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Integrative Molecular Engineering of CAR-T Cells: Emerging Strategies to Enhance Persistence, Targeting, and Tumor Microenvironment Adaptation

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Abstract

Chimeric Antigen Receptor-T cell (CAR-T cell) therapy has become a revolutionary immunotherapeutic strategy for treating hematologic malignancies. However, despite its early success, CAR-T cell therapy continues to experience critical limitations, such as limited cell persistence of CAR-T cells, susceptibility to immune escape by tumor cells, and poor efficacy in solid tumors. This review investigates the role of CAR-T cell therapy in cancer treatment and how recent developments, such as dual-antigen targeting, re-engineering of the tumor microenvironment, introduction of enhancers, and combinatorial delivery systems, are addressing ongoing limitations in the field. Referencing recent developments in both clinical and preclinical research, this review highlights that through the combination of molecular engineering and targeted modulation of the immune system's response to tumors, CAR-T cell therapy is transitioning from a narrowly focused therapy to a broader, more widely applicable therapy across cancer types, potentially reshaping the future of cancer treatment.

Keywords: CAR-T cell therapy, immunotherapy, chimeric antigen receptor (CAR), cancer treatment, hematologic malignancies, solid tumors, tumor microenvironment (TME), antigen escape, dual-antigen targeting

1. Introduction

Chimeric Antigen Receptor-T cell (CAR-T cell) therapy has become a revolutionary therapeutic strategy in the realm of cancer treatment (June et al., 2018). Designed to allow lymphocytes such as T cells to target and eliminate cells expressing a specific surface antigen, Chimeric Antigen Receptors (CARs) are engineered artificial receptors that, unlike normal T cell receptors, do not rely on major histocompatibility complex (MHC) molecules to recognize antigens (Sadelain et al., 2013). Because of CAR's ability to stimulate strong immune responses and effectively attack tumors, the U.S. Food and Drug Administration (FDA) approved anti-CD19 CAR-T cell therapy against B-cell tumors in 2017—an important milestone in the development of immunotherapies (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017). In spite of prior success, CAR-T cell therapy still faces many key challenges: most notably, the limited cell persistence of CAR-T cells, the evolution of cancer cells to avoid detection and destruction by immunotherapies, and the poor effectiveness of the therapy in solid tumors. Hence, in an effort to shape CAR-T cell therapy into a broader and more applicable cancer treatment, ongoing innovations such as bispecific CAR-T cells targeting both CD19 and CD20 antigens, inhalable nanovesicles carrying a STING agonist, integration of a CAR enhancer, and the directing of A1R expression to tumor sites are being developed to address current limitations. This review—focusing on how innovations are addressing its critical limitations—examines current applications and emerging strategies in CAR-T cell therapy.

2. Structure of CAR

For CARs to perform their function, they require these four main components: antigen-binding domain, hinge region, transmembrane domain, and intracellular signaling domain(s) (Fig. 1).

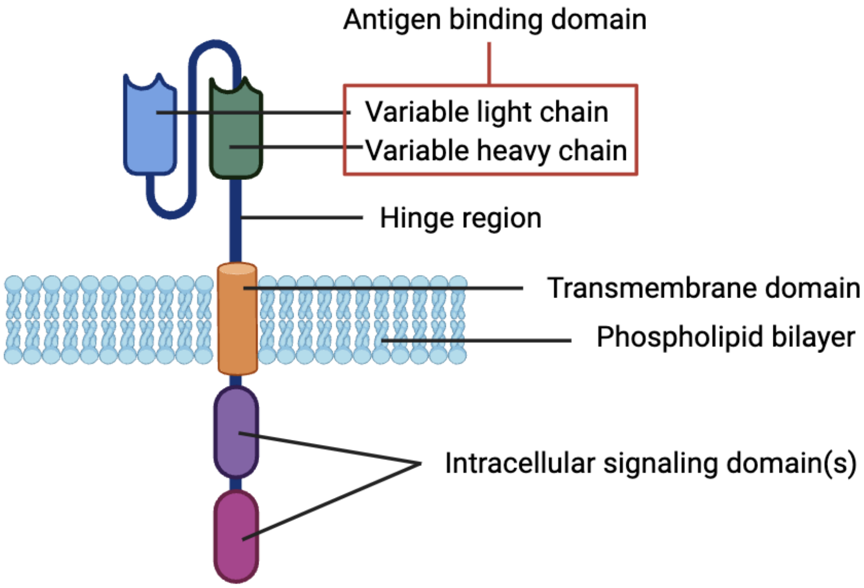

Figure 1: Structure of CAR. CARs contain four main components: (1) antigen binding domain, (2) hinge region, (3) transmembrane domain, and (4) intracellular signaling domain(s)

2.1. Antigen-binding domain

Produced from monoclonal antibodies, specifically their variable heavy (VH) and variable light (VL) chains, the antigen-binding domain is the part of a CAR that directly interacts with cancer cells by allowing the CAR to detect and bind to specific antigens on them. Through a flexible linker, the VH and VL chains are connected to form a single-chain variable fragment (scFv) (Fig. 1). CARs use scFvs to recognize and bind to antigens on the extracellular surface of cancer cells and activate the T cell without the use of MHCs (Zhang et al., 2014). Moreover, beyond binding, binding specificity and affinity of the CAR to its target can also be altered by the way the VH and VL chains interact and the position of the specific parts that bind to epitopes called complementary-determining regions (CDRs) (Chailyan et al., 2011). To activate a CAR-T cell, sufficient affinity of the antigen-binding domain to the epitope is required. However, excessively strong affinity will result in activation-induced cell death or other detrimental side effects in the patient (Caruso et al., 2015; Liu et al., 2015).

