
Gene Modified Immune Cell Therapy: A Survey

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Abstract

Gene modified immune cell therapy, encompassing CAR-T and TCR-T cells, represents a significant advancement in oncology, offering a personalized and targeted approach to cancer treatment. This survey examines the current landscape of these therapies, highlighting their efficacy in hematological malignancies and the challenges faced in solid tumors due to the complex tumor microenvironment (TME). CAR-T cells, particularly those targeting CD19, have achieved notable success in blood cancers, while TCR-T cells show promise in targeting intracellular antigens in solid tumors. Despite these advancements, issues such as antigen escape, T cell exhaustion, and the immunosuppressive TME continue to pose significant hurdles. Innovations in genetic engineering, including CRISPR-Cas9, have enhanced the specificity and safety of these therapies, while advancements in delivery technologies and combination strategies aim to overcome resistance mechanisms and improve therapeutic outcomes. The regulatory landscape has evolved with key approvals, yet manufacturing complexities and high costs remain challenges. Future research directions focus on optimizing T cell persistence, exploring universal CAR-T cells, and integrating advanced imaging and computational models to enhance therapy precision. As these therapies continue to evolve, they hold the potential to transform cancer treatment, offering new hope for patients with resistant or difficult-to-treat malignancies.

1 Introduction

1.1 Significance of Gene Modified Immune Cell Therapy

Gene modified immune cell therapy marks a significant advancement in oncology, providing a personalized approach that contrasts sharply with conventional treatments like chemotherapy and radiotherapy. By genetically engineering T cells to enhance their capacity to recognize and destroy cancer cells, these therapies, particularly Chimeric Antigen Receptor T (CAR-T) cells, have demonstrated remarkable efficacy in hematological malignancies. This progress underscores the potential of genetically modified immune cell therapies to not only improve response rates but also to achieve lasting remissions in challenging malignancies. Innovative delivery technologies, including nanomedicine and advanced biomaterials, aim to optimize immune responses while minimizing adverse effects, signaling a transformative shift in cancer immunotherapy strategies [1, 2].

Despite the successes seen in blood cancers, the application of CAR-T cell therapy to solid tumors presents significant challenges due to efficacy issues and resistance. The heterogeneity of target antigens in adoptive cell transfer (ACT) therapies introduces additional complexities, necessitating innovative strategies to overcome therapeutic resistance and enhance treatment outcomes [3]. Furthermore, the metabolic reprogramming of cancer cells and its interplay with the immune response are crucial factors affecting the success of these therapies [4].

T Cell Receptor-modified T (TCR-T) cell therapies offer another promising strategy, particularly for conditions such as acute myeloid leukemia (AML). The precise targeting of tumor-specific antigens is essential for effective TCR-T immunotherapy, paving the way for more tailored treatment approaches

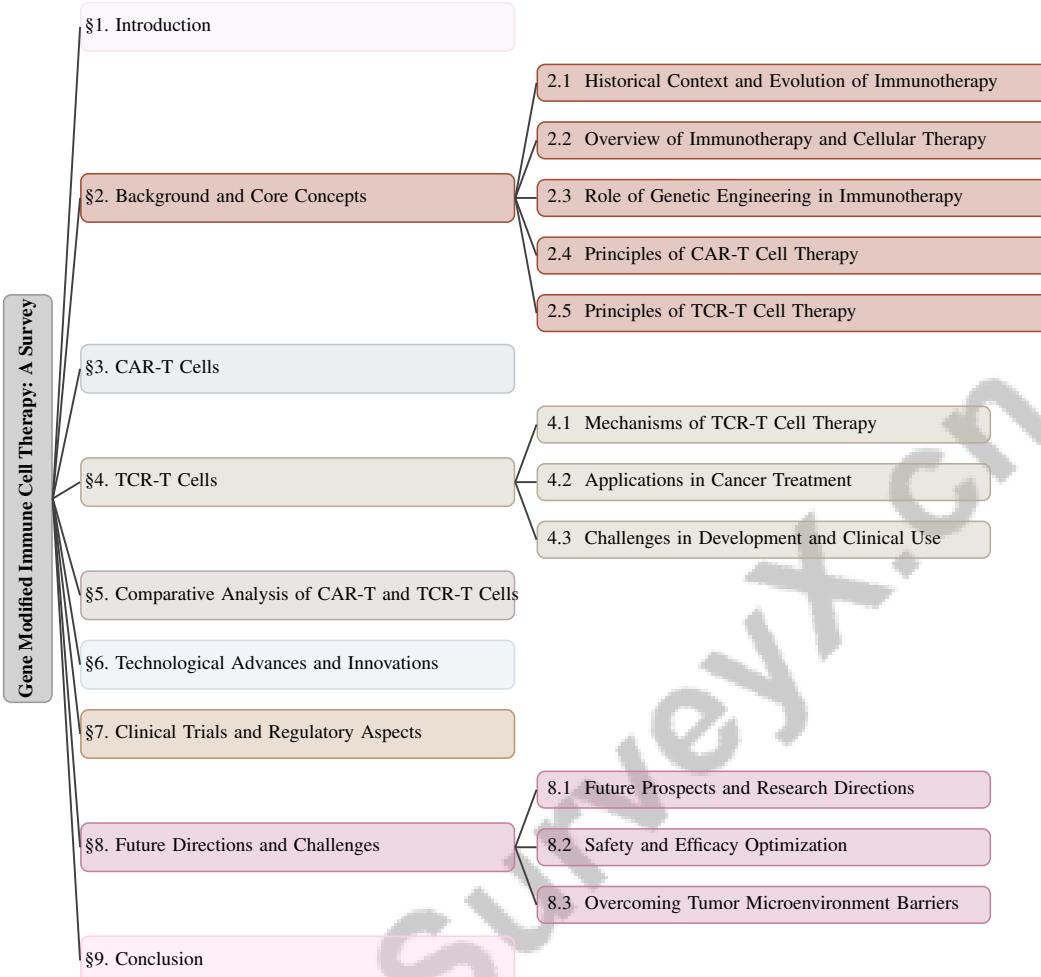


Figure 1: chapter structure

[5]. Nonetheless, challenges related to the immunogenicity of CAR constructs and their influence on treatment efficacy persist [2].

Advancements in biomaterials and drug delivery systems are being developed to enhance the efficacy of these therapies while reducing adverse effects [2]. As research continues to address these challenges, the role of gene modified immune cell therapy in improving cancer outcomes is anticipated to expand, providing new hope for patients with previously untreatable malignancies. The exploration of universal CAR-T (UCAR-T) cell therapy also presents a potential breakthrough to the limitations faced by autologous CAR-T cell therapy [6]. As these therapies evolve, their significance in cancer treatment is likely to increase, creating new opportunities to enhance patient prognosis and quality of life [7].

