LITERATURE REVIEW

CAR-T Cell Therapy in Cancer: Clinical Applications, Challenges in Solid Tumors, and Emerging Strategies

Wenhan Jiang[1]*

[1] Seattle Academy of Arts and Sciences, Seattle, United States
* Corresponding author: jwenhan9@gmail.com

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ABSTRACT

Cancer treatment increasingly incorporates precision and immune-based therapies alongside surgery, chemotherapy, and radiotherapy, shifting from conventional approaches that directly damage or remove tumors toward precision strategies that involve genetically engineering autologous or donor-derived T cells to recognize selected surface antigens on cancer cells. Chimeric antigen receptor T-cell therapy represents one of the most clinically developed examples of this shift by re-engineering T cells to target specific cancer-associated antigens. While CAR-T therapies have achieved durable responses in hematologic malignancies, translating these clinical successes to solid tumors presents a formidable biological barrier. A major challenge is identifying antigens that are consistently expressed by tumor cells but absent or minimally expressed in healthy tissues. Success also hinges on navigating an intricate and hostile tumor microenvironment characterized by antigen heterogeneity, dense extracellular matrices that restrict immune infiltration, pervasive immunosuppressive signaling cascades, and chronic antigen exposure that induces premature T-cell exhaustion. Consequently, optimizing therapeutic efficacy requires a dual approach that addresses both T-cell intrinsic biology and host tissue landscapes. This review examines the applications of CAR-T therapy in hematologic cancers, the barriers limiting its effectiveness in solid tumors, and the emerging strategies that aim to address these limitations. These include multi-antigen logic gating to prevent antigen escape, cytokine-armored vectors to remodel local immunosuppression, and in vivo approaches to simplify manufacturing. Ultimately, overcoming current clinical bottlenecks will require engineering context-aware CAR-T platforms tailored to the complex biological realities of individual solid tumors.

Keywords Cancer, CAR-T cell, antigen, tumor microenvironment

Introduction

Cancer is a complex group of diseases characterized by uncontrolled cell growth and division. These malignant cells can invade nearby tissues, metastasizing to distant sites. Normally, tissue homeostasis is maintained by a precise system that balances how cells grow, divide, repair their DNA, or die when they are damaged or no longer needed. When these regulatory mechanisms become disrupted, abnormal cells may survive and multiply, eventually contributing to tumor development and progression. Both inherited and environmental factors contribute to cancer development, with genetic susceptibility and exposures such as tobacco use, ultraviolet radiation, and chemical carcinogens influencing an individual's risk of malignancy (1-3). Cancer usually develops through the accumulation of genetic alterations that interfere with normal cellular regulation, although the specific changes involved vary between cancer types (1-3).

At the molecular level, cancer often begins when mutations affect genes that control cell growth and stability (4, 5). Three key groups of genes involved in regulating normal cellular function are proto-oncogenes, which normally control cell growth and division but can mutate into oncogenes that drive uncontrolled proliferation; tumor suppressor genes, which help slow cell division or trigger cell death when abnormalities occur; and DNA repair genes, which correct genetic damage and maintain genomic stability by preventing mutations from accumulating over time (4, 6). When these protective mechanisms fail, cells can accumulate further genetic changes that support uncontrolled growth and malignant transformation (4, 5).

Cancer development is also influenced by the tumor microenvironment (TME), which consists of the cells, blood vessels, signaling molecules, and connective tissues surrounding a tumor (7, 8). These components interact with cancer cells and can influence their growth, survival and response to treatment. For example, some tumors alter the activity of nearby immune cells, creating conditions that help malignant cells avoid immune-mediated destruction (7). This interaction partially explains why cancer is more than just issues with cells; it is a complex system that adapts to resist both the immune system and medical treatments (4, 7).

Cancer cells have a set of biological traits called hallmarks of cancer, which describe how tumors develop and survive within the body (4). One important feature is its ability of cancer cells to evade immune surveillance, allowing them to avoid recognition and destruction by the immune system (4, 7). Cancer cells can achieve this in several ways, such as reducing the visibility of tumor associated antigens, altering antigen presentation, and creating an immunosuppressive tumor microenvironment that limits the activity of immune cells (4, 7). These mechanisms are particularly relevant to immunotherapy because treatment effectiveness depends partly on whether immune cells can recognize cancer cells and remain functional after reaching the tumor.

