory Role of miRNAs

To extend CAR-T treatment to solid tumors, the immunosuppressive TME is one of the most difficult obstacles. Solid tumors, as opposed to hematological malignancies, embed CAR-T cells in a hostile milieu that is marked by metabolic stress, hypoxia, suppressive immune subsets, inhibitory cytokines, and external modulators such as immunological checkpoints and exosomes. Together, these elements weaken T cell activity, causing fatigue and a lack of tenacity. MiRNAs are key regulators of these processes, serving as molecular switches that connect environmental stimuli to transcriptional and post-transcriptional regulation of T cell activity, as evidenced by growing data.

3.3.1 Cytokine-Mediated Suppression

One important inhibitory axis within the TME is TGF-β signaling, which profoundly suppresses the cytotoxic activity of $CD8^+$ T cells. MiR-23a, induced downstream of the TGF-β/SMAD pathway, dampens effector function by repressing IFN-γ and granzyme B expression [85]. In contrast, miR-155 counteracts this pathway and promotes proliferation and cytokine production by attenuating TGF-β signaling [86]. The balance between these antagonistic miRNAs dictates whether CAR-T cells maintain effector functionality or become paralyzed within the TME. This reciprocal regulation also converges with broader exhaustion-related programs that shape long-term persistence and functional fate of CAR-T populations.

3.3.2 Immunosuppressive Cell Networks

Tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) grow to create an additional layer of TME resistance. According to Lu et al., miR-146a is essential for maintaining FoxP3 stability and suppressive activity in

Tregs [87]. MiR-494 supports the accumulation and inhibitory potential of MDSCs by targeting PTEN and activating PI3K/Akt signaling [88]. By upregulating PD-1, exosomal miR-21 and miR-222 produced from tumor cells rewire T cells in TAMs, strengthening fatigue characteristics [89,90]. According to this research, miRNAs are molecular messengers of suppression that increase the interaction between immune cells and tumor cells, strengthening resistance to CAR-T therapy.

3.3.3 Metabolic and Hypoxic stress

Solid tumors are characterized by nutritional restriction and hypoxia. According to Noman et al., hypoxia-induced miR-210 reduces mitochondrial respiration and promotes functional fatigue by stabilizing HIF-1α and reprogramming $CD8^+$ T cell metabolism toward glycolysis [78].

HIF-dependent pathways that influence T cell adaptation to low-oxygen settings are fine-tuned by other hypoxia-related miRNAs, such as miR-20b and miR-214 [91]. Loss of miR-101 further worsens functionality by releasing EZH2 activity and compromising effector differentiation [92]. These results demonstrate that metabolic reprogramming under hypoxia is actively coordinated by miRNAs, which function as regulators of CAR-T cell exhaustion programs, rather than being a passive adaptation.

3.3.4 Extrinsic Checkpoint and Exosomal Regulation

Tumor-intrinsic miRNAs such as miR-34a, miR-142-3p, and miR-200c modulate PD-L1 surface expression and thereby influence extrinsic checkpoint control at the tumor–T-cell interface. By adjusting these pathways, miR-142-3p can also restore effector cytokine production and enhance T-cell activation [93]. In addition to tumor-cell regulation, the suppressive network is reinforced through exosomal miRNAs released into the tumor milieu. For instance, miR-24-3p and miR-20a-5p carried by tumor-derived exosomes inhibit T-cell proliferation and cytotoxicity by reducing IL-2 and IFN-γ production [94]. Likewise, exosomal miR-222-3p and miR-940 derived from ovarian cancer cells promote M2-like macrophage polarization, amplifying local immunosuppression [95].

Together, these findings illustrate how both cell-intrinsic and exosome-mediated miRNA networks extend checkpoint regulation beyond direct receptor–ligand interactions, forming systemic barriers to CAR-T-cell efficacy within solid-tumor microenvironments.

3.4 Trafficking and Infiltration Defects

One of the most formidable barriers to CAR-T cell efficacy in solid tumors is the failure of infused cells to efficiently traffic to and infiltrate the tumor bed. Unlike hematologic malignancies, where malignant cells circulate and remain readily accessible, solid tumors impose multiple layers of physical and biochemical constraints, including abnormal vasculature, dense extracellular matrix, immunosuppressive stroma, and hypoxia-driven chemokine gradients. These factors collectively impair the homing, extravasation, and persistence of CAR-T cells within the tumor microenvironment [96]. Preclinical studies have shown that inadequate trafficking significantly limits the therapeutic window even when CAR-T cells retain potent cytotoxic capacity in vitro.

