CAR-T Cell Therapy: Revolutionizing Cancer Treatment

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Abstract

CAR-T cell therapy has emerged as a groundbreaking approach in cancer treatment, offering new hope for patients with refractory and relapsed malignancies. This literature review provides a comprehensive overview of the development, applications, and future directions of CAR-T cell therapy. We explore the principles behind CAR-T cell engineering, highlight the clinical successes and challenges in treating hematologic malignancies, and discuss the potential and hurdles in targeting solid tumors. The review also examines the safety profiles, including adverse effects management, and delves into the mechanisms of resistance and relapse. Furthermore, we address regulatory and ethical considerations, patient perspectives, and the innovative advancements shaping the future of CAR-T cell therapy. By synthesizing current research and clinical data, this review underscores the transformative impact of CAR-T cell therapy in oncology and its promising trajectory in the fight against cancer.

Keywords: *CAR-T cell therapy, cancer treatment, hematologic malignancies, solid tumors, next-generation CAR-T.*

INTRODUCTION

Chimeric Antigen Receptor T-cell (CAR-T) therapy represents a groundbreaking advance in cancer immunotherapy. The concept of CAR-T cells emerged in the late 1980s when researchers began to explore the possibility of genetically engineering T cells to target cancer cells more effectively. The first CARs were developed in 1987 by Kuwana, *et al*., which laid the foundation for future developments in this field (Lin, *et al*., 2022).

The first-generation CARs, which included a single-chain variable fragment (scFv) for antigen recognition and a CD3ζ signaling domain, showed limited success due to their inability to sustain T-cell activation and proliferation. This led to the development of second-generation CARs that incorporated one co-stimulatory domain, such as CD28 or 4-1BB, enhancing T-cell persistence and anti-tumor activity (Xin, *et al*., 2022). Third-generation CARs combined two co-stimulatory domains, further improving their efficacy. The most recent, fourth-generation CARs, also known as TRUCKs (T cells Redirected for Universal Cytokine-mediated Killing), are engineered to secrete additional cytokines, boosting their ability to modulate the tumor microenvironment (NCI, 2022).

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The pivotal moment for CAR-T therapy came in 2017 when the U.S. Food and Drug Administration (FDA) approved the first CAR-T cell therapy, tisagenlecleucel, for the treatment of pediatric acute lymphoblastic leukemia (ALL). This was followed by approvals for other CAR-T therapies targeting different hematological malignancies, such as diffuse large B-cell lymphoma (DLBCL) (Xin, *et al*., 2022).

CAR-T cell therapy involves collecting a patient's T cells, genetically engineering them to express CARs that specifically target cancer cells, and then re-infusing these modified cells back into the patient (Figure 1). The CAR is an artificial receptor that combines an extracellular antigen-recognition domain, usually derived from an antibody, with intracellular signaling domains that activate T cells upon antigen binding (Lin, *et al*., 2022).

The process begins with leukapheresis, where T cells are extracted from the patient's blood. These cells are then genetically modified using viral vectors to express CARs on their surface. The engineered CAR-T cells are expanded in the laboratory before being infused back into the patient (NCI, 2022).

Upon infusion, CAR-T cells home in on and bind to target antigens on the surface of cancer cells. This binding activates the CAR-T cells, triggering their proliferation and the release of cytotoxic molecules that kill the cancer cells. Additionally, the activated CAR-T cells secrete cytokines that enhance the anti-tumor immune response (Lin, *et al*., 2022; Frontiers in Oncology, 2022).

Despite their remarkable success in treating certain cancers, CAR-T cell therapies face challenges, including severe side effects such as cytokine release syndrome (CRS) and neurotoxicity. Moreover, issues related to antigen escape, where cancer cells lose the target antigen, can lead to relapse. Researchers are actively developing strategies to overcome these hurdles, including the use of next-generation CARs and combination therapies (NCI, 2022; Xin, *et al*., 2022).