The theoretical framework and historical evolution of immune-based cancer therapies further emphasize the importance of gene modified immune cell therapy. These therapies have shown efficacy across various cancer types, reinforcing their potential impact on the future of cancer treatment [8]. Additionally, addressing the limitations of high-content imaging in drug discovery, particularly regarding single-cell variability and noise, highlights the need for improved methodologies that can enhance the precision and effectiveness of these therapies [9].

1.2 Scope and Objectives of the Review

This survey provides a comprehensive examination of gene modified immune cell therapies, focusing primarily on Chimeric Antigen Receptor T (CAR-T) cells and T Cell Receptor-modified T

(TCR-T) cells while excluding unrelated immunotherapies such as oncolytic viruses and bispecific antibodies [2]. The scope includes advancements in CAR-T cell design, mechanisms of action, and challenges related to resistance, particularly antigen loss and CAR-T cell persistence. The review also addresses engineering strategies in TCR-T cell therapies and their applications in both hematological malignancies and solid tumors [5].

Moreover, the review explores challenges posed by intratumor heterogeneity and the tumor microenvironment, which significantly influence the efficacy of ACT therapies [3]. It investigates the potential of universal CAR-T (UCAR-T) cell therapy as a solution to the limitations of autologous CAR-T therapy, highlighting recent advancements and ongoing challenges in this domain [6].

The objectives of this survey are to delineate the current landscape of gene modified immune cell therapies, identify knowledge gaps, and propose future research directions to enhance the efficacy and safety of these innovative treatments. By focusing on mechanisms of toxicity, resistance, and potential future directions for CAR-T cell therapy, the paper aims to provide insights into optimizing these therapies for improved clinical outcomes. Additionally, the survey examines how glycoengineering can enhance the efficacy and safety of immunotherapeutics [7]. This comprehensive review seeks to contribute to the advancement of gene modified immune cell therapies and improve patient outcomes in oncology.

1.3 Structure of the Survey

The organization of this survey systematically explores the multifaceted aspects of gene modified immune cell therapy, specifically CAR-T and TCR-T cell therapies. The initial sections establish a foundational understanding, beginning with an introduction that outlines the significance and objectives of the review. This is followed by a detailed background on the evolution and core concepts of immunotherapy, emphasizing the pivotal role of genetic engineering in advancing these therapies.

Subsequent sections delve into the historical context and evolution of immunotherapy, offering insights into its development as a treatment modality [10]. This is complemented by an overview of immunotherapy and cellular therapy, elucidating the methodologies and principles underlying these approaches. The survey further investigates the revolutionary impact of genetic engineering in immunotherapy, paving the way for advanced therapeutic strategies.

The core of the paper dedicates itself to an in-depth analysis of CAR-T and TCR-T cell therapies. The section on CAR-T cells discusses their development, engineering, and clinical applications, focusing on the successes and challenges encountered in their use. This includes an exploration of the different generations of CAR-T cell therapy, categorized based on design and functionality, emphasizing advancements in enhancing T cell persistence and efficacy [11]. Concurrently, the section on TCR-T cells examines their mechanisms, applications, and challenges faced during development and clinical application.

A comparative analysis of CAR-T and TCR-T therapies highlights their respective mechanisms, advantages, and limitations. The subsequent section provides an in-depth analysis of recent technological advancements and innovative strategies in genetic engineering, particularly focusing on their transformative effects on the development and efficacy of cancer immunotherapies. These innovations include advanced biomaterials, drug delivery systems, and glycoengineering techniques, which aim to enhance therapeutic effectiveness while minimizing adverse effects associated with traditional treatments [12, 7, 2].

The survey also reviews the landscape of clinical trials and regulatory aspects, addressing the challenges and milestones in transitioning these therapies from research to clinical practice. The concluding sections focus on future directions and challenges, providing insights into potential new applications and strategies to overcome existing obstacles in optimizing the safety and efficacy of these therapies.

This structured approach facilitates an in-depth analysis of the current landscape of gene-modified immune cell therapies, highlighting their transformative potential in cancer treatment while addressing the complexities of immune modulation, diverse patient responses, and implications for future research directions, particularly in enhancing therapeutic efficacy and minimizing adverse effects through advanced delivery technologies and comprehensive profiling of tumor-infiltrating immune cells [13, 2, 10]. The following sections are organized as shown in Figure 1.

2 Background and Core Concepts

2.1 Historical Context and Evolution of Immunotherapy

The evolution of immunotherapy has transformed cancer treatment, shifting from initial skepticism to becoming a cornerstone of modern oncology. Immune checkpoint inhibitors (ICIs), targeting pathways like CTLA-4 and PD-1/PD-L1, have been pivotal in overcoming immune tolerance and enhancing anti-tumor immunity [14]. Despite these advancements, issues such as treatment resistance due to tumor heterogeneity and immunosuppressive tumor microenvironments persist, necessitating innovative strategies to improve outcomes [14]. The asymptomatic nature of early-stage hepatocellular carcinoma (HCC) complicates timely diagnosis, highlighting the need for better detection methods and interventions [15].

The integration of genetic engineering into immunotherapy, particularly through chimeric antigen receptor T (CAR-T) and T cell receptor T (TCR-T) cell therapies, marks a transformative advancement. These engineered T cells target specific cancer cells and have shown remarkable efficacy in clinical trials, especially against hematological cancers. However, challenges such as antigen escape, severe toxicities, and limitations in targeting solid tumors persist, driving ongoing research into innovative strategies to enhance their effectiveness [16, 5, 17]. These advancements offer promise for improving the specificity and efficacy of immunotherapies, providing new hope for patients with resistant cancers.

2.2 Overview of Immunotherapy and Cellular Therapy

Immunotherapy and cellular therapy are integral components of cancer treatment, leveraging the immune system to recognize and eliminate cancer cells. These therapies include adoptive cell transfer (ACT), immune checkpoint inhibitors (ICIs), cancer vaccines, and oncolytic virus therapies [10]. ICIs, by disrupting pathways like CTLA-4 and PD-1/PD-L1, have restored effective anti-tumor responses and improved survival rates across various malignancies [18].

Adoptive cell therapy involves the ex vivo manipulation and expansion of immune cells for reinfusion, including tumor-infiltrating lymphocyte (TIL) therapy, T Cell Receptor (TCR) gene therapy, and CAR-T cell therapy, each with distinct mechanisms and therapeutic potential [10]. CAR-T cell therapy has achieved significant success in hematological cancers by engineering T cells to express receptors targeting tumor-associated antigens.

The complexity of cancer immunotherapy is compounded by variability in patient responses, necessitating a nuanced understanding of individual profiles and tumor characteristics [18]. Innovative approaches, such as the time-to-event Bayesian optimal phase II (TOP) design, aid in real-time decision-making and accommodate various clinical endpoints [19]. Current strategies in immunotherapy focus on three main approaches: directly targeting cancer cells to induce immunogenic cell death, modulating the tumor immune microenvironment, and enhancing peripheral immune responses through advanced techniques [2, 20, 21, 13, 22]. These strategies aim to overcome treatment resistance and variability in patient responses, ultimately improving the efficacy of cancer treatments across diverse populations.