3.4.1 Chemokine-Receptor Matching: Recalibrating the Compass

Effective homing of CAR-T cells into solid tumors depends on the alignment between tumor-secreted chemokines and T-cell chemokine receptors. The CXCR3-CXCL9/10/11 axis is essential for CD8+ effector T-cell trafficking into tumors, and its disruption or insufficient intertumoral chemokine expression is associated with poor T-cell infiltration and limited tumor control. Mechanistic studies in murine melanoma have shown that CXCR3 is non-redundant for CD8+ T-cell recruitment, while immune profiling across multiple tumor types links high expression of CXCR3 ligands with improved effector T-cell access and antitumor activity. These data collectively establish chemokine–receptor alignment as a prerequisite for CAR-T cells to access the tumor microenvironment [97,98].

MicroRNAs have emerged as key regulators of this chemokine–receptor network. In donor T cells, miR-155 sustains CCR5 and CXCR4 expression, and its loss leads to impaired chemokine-driven migration and reduced tissue infiltration, although excessive miR-155 expression can promote hyperactivation and exhaustion [99]. In contrast, miR-150 directly targets CXCR4, and its downregulation enhances CXCR4/CXCL12 signaling, increasing T-cell responsiveness to stromal chemokine gradients [100]. Activation-induced miR-21 modulates CCR7 expression in primary human T cells, diverting homing patterns away from lymphoid tissues and favoring migration into peripheral sites [101]. Similarly, miR-146a attenuates NF-κB signaling through TRAF6 and IRAK1, indirectly dampening chemokine receptor activity and inflammatory chemotaxis, a mechanism that could fine-tune CXCR3-mediated responses [102]. Additional work in hepatocellular carcinoma has identified miR-940 as a negative regulator of CXCR2, suggesting that miRNAs can shape leukocyte recruitment into tumors enriched for ELR+ chemokines [103]. Evidence from human inflammatory disease supports these findings: in pulmonary tuberculosis, patient tissues show elevated miR-30a-3p and reduced let-7b-5p alongside increased CXCL10 expression, linking miRNA dysregulation with aberrant CXCR3-CXCL10 signaling in vivo [104].

Together, these studies describe a miRNA-governed chemokine tuning network that determines the migratory

competence of CAR-T cells. Targeted modulation of this network-for example, preserving CXCR3 pathways while relieving miRNA-mediated repression of CCR5, CXCR4, or CCR7, or preventing excessive NF-κB inhibition-may restore chemokine-receptor alignment and improve CAR-T trafficking into poorly accessible solid tumors.

3.4.2 Adhesion and Extravasation: Tightening the Molecular Velcro

CAR-T cells must halt on the endothelium and transmigrate into tissue even with precise chemokine sensing. LFA-1-ICAM-1 engagement and the relationship between endothelial VCAM-1 and VLA-4 (ITGA4/ITGB1) are crucial [105]. MiR-30a silences ITGA4, reducing VLA-4-VCAM-1 adhesion and preventing extravasation [106]. The repression of ITGB1 by the miR-29 family (a, b, and c) compromises integrin-dependent adhesion and ECM navigation [107]. Endothelial miRNAs also serve as barriers: miR-126 inhibits VCAM-1 and interferes with VLA-4 connections [107], while miR-221/222 and miR-339 decrease ICAM-1 production, which restricts lymphocyte binding and transendothelial migration [65]. These miRNAs work together to prevent CAR-T cells from "stopping and crossing" vascular barriers, which is a crucial checkpoint that can be circumvented through targeted miRNA modification.

3.4.3 Cytoskeletal Remodeling and Motility: Enforcing Polarity and Directionality

T cells must take on a polarized shape, expand their lamellipodia, and produce traction against the stiff extracellular matrix once they are inside the tumor parenchyma. Targeting RAC1, miR-142-3p interferes with directed motility and actin polymerization [108]. RhoA/CDC42 is modulated by miR-31, which increases motility but at the expense of coupling to programs linked to exhaustion [109]. Defective polarity and ineffective migration result from miR-24's destabilization of CDC42 [110]. Collectively, these miRNAs determine the "quality" of migration, determining whether CAR-T cells meander aimlessly through stromal tissue or follow fruitful, antigen-seeking pathways.

3.4.4 Hypoxia and Stromal Adaptation: Rewriting the Tissue Barrier

Even after extravasation, the metabolic and stromal obstacles presented by the tumor microenvironment can inhibit invasion. Disoriented migration is caused by hypoxia-induced miR-210, which skews CXCR4 signaling and interferes with mitochondrial metabolism [111]. miR-132 affects integrin-ligand availability by modulating vascular tone and normalization through the suppression of p120RasGAP-21 [112]. miR-200c remodels stromal retention signals via interacting with CXCL12/CXCR4 signaling and the epithelial-to-mesenchymal transition (EMT) [113]. Last but not least, miR-34a suppresses PD-L1, providing an extra benefit by combining infiltration with checkpoint resistance [114].