For many years, cancer treatment has relied primarily on traditional approaches, including surgery, chemotherapy, and

radiotherapy (9-11). Surgery remains one of the most effective approaches for removing localized tumors before cancer spreads throughout the body (11). Chemotherapy, in contrast, uses cytotoxic drugs that interfere with cellular processes essential for replication, particularly targeting rapidly dividing cells by disrupting DNA synthesis, mitosis, or metabolic activity required for cell survival (9). Although chemotherapy has significantly improved outcomes in many cancer types, its systemic nature often causes damage to healthy rapidly dividing cells, resulting in side effects such as immune suppression, gastrointestinal toxicity, and hair loss (9). Radiation therapy functions through a different mechanism by using radiation to directly induce DNA damage within cancer cells located in specific targeted tissues (10). This damage prevents cancer cells from successfully replicating and eventually leads to cellular death, thereby limiting further tumor growth and reducing the potential for metastasis (10). Because radiotherapy targets a specific tumor site rather than affecting the entire body, it often reduces systemic exposure compared with chemotherapy (9, 10). However, surrounding healthy tissues may still be exposed to radiation, and many cancers eventually develop mechanisms that reduce the long-term effectiveness of treatment (10).

More recently, immunotherapy has transformed cancer treatment by shifting focus from directly attacking tumors toward strengthening the body's own immune system to eliminate malignant cells (12, 13). Among these methods, chimeric antigen receptor T-cell (CAR-T) therapy has attracted significant attention due to its innovative mechanism and promising clinical outcomes (12). CAR- T therapy involves genetically engineering a patient's own T cells, a central component of the adaptive immune system, to express chimeric antigen receptors that allow them to recognize specific antigens directly on the surface of cancer cells, without requiring antigen presentation by major histocompatibility complex (MHC) molecules (12, 13). The choice of target antigen is essential in this process, as it must be sufficiently expressed on cancer cells while having limited expression on healthy tissues to support effective tumor targeting and reduce unintended damage to normal cells (12, 13). Once reintroduced into the patient, these modified T cells can recognize cells expressing the chosen target antigen and destroy them (12). This may provide a more targeted approach than conventional chemotherapy, although its effectiveness and safety depend partly on the characteristics of the selected antigen (12, 13). Early clinical success, particularly in hematologic cancers such as leukemia and lymphoma, has positioned CAR-T therapy as an important development in modern cancer immunotherapy (12, 14).

As research in cancer biology and immunotherapy continues to advance, CAR-T cell therapy has become a crucial field of study due to both its remarkable therapeutic potential and the challenges that remain in improving accessibility and effectiveness across different cancer types. This literature review aims to examine the biological foundation of CAR-T cell therapy, summarize current clinical applications, evaluate its major limitations, and explore its future potential in transforming the landscape of cancer treatment.

CAR-T Cell Therapy And Blood Cancers

Chimeric Antigen Receptor T-cell (CAR-T) therapy represents a landmark advancement in cancer immunotherapy, primarily owing to its capacity to redirect immunological effector functions toward neoplastic cells (12, 13). In contrast to conventional chemotherapeutic agents or radiotherapeutic interventions, which exert their effects through non-specific chemical or physical cytotoxicity against proliferating cells, CAR-T cell therapy adopts a mechanistically distinct strategy that leverages the host's immune system as an active participant in tumor elimination (13). Rather than relying on exogenous treatment modalities to directly damage malignant tissues, this approach entails the ex vivo engineering of T lymphocytes to enhance their intrinsic capability for precise tumor cell recognition and eradication. This approach combines genetic engineering with T-cell biology to direct immune cells toward selected antigens on cancer cells (13, 14).