2.2. Hinge region

Connecting the antigen-binding domain to the transmembrane domain, the hinge region, also known as the spacer region, is the part of a CAR that extends and positions the binding domain far enough from the surface of the T cell, allowing the binding domain to reach and bind to the epitope of a specific antigen (Fig. 1). Although the hinge region can provide flexibility, which helps the CAR overcome steric hindrance and reach epitopes that might otherwise be hard to access, a CAR's efficacy depends on the length and composition of the hinge. Potentially, it can affect how flexible the receptor is, how well a CAR is expressed on a T cell, how strongly a CAR signals after binding an antigen, how effectively a CAR recognizes an epitope, and the strength of the activation of the T cell (Hudecek et al., 2015; Jensen & Riddell, 2015). As it controls the distance between the CAR-T cell and the target cell, the hinge is also important for the formation of an immunological synapse, which is needed for proper T cell activation (Srivastava & Riddell, 2015). According to the kinetic segregation model, T cell activation is dependent on the spatial exclusion of large inhibitory phosphatases like CD45 from the close-contact zone between the T cell and its target. The size of the hinge determines how closely the two cell membranes can approach each other. If the hinge is too short, steric hindrance may prevent optimal receptor-ligand engagement. If the hinge is too long, the intermembrane distance may be too wide to exclude CD45 efficiently. In either case, improper spacing can disrupt the subsecond evacuation of CD45 that initiates signaling, which could lead to reduced activation or signaling errors. Thus, the hinge length directly influences the molecular organization and signaling kinetics of the CAR-T immunological synapse (Taylor et al., 2022). Additionally, longer hinges are generally more suitable for reaching epitopes closer to the surface of the target cell or those that are part of bulky, glycosylated antigens, while shorter hinges are more suitable for epitopes that are farther away from the surface of the target cell (Guest et al., 2005; Hudecek et al., 2015; James et al., 2008; Wilkie et al., 2008). Because different hinge lengths are commonly required for each antigen and epitope, researchers often have to test different spacer lengths to determine what functions best with a specific antigen-binding domain. Most hinge regions come from parts of CD8, CD28, IgG1, or IgG4 proteins. However, deriving hinge regions from parts of IgG proteins can be risky, as they may interact with Fcγ receptors in the body, which not only results in CAR-T cell depletion but also reduced CAR-T cell persistence (Almåsbak et al., 2015; Hombach et al., 2010).

2.3. Transmembrane domain

Apart from anchoring it into the T cell membrane, the transmembrane domain is a part of the CAR that can also influence how much a CAR is expressed on the surface of a T cell, affect the stability of the CAR, aid with signaling or forming of the immunological synapse, and dimerize with other natural signaling proteins in a T cell (Bridgeman et al., 2010; Guedan et al., 2018; Zhang et al., 2012) (Fig. 1). Because different transmembrane domains are often used depending on the hinge or signaling domains being used in a specific CAR, researchers have yet to fully understand how switching out one transmembrane domain for another affects the function of CARs. Moreover, scientists have discovered that since it can result in CARs forming dimers and joining with the cell's natural T cell receptors (TCRs), using the CD3ζ transmembrane domain could possibly help more effectively activate T cells. This is because this structural coupling brings the CAR into close proximity with the TCR signaling complex, which allows it to recruit the same intracellular kinases that normally activate T cells (Bridgeman et al., 2010). With that said, due to the possibility of this domain interacting with endogenous CD3ζ chains, it may reduce receptor stability and surface expression, making CARs with a CD3ζ transmembrane domain less stable than those with CD28 transmembrane domains (Dotti et al., 2014). Furthermore, studies have shown that depending on the combination of the transmembrane and hinge regions, CAR-T cells could behave differently.

2.4. Intracellular signaling domain(s)

To figure out how to design CARs that trigger strong and lasting T cell responses, researchers have placed immense emphasis on studying the intracellular signaling domain, also known as the endodomain (Fig. 1). Produced in the late 1990s, the first-generation CARs only consisted of a CD3ζ or FcRγ signaling domain that contained immunoreceptor tyrosine-based activation motifs (ITAMs), which helped activate T cells when the CAR binds to its target antigen (Gross et al., 1989; Rafiq et al., 2020) (Fig. 2). However, with just CD3ζ signaling alone, many issues arose: the T cells did not proliferate or maintain functionality after they recognized antigens, the CARs did not produce strong responses in the lab, and early clinical trials showed that these CARs had little to no therapeutic effect (Brocker & Karjalainen, 1995; Hege et al., 2017; Till et al., 2008). Hence, to address these limitations, researchers added a co-stimulatory domain to the first-generation CAR (Imai et al., 2004; Maher et al., 2002). The two most commonly used co-stimulatory domains are CD28 and 4-1BB (CD137). These two co-stimulatory domains were specifically chosen due to their enhancing CAR-T cell function through distinct signaling pathways. CD28 provides rapid T cell activation and cytokine production by promoting PI3K and NF-κB signaling, which leads to stronger but shorter-lived responses. In contrast, 4-1BB signaling enhances mitochondrial biogenesis and memory formation, which results in more sustained CAR-T cell persistence and reduced exhaustion (Long et al., 2015). Because of the addition of these domains, the second-generation CARs demonstrated improved T cell persistence, cytokine production (i.e., IL-2), and response to repeated antigen exposure (Maher et al., 2002). Ultimately, these CARs functioned well in many blood cancers such as chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma (DLBCL), and multiple myeloma (van der Stegen et al., 2015). They are now being tested in solid tumors like glioblastoma, advanced sarcoma, liver metastases, mesothelioma, ovarian cancer, and pancreatic cancer (van der Stegen et al., 2015). With that said, as some scientists believed that one co-stimulatory domain may not fully activate the T cell, third-generation CARs were developed to include two co-stimulatory domains (CD28 and 4-1BB) in a row along with CD3ζ (Pulè et al., 2005). Moreover, the effectiveness of this generation of CARs depends on the cancer type. While hematologic cancers like B-cell malignancies present uniform antigen targets and allow for CAR-T cells to circulate freely, solid tumors often display heterogeneous antigen expression and contain dense extracellular matrices, immunosuppressive cells, and inhibitory cytokines that limit the infiltration and persistence of CARs (Kloss et al., 2018; Yin et al., 2018). Consequently, the field hypothesizes that CAR-T efficacy is determined by the interplay between tumor composition and the design of CAR signaling. This explains why second- and third-generation CARs show strong results in blood cancer but variable success in solid tumors (Abate-Daga et al., 2014; Milone et al., 2009; Zhong et al., 2010).