Classifying immunotherapy into distinct categories—such as checkpoint inhibitors, CAR T-cell therapy, monoclonal antibodies, and oncolytic viral therapies—enhances understanding of their clinical applications. This structured approach facilitates identifying suitable therapies for diverse patient populations and underscores the importance of ongoing education for healthcare providers, ultimately improving treatment outcomes and addressing complexities associated with immune modulation and potential adverse effects [2, 13, 10].

2.3 Role of Genetic Engineering in Immunotherapy

Genetic engineering has revolutionized immunotherapy, enhancing the immune system's ability to target and eliminate cancer cells with precision. Chimeric Antigen Receptor (CAR) T cell therapy exemplifies this transformation, where T cells are engineered to express synthetic receptors that specifically recognize tumor-associated antigens. This innovation has shown significant efficacy in treating hematological malignancies, yet challenges persist in applying immunotherapy to solid tumors, including severe toxicities, complex manufacturing processes, and antigen escape [23, 24],

2, 20, 13]. The tumor microenvironment (TME) further complicates these therapies, necessitating advanced genetic modifications to enhance T cell persistence and efficacy.

T Cell Receptor-modified T (TCR-T) cell therapies target intracellular antigens presented by major histocompatibility complex (MHC) molecules. However, TCR-T therapies face challenges such as TCR mis-pairing and the TME's immunosuppressive nature, which can hinder T cell persistence and effectiveness post-adoptive transfer. Addressing these challenges requires sophisticated gene editing technologies to improve TCR specificity and functionality, although high costs and time-intensive experimental methods present barriers to widespread adoption [6].

The introduction of gene editing tools like CRISPR has enabled precise genetic modifications, enhancing therapeutic efficacy while managing associated toxicities [25]. Accurate prediction of on-target and off-target effects is crucial for the successful application of these technologies [7]. Moreover, integrating cytokines into cancer treatment poses challenges due to the need to balance their therapeutic potential against associated toxicities [8].

Innovations in genetic engineering are further supported by advancements such as Optical Pooled Screening (OPS), which provides a cost-effective means for capturing cellular responses, facilitating the development and optimization of immunotherapies [9]. As research progresses, genetic engineering remains at the forefront of immunotherapy, driving the development of more effective and personalized treatment strategies and offering new hope for patients with resistant or difficult-to-treat cancers.

2.4 Principles of CAR-T Cell Therapy

Chimeric Antigen Receptor T (CAR-T) cell therapy is a significant advancement in cancer immunotherapy, utilizing the immune system to specifically target and eliminate cancer cells. This therapy involves genetically modifying a patient's T cells to express synthetic receptors, known as CARs, engineered to recognize specific antigens on cancer cell surfaces. CAR-T therapy's success is particularly notable in hematological malignancies, such as chronic lymphocytic leukemia (CLL), where CD19-directed CAR-T cells have demonstrated long-term persistence and sustained remission [26].

The development process involves extracting T cells from the patient's blood, genetically engineering them—often using viral vectors—to express CARs, expanding the modified cells *ex vivo*, and reinfusing them into the patient, where they proliferate and target cancer cells expressing the specific antigen [20]. The therapy's efficacy stems from its ability to induce robust and sustained immune responses, although challenges such as antigen escape and tumor heterogeneity necessitate ongoing innovation [17].

CAR-T cells recognize and bind to target antigens, triggering T cell activation, proliferation, and cancer cell destruction. Interactions within the tumor microenvironment, especially involving cytokines like IFN, complicate the therapeutic landscape, playing dual roles in immune activation and suppression [27]. Continuous stimulation of CAR-T cells by B-cells is crucial for therapy success, highlighting the tumor microenvironment's role in modulating outcomes [28].

Advancements in synthetic biology and multi-targeting strategies are being integrated into CAR-T therapies to address limitations and improve outcomes. The evolution from first-generation to next-generation CAR-T cells incorporates immune modifiers and advanced engineering strategies to enhance specificity and reduce off-target effects [17]. Additionally, incorporating cytokines poses challenges due to their short half-life and potential adverse effects, necessitating careful management to balance benefits against risks [29].

Agent-based models have been proposed to rationalize outcomes of antigen-specific and multi-antigen recognition therapies in heterogeneous tumors, providing insights into optimizing therapeutic strategies [3]. Furthermore, distinguishing between autologous and universal CAR-T (UCAR-T) therapies highlights differences in manufacturing processes, costs, and safety profiles, which are critical in clinical applications [6].

CAR-T cell therapy exemplifies the transformative potential of genetic engineering in cancer treatment. By advancing our understanding of T cell biology and integrating innovative strategies, CAR-T therapy continues to evolve, offering new hope for patients with previously intractable malignancies. As research progresses, incorporating cutting-edge modeling techniques and novel strategies will be

essential for enhancing CAR-T therapy's efficacy and safety, particularly in addressing limitations such as high costs, manufacturing delays, and challenges posed by tumor microenvironments and antigen heterogeneity [17, 16, 6].

2.5 Principles of TCR-T Cell Therapy

T Cell Receptor-modified T (TCR-T) cell therapy is a sophisticated form of adoptive cell therapy that utilizes the specificity of T cell receptors (TCRs) to target cancer cells. This method involves genetically engineering T cells to express TCRs that recognize specific peptide antigens presented by major histocompatibility complex (MHC) molecules on tumor cells. TCR-T therapy's precision allows it to target intracellular antigens, extending the range of targetable tumor-associated antigens beyond those accessible by CAR T cells [30].

Developing TCR-T therapy encompasses critical steps, starting with identifying and validating tumor-specific antigens. Recent advancements in protein engineering have facilitated the discovery of novel tumor antigens and the optimization of TCRs for enhanced affinity and specificity, crucial for effective tumor targeting [31]. Engineering TCRs involves modifications to the TCR alpha and beta chains to improve binding affinity to the antigen-MHC complex while minimizing off-target effects and cross-reactivity with normal tissues [30].

A significant challenge in TCR-T therapy is treating solid tumors, which often possess hostile microenvironments that hinder T cell infiltration and function. Optimizing dosing strategies is essential to enhance TCR-T efficacy in solid malignancies [32]. Additionally, TCR mis-pairing and the immunosuppressive nature of the TME necessitate developing strategies to improve T cell persistence and functionality post-adoptive transfer [30].

Clinical applications of TCR-T therapy have progressed significantly, with various trials exploring its efficacy across different cancer types. Notably, CMV-TCR-T therapy represents a promising approach for targeting cytomegalovirus (CMV)-infected cells, demonstrating TCR-T cells' versatility in addressing oncological and infectious diseases [33]. Ongoing research focuses on refining TCR specificity and enhancing TCR-T therapeutic potential [31].