The biological basis of CAR-T therapy comes from the role of T cells in immune surveillance. Under normal physiological conditions, T cells recognize and eliminate infected or abnormal cells through the interaction between their T-cell receptors (TCRs) and peptide antigens presented by major histocompatibility complex (MHC) molecules on the cell surface. Cancer cells can evade normal T-cells by reducing MHC class I antigen presentation (15). In contrast, CARs are engineered to recognize specific antigens directly on the surface of target cells, without requiring the antigens to be presented by MHC molecules. CAR-T cells therefore bypass this type of escape mechanism. However, cancer cells can still evade immune recognition by CAR-T cells by reducing or losing the target antigen on their surface or creating an immunosuppressive environment that limits immune cell activity even when the antigen is present (16, 17).

CAR-T therapy has been specifically devised to overcome these challenges (12, 13). The therapeutic workflow starts with the isolation of autologous T cells from peripheral blood, followed by genetic modification in vitro to express chimeric antigen receptors–synthetic constructs tailored to identify tumor-associated surface antigens (12, 13). After modified T cells are expanded to large numbers, patients undergo a lymphodepleting pre-conditioning regimen to deplete endogenous host lymphocytes (18). After which the engineered cells are reinfused into the patient (Figure 1)(13, 18). Upon reintroduction, these CAR-expressing T cells can directly engage their cognate antigens on neoplastic cells, triggering cytolytic effector functions that ultimately culminate in targeted tumor destruction (12, 13). By bypassing some of the natural constraints on immune recognition, CAR-T therapy enhances T cells' ability to identify and respond to cancer (12, 13).

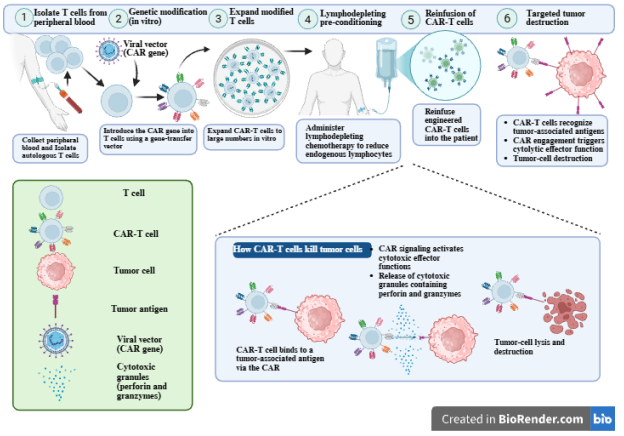

Figure 1. Conventional autologous CAR-T cell therapeutic workflow.

One of the most important advantages of CAR-T therapy is its specificity. Traditional cancer treatments often lack precise targeting, affecting both cancerous and healthy cells. Chemotherapy, for example, works by attacking fast-dividing cells, but this also harms healthy structures such as hair follicles, the digestive tract lining, and bone marrow, which accounts for many of its well-known side effects (13). In contrast, CAR-T therapy is built around molecular recognition, allowing the reengineered immune cells to seek out and attack only those cells carrying a specific surface marker (12, 13). This refined approach improves treatment accuracy and may reduce unintended damage to normal tissues. Moreover, some CAR-T cells can persist in the body for years post-infusion, providing long-term immune surveillance that substantially reduces the risk of disease recurrence (14). While long-term CAR-T persistence establishes ongoing immune surveillance that can lower the risk of recurrence, it does not completely prevent relapse, as tumors may still evade detection through target antigen loss or T-cell exhaustion. Indeed, this enduring immune surveillance is one of the key advantages that distinguishes CAR-T therapy from conventional options like chemotherapy or radiation, which typically do not provide sustained activity after administration. For instance, a long-term follow-up study of two patients with chronic lymphocytic leukemia (CLL) treated with CD19-specific CAR-T cells found that both patients (2/2) maintained complete remission more than 10 years after infusion, with CAR-T cells remaining detectable in their blood at 9 and 10 years of follow-up, respectively (14).

The earliest and most striking clinical achievements with CAR-T therapy have come in blood cancers, particularly those affecting the immune system itself.(13) One reason these cancers have responded well is that they often display uniform surface markers, making them easier for engineered cells to recognize (13). A key example is CD19, a protein commonly found on B cells (13, 19). CAR-T therapies targeting CD19 have shown strong clinical responses in diseases such as B-cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma (DLBCL), and chronic lymphocytic leukemia (CLL) (13, 19, 20). In many cases, patients who had stopped responding to previous treatments or experienced disease relapse achieved long-lasting remission after receiving CAR-T therapy (19, 20). These early results provided some of the most convincing evidence that genetically altered immune cells could function as a powerful weapon against cancer (13, 19).