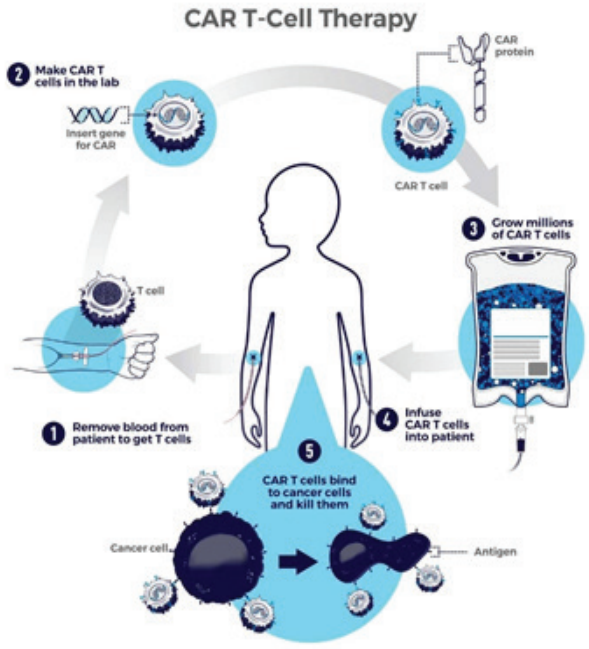

Figure 1 shows the steps for creating CAR T-cell therapy, adapted from cancer.gov (https://www.cancer.gov/about-cancer/treatment/research/car-t-cell-therapy-infographic).

Current Applications of CAR-T Cell Therapy

CAR-T cell therapy has become a transformative approach in the treatment of certain hematologic malignancies, with several therapies approved by the U.S. Food and Drug Administration (FDA) for specific cancer types and patient characteristics (Table 1). Tisagenlecleucel (Kymriah) is approved for relapsed or refractory (r/r) B-cell acute lymphoblastic leukemia (ALL) in patients up to 25 years old and for adults with r/r large B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma (Gauthier, *et al*., 2022). Similarly, Axicabtagene ciloleucel (Yescarta) is indicated for adult patients with r/r large B-cell lymphoma after two or more lines of systemic therapy, covering DLBCL, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. It is also approved for adults with r/r follicular lymphoma (Neelapu, *et al*., 2020).

Lisocabtagene maraleucel (Breyanzi) is another therapy for adults with r/r large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B. The FDA expanded its approval in March 2024 to include patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) (FDA, 2024). Additionally, Idecabtagene vicleucel (Abecma) is the first CAR-T cell therapy approved for multiple myeloma, indicated for adults with r/r multiple myeloma after four or more prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody (Schuster, *et al*., 2019).

CAR-T cell therapy has shown significant success in treating hematologic malignancies such as leukemia and lymphoma, often achieving complete remission where other therapies have failed. In B-cell acute lymphoblastic leukemia (ALL), a young patient with refractory B-cell ALL achieved complete remission after treatment with Tisagenlecleucel (Kymriah), exemplifying the therapy's potential to induce long-term remission and even cure in pediatric and young adult patients who have exhausted conventional treatments (Schuster, *et al*., 2019). For diffuse large B-cell lymphoma (DLBCL), the ZUMA-1 trial highlighted the efficacy of Axicabtagene ciloleucel (Yescarta), where a significant proportion of patients achieved complete remission, with some maintaining their response for years, underscoring the durable nature of CAR-T cell therapy in aggressive lymphomas (Neelapu, *et al*., 2020). The recent approval of Lisocabtagene maraleucel (Breyanzi) for CLL has opened new avenues for patients with this challenging disease. Clinical trials have shown promising results, with many patients achieving deep and lasting remissions, particularly those who have previously failed multiple lines of therapy (FDA, 2024; Gauthier, *et al*., 2022). In multiple myeloma, the KarMMa trial demonstrated the effectiveness of Idecabtagene vicleucel (Abecma) in heavily pre-treated patients, inducing high overall response rates, including stringent complete responses, showcasing its potential as a game-changer for a disease traditionally considered difficult to treat (Schuster, *et al*., 2019).

CAR-T cell therapy continues to revolutionize the treatment landscape for hematologic malignancies, providing hope for patients with otherwise limited options. Its success in clinical practice underscores the importance of ongoing research and development to expand its applications and improve patient outcomes.