Hypoxia also activates tumor-intrinsic ncRNA programs, which further strengthen immunological exclusion, in addition to T cell–intrinsic circuits. When hypoxia signaling interferes with the circ_0000977/miR-153 axis in pancreatic cancer, membrane MICA is downregulated and soluble MICA (sMICA), an immune evasion factor that desensitizes NK and T cells, is released concurrently [13,64]. Similarly, exosomes enriched in miR-21-3p, miR-125b-5p, and miR-181d-5p are secreted by hypoxic epithelial ovarian cancer cells. These exosomes skew macrophages toward an M2-like phenotype, enhancing tumor development and stromal suppression [115]. Hypoxic glioma cells produce exosomal miR-29a and miR-92a, which increase the activity and proliferation of myeloid-derived suppressor cells (MDSCs) by suppressing Hbp1 and Prkar1a [116]. Hypoxia-induced miR-10a and miR-21 also encourage MDSC growth and immunosuppressive function [116].

These tumor-derived ncRNA programs and T cell-intrinsic miRNAs work together to coordinate angiogenesis, stromal suppression, and hypoxia adaptation. The ultimate effect is a layered hypoxic barrier: CAR-T cells have to face exosome-mediated recruitment of suppressive myeloid and macrophage populations, activating ligand shedding, and impaired metabolic fitness in addition to misdirected chemokine responses. To enable persistent CAR-T infiltration and persistence in solid tumors, it may be imperative to target these ncRNA-driven hypoxic adaptations.

4. MicroRNA Modulation as a Precision Layer in Next-Generation CAR-T Cells

During the past decade, the understanding of miRNAs in adoptive T-cell therapies has moved well beyond theoretical interest, showing increasing promise as a practical tool in cellular engineering. Rather than acting as simple on–off switches, miRNAs fine-tune post-transcriptional networks in a dose-dependent manner, influencing multiple aspects of T-cell biology such as survival, differentiation patterns, metabolic fitness under stress, trafficking behavior, and susceptibility to exhaustion. Because these processes collectively limit CAR-T efficacy in solid tumors, the ability of miRNAs to regulate entire gene networks-rather than single genes-offers a potential route to overcome several barriers simultaneously.

Experimental studies provide a foundation for this approach. Work on the miR-17~92 cluster demonstrated that silencing pro-apoptotic and inhibitory molecules like Bim and PTEN improves T-cell survival and persistence, as confirmed in both knockout and transgenic mouse models [117]. There is

also growing evidence that miRNAs can reshape chemokine receptor expression to improve homing of T cells into tumor beds. For example, miR-103 has been shown to suppress CCR5 expression in CD4+ T cells, while miR-147-3p influences CXCR3 expression in inflammatory Th1 populations [118,119]. In parallel, studies on miR-155 highlight its central role in sustaining T-cell activation, proliferation, and persistence, features directly relevant to CAR-T cell durability [120]. Recent research has demonstrated that miR-379-5p induces a memory-like state and reduces $CD8^+$ T-cell exhaustion in tumor environments [121], whereas blocking miR-23a prevents TGF-β-induced paralysis of CTLs by reestablishing BLIMP-1, granzyme B, and IFN-γ [121]. It has been suggested that inhibiting hypoxia-induced miR-210 can increase directional migration and uncouple hypoxic stress from metabolic collapse by connecting HIF signaling to mitochondrial reconfiguration and chemokine responsiveness [78].

Clinical translation of these insights has already begun. In the IMMUNICY-1 trial (NCT04613557), the allogeneic CAR-T product CYAD-211 incorporated a miRNA-based shRNA cassette to silence endogenous TCR/CD3ζ expression [122]. Early-phase results demonstrated both the safety and feasibility of this RNA interference approach for allogeneic CAR-T manufacturing. Additional studies using shRNA modules to downregulate PD-1 in CAR-T cells further support the potential of RNA-based strategies to prolong activation and enhance anti-tumor effects [123].

Taken together, these findings suggest that miRNAs function as a regulatory layer at the network level, capable of stabilizing adhesion dynamics, cytoskeletal polarity, exhaustion thresholds, and chemotactic programming in engineered T cells. The following section will build on this rationale by examining specific examples where miRNA manipulation has directly improved CAR-T performance in preclinical and clinical settings.