As research progressed, the scope of CAR-T therapy expanded to include other blood-based cancers beyond those initially treated with CD19-targeting approaches (13). Multiple myeloma, a cancer arising from abnormal plasma cells in the bone marrow, became another area of focus (21, 22). In this setting, the protein B-cell maturation antigen (BCMA) was identified as a suitable target because it is highly expressed on malignant plasma cells (21, 22). Clinical studies investigating BCMA-targeted CAR-T therapy have demonstrated encouraging outcomes, with high response rates even in patients with advanced disease that no longer responded to conventional treatments (21, 22). However, long-term therapeutic durability remains constrained, and relapse remains common, primarily driven by factors such as reduced BCMA expression, antigen escape, limited CAR T-cell persistence, and T-cell exhaustion (21, 22). Despite these challenges, these observations illustrate how CAR-T cell therapy can be adapted to recognize different cancer-associated antigens (13). This adaptability has driven growing interest in applying CAR-T-based approaches to a wider range of malignancies (13).

Despite these achievements, blood cancer accounts for only a fraction of the overall cancer cases worldwide. The majority of cancer-related fatalities stem from solid tumors, which grow as organized masses within particular organs or tissues (12, 13). This category encompasses some of the most prevalent and challenging malignancies, including breast, lung, pancreatic, ovarian, liver, and colorectal cancers, along with brain tumors like glioblastoma and skin cancers such as melanoma (13, 23). Since conventional treatments frequently fail to fully eradicate advanced solid tumors, researchers have increasingly turned their attention to whether CAR-T cell therapy might be adapted to address these diseases (12, 13, 23).

CAR-T Cell Therapy And Solid Cancers

Although CAR-T cell therapy has demonstrated notable clinical efficacy in treating hematologic malignancies, its application to solid tumors has encountered substantial obstacles (13, 24). In contrast to hematological cancers, which are generally more accessible to circulating immune cells, solid tumors exhibit a heterogeneous architecture composed of diverse malignant cells interspersed with stromal elements, aberrant vasculature, extracellular matrix components, and various infiltrating immune populations (12, 13, 24). Moreover, numerous solid tumors evolve an immunosuppressive TME that impedes T-cell activation, persistence, and effector functions (12, 13, 24). These intrinsic features partially account for why the therapeutic achievements observed in liquid tumors have not been equivalently realized in solid forms. Understanding these barriers is essential for developing CAR-T strategies that can function more effectively in solid tumors (12, 24).

Multiple biological barriers limit the effectiveness of CAR-T therapy against solid malignancies. A principal constraint is intratumoral heterogeneity, wherein malignant cells—both within a single lesion and across disparate patients—display marked variability in genomic profiles and surface epitope expression (12, 13). Consequently, a uniformly engineered CAR-T product may fail to recognize all tumor clones. Even if CAR-T cells efficiently eliminate antigen-expressing tumor cells, those variants with diminished or absent target antigen can persist and propagate, a process termed antigen escape (13, 25). Clinically, this phenomenon is widely documented in B-cell ALL, where 30-50% of patient relapses post-CD19 CAR-T therapy are characterized by CD19-negative tumor clones (25). This pattern highlights the importance of developing strategies that can recognize tumor cells even when CD19 expression is lost or reduced (23, 25).

Beyond antigen heterogeneity, the structural organization of solid tumors poses a second major physiological barrier. In contrast to the circulating nature of hematological malignancies, solid tumors are often characterized by dense fibrotic stroma, aberrant vasculature, and an extensive extracellular matrix, all of which physically obstruct the infiltration of immune effectors (14, 26). As a result, even after intravenous infusion, CAR-T cells often struggle to navigate through these physical obstacles and reach tumor tissue effectively; those that do manage to enter may exhibit uneven spatial distribution (26-28). This compromised trafficking and limited intratumoral accumulation thereby reduce the frequency of CAR-T tumor cell interactions, undermining overall treatment potency (27, 28).