TCR-T cell therapy exemplifies genetic engineering's potential to create highly specific and effective cancer treatments. By building on advances in TCR biology, antigen discovery, and protein engineering, TCR-T therapy continues to evolve, offering new avenues for treating malignancies resistant to conventional therapies. As research in adoptive cell therapy advances, incorporating cutting-edge strategies, such as TCR-signaling-responsive nanoparticle drug delivery systems, will be essential for overcoming current challenges and enhancing TCR-T therapy's effectiveness, particularly in solid tumors. This integration aims to optimize T cell functionality by enabling precise and controlled drug release in response to T cell receptor activation, thereby improving therapeutic outcomes [34, 24].

3 CAR-T Cells

Immunotherapy has seen significant progress, notably with Chimeric Antigen Receptor T (CAR-T) cells, which have transformed cancer treatment options. This section delves into CAR-T cell development and engineering, highlighting methodologies that enhance their therapeutic efficacy, paving the way for clinical applications and successes. As illustrated in Figure 2, the hierarchical structure of CAR-T cell development encompasses various stages, including the innovative processes involved in their creation, such as peripheral blood mononuclear cell (PBMC) collection and dual-epitope targeting. The figure also delineates clinical applications, showcasing notable successes in treating hematological malignancies while addressing the challenges faced in solid tumors, thereby underscoring the necessity for combination therapies. Furthermore, it highlights the complexities inherent in CAR-T therapy, particularly the treatment-related issues and manufacturing constraints that pose significant barriers to broader application. This comprehensive overview serves to contextualize the advancements and ongoing challenges within the field of CAR-T cell therapy.

3.1 Development and Engineering of CAR-T Cells

CAR-T cell engineering involves intricate processes to maximize anticancer potential. Peripheral blood mononuclear cells (PBMCs) are collected from patients through leukapheresis, a critical step

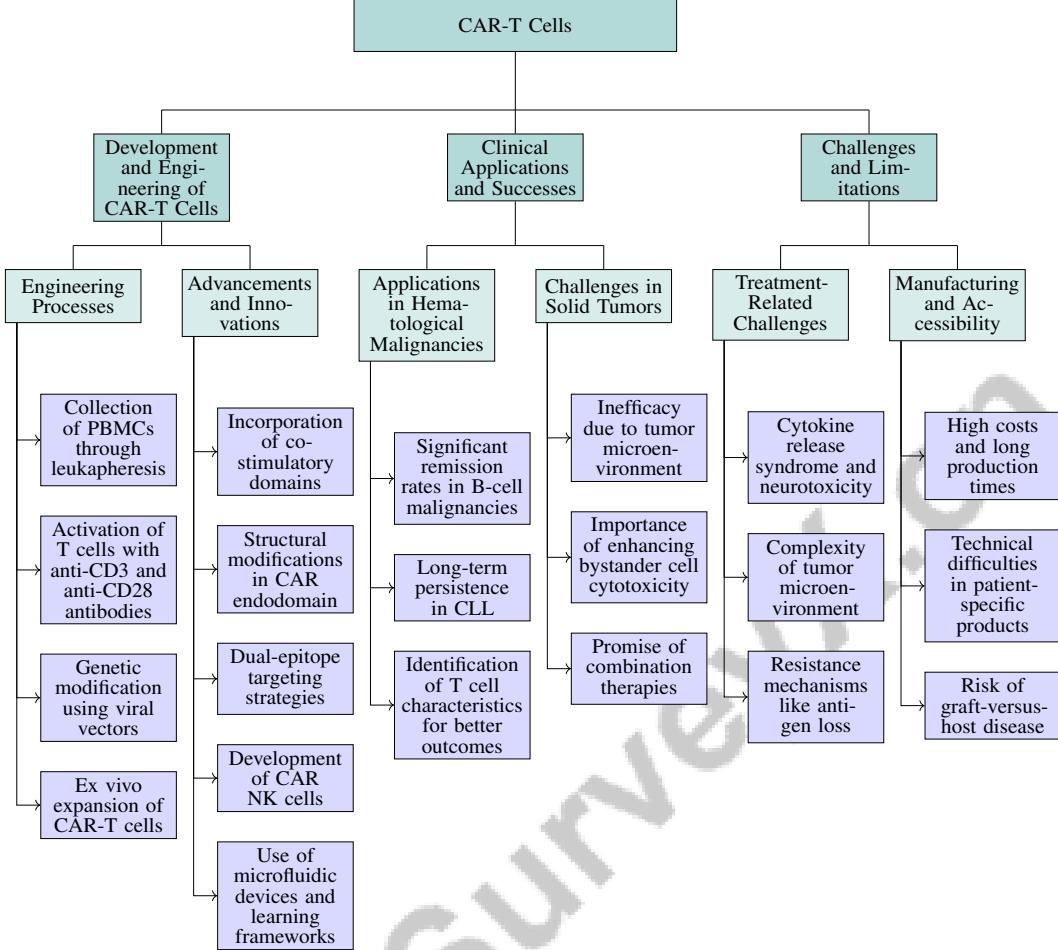


Figure 2: This figure illustrates the hierarchical structure of CAR-T cell development, clinical applications, and challenges. The development and engineering section details the processes and innovations in CAR-T cell creation, such as PBMC collection and dual-epitope targeting. Clinical applications highlight successes in hematological malignancies and challenges in solid tumors, emphasizing the need for combination therapies. Challenges and limitations focus on treatment-related issues and manufacturing constraints, underscoring the complexities in CAR-T therapy's broader application.

for developing adoptive cell therapies, including TCR redirected therapies for acute myeloid leukemia (AML) [35, 24, 33, 2]. Post-isolation, T cells are activated with anti-CD3 and anti-CD28 antibodies to promote proliferation and prepare them for genetic modification. Viral vectors, such as lentiviral or retroviral, introduce genes encoding CAR constructs targeting tumor-associated antigens like CD19 and BCMA [36]. Following transduction, CAR-T cells undergo ex vivo expansion to achieve therapeutic cell numbers under stringent conditions to ensure functionality, viability, and safety [37].

Advancements in CAR-T cell engineering have incorporated additional co-stimulatory domains to enhance activation, persistence, and efficacy. Structural modifications in the CAR endodomain have improved outcomes, with recent innovations focusing on optimizing CAR-T cell design to enhance targeting capabilities and reduce adverse effects [17]. Strategies such as dual-epitope targeting address challenges like antigen escape and tumor heterogeneity. Engineered T cells with dual-epitope BCMA-targeting CARs have shown increased efficacy in preclinical models [36]. Mathematical modeling and high-throughput screening methods guide the development of more effective therapeutic strategies.

Innovative approaches, such as CAR NK cells, offer alternatives with innate immune properties and reduced graft-versus-host disease (GvHD) risk, paving the way for off-the-shelf therapies [38].