CAR-T Cell Therapy in Solid Tumors

CAR-T cell therapy, while demonstrating significant success in treating hematologic malignancies, faces several challenges when applied to solid tumors. The unique tumor

Table 1. shows a summary of the current applications of CAR-T cell therapy.

CAR-T Cell Therapy	*FDA Approval Indications*	*Patient Population*	*Clinical Trials/Notes*
Tisagenlecleucel (Kymriah)	- Relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (ALL) in patients up to 25 years old - Adults with r/r large B-cell lymphoma (DLBCL, high-grade B-cell lymphoma, DLBCL arising from follicular lymphoma)	Pediatric and young adult patients w ith ALL A dults with DLBCL	Achieved complete remission in a significant proportion of patients with ALL and DLBCL (Gauthier, *et al*., 2022)
Axicabtagene ciloleucel (Yescarta)	- Adult patients with r/r large B-cell lymphoma after two or more lines of systemic therapy - Primary mediastinal large B-cell lymphoma - High-grade B-cell lymphoma - DLBCL arising from follicular lymphoma - Adults with r/r follicular lymphoma	Adult patients w ith DLBCL and follicular lymphoma	ZUMA-1 trial showed significant complete remission rates in DLBCL patients (Neelapu, *et al*., 2020)
Lisocabtagene maraleucel (Breyanzi)	- Adults with r/r large B-cell lymphoma after two or more lines of systemic therapy - DLBCL not otherwise specified - High-grade B-cell lymphoma - Primary mediastinal large B-cell lymphoma - Follicular lymphoma grade 3B - Chronic lymphocytic leukemia (CLL) - Small lymphocytic lymphoma (SLL)	Adults w ith various forms of r/r l arge B-cell l ymphoma, C LL, and SLL	Recent FDA approval for CLL in March 2024 with promising results in clinical trials (FDA 2024; Gauthier, *et al*., 2022)
Idecabtagene v icleucel (Abecma)	- Adults with r/r multiple myeloma after four or more prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody	Adults w ith multiple myeloma	KarMMa trial demonstrated significant efficacy in multiple myeloma patients (Schuster, *et al*., 2019)

microenvironment, antigen heterogeneity, and physical barriers of solid tumors pose significant obstacles. The immunosuppressive nature of the solid tumor microenvironment, characterized by factors such as regulatory T cells, myeloid-derived suppressor cells, and cytokines like TGF-β, hinders the efficacy of CAR-T cells (Marofi, *et al.*, 2021). Moreover, the physical barriers of the dense extracellular matrix and abnormal vasculature in solid tumors restrict the infiltration and penetration of CAR-T cells (Sorkhabi, *et al*., 2023).

Despite these challenges, there have been significant advancements in the development of CAR-T cell therapies for solid tumors. Researchers are exploring various strategies to enhance the efficacy of CAR-T cells in this context. These include engineering CAR-T cells to express chemokine receptors that facilitate better tumor homing, designing CAR-T cells with dual or multiple targeting receptors to address antigen heterogeneity, and employing gene-editing techniques like CRISPR to enhance the persistence and functionality of CAR-T cells (Newick, *et al*., 2021).

Clinical trials are underway to evaluate the safety and efficacy of these novel approaches. For instance, studies are investigating CAR-T cells targeting solid tumor antigens such as HER2 in breast cancer, EGFR in glioblastoma, and mesothelin in mesothelioma and pancreatic cancer (Guzman, *et*

al., 2023). Additionally, the combination of CAR-T cell therapy with other treatment modalities, such as checkpoint inhibitors and oncolytic viruses, is being explored to overcome the immunosuppressive tumor microenvironment and enhance anti-tumor responses (Fischer, *et al*., 2021).

Manufacturing and Engineering of CAR-T Cells

The process of manufacturing CAR-T cells involves several critical steps to ensure the efficacy and safety of the final therapeutic product. The production begins with the collection of T cells from the patient, typically through leukapheresis, a procedure where white blood cells are separated from the rest of the blood components. These collected T cells are then genetically modified in the laboratory to express chimeric antigen receptors (CARs) on their surface. CARs are engineered molecules that allow T cells to recognize and attack cancer cells.