Once CAR-T cells have infiltrated the tumor site, the local microenvironment imposes additional layers of immunosuppression. Within many solid tumors, there exists an abundance of regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), all of which actively restrain T-cell activation and lytic activity (13, 29). Concurrently, secreted suppressive cytokines-including transforming growth factor-β (TGF-β) and interleukin- 10 (IL-10), further suppress immune activity and can impair CAR-T cell persistence and function (13). Continuous exposure to tumor antigens within this immunosuppressive environment can eventually lead to T-cell exhaustion, a hypofunctional state marked by declining proliferative capacity, diminished cytokine output, and impaired tumor-killing function (12, 29). These mechanisms can limit CAR-T cell activity even after the cells reach the tumor, making sustained responses more difficult to achieve (13, 23).

Table 1. Common differences between CAR-T cell therapy in blood versus solid cancers

Hematological malignancies	Solid tumors
Relatively accessible malignant cells	Restricted trafficking to malignant cells (due to dense tumor tissue and/or abnormal blood vessels)
Some established target antigens (although expression can vary)	High antigen heterogeneity / Shortage of unique tumor-specific antigens
Generally more favorable microenvironment for T-cell expansion and activity	Immunosuppressive tumor microenvironment (TME) including both physical barriers and inhibitory signals
Lower risk of severe "on-target, off-tumor" toxicity, yet healthy cells expressing target antigens may also be affected	Higher risk of "on-target, off-tumor" toxicity due to shared antigens on healthy tissues

In light of these obstacles, considerable research effort has been directed toward identifying suitable tumor-associated antigens for CAR-T targeting (30, 31). An optimal candidate would exhibit robust expression on neoplastic cells while showing minimal presence on vital normal tissues, thereby mitigating the risk of on-target/off-tumor adverse effects (30, 31). However, truly tumor-restricted surface markers are relatively scarce; consequently, most current CAR-T designs rely on tumor-associated antigens that are not entirely exclusive to malignant cells and may be detected at lower levels on certain healthy tissues (30, 32).

Accordingly, a number of candidate antigens have advanced into clinical evaluation.(33) In breast cancer, human epidermal growth factor receptor (HER2) has garnered interest owing to its overexpression in a proportion of tumors (33). Although HER2-targeted CAR-T constructs have exhibited antitumor activity in preclinical models and preliminary human studies, their clinical application remains at an exploratory stage. A more compelling example comes from neuroblastoma, where the surface glycolipid GD2 is abundantly present on malignant cells (34). Clinical trials of GD2-specific CAR-T products have demonstrated in vivo expansion and persistence of the engineered cells, together with measurable antitumor responses in patients (34). In a phase 1-2 clinical trial of GD2-CART01, 17 of 27 treated patients had an objective response, including 9 complete responses and 8 partial responses (34). These observations underscore both the promise of appropriate antigen selection and the ongoing challenges of achieving durable disease control (34).

Glioblastoma, in contrast, exemplifies additional layers of complexity, particularly regarding intratumoral antigen diversity and restricted physical accessibility (35). Investigational efforts have focused on targets such as the mutant variant EGFRvIII and IL13Rα2 (35, 36). EGFRvIII is an attractive target because it is a tumor-specific mutant form of EGFR; however, its heterogeneous expression can permit antigen-negative tumor cells to escape CAR-T-mediated killing (35, 36). In parallel, IL13Rα2-directed CAR-T therapies have moved forward into clinical testing, with some trials incorporating locoregional administration strategies to circumvent delivery barriers and address antigenic variability (35, 37). A 2024 phase 1 study of locoregionally administered IL13Rα2-CAR-T cells in recurrent high-grade glioma reported stable disease or better in 29 of 58 response-evaluable patients, including two partial responses and one complete response (37). A separate phase 1 trial evaluated intrathecally delivered bivalent CAR-T cells targeting EGFR and IL13Rα2; although all six patients in the interim analysis displayed early reduction in tumor enhancement or size, none fulfilled the criteria for an objective response (38). These findings indicate biological activity while also highlighting that refined targeting and administration strategies have not yet consistently translated into durable clinical responses (37, 38).