Advanced methodologies, including microfluidic devices and weakly supervised learning frameworks like Set-DINO, enhance the assessment of TCR-engineered T cell efficacy and representation learning, contributing to CAR-T cell therapy refinement.

This section is further complemented by Figure 3, which illustrates the key processes and advancements in CAR-T cell engineering, highlighting the engineering process, recent advancements, and innovative approaches. The development and engineering of CAR-T cells involve rigorous genetic modifications, manufacturing protocols, and cutting-edge therapeutic strategies aimed at optimizing efficacy and safety, offering new hope for patients with resistant cancers [39].

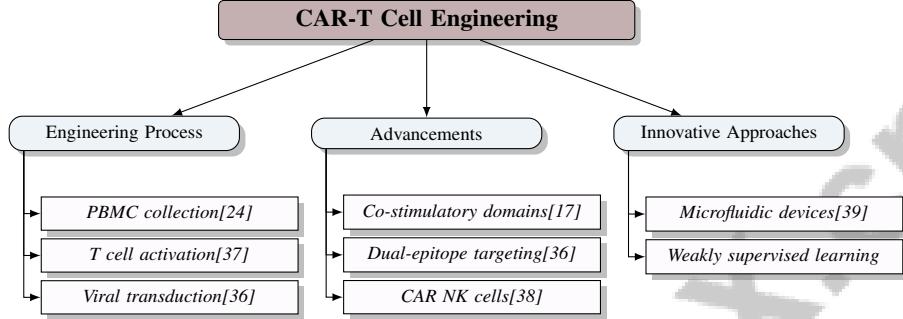


Figure 3: This figure illustrates the key processes and advancements in CAR-T cell engineering, highlighting the engineering process, recent advancements, and innovative approaches.

3.2 Clinical Applications and Successes

Method Name	Clinical Applications	Therapeutic Strategies	Predictive Modeling
MM-CART[40]	Solid Tumors	Combination Therapies	Mathematical Model Tcr Specificity Prediction
TCR-GSDF[41]	-	-	-

Table 1: Overview of CAR-T cell therapy methods, their clinical applications, therapeutic strategies, and predictive modeling approaches. This table highlights the current advancements in CAR-T therapy, focusing on specific methods such as MM-CART and TCR-GSDF, and their roles in addressing challenges in solid tumors and enhancing T cell receptor specificity prediction.

CAR-T cell therapy has become a groundbreaking cancer treatment, especially for hematological malignancies. Its clinical applications are most notable in B-cell malignancies, such as acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL), where CAR-T cells targeting CD19 have achieved significant complete remission rates [8]. Long-term persistence of CD4+ CAR-T cells, lasting over a decade, alongside sustained activation and cytotoxic potential, is critical for maintaining long-term remission in CLL patients [26]. Identifying T cell characteristics linked to better outcomes, such as CD27+ PD-1CD8+ cells, is pivotal for refining patient selection and optimizing therapeutic strategies [42].

However, applying CAR-T cell therapy to solid tumors presents significant challenges. The tumor microenvironment (TME) often hinders CAR-T cell efficacy, necessitating innovative strategies to enhance therapeutic potential. Research indicates that increasing CAR-T cell doses does not guarantee better outcomes; enhancing the cytotoxic capability of bystander cells is crucial [40]. Combination therapies have shown promise in augmenting CAR-T efficacy in solid tumors. For example, integrating immunotherapy with targeted agents has yielded encouraging results in hepatocellular carcinoma (HCC) [15].

Advances in predictive modeling and specificity detection frameworks complement CAR-T therapy successes. A TCR generative specificity detection framework demonstrates superior performance in predicting T cell receptor specificity, tailoring cancer immunotherapy to individual needs [41]. The clinical applications of CAR-T therapy continue to expand, driven by ongoing research and innovation. Achievements in treating hematological malignancies lay a robust groundwork for applying this therapy to solid tumors and other complex cancers. These successes highlight the potential for developing novel therapeutic targets and addressing challenges like the immunosuppressive TME and CAR-T persistence, critical for enhancing efficacy in resistant cancers [36, 43, 6].

As illustrated in Figure 4, which depicts the hierarchical structure of CAR-T cell therapy applications, the therapy's utility is prominently highlighted in hematological malignancies while also addressing the challenges faced in treating solid tumors and the role of predictive modeling. The accompanying figures provide insights into the manufacturing process and challenges faced during clinical trials. The first image presents a detailed flowchart outlining the steps in creating T cell transduced products, from PBMC collection to activation, transduction with lentiviral vectors, expansion, and characterization. This meticulous process ensures the production of high-quality CAR-T cells for therapeutic use. The second image highlights complexities encountered in clinical trials, categorizing challenges such as ethical and regulatory issues and participant recruitment difficulties, alongside corresponding response strategies. Together, these images underscore the balance between scientific innovation and clinical pragmatism that underpins the successful application of CAR-T cell therapies. As research progresses, integrating advanced technologies and combination strategies will be essential in unlocking CAR-T therapy's full potential and improving patient outcomes across diverse cancer landscapes. Additionally, Table 1 provides a comprehensive overview of the methodologies employed in CAR-T cell therapy, detailing their clinical applications, therapeutic strategies, and advancements in predictive modeling.

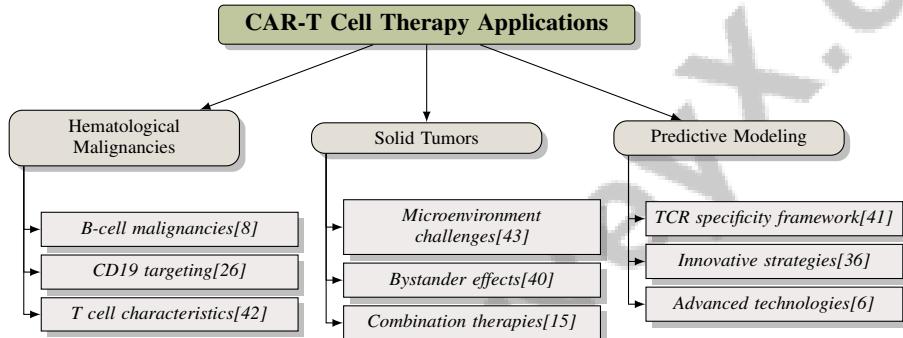


Figure 4: This figure illustrates the hierarchical structure of CAR-T cell therapy applications, highlighting its use in hematological malignancies, challenges in solid tumor treatment, and the role of predictive modeling. Key areas include targeting B-cell malignancies, overcoming the tumor microenvironment, and employing advanced technologies for improved outcomes.

3.3 Challenges and Limitations

Method Name	Treatment Challenges	Manufacturing Constraints	Therapeutic Efficacy
GCD[7]	Resistance Mechanisms	High Costs	Antigen Heterogeneity
SD[9]			

Table 2: Comparison of CAR-T Cell Therapy Methods: Treatment Challenges, Manufacturing Constraints, and Therapeutic Efficacy.