The genetic modification is most commonly achieved using viral vectors, such as lentiviruses or retroviruses, which integrate the CAR gene into the T cell genome. This process enables the T cells to produce CARs that can target specific antigens on the surface of cancer cells. Once the T cells are genetically modified, they are expanded in culture to produce a sufficient number of CAR-T cells for infusion back into the patient. The expansion phase is crucial, as it ensures an adequate number of functional CAR-T cells to mount an effective anti-tumor response (Lantidra, 2024).

One of the significant advancements in CAR-T cell engineering is the development of dual-targeting CARs, which are designed to recognize two different antigens on cancer cells. This innovation aims to reduce the risk of antigen escape, where cancer cells lose the targeted antigen and evade the CAR-T cells. Dual-targeting CARs enhance the specificity and efficacy of the therapy by broadening the range of recognizable cancer cell markers (Sana Biotechnology, 2022).

Another innovative approach in CAR-T cell design is the creation of "armored" CAR-T cells. These cells are engineered to secrete additional molecules, such as cytokines, that enhance their anti-tumor activity and persistence within the tumor microenvironment. Armored CAR-T cells are particularly advantageous in overcoming the immunosuppressive conditions often found within tumors, thereby improving the therapeutic outcomes (Rath and Arber, 2020).

The manufacturing process also faces challenges, including maintaining the consistency and quality of the CAR-T cell product. Advanced technologies, such as real-time metabolic analyzers and single-use systems, have been integrated into the production workflow to enhance product quality and reduce contamination risks. These technologies ensure that the CAR-T cells remain viable and functional throughout the manufacturing process (Outlook on Cell and Gene Therapy, 2024).

Safety and Efficacy of CAR-T Cell Therapy

CAR-T cell therapy has demonstrated significant efficacy, particularly in hematological malignancies. The clinical outcomes and long-term efficacy data for CAR-T cell therapy highlight its transformative potential in oncology, but also underscore the complexities involved in its administration and patient management.

CAR-T cell therapy has shown remarkable success in treating certain types of cancers, especially hematologic malignancies such as B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL). Clinical trials have reported high rates of complete remission, with studies indicating that approximately 80-90% of pediatric and young adult patients with relapsed or refractory B-ALL achieve complete remission after treatment with CAR-T cells (Maakaron, *et al*., 2022). Additionally, for patients with DLBCL, complete response rates of around 40-50% have been observed, with sustained remissions extending beyond two years in many cases (Cai, *et al*., 2021).

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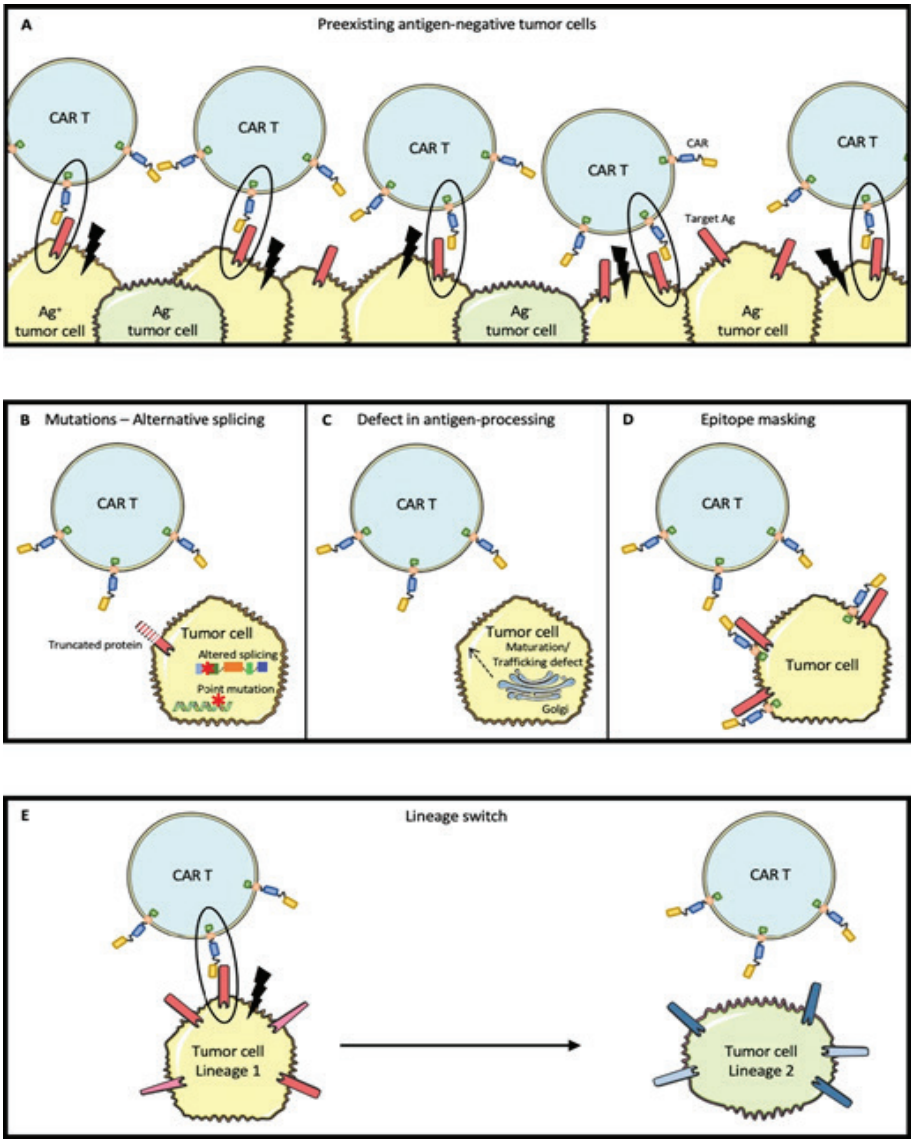