Beyond these, additional tumor-associated antigens–such as mesothelin in pancreatic and ovarian cancer, and glypican-3 (GPC3) in hepatocellular carcinoma–have also entered preclinical and early clinical investigation (39-41). Nevertheless, preliminary evidence suggests that antigen recognition alone may be insufficient to surmount the multifaceted barriers characteristic of solid malignancies (39). Consequently, research has increasingly expanded beyond target discovery to include engineering the CAR-T cells themselves, enhancing their capacity to withstand and operate within the immunosuppressive and structurally complex tumor environment (42). Emerging approaches–including multi-antigen targeting, optimized receptor architectures, improved homing capabilities, and resistance to inhibitory signals–constitute important strategies for advancing next-generation CAR-T therapies against solid tumors (42, 43).

Despite the ability of CAR-T cells to recognize specific tumor-associated antigens, their clinical use can cause several serious adverse effects. One major complication is cytokine release syndrome (CRS), which occurs when extensive CAR-T-cell activation triggers the excessive release of inflammatory cytokines. Depending on its severity, CRS can cause fever and systemic inflammation, as well as more serious complications such as low blood pressure and organ dysfunction. Another important toxicity is immune effector cell-associated neurotoxicity syndrome (ICANS), a neurological complication that can involve confusion, difficulty speaking, and, in severe cases, seizures or cerebral edema (13). These complications have been observed in clinical studies. For example, in a study of 50 children and young adults treated with CD19-directed CAR-T cells for B-cell acute lymphoblastic leukemia, 35 patients (70 %) developed CRS and 10 (20%) experienced neurotoxicity, including four cases of severe neurotoxicity (19).

A further safety concern is on-target, off-tumor toxicity, which occurs when CAR-T cells recognize their target antigen on healthy cells as well as cancer cells. CD19-directed CAR-T therapy provides a clear example: because CD19 is also expressed on normal B cells, treatment can eliminate these cells along with malignant B cells, resulting in B-cell aplasia. This

can reduce antibody production and increase susceptibility to infections, sometimes requiring immunoglobulin replacement therapy (13 30). The extent of this risk depends partly on the distribution of the target antigen in normal tissues, making careful antigen selection particularly important when developing CAR-T therapies for solid tumors (30).

Emerging Strategies

Although conventional CAR-T cell therapy has produced remarkable results in hematologic malignancies, its applications to solid tumors remain limited by antigen heterogeneity, poor T-cell infiltration, and the immunosuppressive tumor microenvironment (44, 45). These challenges have inspired researchers to move beyond the conventional approach of engineering T cells to recognize a single antigen. Instead, newer CAR-T designs aim to improve tumor recognition, reduce antigen escape, increase selectivity, and allow the engineered cells to function more efficiently within the TME. Among these approaches, multi-target CAR-T cells, logic-gated CAR-T cells, in vivo CAR- T generation, and armored CAR-T cells represent some important directions in the development of next-generation cellular therapies (44, 45).