5. Delivery Platforms and Clinical Translation of miRNA Modulation in CAR-T

Effective, regulated, and clinically scalable delivery methods are ultimately necessary to transform miRNA biology into therapeutic benefit for CAR-T cells. A growing corpus of research indicates that miRNA manipulation might boost trafficking, provide safety logic to cellular therapies, resist exhaustion/TGF-β/hypoxia, and improve persistence across synthetic oligonucleotides, vectorized expression cassettes, and circular-RNA (circRNA) formats and exosome carriers.

The effective delivery of miRNA modulators into CAR-T cells remains a major technical hurdle. In addition to conventional electroporation and viral transduction, a wide array of nanoparticle-based, liposomal, and polymeric systems is being investigated to enhance intracellular uptake and durability. Cationic liposomes and lipid nanoparticles (LNPs) provide efficient encapsulation and membrane fusion, while polymeric carriers such as polyethyleneimine (PEI) and poly (lactic-co-glycolic acid) (PLGA) offer tunable charge density and biodegradability for sustained release. Optimization of these vectors—through surface modification, charge balancing, and PEGylation-improves serum stability and minimizes premature degradation. Moreover, strategies such as targeted ligand conjugation and controlled-release scaffolds can increase cell-type specificity and reduce systemic exposure. To mitigate off-target and immunogenic effects, rational design of sequence complementarity, transient exposure windows, and incorporation of immune-silent chemical modifications (e.g., 2′-O-methyl or phosphorothioate backbones) are being increasingly applied. These engineering refinements collectively advance the stability, efficiency, and biosafety of miRNA delivery in CAR-T-cell applications.

5.1 Synthetic Oligonucleotide Approaches

Synthetic oligonucleotides which can act as antagonists or mimics, provide a temporary and accurate method for reprogramming miRNA activity. Oligonucleotide inhibitors and mimics offer a reversible method of "dialing" miRNA activity during ex-vivo manufacture or the initial post-infusion phase. Since TGF-β is a dominant suppressive axis in the majority of solid tumors, blocking miR-23a, a TGF-β-induced brake in human CTLs, restores BLIMP-1, granzyme B, and IFN-γ and enhances tumor control in vivo [12,121,124]. This benefit is immediately transferable to CAR-T. In tumor-antigen-specific CD8 T lymphocytes, transient miR-155 gain-of-function promotes tumor control, glycolytic competence, and proliferation while facilitating brief "pulses" of a pro-fitness miRNA during cell preparation [54,76].

Based on mechanistic research demonstrating ISCU1/2 suppression and mitochondrial collapse, hypoxia-linked miR-210 antagomirs make sense as a defense mechanism against hypoxic environments for CAR-T cells [78]. In actuality, these oligos can be combined with lipid systems already employed in cancer or with the conventional electroporation method utilized for mRNA-CARs, which is consistent with the larger RNA-therapeutics strategy emphasized by recent studies [125]. Additionally, CTL experiments indicate that anti-miR treatment prior to ACT was sufficient to imprint long-lasting functional improvements, resulting in preclinical ex vivo dosing windows [126]. This temporary method provides control and reversibility, which are benefits during first-in-human translation when combined with quality-by-design release criteria.

5.2 Vectorized miRNA Circuits for Stable Reprogramming

Incorporating genetic regulatory elements-whether delivered through viral or non-viral systems-into CAR constructs enables durable and cell-intrinsic reprogramming. The integration of miRNA logic, in particular, allows for sustained multi-axis modulation. For instance, a landmark GBM study demonstrated that co-expression of the miR-17~92 cluster with an EGFRvIII-CAR enhanced T-cell persistence by suppressing the pro-apoptotic factor Bim, elevating IFN-γ production, and maintaining antitumor efficacy even in the presence of temozolomide [127]. Enforced miR-155 also enhanced proliferation and antitumor performance in CD19 CAR-T cells [77]. Translationally, CYAD-211 (allogeneic anti-BCMA) suppresses endogenous TCR/CD3ζ and reduces GvHD by using a miRNA-based shRNA module in a single vector-representing first-in-human proof that miRNA circuits may be securely placed in a clinical-grade CAR-T without genome editing [122]. Non-viral systems (like transposons) and activation-linked promoters further expand this region, enabling expression to align with T-cell activation stages. This strategy has been often suggested in RNA-engineering reviews as a way to reduce off-pathway danger. These findings collectively demonstrate that vectorized miRNA programs can increase exhaustion thresholds, increase durability, and incorporate safety logic into the CAR architecture.