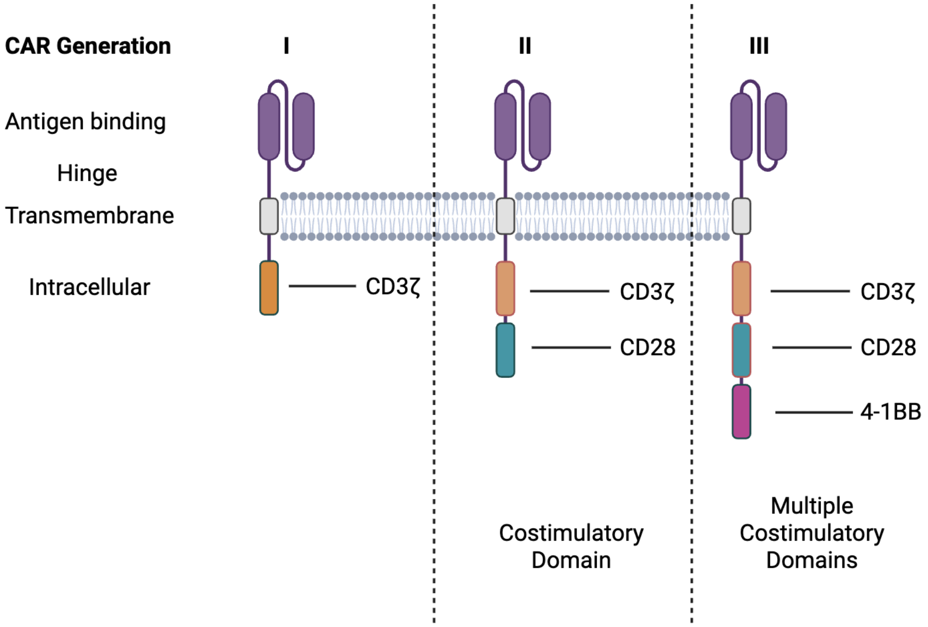

Figure 2: Generations of CARs. The first generation of CARs consisted of only a CD3ζ signaling domain. In addition to the CD3ζ signaling domain, the second generation of CARs consisted of a CD28 costimulatory domain, and the third generation of CARs consisted of both CD28 and 4-1BB costimulatory domains.

3. Introduction to CAR-T Cell Therapy

CAR-T cell therapy is an immunotherapy that modifies one's body's own T cells (a type of white blood cell) to better recognize and destroy cancer cells. Although conventional treatments such as chemotherapy and stem cell transplantation have been successful in helping many achieve remission, they often fail in a significant subset of cases. For example, nearly 40% of acute myeloid leukemia (AML) patients experience induction failure after standard chemotherapy that combines cytarabine and anthracyclines (Culver-Cochran et al., 2024). Furthermore, many of these refractory cases do not respond to salvage therapy or allogeneic stem cell transplantation. Similarly, in patients with high-risk leukemia undergoing haploidentical stem cell transplants, despite intensified conditioning regimens, graft failure and relapse are common (Passweg et al., 2000). In contrast, even in patients who have not responded to prior chemotherapy or transplant, CAR-T cell therapy has achieved remarkable success in treating relapsed or refractory blood cancers.

While normal T cells can recognize and attack infected or abnormal cells, cancer cells often escape detection by upregulating inhibitory ligands like PD-L1 or downregulating antigen presentation, which suppresses T cell activation and masks their immunogenic identity (Perales et al., 2018). To address this problem, instead of relying on MHCs, CAR-T cells are genetically modified to carry a synthetic receptor that directly binds to antigens, which are specific proteins found on cancer cells (i.e., CD19 in B-cell cancers) (Fig. 3). Because they are genetically engineered to recognize specific antigens independently of MHC presentation, CAR-T cells exhibit enhanced anti-tumor activity. This allows CAR-T cells to rapidly engage tumor cells, secrete cytotoxic molecules such as perforin and granzyme B, and even induce apoptosis in tumors that evade normal immune detection.

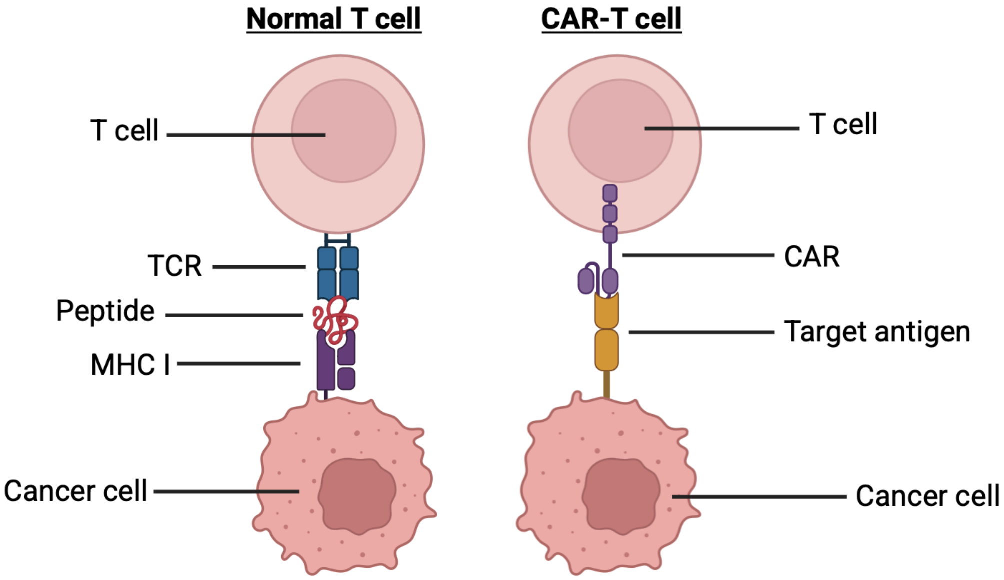

Figure 3: Normal T cell vs CAR-T cell. Normal T cells rely on the MHCs of the cancer cell to present the antigen for the TCR to bind to it, whereas CAR-T cells can directly bind to the target antigen using their CAR.