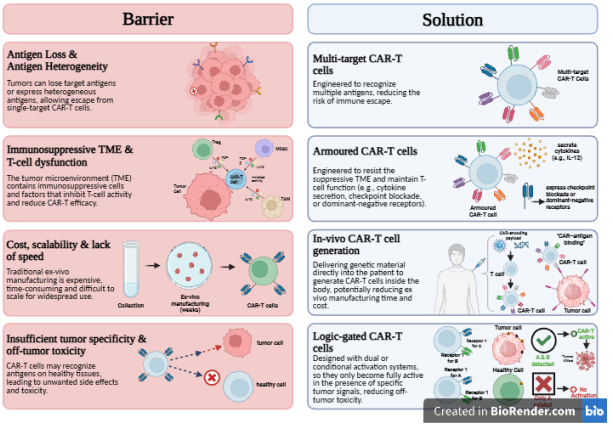

Figure 2. Key Barriers and Next-Generation Solutions in CAR-T Cell Therapy

Dual/multi-CAR-T cells

One major limitation of the conventional CAR-T therapy is its dependence on a single tumor-associated antigen. If the target antigen is expressed unevenly across a tumor or is lost during treatment, cancer cells that no longer express the antigen may escape CAR-T-mediated killing (46, 47). Dual-or multi-target CAR-T strategies have therefore been developed to recognize more than one antigen, with the goal of reducing the likelihood of antigen-negative tumor escape. In these approaches, a single CAR-T product may be engineered to express two CARs or a receptor that is able to recognize multiple antigens. Depending on the design, the T cells may respond when either target is present, or may require the recognition of multiple targets to achieve a stronger activation signal (47, 48). The first strategy can increase tumor coverage when antigen expression is heterogeneous, whereas the second can improve selectivity when individual antigens are also expressed on normal tissues. This approach is particularly relevant to solid tumors, where different cancer cells within the same tumor may express different levels of surface antigens. Preclinical studies have shown that dual-target CAR-T cells can improve tumor control compared with single-target approaches in selected models, although determining the appropriate combination of antigens remains challenging (46, 47). Therefore, multi-targeting may help address antigen heterogeneity, but it does not completely eliminate the biological barriers created by the tumor microenvironment.

Logic-gated CAR-T cells

A related but more sophisticated strategy is the development of logic-gated CAR-T cells, which use combinations of biological signals to control T-cell activation. Rather than just recognizing one antigen, these systems are designed according to principles similar to Boolean logic, such as AND, OR, and NOT gates (36, 49). For example, an AND-gated CAR-T cell can be designed to require recognition of two tumor-associated antigens before producing a full activation response. This may improve tumor selectivity when neither antigen alone is sufficiently specific for cancer cells (49, 50). In contrast, an OR-gated system allows recognition of either of two antigens, which can increase the number of tumor cells that can be targeted and reduce antigen escape (49, 51). NOT-gated systems can provide an additional layer of protection by using an inhibitory receptor to prevent T-cell activation when a marker associated with healthy tissue is detected (48). More recently, researchers have also explored contextual logic gates that use characteristics of the TME, such as hypoxia, pH, protease activity, or immunosuppressive cytokine signals, as additional inputs (48). These approaches are especially attractive for solid tumors because they attempt to distinguish malignant tissues from healthy tissue based on combinations of signals instead of a single antigen (48, 49). However, the presence of logic-gated CAR-T cells requires more complex genetic designs, and their safety and effectiveness still need to be established through future preclinical and clinical studies (36, 48).

In vivo CAR-T cell generation

Unlike the previous two approaches, which primarily modify how CAR-T cells recognize and respond to tumor cells, in vivo CAR-T generation focuses on changing how these therapeutic cells are produced. Rather than collecting T cells from a patient, genetically engineering and expanding them outside the body, and then reinfusing the final product, this approach aims to deliver CAR-encoding genetic material directly to T cells within the patient (52). Importantly, these advantages relate primarily to the manufacturing and delivery of CAR-T therapy rather than inherently improving tumor recognition or overcoming the biological barriers within solid tumors (52). Traditional CAR-T therapy requires T cells to be collected from the patient, genetically modified and expanded outside the body, and then reinfused. This manufacturing process is technically complex, time-consuming, and expensive, and the prolonged ex vivo expansion of T cells may also influence their functional state (52, 53). In vivo CAR-T therapy aims to bypass much of this process by delivering CAR-encoding genetic material directly to T cells within the patient, which allows CAR-T cells to be generated inside the body (52). Several delivery systems, including targeted viral vectors, lipid nanoparticles, and biomaterial-based platforms, have been investigated to deliver CAR-encoding genetic material to T cells in vivo. Preclinical studies have demonstrated that T-cell-targeted vectors can generate functional CAR-T cells in vivo (52-55). For example, Ho, in his primary study “In vivo generation of CAR T cells in the presence of human myeloid cells,” demonstrated in humanized mouse models that a single intravenous

administration of T-cell-targeted lentiviral vectors could generate CD19-specific CAR-T cells in vivo, although CAR-T generation was affected by innate immune responses and the presence of myeloid cells (53). Meanwhile, Agarwalla and colleagues loaded a scaffold with human peripheral blood mononuclear cells and retroviral particles carrying the CAR gene, then implanted it in mice. The scaffold allowed CAR-T cells to be generated and released into the body (54). These approaches could reduce the extent to which ex vivo processing is needed and therefore also simplify manufacturing. This could, in turn, make treatment faster, more scalable, and less dependent on individualized manufacturing (52, 54). However, this strategy also introduces new challenges, especially the need to control which cells receive the genetic material, regulate the level and duration of CAR expression, and minimize unintended gene delivery (52, 55). As a result, although the in vivo CAR-T generation has progressed from a conceptual approach to increasingly advanced preclinical studies and early translational development, substantial work remains before it can become a broadly applicable treatment strategy (52, 55).