5.3 Circular RNA Systems for Enhanced Stability and miRNA Sponge Logic

In contrast to linear mRNA, circRNAs provide exceptional stability and decreased innate immune detection. They can also function as miRNA sponges and expression templates. Compared to linear mRNA, electroporated circRNA promoted more robust CAR production and better tumor clearance in DLL3-targeted circRNA-CAR-T for SCLC [128]. In addition to encoding the CAR, synthetic circRNAs can be engineered to absorb hypoxamiRs (miR-210) or exhaustion-linked miRNAs (e.g., miR-31/miR-23a), safeguarding important transcripts during hypoxic stress [78,121,129]. Therefore, circRNA formats provide a condensed path to multi-target miRNA control while addressing two translation bottlenecks: immunogenicity and durability.

5.4 Exosome-Mediated miRNA Delivery

A growing body of review literature underscores how such exosomes can transcend traditional CAR-T/tumor immunological synapses, facilitating broader paracrine modulation of the tumor milieu [130]. In functional studies, exosomes isolated from activated CAR-T cells have demonstrated inherent antitumor activity and mimic parent-cell cytotoxic functions through encapsulated granzyme-containing vesicles [131]. Innovatively engineered systems now enable exosome-mediated delivery of CAR mRNA alongside T-cell activation signals: Si et al. developed exosomes expressing anti-CD3/CD28 scFvs and packaging CAR mRNA via LAMP-2B/MS2 systems to both stimulate and reprogram T cells ex vivo [132]. Exosomes-owing to their low immunogenicity, ability to traverse physiological barriers, and customizable cargo-can recruit endogenous immune effectors, reshape the tumor microenvironment, and deliver functional payloads with potentially lower systemic toxicity compared to free oligonucleotides [133,134].

5.5 Safety Engineering and Clinical Translation

Clinical evidence has demonstrated that non–gene-edited shRNA/miRNA modules can be successfully integrated into allogeneic CAR-T cell constructs. A notable example is CYAD-211, which co-expresses an anti-BCMA CAR alongside a miRNA-based shRNA targeting CD3ζ, effectively suppressing TCR/CD3 expression and preventing graft-versus-host disease, as confirmed in a Phase I clinical evaluation [122]. Moreover, miRNA-guided detargeting strategies-such as inserting binding sites for tissue-specific miRNAs like liver-enriched miR-122 into the 3′ untranslated region (3′ UTR) of therapeutic transcripts-have been shown to significantly reduce off-target expression, enhancing tissue specificity across multiple delivery platforms [135]. As the field advances, exosome-based delivery mechanisms and circRNA constructs are maturing, enabling the CAR-T engineering toolkit to include both durable, circuit-level reprogramming and transient, dose-controlled modulation. Collectively, RNA-based modalities—including miRNA, siRNA, and circRNA-afford a graded and context-aware regulatory framework, moving beyond binary on/off control and paving the way for safer, more physiologically balanced cellular therapies (Table 1).

Table 1. Representative miRNAs, delivery platforms, mechanistic goals, and translational evidence in CAR-T cell engineering.

Delivery platform	Example miRNAs / circuit	Mechanistic goal	Modulation type	Preclinical / Clinical evidence	Notable product / trial	References (corrected)
Synthetic oligonucleotides	miR-23a, miR-155, miR-210	TGF-β resistance, memory programming, hypoxia adaptation	mimic / antagomir	Preclinical (murine & CAR-T models)	Lipid carriers, electroporation	[12,54,76,78, 121,124-126]
Vectorized miRNA circuits	miR-17~92, miR-155, PD-1 shRNA	Persistence, checkpoint modulation	shRNA in viral / non-viral vectors	Preclinical + Early clinical evidence	CYAD-211 (NCT04613557)	[77,122,127]
Circular RNA systems	miR-210 sponge, CAR circRNA	Hypoxia adaptation, CAR durability	circRNA sponges / templates	Preclinical (DLL3-CAR-T mouse models)	DLL3 circRNA-CAR-T	[78,121,128, 129]
Exosome-mediated delivery	miR-21 antagomir, CAR mRNA + miRNA	In situ TME modulation, local reprogramming	Engineered CAR-T exosomes	Preclinical (CAR-T + exosome studies)	Exosome CAR-T prototypes	[130-134]
Safety / detargeting modules	miR-122 sites, CD3ζ shRNA	Off-tumor detargeting, GVHD control	shRNA within miRNA scaffold	Early clinical (CYAD-211, AB-1015)	IMMUNICY-1, AB-1015 (NCT05617755)	[122,135]

6. Clinical Translation Gap in miRNA-Engineere CAR-T Cells

MiRNA-based engineering of CAR-T cells represents a compelling strategy for achieving tunable, multiplexed, and physiologically compatible gene regulation. By exploiting endogenous regulatory circuits, miRNA scaffolds enable graded rather than binary gene silencing, thereby lowering the risk of deleterious loss-of-function effects while preserving essential cellular functions [135]. The natural clustering of miRNAs further allows for simultaneous regulation of multiple targets, a feature well-suited to addressing the multifactorial barriers in adoptive cell therapy such as immunosuppression, T-cell exhaustion, and allo-reactivity [136].