Figure 2 shows Mechanisms responsible for loss of target antigen, conferring resistance to CAR-T cell therapy. A. Due to tumor heterogeneity before any treatment, pre-existing antigen-negative tumor cells may be responsible for resistance to CAR-T cell therapy; B. Point mutations or altered alternative splicing may lead to a truncated target antigen that can no longer be recognized by CAR-T cells; C. Defect in target antigen maturation and trafficking due to lack of appropriate chaperon proteins may be responsible for target antigen membrane expression loss; D. Exceptionally, a tumor cell may be transfected with the CAR vector leading to an epitope masking by the CAR itself and hiding the target antigen from the CAR T cells; E. Lineage switch may be responsible for a complete phenotypic markers remodeling including loss of the target antigen. Adapted from nature.com, https://jhoonline.biomedcentral.com/articles/10.1186/s13045-021-01209-9/figures/1.

However, the efficacy of CAR-T cell therapy can vary, and not all patients experience long-term benefits. Relapse is a significant issue, particularly in solid tumors where CAR-T cells face challenges such as tumor microenvironment resistance and antigen heterogeneity (Kosti, *et al*., 2021). Long-term follow-up studies are crucial for understanding the durability of responses and the potential need for retreatment or additional therapies.

The administration of CAR-T cell therapy is associated with several adverse effects, the most notable being cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). CRS is a systemic inflammatory response caused by the rapid proliferation of CAR-T cells and the consequent release of large quantities of cytokines. Symptoms can range from mild flu-like symptoms to severe, life-threatening conditions such as multi-organ failure (Maakaron, *et al*., 2022).

Management of CRS typically involves supportive care and, in severe cases, the administration of cytokine inhibitors like tocilizumab, an IL-6 receptor antagonist. Tocilizumab has been shown to effectively mitigate severe CRS without impeding the anti-tumor efficacy of CAR-T cells (Shimabukuro-Vornhagen, *et al*., 2018).

Neurotoxicity, manifesting as ICANS, is another significant concern. Symptoms can include confusion, aphasia, seizures, and cerebral edema. The pathophysiology of ICANS is not fully understood, but it is thought to be related to endothelial activation and disruption of the blood-brain barrier. Management strategies for ICANS include corticosteroids and supportive neurological care (Cai, *et al*., 2021).

Mechanisms of Resistance and Relapse in CAR-T Cell Therapy

While CAR-T cell therapy has shown remarkable success in treating hematologic malignancies, a significant challenge remains: resistance and relapse. Understanding the mechanisms behind these issues is crucial for improving the long-term efficacy of this innovative therapy.