Armored CAR-T cells

Even when CAR-T cells can successfully reach a solid tumor and recognize their target, the immunosuppressive tumor microenvironment can continue to limit their activity. Armored CAR- T cells are engineered with additional transgenes to express functional proteins–such as cytokines, chemokine receptors, or checkpoint inhibitors–that enhance cell persistence, resist immunosuppression, and actively remodel the TME (56-58). For example, some designs incorporate cytokines such as interleukin-12(IL-12), which can enhance T-cell activity and stimulate other components of the immune system (56, 59). Other approaches have introduced dominant-negative receptors or switch receptors designed to reduce the inhibitory effects of signals such as transforming growth factor-β (TGF-β) or immune checkpoint pathways (56, 58, 60). Some armored CAR-T cells have also been designed to secrete proteins that modify the tumor environment, such as enzymes that are capable of altering the extracellular matrix, potentially improving T-cell penetration into dense tumor tissues (56). These additional functions are intended to make CAR-T cells more capable of surviving and functioning in conditions that would normally suppress their activity (57, 58). However, increasing the number of functions engineered into a CAR-T cell can also increase the complexity of therapeutic products and may introduce additional safety concerns (56, 58, 59). Despite their potential, many armored CAR-T approaches remain at the preclinical stage, and their safety and effectiveness need further evaluation. Their clinical value will depend on whether these added functions improve tumor control without causing unacceptable toxicity or damage to healthy tissues (57, 58). Overall, these newer approaches demonstrate that the future development of CAR-T therapy may depend not on a single improvement, but on combining better tumor recognition with enhanced control, persistence, trafficking, and resistance to the tumor microenvironment.

Conclusion

This review shows that CAR-T cell therapy represents a major shift in cancer treatment by using genetically engineered immune cells to directly recognize and eliminate malignant cells. Its clinical success in hematologic cancers has demonstrated that T cells can be modified to produce strong and sometimes long-lasting antitumor responses (12, 13). Studies targeting CD19 and BCMA have shown that CAR-T cells can induce durable remission even in patients with relapsed or treatment-resistant disease (13, 20-22). These results have provided an important foundation for applying CAR-T therapy to other types of cancer.

At the same time, the challenges have been seen in the solid tumors show that successful CAR-T therapy involves more than finding a tumor-associated antigen. Solid tumors can have heterogeneous antigen expression, dense extracellular matrix, abnormal blood vessels, and an immunosuppressive TME, all of which can limit CAR-T cell infiltration, persistence, and eventually its function (13, 24, 26-28). Clinical studies targeting antigens such as GD2, IL13Rα2, and GPC3 have shown that CAR-T cells can produce responses in some solid tumors, but their effectiveness is still limited compared with their success in hematologic cancers (23, 37, 40). This overall highlights the need to consider the biology and environment of each tumor when developing CAR-T therapies.

Newer CAR-T strategies are being developed to address these limitations from different directions. Multi-target and logic-gated CAR-T cells are aimed at improving tumor recognition and reducing antigen escape, while armored CAR-T cells are designed to help T cells survive and function in the immunosuppressive TME (24, 36, 48, 49, 57). In vivo CAR-T generation focuses on a different problem by potentially making the manufacturing process faster, simpler, and more scalable (52- 55). Together, these approaches show that improving CAR-T therapy will likely require several strategies working together rather than one single technological change.

Overall, CAR-T cell therapy has demonstrated the potential of using engineered immune cells as a personalized approach to cancer treatment. Although many limitations still remain, continued research into tumor biology, CAR design, T-cell function, and manufacturing could help make CAR-T therapy more effective and applicable to a wider range of cancers, and benefiting a larger number of cancer patients (12, 14, 25).

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Ethics and consent

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