Additionally, CAR-T cells can develop memory-like phenotypes, which allow them to persist longer and respond faster to relapsing cancer cells. Although CAR-T cells still interact with other components of the tumor microenvironment like endogenous cytotoxic T cells and natural killer cells, their design provides a direct, high-affinity mechanism for the killing of tumor cells that enhances efficacy even in cancers that are more difficult to treat (Maurya et al., 2025). Once inside the body, CAR-T cells continue to survive and proliferate. They "patrol" the bloodstream and look for any cancer cells that return over time, providing patients with long-term protection. Some CAR-T cells even form memory T cells and stay in the body permanently and attack relapsed cancer. Because of its effectiveness, CAR-T cell therapy has been FDA-approved to treat several blood cancers like acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), and multiple myeloma (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017). Some of the approved CAR-T products include Kymriah (tisagenlecleucel) by Novartis, Yescarta (axicabtagene ciloleucel) by Kite Pharma, Breyanzi, Abecma, and other newer products that are still under clinical trials. Especially for patients who had relapsed after several treatments, CAR-T cell therapy has shown incredibly high success rates. For example, the ELIANA trial showed an 83% complete remission at 3 months with Kymriah, and the ZUMA-1 trial showed a 54% complete remission rate in patients treated with Yescarta (Almond et al., 2017; Locke et al., 2017).

While effective, the process for CAR-T cell therapy is personalized and takes several weeks (Fig. 4).

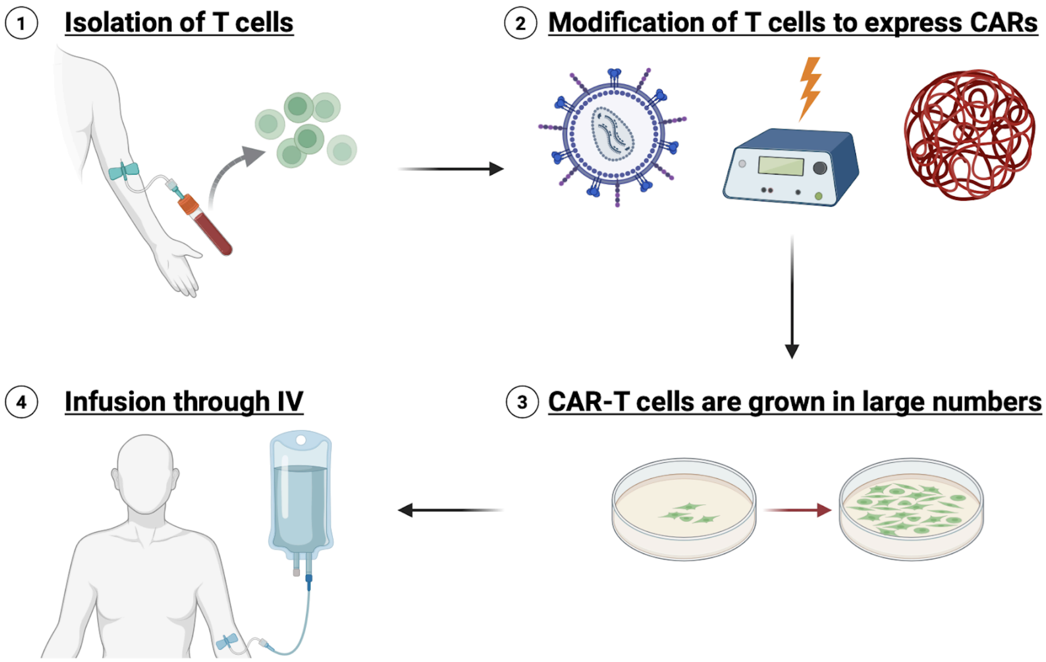

Figure 4: Administration of CAR-T cells to patients. Patients first undergo leukapheresis to isolate their T cells from other blood components. After isolation, the T cells are modified to express CARs through viral vectors, transposon systems, electroporation, or nanoparticles. Once modified, the CAR-T cells are grown in large numbers in labs to be used for treatment. The CAR-T cells are then infused into the patient's bloodstream through an IV.

First, through leukapheresis, doctors collect a patient's blood and, using a machine that separates blood components, isolate the patient's T cells (Miliotou & Papadopoulou, 2018; Mirzaei et al., 2019). Then, the isolated T cells are modified to express CARs. This can be done using viral vectors (i.e., γ-retroviruses or lentiviruses) that are engineered to carry the CAR gene into the T cell's DNA, transposon systems (i.e., Sleeping Beauty or piggyBac) which use "cut-and-paste" DNA methods with a transposase enzyme, electroporation that uses electric pulses to open cell membranes so DNA can enter, or nanoparticles which are tiny carriers that deliver CAR genes safely without the use of viruses (Kidd et al., 2012; Munoz-Lopez & Garcia-Perez, n.d.; Smith et al., 2017; Yarmush et al., 2014; Yi et al., n.d.). Once modified, the T cells are grown in large numbers in the lab until there are millions of CAR-T cells prepared for treatment. To lower their existing immune cells, called lymphodepletion, the patient may first receive a short round of chemotherapy. This helps the CAR-T cells work better. Following this, the CAR-T cells are infused through an intravenous line (IV). After the treatment is complete, for the first 2–3 weeks, patients are observed closely for side effects.

4. Key Limitations in Current CAR-T Cell Therapy

Despite the initial success of CAR-T cell therapy, it continues to face many limitations—namely, the limited cell persistence of CAR-T cells, the evolution of cancer cells to avoid detection and destruction, and poor efficacy of the therapy in solid tumors.