One primary reason for resistance and relapse is the loss or downregulation of the target antigen on cancer cells. For instance, in patients treated with CD19-targeted CAR-T cells, a common relapse mechanism involves the loss of CD19 expression on leukemic cells (Lee, 2023). Similarly, in multiple myeloma, resistance can develop through biallelic loss of the B-cell maturation antigen (BCMA) (Samur, *et al.*, 2021). This antigen loss renders the CAR-T cells unable to recognize and attack the cancer cells, leading to disease recurrence.

Another contributing factor is the limited persistence of CAR-T cells within the patient. Effective long-term surveillance against cancer cells requires these engineered T cells to survive and remain active for extended periods. However, many patients experience a decline in CAR-T cell levels over time, reducing the therapy's durability (Gogishvili, *et al*., 2017).

Additionally, the tumor microenvironment can create an immunosuppressive setting that hampers the activity of CAR-T cells. Factors such as regulatory T cells, myeloid-derived suppressor cells, and certain cytokines can inhibit CAR-T cell function and proliferation (Neelapu, *et al.*, 2021). Overcoming these immunosuppressive barriers is essential for enhancing the efficacy of CAR-T therapy in solid tumors and resistant hematologic cancers.

To address these challenges, several strategies are being explored. One approach involves the development of dual-targeting CAR-T cells that can recognize multiple antigens on cancer cells. This method aims to prevent tumor escape through antigen loss by ensuring that if one target is downregulated, the CAR-T cells can still attack another (Jiang, *et al*., 2023). Additionally, engineering CAR-T cells to express cytokines or receptors that enhance their survival and function in

the tumor microenvironment is being investigated (Nerreter, *et al.*, 2021).

Combination therapies also hold promise in overcoming resistance. For example, pairing CAR-T cells with immune checkpoint inhibitors can help alleviate the immunosuppressive effects of the tumor microenvironment, potentially enhancing CAR-T cell activity and persistence (Maude, *et al.,* 2022). Furthermore, ongoing research into the genetic and molecular profiling of resistant tumors is providing insights into new therapeutic targets and approaches to counteract resistance mechanisms.

Regulatory and Ethical Considerations

Regulatory and ethical considerations in CAR-T cell therapy are crucial for its successful implementation and equitable access. The approval process for CAR-T therapies is rigorous and multifaceted, involving several stages to ensure safety and efficacy. Regulatory bodies such as the FDA have introduced pathways like accelerated approval to expedite the availability of CAR-T therapies. However, these pathways present challenges, such as the potential for initial data to be insufficient for long-term clinical benefit verification, leading to possible withdrawal of the therapy post-approval (Marks, 2024).

The FDA's role extends to offering continuous feedback to sponsors through programs like RMAT (Regenerative Medicine Advanced Therapy) designation, which facilitates early interaction to discuss surrogate endpoints and trial designs, thereby aligning expectations and minimizing development delays (FDA, 2023). Despite these supportive measures, regulatory hurdles remain significant, including complex manufacturing requirements and the need for extensive quality checks, which can delay the "vein-to-vein" time and increase production costs (Pharmaceutical Medicine, 2024).

Ethically, CAR-T cell therapy raises significant issues regarding access, cost, and equity. The high cost of CAR-T therapies, often exceeding $373,000, limits their accessibility to a broader patient population, highlighting the need for policies that address cost barriers and promote equitable distribution (Imbach, *et al.*, 2018). Additionally, the ethical implications of patient selection for these expensive treatments require careful consideration to avoid biases and ensure that the benefits of CAR-T therapies are available to all eligible patients, regardless of socioeconomic status.

Future Directions and Innovations in CAR-T Cell Therapy

Next-generation CAR-T cell therapies are being developed to enhance the efficacy and safety of current treatments. One major area of focus is the design of next-generation CARs, which include dual-targeting CARs and armored CARs. Dual-targeting CARs are engineered to recognize two different antigens simultaneously, thereby reducing the likelihood of tumor escape due to antigen loss (Rafiq, *et al.,* 2020). Armored CARs are designed to secrete cytokines or other immune-modulating agents, enhancing their anti-tumor activity and persistence in the hostile tumor microenvironment (Brudno and Kochenderfer, 2020).