Despite CAR-T cell therapy's transformative potential in hematological malignancies, significant challenges and limitations hinder broader application. Managing treatment-related toxicities, such as cytokine release syndrome (CRS) and neurotoxicity, requires standardized grading and management protocols to enhance patient safety and outcomes [39]. The complexity of the tumor microenvironment (TME) exacerbates these challenges, particularly in solid tumors, where immunosuppressive conditions and tumor antigen heterogeneity impede therapeutic efficacy [39]. Metabolic competition for nutrients between tumor and immune cells can lead to immune evasion, further reducing CAR-T efficacy [39]. Understanding TME interactions is crucial for developing strategies that enhance CAR-T function and longevity. Resistance mechanisms, including antigen loss and T cell exhaustion, complicate the therapeutic landscape. Antigen loss and insufficient CAR-T persistence limit response durability and increase relapse risk. Variability in patient responses and difficulties predicting these responses add complexity to clinical application [8]. Table 2 illustrates the challenges and limitations associated with different CAR-T cell therapy methods, focusing on treatment challenges, manufacturing constraints, and therapeutic efficacy.

Manufacturing CAR-T cells presents challenges, including high costs, long production times, and technical difficulties in generating patient-specific products. These factors restrict accessibility and raise concerns about scalability and feasibility for widespread adoption [8]. The risk of graft-versus-host disease (GVHD) in allogeneic therapies further complicates the safety profile. Ongoing research in combination therapies and advanced modeling techniques offers promising avenues to overcome limitations. Integrating immune checkpoint inhibitors and adoptive cell therapies shows potential in addressing resistance. However, current models often fail to replicate all tumor biology aspects, such as immune cell presence and functional vasculature [25]. The complexity of glycan biosynthesis and potential off-target effects during glycoengineering present further challenges [7].

Recent studies highlight significant cell-to-cell variation in single-cell data, complicating reliable representation learning and potentially misleading analyses [9]. Understanding long-term CAR-T therapy responses—whether sustained remission, relapse, or progression—is crucial for optimizing strategies and advancing the field. Addressing these challenges is essential for unlocking CAR-T therapy’s full potential and improving patient outcomes across diverse cancer landscapes.

4 TCR-T Cells

T Cell Receptor-modified T (TCR-T) cell therapy has gained substantial attention as a transformative cancer treatment, harnessing engineered T cells to target and destroy malignant cells. This section explores the complex mechanisms of TCR-T cell therapy, detailing how these cells recognize tumor-associated antigens via major histocompatibility complex (MHC) molecules. Understanding these processes enhances our appreciation of TCR-T cells’ therapeutic potential and the ongoing advancements in this innovative treatment.

4.1 Mechanisms of TCR-T Cell Therapy

TCR-T cell therapy is a specialized form of adoptive cell therapy that utilizes T cell receptors (TCRs) for cancer cell targeting. It involves genetically modifying T cells to express TCRs that recognize peptide antigens presented by MHC molecules on tumor cells, thus extending targetable antigens beyond those accessible by CAR-T cells [44]. This ability to target intracellular antigens is particularly beneficial in treating acute myeloid leukemia (AML), where TCR-T therapies have shown promising responses [35].

The efficacy of TCR-T cells lies in their precision, targeting neoantigens unique to cancer cells. Advances in neoantigen identification have enhanced TCR-T therapies, showing superior outcomes over traditional methods [44]. Efficient manufacturing processes preserve functional characteristics, crucial for clinical application [24]. Real-time monitoring via droplet microfluidics allows precise assessment of TCR-T interactions with tumor cells [45].

While sharing similarities with CAR-T therapy, TCR-T therapy relies on natural antigen processing, necessitating a deep understanding of the tumor microenvironment. Mathematical models, primarily used for CAR-T interactions, offer insights into factors influencing TCR-T efficacy [46]. Recent breakthroughs in TCR biology and manufacturing techniques continue to advance TCR-T therapy, offering personalized cancer immunotherapy options for resistant malignancies [24, 31, 30, 47].

Figure 5 illustrates the multifaceted mechanisms of TCR-T cell therapy, showcasing CD8+ T cells with protein nanogels for drug delivery, the molecular intricacies of TCR complexes, and the dynamics between cancer and CAR-T cells. These examples highlight TCR-T therapy’s sophisticated strategies to harness and amplify natural defenses against cancer [34, 31, 32].

4.2 Applications in Cancer Treatment

TCR-T cell therapy is a promising strategy for treating cancers where conventional therapies fall short. Its specificity for intracellular antigens presented by MHC molecules allows targeting a wide range of tumor-associated antigens, advantageous for cancers like AML [35, 44]. Advances in neoantigen identification and TCR optimization have improved responses in solid tumors, such as melanoma, by targeting tumor-specific antigens [44].

TCR-T therapy has also been explored for viral-associated cancers, like those linked to Epstein-Barr virus and human papillomavirus, using tumor-specific neoantigens as targets [44, 35, 31]. Ongoing

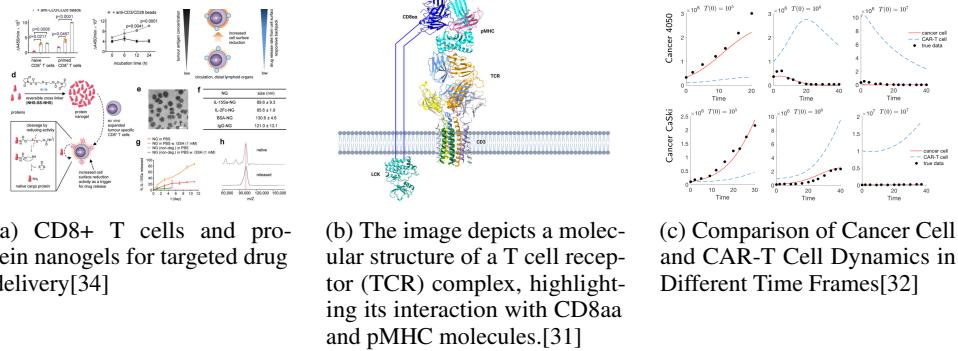


Figure 5: Examples of Mechanisms of TCR-T Cell Therapy

research and clinical trials focus on optimizing TCR-T efficacy and safety, supported by advanced manufacturing and real-time monitoring technologies [45].

Overall, TCR-T therapy exemplifies the potential of genetic engineering in creating precise cancer treatments. As research progresses, integrating innovative strategies like TCR-signaling-responsive nanoparticle drug delivery systems will enhance TCR-T therapies, particularly for solid tumors [34, 24].