4.1. Limited cell persistence of CAR-T cells

With the inability to persist for long periods of time, sustained anti-tumor activity and durable patient responses are hindered. Although CAR-T cells can expand and mediate tumor clearance at first, many patients are susceptible to relapse due to a decline in CAR-T cell levels over time. This decline in cell levels could be due to several reasons. Firstly, suboptimal function of the co-stimulatory domains within the CAR could reduce T cell proliferation, survival, and memory formation after interaction with the antigen. Without sufficient co-stimulation, CAR-T cells will fail to sustain robust activation, leading to limited persistence. Furthermore, due to age, prior chemotherapy, chronic infections, or exhaustion induced by the tumor, the function of patient-derived T cells may already be compromised. This results in lower proliferation capacity, increased susceptibility to apoptosis, and impaired metabolic fitness. The diminished persistence causes issues, specifically in hostile tumor microenvironments (TME) where immunosuppressive signals like TGF-β, IL-10, and checkpoint pathways like PD-1/PD-L1 rapidly exhaust CAR-T cells and impair their survival (Kloss et al., 2018; Yin et al., 2018). Furthermore, the short lifespan of CAR-T cells may be due to inadequate co-stimulatory signaling or poor T cell fitness at the time of infusion. Given that early-generation CARs lacked co-stimulatory domains, they failed to provide the necessary signals for robust memory formation, which contributed to rapid attrition in vivo (Brocker & Karjalainen, 1995; Hege et al., 2017; Till et al., 2008). Even with newer generations of CARs being developed, the persistence of CAR-T cells remains variable and patient-dependent (Abate-Daga et al., 2014; Milone et al., 2009; Zhong et al., 2010).

4.2. Evolution of cancer cells to avoid detection and destruction

Because of the adaptive evolution of tumor cells, this can lead to antigen escape. Antigen escape is a process where cancer cells downregulate or completely lose expression of the target antigen recognized by CAR-T cells. Although initial single-antigen CAR-T therapies—such as CD19 or BCMA targeting—achieved high response rates in hematologic cancers, over time, a significant number of patients relapse as the tumor cells modify themselves to avoid immune recognition. For example, due to a loss or mutation of the CD19 antigen, up to 30–70% of relapsed B-ALL patients experience recurrence (Majzner & Mackall, 2018; Maude et al., 2015). Likewise, following anti-BCMA CAR-T treatment, BCMA downregulation has been observed in multiple myeloma (Brudno et al., 2018; Cohen et al., 2019; Green et al., 2018). Additionally, solid tumors exhibit this immune escape pattern, like reduced IL13Ra2 expression following CAR-T therapy in glioblastoma (Brown et al., 2016). This could lead to an undermining of long-term treatment success because of the ability of tumor cells to effectively "hide" from the immune attack.

4.3. Poor efficacy in solid tumors

Due to several interrelated biological and structural barriers, the effectiveness of CAR-T cell therapy in solid tumors remains limited. Firstly, solid tumors lack ideal antigen targets that are both tumor-specific and not expressed on normal tissues. Because many antigens targeted in solid tumors are also found on healthy cells, although at low levels, this leads to on-target off-tumor toxicity and reduced therapeutic windows. Additionally, in solid tumors, CAR-T cells face significant trafficking and infiltration challenges. Unlike blood cancers, solid tumors have dense physical barriers like the extracellular matrix and tumor stroma that impede T cell penetration (Zhang et al., 2016). Finally, the immunosuppressive TME, which is rich in regulatory T cells, myeloid-derived suppressor cells (MSDCs), and tumor-associated macrophages (TAMs) that secrete inhibitory cytokines and engage immune checkpoint pathways (i.e., PD-1/PD-L1, CTLA-4), results in T cell exhaustion and poor persistence of the CAR-T cells (Quail & Joyce, 2013; Yin et al., 2018).

5. Advancements and Strategies to Overcome Limitations in CAR-T Cell Therapy

Hence, to enhance the overall effectiveness of CAR-T cell therapy in cancer treatment, scientists have pursued multiple strategies to overcome poor efficacy in solid tumors, susceptibility to immune escape by tumor cells, and limited cell persistence.

5.1. Targeting poor efficacy in solid tumors: Re-engineering of the tumor microenvironment and combinatorial delivery systems

Although CAR-T cell therapy has been successful for some blood cancers such as leukemia and lymphoma, it has not yet shown the same success in treating solid tumors like lung, breast, and ovarian cancers (Albelda, 2024; Flugel et al., 2023). When treating solid tumors, the main challenge lies in the fact that the TME, which is the space around tumor cells, is a hostile environment for immune cells (Hou et al., 2021; Landoni et al., 2024; Lee et al., 2023). In solid tumors, the TME creates multiple layers of defense that shut down immune responses (Table 1).

Table 1: Components of the TME. The TME consists of multiple components: checkpoint proteins, suppressive immune cells, and adenosine.

Component	Example(s)	Function
Checkpoint proteins	PD-L1	They act as key immune regulatory molecules that inhibit CAR-T cell activity by binding to PD-1 receptors. While they maintain immune tolerance and prevent damage to healthy tissues, they also enable tumor cells to evade immune-mediated destruction.
Suppressive immune cells	Regulatory T cells (Tregs) and Myeloid-derived suppressor cells (MDSCs)	They release anti-inflammatory signals that suppress CAR-T cell function.
Adenosine	–	High levels of it accumulate in the low-oxygen TME and signal through the A2AR receptor on CAR-T cells, thereby diminishing their functional capacity

Overall, these components shorten the lifespan and reduce the efficacy of CAR-T cells when destroying solid tumor cells.

Hence, to tackle the limited efficacy of CAR-T cell therapy in solid tumors, the most direct approach was to target modifying the TME to make it more suitable for CAR-T cell activity. For example, inhalable nanovesicles have been engineered to overcome the hostile TME (Zhu et al., 2025) (Fig. 5).