Emerging technologies such as gene editing and synthetic biology are also being harnessed to improve CAR-T cell therapies. CRISPR/Cas9 technology, for example, allows precise modifications to be made to the T-cell genome, potentially increasing the safety and effectiveness of CAR-T cells by knocking out genes that cause adverse effects or resistance (Khoshandam, *et al*., 2021). Synthetic biology approaches are being used to create more sophisticated CAR constructs, including those that can respond to the tumor microenvironment or other stimuli, offering more controlled and targeted therapeutic responses (Schmidt, *et al.*, 2020).

Beyond cancer, there is growing interest in applying CAR-T cell technology to other diseases, including autoimmune disorders. CAR-T cells could be engineered to target and eliminate pathogenic immune cells, providing a novel approach to

treating diseases such as lupus or rheumatoid arthritis (Aghajanian, *et al.*, 2020). This expansion of CAR-T cell therapy beyond oncology represents a significant innovation, potentially transforming the treatment landscape for a variety of diseases.

Patient Perspectives and Quality of Life

CAR-T cell therapy has brought about significant changes in the landscape of cancer treatment, not only from a clinical standpoint but also in terms of patient quality of life. Understanding the impact of this therapy from the patients' perspectives provides a comprehensive view of its benefits and challenges.

Patients undergoing CAR-T cell therapy often experience a profound change in their quality of life, especially those who have not responded to conventional treatments. The initial response to CAR-T cell therapy can be dramatic, with many patients experiencing complete remission. For instance, John Cadwallader, a multiple myeloma patient, saw a significant improvement in his condition following CAR-T cell therapy. His story highlights the potential for CAR-T therapy to induce remission even in cases where other treatments have failed (Sidana, *et al.*, 2022).

However, the journey is not without challenges. Patients frequently face severe side effects, including cytokine release syndrome (CRS) and neurotoxicity, which can significantly impact their daily lives. Effective management of these adverse effects is crucial for improving patient outcomes and quality of life. Innovations in monitoring and treating these side effects, such as the use of interleukin-6 inhibitors and corticosteroids, have been instrumental in making the therapy more tolerable (Chou and Turtle, 2020).

The long-term quality of life for patients who achieve remission is another critical aspect. While some patients remain in remission and can return to their normal lives, others may experience a relapse, requiring further treatment. The emotional and psychological burden of potential relapse is substantial, and ongoing support from healthcare providers and patient advocacy groups is essential (Gauthier, *et al*., 2022).

Patient advocacy has also played a pivotal role in improving access to CAR-T cell therapy and addressing issues related to cost and equity. The high cost of CAR-T therapy remains a significant barrier, limiting its availability to a broader patient population. Efforts by advocacy groups to push for better insurance coverage and funding for CAR-T therapies are crucial for making these life-saving treatments accessible to all who need them (Gauthier, *et al*., 2022).

CONLUSION

CAR-T cell therapy has revolutionized the treatment landscape for various cancers, particularly hematologic malignancies such as leukemia and lymphoma. The therapy's mechanism, which involves reprogramming a patient's T cells to target cancer cells, has demonstrated remarkable efficacy, leading to significant remission rates even in patients with refractory or relapsed disease. Despite these successes, challenges remain, including the management of severe side effects, the high cost of treatment, and the complexities associated with manufacturing and regulatory approval. Innovations in CAR-T cell design, such as dual-targeting and armored CARs, alongside advancements in gene editing technologies, hold promise for overcoming these hurdles and enhancing the therapy's safety and effectiveness.

Looking forward, the potential of CAR-T cell therapy extends beyond oncology, with ongoing research exploring its application in autoimmune diseases. The future of CAR-T therapy in oncology appears promising, with continuous improvements in design and delivery expected to broaden its applicability and accessibility. Efforts to reduce costs and streamline production will be crucial in

making these therapies available to a wider patient population. As research progresses, the integration of CAR-T cell therapy into standard cancer treatment protocols will likely increase, offering hope to many patients and significantly advancing the field of oncology.

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"NOT APPLICABLE"

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