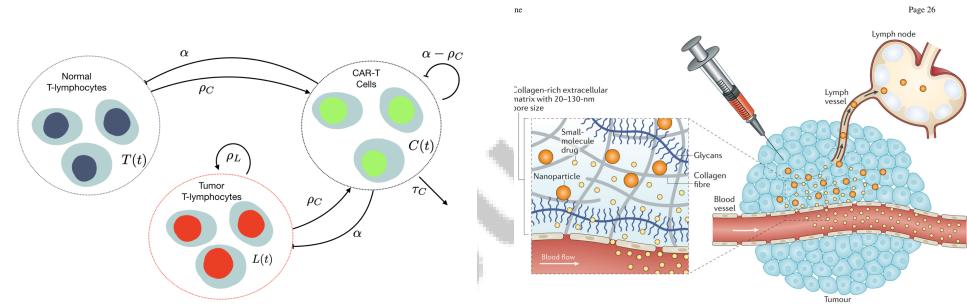


Figure 6: Examples of Applications in Cancer Treatment

Figure 6 highlights TCR-T's potential in cancer therapy, demonstrating its ability to target tumor-specific antigens and initiate immune responses. These advancements represent significant progress in cancer treatment, offering hope for more effective and personalized therapies [48, 1].

4.3 Challenges in Development and Clinical Use

Developing TCR-T cell therapy faces several challenges, including the need for standardized manufacturing across diverse TCRs and patient populations to ensure consistent clinical outcomes [24]. Selecting optimal antigens for targeting is crucial to maximize efficacy and minimize off-target effects. Long-term effects of TCR-T therapy and T cell exhaustion mechanisms within the tumor microenvironment are under active investigation [49, 50].

TCR mis-pairing, where engineered TCRs form unintended pairings with endogenous chains, poses a risk of off-target reactivity. Addressing this requires advanced engineering to ensure specificity and safety [50]. The tumor microenvironment's immunosuppressive nature also hinders treatment effectiveness, necessitating strategies to overcome these barriers.

The limited understanding of TCR-disease associations and challenges in isolating responding TCRs from static data further complicate therapy optimization [51]. Advanced methods for real-time functional analysis at the single-cell level offer potential solutions [45].

Grading cytokine release syndrome (CRS), a common adverse effect, remains challenging due to its delayed onset and variable clinical presentation [52]. Developing precise grading and management protocols is essential for improving safety and outcomes.

Addressing these challenges is crucial for advancing TCR-T therapy. By optimizing antigen targeting and overcoming the immunosuppressive tumor microenvironment, TCR-T therapy can evolve into a highly effective and personalized option for treating diverse malignancies [31, 53, 49, 47].

5 Comparative Analysis of CAR-T and TCR-T Cells

5.1 Comparative Analysis of CAR-T and TCR-T Therapies

Chimeric Antigen Receptor T (CAR-T) and T Cell Receptor-modified T (TCR-T) cell therapies represent pivotal advancements in adoptive cell therapy, each with distinct mechanisms and therapeutic potential. A comparative analysis underscores their similarities and differences, impacting clinical applications, efficacy, and patient outcomes. In immunotherapy, understanding the tumor microenvironment and employing advanced delivery technologies are crucial for personalizing treatment and minimizing adverse effects [54, 55, 2, 56].

As illustrated in Figure 7, which presents a hierarchical classification of CAR-T and TCR-T therapies, key attributes such as antigen targeting strategies, therapeutic success in hematological cancers, and the potential of universal CAR-T therapies in addressing cost and availability issues are highlighted. CAR-T therapy targets surface antigens on tumor cells, with CD19-targeted therapies achieving notable success in B cell malignancies [57]. This strategy has resulted in high efficacy rates for hematological cancers [58]. However, CAR-T encounters challenges in solid tumors due to the complex tumor microenvironment and antigen heterogeneity [57]. To address these issues, dual-targeting and multi-specific strategies have been developed, showing promise in reducing antigen escape and improving treatment outcomes.

TCR-T therapy, on the other hand, targets intracellular antigens presented by major histocompatibility complex (MHC) molecules, allowing a broader spectrum of targetable tumor-associated antigens [35]. This is advantageous for malignancies without accessible surface antigens. TCR-T therapies exhibit high specificity and can target unique neoantigens specific to cancer cells. However, their dependence on MHC presentation introduces variability in patient responses due to different HLA types, which may limit the applicability of TCR-T therapies compared to CAR-T therapies [35].

Differences between autologous CAR-T therapies and universal CAR-T (UCAR-T) therapies further highlight variations in cost, availability, and safety. UCAR-T therapies are more cost-effective and accessible, offering an appealing alternative to autologous therapies, yet they face challenges related to safety and efficacy, particularly concerning graft-versus-host disease (GVHD) and immune rejection risks [6].

The comparative analysis of CAR-T and TCR-T therapies emphasizes the need to tailor therapeutic strategies to the specific characteristics of both the cancer and the patient. CAR-T therapies have demonstrated significant effectiveness in treating hematological malignancies by targeting specific surface antigens such as CD19 and BCMA, though they are hindered by severe toxicities and limited efficacy in solid tumors. In contrast, TCR-T therapies provide a promising alternative by enabling the targeting of intracellular antigens with high specificity, potentially expanding treatment options for cancers resistant to conventional therapies. This capability addresses unmet needs in cancer treatment by targeting unique neoantigens associated with various tumors [59, 60, 36, 31]. Continued research and the integration of innovative strategies and technologies will be crucial in optimizing the efficacy and safety of both CAR-T and TCR-T cell therapies, ultimately enhancing patient outcomes across diverse cancer landscapes.

6 Technological Advances and Innovations

6.1 Innovations and Technological Advances

Recent technological innovations have propelled the advancement of CAR-T and TCR-T cell therapies, crucial for cancer immunotherapy. Enhanced delivery methods, such as nanoparticles and T cell-based systems, significantly improve therapeutic outcomes [2]. Molecular MRI techniques now

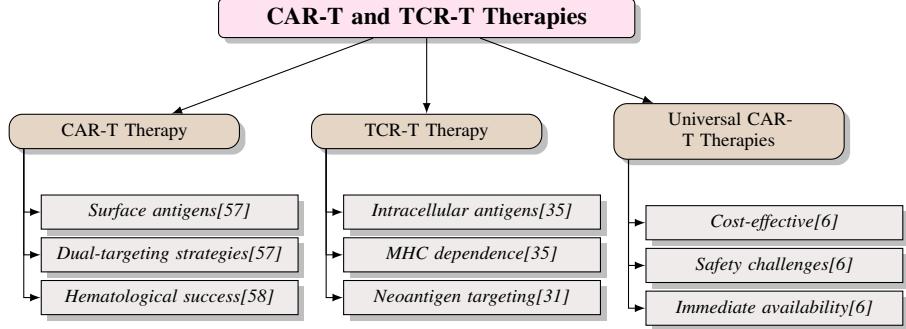


Figure 7: This figure illustrates the hierarchical classification of CAR-T and TCR-T therapies, highlighting key attributes such as antigen targeting strategies, therapeutic success in hematological cancers, and the potential of universal CAR-T therapies in addressing cost and availability issues.

allow real-time visualization of tumor microenvironment dynamics and treatment responses, aiding in comprehensive monitoring of immune cell therapies [61].