Embedding shRNA cassettes within miRNA backbones preserves physiological processing pathways and avoids cytoplasmic saturation, as shown in preclinical studies, resulting in safer and more controllable gene silencing [136]. Collectively, these characteristics make miRNAs a flexible and clinically scalable toolkit for immune cell programming. The first proof-of-concept in humans came from *CYAD-211*, an allogeneic BCMA-directed CAR-T product incorporating an shRNA module within a miRNA scaffold to silence endogenous TCR/CD3ζ expression and mitigate graft-versus-host disease-without requiring genome editing. In the Phase I IMMUNICY-1 trial, CYAD-211 showed a favorable safety profile with no GvHD events and early antitumor activity [122].

Additional early-phase studies (e.g., NCT04613557, NCT03466320) have supported the safety and feasibility of RNAi-based circuits in immune cells, with multiplex miRNA/shRNA modules enabling both immune checkpoint modulation and cytokine tuning [137-139]. For example, IL-6 knockdown cassettes inserted into CD19 CAR constructs reduced cytokine release syndrome risk without compromising cytotoxicity in NCT04825496 [140], while GM-CSF silencing attenuated inflammatory cascades in preclinical studies [136]. Moreover, integrated circuit T-cell platforms such as AB-1015 (NCT05617755) and AB-2100 (NCT06245915) combine multiplex shRNA-miR modules with antigen-gated logic to achieve enhanced expansion and persistence in preclinical models and are currently under clinical evaluation for ovarian and renal cell carcinomas [141,142] (clinicaltrials.gov/NCT05617755, clinicaltrials.gov/NCT06245915).

Despite these advances, translation beyond early-phase studies remains limited. No completed trials have conclusively shown that miRNA circuits enhance tumor trafficking, persistence, or resistance to exhaustion. For instance, elevated tumor-derived miR-125a levels in extracellular vesicles have been associated with T-cell exhaustion and CAR-T failure in non-responders [143], but causal links remain unproven. Finally, challenges persist regarding delivery durability, pleiotropic effects, and pathway interactions, as transient oligonucleotide systems lack persistence, while viral and circRNA-based delivery approaches require further safety validation. Given the complexity of solid tumors-including hypoxia, stromal barriers, and antigen heterogeneity-combinatorial and multiplexed strategies will likely be necessary for robust therapeutic benefit. In summary, miRNA-based CAR-T engineering has moved from conceptual frameworks to clinical feasibility, with *CYAD-211* serving as a milestone [122]. However, overcoming barriers in delivery systems,

functional augmentation beyond safety switches, and pharmacodynamic characterization will be essential for these tools to become integral components of next-generation adoptive cell therapies.

While early-phase studies such as CYAD-211 have demonstrated the feasibility and safety of incorporating miRNA circuits into CAR-T constructs, broader clinical validation remains essential. A greater number of well-designed clinical trials are needed to evaluate miRNA-engineered CAR-T therapies across diverse solid and lymphoid malignancies. Such studies should include comprehensive long-term follow-up to assess sustained efficacy, durability, and potential delayed toxicities. Expanding the clinical evidence base will be critical for establishing the translational robustness and safety profile of miRNA modulation in CAR-T therapy.

7. Considerations of miRNA-Engineered CAR-T Cells

7.1 Functional and Context-Dependent Limitations

Despite the growing promise of miRNA-based engineering, the biological functions of individual miRNAs are strongly context-dependent and may vary between tumors, T-cell subsets, and activation states. For example, miR-21 promotes glycolysis and immunosuppression downstream of PTEN/AKT in tumors [144-146], yet transiently supports effector differentiation in activated T cells [147]. Similarly, miR-155 enhances persistence and antitumor function but, when overexpressed, may trigger hyperactivation and exhaustion [76,148,149]; hypoxia-induced miR-210 supports survival under low oxygen yet reinforces glycolytic dependence [146]; and miR-17~92 boosts proliferation at the cost of early exhaustion [150]. These bidirectional effects underscore the pleiotropy of miRNA networks and the need for dosage and timing control.

To mitigate these risks, rational design should employ dose-controlled or inducible expression systems, transient oligonucleotide delivery during ex vivo manufacturing, and multiplex “soft-tuning” across several miRNAs rather than drastic modulation of a single target [151]. Comprehensive pharmacodynamic monitoring-encompassing cytokine production, checkpoint expression, and metabolic profiles-remains essential to ensure physiological balance and avoid functional collapse of engineered T cells.