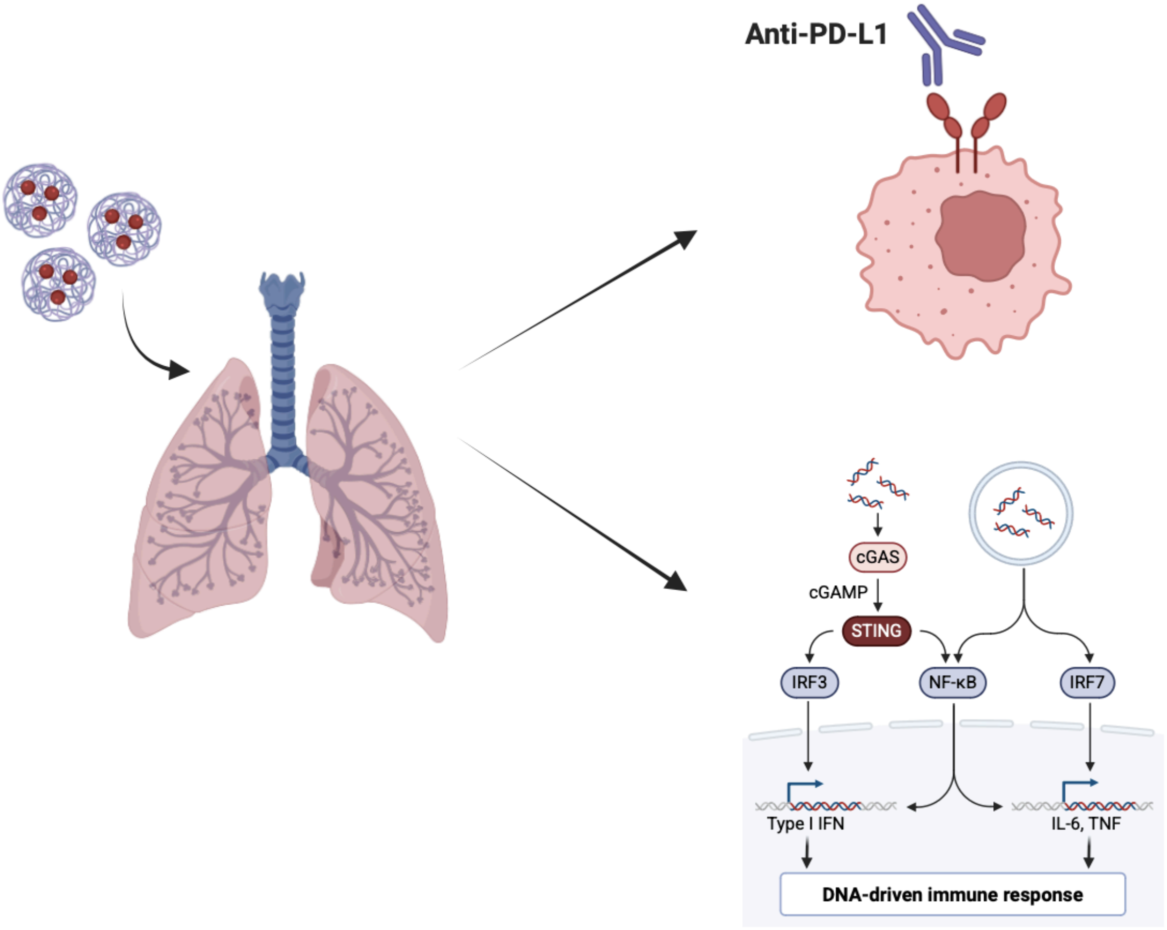

Figure 5: Using inhalable nanovesicles to overcome the hostile TME. Inhalable nanovesicles that contain therapeutic molecules are inhaled into the patient's lungs. Upon inhaling these nanovesicles, they block the PD-1/PD-L1 interaction, allowing CAR-T cells to remain active, and they activate the STING pathway, initiating a strong immune response.

Inhalable nanovesicles are small, biodegradable vesicles that contain therapeutic molecules that could be directly inhaled into the lungs. Each nanovesicle carries an anti-PD-L1 single-chain variable fragment (scFv) and a STING agonist (cGAMP). The scFv is a small antibody piece that binds to PD-L1 on tumor cells and blocks the PD-1/PD-L1 interaction, allowing the CAR-T cells to remain active. The cGAMP is a molecule that activates the Stimulator of Interferon Genes (STING) pathway and leads to the production of type I interferons such as IFN-β and the attraction of antigen-presenting cells such as dendritic cells to help amplify the immune response. Because these vesicles are delivered directly into the lungs, their therapeutic components become highly bioavailable in pulmonary tissue, making this strategy particularly suited for lung cancer CAR-T applications and potentially more effective than systemic intravenous administration of anti-PD-L1 antibodies. Success of these inhalable nanovesicles was observed in mouse models of lung cancer where they triggered local immune activation without systemic toxicity, increased levels of molecules (i.e., IFN-β, IL-2, and CXCL9) that help attract and activate more CAR-T cells, reduced exhaustion markers (i.e., PD-1, LAG3, and TIGIT) on CAR-T cells, promoted the formation of $TCF1^+$ memory-like T cells which are known for their persistence and self-renewal properties, and resulted in stronger tumor shrinkage, delayed relapse, and improved survival of CAR-T cells.

Besides targeting the TME, efforts have also been made in changing the CAR-T cells themselves to resist suppressive signals from within the TME. Research has shown that adenosine, which is released in high amounts by tumors, functions by signaling through the A2AR receptor, a G protein-coupled receptor that suppresses T cell proliferation, cytokine production, and cytotoxic activity, to deactivate CAR-T cells (Sek et al., 2025). In response to this, because A1R, another adenosine receptor whose signaling counteracts A2AR, enhances T cell activation and survival when activated, CRISPR-Cas9 gene editing technology was used to insert the gene for the A1R receptor into CAR-T cells. These newly engineered CAR-T cells with the A1R receptor resulted in higher production of immune-stimulating cytokines (i.e., IFNγ, TNFα, and IL-2), reduced expression of exhaustion markers (i.e., PD-1 and TIM-3), and increased expression of CD69 and IRF8, which indicated stronger immune readiness and potential for longer-lasting memory. To stabilize the beneficial effects, the transcription factor NR4A2 has been added, which helps CAR-T cells, especially in environments full of stress and inflammation, maintain anti-suppressive gene expression over time. In mice with tumors, CAR-T cells engineered with A1R and NR4A2 resulted in sustained tumor disappearance, preserved stem-like and central memory T cell populations that are essential for long-term immunity, and eliminated the need for repeated dosing of CAR-T cells.

In combination, these strategies could potentially revolutionize CAR-T cell therapy for increased efficacy in solid tumors, which make up the vast majority of cancers.

5.2. Targeting susceptibility to immune escape by tumor cells: Dual-antigen targeting

Another major limitation in CAR-T cell therapy is the downregulation or loss of the target antigen on tumor cells (Neelapu et al., 2017; Tong et al., 2020). This facilitates tumor cells escaping from immune cells, leading to relapse. This phenomenon is well-documented in B-cell malignancies (i.e., DLBCL and B-ALL) where alterations in target antigens like CD19—caused by missense mutations, frameshifts, or alternative splicing—allow tumor cells to evade recognition by CARs (Sotillo et al., 2015).