7.2 Biosafety and Ethical Aspects

The translation of miRNA-engineered CAR-T therapies also require rigorous biosafety evaluation. Introducing exogenous RNA molecules or nanoparticle carriers may elicit innate and adaptive immune activation distinct from DNA- or protein-based systems, potentially exacerbating cytokine-release or neurotoxicity syndromes already associated with CAR-T therapy [1,2]. Use of integrating viral vectors introduces the risk of insertional mutagenesis and clonal expansion, mandating standardized vector-copy-number (VCN) quantification and long-term genomic surveillance [3,4].

Moreover, miRNA constructs can affect multiple endogenous transcripts, creating potential for off-target repression or on-target/off-tumor toxicity when target genes overlap with normal hematopoietic or stromal compartments [5]. These risks can be minimized by adopting transient, non-integrating delivery systems such as mRNA electroporation or lipid nanoparticles [6], along with transcriptome-wide off-target mapping and immunogenicity testing [7].

Ethically responsible translation depends on transparent risk–benefit assessment, adherence to regulatory guidance, and inclusion of longitudinal follow-up programs to monitor genotoxicity, immune recovery, and patient-reported outcomes [8]. Collectively, these measures ensure that the advancement of miRNA-engineered CAR-T therapies proceed safely, balancing innovation with patient welfare.

8. Comparative Analysis of Gene-Regulation Platforms in CAR-T-Cell Engineering

A growing spectrum of gene-regulatory platforms has been explored to optimize the performance and safety of CAR-T cells, including microRNA (miRNA) modulation, CRISPR/Cas9-based genome editing, RNA interference (siRNA/shRNA), and protein- or pharmacologic-level interventions. Each of these approaches operates at a distinct molecular tier-ranging from transcriptional to post-transcriptional and post-translational control-and presents specific advantages and constraints in terms of reversibility, specificity, multiplexing capability, manufacturability, and clinical translation.

8.1 Mechanistic Scope and Modality

miRNA-based modulation functions at the post-transcriptional level, enabling coordinated regulation of multiple genes and pathways through endogenous RNA-processing machinery. This network-level property has been particularly valuable for the metabolic and mitochondrial reprogramming of CAR-T cells, where harmonized control of oxidative and biosynthetic programs enhances persistence and anti-tumor fitness [77,152]. RNA-interference platforms, based on shRNA or siRNA, achieve transcript-specific silencing via the RNA-induced silencing complex (RISC). When integrated into viral vectors, shRNA constructs can provide stable, long-term repression after tumor infiltration [153-155]. By contrast, CRISPR/Cas9 enables permanent gene disruption at the genomic level and has been employed to ablate inhibitory receptors such as A2AR, yielding enhanced CAR-T efficacy

[156]. However, its irreversible nature and potential for genotoxic or off-target effects remain translational challenges [157]. Transient mRNA/siRNA strategies, often delivered via lipid nanoparticles (LNPs), allow non-integrating, time-limited programming-such as co-delivery of CAR mRNA with PD-1 siRNA-to achieve potent yet reversible re-engineering [158]. Protein-level and pharmacologic interventions, including small-molecule inhibitors [159] or CAR-secreted checkpoint-blocking scFvs [160], act directly on signaling networks and provide fast, adjustable modulation without genetic alteration.

8.2 Functional Outcomes and Safety Considerations

Functionally, miRNA or shRNA co-expression has been shown to enhance CAR-T cytotoxicity, persistence, and resistance to exhaustion through sustained epigenetic and metabolic tuning [152,161]. Gene-editing strategies targeting A2AR or GM-CSF via CRISPR or TALEN eliminate dominant inhibitory or inflammatory pathways, mitigating cytokine-release-syndrome (CRS) risk and improving therapeutic durability [156,157]. Likewise, RNA-silencing of IL-6 has demonstrated preclinical and clinical benefit in reducing CRS-associated inflammation [162-164]. Protein-level modifications can achieve spatially restricted checkpoint blockade, concentrating immunomodulation at the tumor site and minimizing systemic toxicity [160,165]. Moreover, a miRNA-based shRNA cassette has reached clinical proof-of-concept in the CYAD-211 allogeneic CAR-T product, validating the feasibility of RNA-mediated TCR silencing for graft-versus-host-disease prevention [122].