Hence, to reduce the likelihood of relapse due to the loss of target antigens on tumor cells, dual-antigen targeting strategies—such as by simultaneously engaging two distinct antigens associated with tumors—have been developed (Dai et al., 2020; Grada et al., 2013; Hamieh et al., 2019; Hegde et al., 2016; Majzner & Mackall, 2018; Zah et al., 2016).

For example, in the phase I/II of a clinical trial, researchers developed a tandem CAR construct with the ability to bind to both CD19 and CD20 (Wang et al., 2024). CD19 and CD20 surface proteins are ideal targets for cancer therapy as they are highly expressed almost exclusively on B lymphocytes and absent on most other cell types. The construct, connected via a flexible linker, integrated scFvs specific for CD19 and CD20 and incorporated the hinge and transmembrane domains of CD8 with 4-1BB and CD3ζ signaling domains (Fig. 6).

In preclinical assays, the CD19/20 CAR-T cells demonstrated efficacy in destroying cancer cells that expressed either CD19, CD20, or even both, and they released similar levels of cytokines as single-target CAR-T cells. The release of cytokines is important as they function as chemical messengers that enable immune cells to communicate and coordinate an immune attack against harmful substances such as cancer cells.

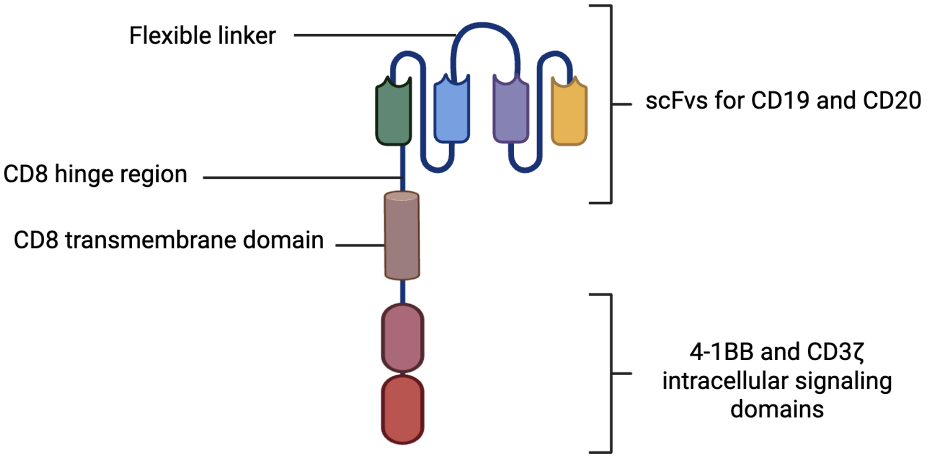

Figure 6: Structure of CD19/20 CAR-T cell. The CD19/20 CAR-T cells have tandem CAR constructs that contain an antigen-binding domain with single-chain variable fragments (scFvs) for CD19 and CD20, a CD8 hinge and transmembrane domain, and 4-1BB and CD3ζ intracellular signaling domains.

Clinically, this dual-targeting approach showed promising efficacy. In the group of patients with relapsed or refractory (R/R) B-cell NHL, with a 12-month overall survival rate of 81.82% and progression-free survival of 60%, 81.8% of patients experienced complete remission. Importantly, even in the presence of antigen loss for one target, the bispecific CAR-T cells remained robust. This suggests a decreased risk of immune escape in these CAR-T cells compared to single-target CAR-T therapy. Through single-cell RNA sequencing, results showed that the main CAR-T cell groups after infusion contained high levels of genes that are involved in immune signaling, especially those that help activate the body's first-line immune defenses and those that aid in the destruction of cancer cells. Despite changes in the way cancer cells display their surface antigens to allow them to avoid detection from the immune system, likely due to the mentioned features, these CAR-T cells sustained anti-tumor activity.

Furthermore, beyond mitigating relapse driven by antigen loss, this dual-antigen targeting strategy also broadens the therapeutic window for patients with heterogeneous antigen expression profiles. Through the maintenance of targeting capability against tumor cells that have downregulated one antigen, these CD19/20 CAR-T cells exemplify how rational CAR design can directly address a critical resistance mechanism in immunotherapy.

5.3. Targeting limited cell persistence: CAR enhancer (CAR-E)

After the initial CAR-T cell treatment, CAR-T levels often deplete, resulting in patients being vulnerable to relapse. Hence, new ways to target the limited cell persistence of CAR-T cells are being developed to improve the long-term effectiveness of the therapy.

For example, scientists have designed a CAR enhancer (CAR-E) to prolong CAR-T durability without broad cytokine exposure (Rakhshandehroo et al., 2025). CAR-E is a recombinant protein that exists in the extracellular space and can diffuse to interact with CAR-T cells that are bound to the target antigen. It functions by coupling a tumor-antigen binder to a low-affinity Interleukin 2 (IL-2) dimer that engages only antigen-bound CAR-T cells. This ensures that the cis IL-2 receptor (cis IL-2R) signaling is delivered precisely where it is required. In this context, rather than activating neighboring T cells in the environment (trans signaling), "cis" signaling means that the IL-2 dimer acts locally within the same CAR-T cell that has engaged its target antigen. While not compromising its efficacy by preserving cytotoxicity and not activating other cells, such as non-transduced T cells that might hurt the safety and efficacy of the therapy, CAR-E switches on STAT5 and early activation in CAR-T cells (Fig. 7). By switching on STAT5 and early activation in CAR-T cells, this will result in bigger, longer-lived, and more effective CAR-T responses. The CAR-E only functions when both the CAR endodomain and the IL-2 receptor signal, and it's pulled inside the CAR-T cell itself via the IL-2R, which helps keep the effect contained.