8.3 Multiplexing, Delivery, and Integration Potential

miRNA/shRNA cassettes inherently enable multiplexed, compact regulation compatible with lentiviral or retroviral CAR constructs, facilitating simultaneous tuning of several functional nodes within limited vector capacity [77,166]. CRISPR can also be multiplexed through the delivery of multiple guide RNAs, allowing concurrent deletion of inhibitory receptors or cytokine genes; however, this requires stringent safety evaluation due to permanent genomic alteration [157]. Transient mRNA or LNP platforms, by contrast, are ideal for ex vivo manufacturing, dose iteration, and reversible testing of synthetic designs [158]. Protein-based or pharmacologic methods may be layered with genetic interventions-for instance, combining cytokine knockdown (IL-6 or GM-CSF) with CAR-secreted checkpoint scFvs-to reinforce efficacy while maintaining local immune control [160,167].

8.4 Comparative Synthesis and Platform Selection

Overall, no single modality universally outperforms others. miRNA/shRNA platforms provide compact, low-immunogenic, and multitarget regulation suitable for metabolic and epigenetic reprogramming. CRISPR and TALEN systems offer definitive, heritable remodeling of key suppressive genes for long-term efficacy. RNAi and transient mRNA delivery confer reversible and non-integrating modulation advantageous for safety and manufacturing scalability. Protein-level and pharmacologic strategies offer immediate, tunable control with minimal genomic risk. The most promising CAR-T architectures will likely integrate two or more of these technologies, matching each regulatory layer to the temporal, functional, and safety requirements of the intended therapeutic application [122,152,160,168].

9. Future Perspectives

The preclinical success of incorporating microRNA (miRNA) modulation into CAR-T engineering is rapidly progressing toward translational application. Over the next decade, several strategic opportunities and challenges are expected to shape this field.

First, patient-tailored miRNA profiling should become integral to adoptive cell therapy design, enabling the rational selection of mimics or inhibitors aligned with individual tumor signatures and T-cell phenotypes. This precision approach may allow miRNA networks to serve as predictive biomarkers for CAR-T persistence, exhaustion thresholds, and microenvironmental adaptation.

Second, safe and scalable delivery remains a critical barrier to clinical translation. The reversibility of transient oligonucleotides is advantageous, while vectorized circuits and circular RNA payloads can sustain expression with reduced innate immune activation. However, in vivo delivery continues to face limitations in stability, targeted biodistribution, and immunogenicity. Innovative platforms such as exosome-based systems may enable localized or bystander modulation of the tumor milieu in real time. Incorporating these modalities into GMP manufacturing will require validated release assays to ensure potency, reproducibility, and minimal off-pathway toxicity.

Third, synthetic-biology convergence offers new combinatorial strategies-combining miRNA-based reprogramming with CRISPR knockouts, epigenetic modulators, or logic-gated circuits to generate CAR-T products resistant to immunosuppressive cytokines and hypoxia. Tissue-specific miRNA safety switches, such as miR-142-3p in hematopoietic cells and miR-122 in hepatocytes, provide additional avenues for spatial detargeting and systemic safety control [93,136].

Finally, clinical validation in solid malignancies remains the most formidable step. The first-in-human success of CYAD-211 demonstrates feasibility of non-gene-editing RNA approaches, yet further trials must address desmoplastic and hypoxic tumor settings through improved in vivo models, real-time miRNA monitoring, and

standardized regulatory frameworks [135]. Together, these advances will determine whether miRNA engineering evolves from an adjunctive tactic into a cornerstone of next-generation CAR-T therapy.

10. Conclusion

Collectively, current evidence establishes miRNAs as programmable rheostats capable of fine-tuning CAR-T cell function across multiple barriers-including persistence, metabolic adaptation, and resistance to tumor microenvironmental suppression. Preclinical findings consistently show that modulation of key miRNAs such as miR-155, miR-17~92, miR-23a, and miR-210 reshapes T-cell metabolism, enhances cytotoxic durability, and improves antitumor efficacy.

The convergence of delivery platforms-ranging from synthetic oligonucleotides and viral/non-viral vectors to circRNA and exosome-based carriers-together with precision miRNA profiling and synthetic biology integration, outlines a multilayered framework for rational CAR-T design. Unlike binary gene editing, miRNA modulation offers reversible, graded control over complex regulatory circuits, enabling context-dependent adaptability within dynamic tumor ecosystems.

As the field advances, large-scale validation, standardized safety assessment, and harmonized regulatory pathways will be essential to translate this mechanistic promise into durable clinical benefit. With continued innovation in delivery, manufacturing, and multi-omics validation, miRNA engineering is poised to become a central pillar in the evolution of next-generation CAR-T therapies.

Disclosure of Potential Conflicts of Interest

The author has not any conflict of interest to disclose.

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