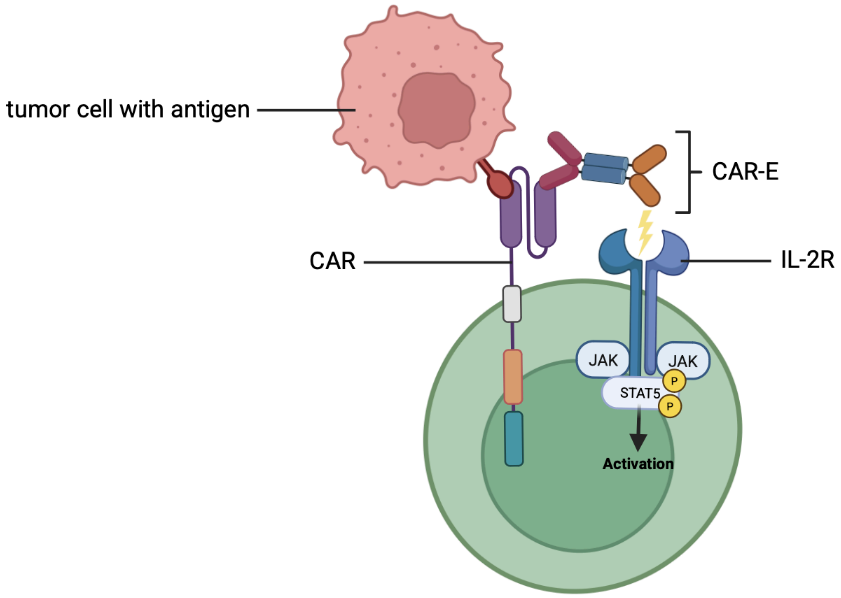

Figure 7: CAR-E mechanism. CAR-E couples tumor-antigen recognition with a low-affinity IL-2 dimer that engages the IL-2R only in CAR-T cells bound to antigens. This activates the JAK/STAT5 pathway, promoting early activation, enhanced persistence, and stronger cytotoxic responses without broad cytokine exposure.

In vivo, dose-dependent multiplication and enhanced cell persistence of CAR-T cells in blood, spleen, and bone marrow, as well as enrichment of memory phenotypes such as central memory (T_CM) and stem-like memory (T_SCM), are produced through brief, pulsed dosing (serum t½ ~1.5 h). Importantly, CAR-T cells that are treated with CAR-E can be maintained even at lower CAR-T doses or in the absence of a tumor and can re-proliferate upon interaction with cancer cells that reappear. This supports an on-demand "booster" paradigm to sustain CAR-T cell levels after they peak and drop.

Overall, CAR-E is a safe method that improves cell persistence of CAR-T cells, as its low-affinity IL-2 is weak enough that it prevents activation of nearby, non-CAR-T cells, and it clears from the body quickly, allowing doctors to fine-tune the dosage. Furthermore, since the CAR-E mainly consists of components derived from humans, it minimizes predicted immunogenicity. The design of the CAR-E is also modular, meaning the binding piece can be swapped to use CAR-E with other CAR targets (i.e., CD19/CD22 or GD2), potentially even in solid tumors. In practice, scientists can initially carry out manufacturing that starts with memory-like T cells (T_SCM/T_CM) and keeps the culture time short to ensure cells stay youthful and durable. Then, CAR-E provides targeted IL-2 signals after infusion to maintain and expand those memory-leaning CAR-T cells in patients. Additionally, as CAR-E clears fast, clinicians can briefly give small pulses of CAR-E when CAR-T cell levels start to dip or when tumor signals reappear, then stop. This ensures the maintenance of cell persistence while minimizing negative side effects like cytokine toxicity, where the immune system releases too many cytokines all at once and triggers undesirable outcomes, or unwanted T cell activation.

When taken together, it is a targeted, scalable method to ensure CAR-T cell persistence while ensuring maintenance of control over systemic cytokine exposure.

6. Conclusion

Offering unprecedented remission rates in hematologic malignancies and giving rise to personalized, immune-based treatment for cancer, CAR-T cell therapy is one of the most transformative innovations in modern oncology. However, because of challenges such as limited cell persistence, susceptibility to immune escape, and poor efficacy in solid tumors, its potential to be used as a treatment in clinical practice has been hindered. Recent advances, such as re-engineering the tumor microenvironment through the use of inhalable nanovesicles and A1R-directed CAR-T cells, developing dual-antigen targeting constructs to counter antigen loss, and integrating CAR enhancers to sustain memory-like T cell populations, demonstrate that these limitations are neither overcome nor insurmountable. Together, the rise of these strategies indicates a shift in the therapy from a reactive treatment toward an increasingly proactive, precision-focused CAR design where CAR-T cell therapy is tailored to the tumor's biology and individual patients' differing needs. As deeper immunological insights are being discovered and innovations are arising, CAR-T cell therapy is anticipated to evolve from a niche intervention method for specific blood cancers into a more versatile and adaptable platform that is capable of overcoming heterogeneous antigen expression, hostile microenvironments, and short-term durability. Looking ahead, the field is increasingly expected to incorporate soluble immunomodulatory proteins to enhance specificity, switch-receptor systems such as PD-1/CD28 to counter T cell exhaustion, and additional modular add-ons that improve long-term surveillance and durability in both hematologic and solid tumor settings. Whether CAR-T cell therapy fulfills its promise of becoming a universal, durable, and safe cancer treatment depends on the continued integration of preclinical breakthroughs into clinical practices accessible for patients worldwide.

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Kaira Tan is a rising senior at Livingston High School with a keen interest in science. Originally hailing from Singapore, she has since moved to New Jersey as of a year ago.

Mentor Contribution Statement

Dr. Hamidreza Shaye served as the primary mentor for the development of this review work and provided guidance throughout the writing process. Before the drafting phase, he met with the author to outline the typical structure, standards, and expectations of a scientific review article. Throughout the project, he offered ongoing feedback, encouragement, and support to ensure that the manuscript met the highest standards of scientific accuracy and scholarly rigor. The author developed the hypothesis, conceived the project, and wrote the manuscript independently.

Dr. Lauren Tetz and the author met one-on-one several times during the research and writing of her manuscript. The author consistently arrived well prepared, with complete section drafts, questions, and thoughtful reflections. Dr. Tetz supported her work by providing access to academic resources requiring institutional permissions, enabling her to review a wide and current range of peer-reviewed literature on CAR-T cell therapy. She also assisted during the revision stages by reviewing the manuscript's organization, clarity, and language. Her feedback helped strengthen readability and ensure the writing met the standards of a formal scientific review.