As illustrated in Figure 8, the key technological advancements in cancer immunotherapy can be categorized into three main areas: delivery methods, genetic engineering, and computational models. Each category highlights specific innovations and their contributions to enhancing therapeutic efficacy and safety. For instance, CRISPR-Cas9 technology has revolutionized immune cell engineering, enabling precise genetic modifications that enhance the specificity and functionality of CAR-T and TCR-T cells [62]. This has led to the development of universal CAR designs, including SUPRA CARs, which address scalability and safety concerns in manufacturing and clinical applications [62]. The integration of synthetic biology and genetic engineering holds promise for extending CAR therapies beyond oncology [63].

Computational models, such as multiscale agent-based models, play a pivotal role in simulating tumor responses to therapies, offering insights for optimizing treatment strategies [3]. Mathematical modeling advancements have also facilitated the creation of safe dosing nomograms for CAR-T therapies, allowing for personalized treatments based on individual tumor characteristics [64].

Machine learning enhances immunological research by optimizing gRNA sequences for CRISPR applications, improving prediction accuracy and genetic engineering outcomes [12]. The Set-DINO framework effectively learns from noisy samples through weakly supervised learning, utilizing the structure of replicate experiments [9].

Understanding CAR T-cell toxicities has improved with the categorization of cytokine release syndrome (CRS) and neurologic toxicities, essential for developing targeted interventions to mitigate these adverse effects [65]. Future research should prioritize combination therapies, personalized approaches based on genetic profiling, and further exploration of the tumor microenvironment and microbiome's roles in treatment outcomes [14].

Advancements in delivery technologies and profiling methods, including single-cell RNA sequencing, highlight the dynamic landscape of cancer immunotherapy. These innovations enhance gene-modified immune cell therapies and facilitate personalized approaches by deepening the understanding of tumor microenvironments and infiltrating immune cells, ultimately improving patient outcomes [55, 2, 10].

6.2 Gene Editing Technologies

Gene editing technologies, notably CRISPR-Cas systems, are pivotal in optimizing CAR-T and TCR-T cell therapies. CRISPR-Cas9's precision allows targeted T cell modifications, enhancing their ability to recognize and combat cancer cells while minimizing off-target effects, crucial for treating solid tumors [12].

CRISPR technology has facilitated the development of universal CAR designs, addressing limitations of patient-specific therapies. These universal CAR-T cells, capable of mass production and storage, offer a cost-effective and readily available therapeutic option. Moreover, CRISPR-mediated gene

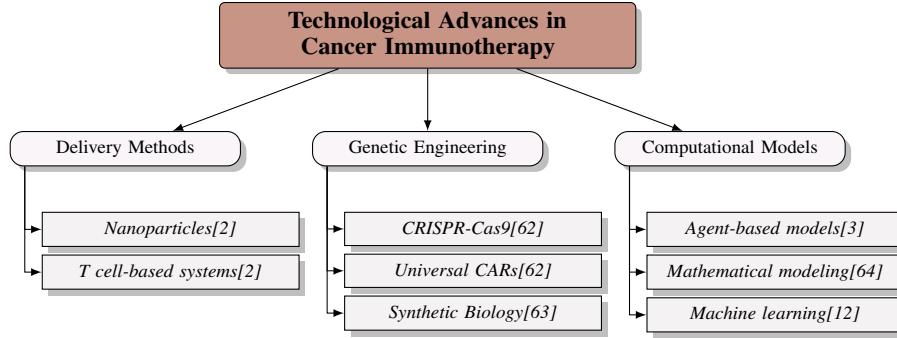


Figure 8: This figure illustrates the key technological advancements in cancer immunotherapy, categorizing them into delivery methods, genetic engineering, and computational models. Each category highlights specific innovations and their contributions to enhancing therapeutic efficacy and safety.

editing enables the knockout of genes associated with immune evasion or adverse effects, improving the efficacy and safety of CAR-T and TCR-T therapies [24].

Innovations like the Wireless Multicolor Fluorescence Image Sensor (WMFIS) demonstrate the integration of advanced imaging with gene editing technologies. WMFIS captures multicolor fluorescence images using ultrasound energy, allowing real-time monitoring of genetically engineered T cells' biodistribution and activity [66].

The comparative analysis of machine learning algorithms has enhanced CRISPR applications, particularly in guide RNA (gRNA) design. By evaluating various algorithms' strengths and weaknesses, researchers can improve gRNA prediction accuracy, thus refining gene editing efficiency [12]. This synergy between machine learning and gene editing technologies marks a significant advancement, refining therapeutic strategies and fostering the development of effective gene-modified immune cell therapies.

Gene editing technologies, particularly CRISPR, are leading innovations in immunotherapy, enhancing targeted cancer treatments. These advancements aim to improve therapy specificity and efficacy while addressing challenges such as immune modulation to reduce adverse effects like autoimmunity and nonspecific inflammation. By integrating advanced biomaterials and delivery systems, including nanoparticles and T cell-based therapies, researchers are exploring new avenues to optimize immunotherapeutic strategies, paving the way for more effective and personalized cancer treatment options [12, 20, 2, 10]. As research progresses, integrating these technologies with advanced imaging and computational tools will be essential for overcoming challenges and optimizing CAR-T and TCR-T cell therapies.

6.3 Advancements in CAR-T and TCR-T Cell Engineering

Recent advancements in engineering CAR-T and TCR-T cells have significantly enhanced their therapeutic potential, particularly in overcoming resistance mechanisms and improving efficacy. A critical challenge in CAR-T therapy is antigen escape, exemplified by the transduction of a single leukemic cell with anti-CD19 CAR lentivirus, leading to CD19 epitope masking. This highlights the need for improved manufacturing technologies to ensure sustained therapeutic efficacy [67].

The evolution of CAR-T cell engineering has progressed through multiple generations, incorporating additional co-stimulatory domains to enhance T cell activation, persistence, and efficacy. Recent innovations focus on optimizing CAR-T cell design to improve targeting capabilities and mitigate adverse effects. Customizing the tumor microenvironment using 3D models allows for investigating T cell behavior under varying conditions, such as oxygen levels and inflammatory cytokines, thus providing insights into optimizing CAR-T cell functionality across diverse tumor settings [25].

In TCR-T cell engineering, advancements concentrate on enhancing TCR specificity and minimizing off-target effects. Techniques such as optimizing TCR affinity and incorporating safety switches have been developed to improve therapeutic outcomes while reducing adverse effects. The integration of real-time monitoring platforms and sophisticated computational models, including a TCR generative

specificity detection framework utilizing the Random Forest algorithm, has accelerated TCR-T product development and evaluation, addressing specificity and performance challenges in personalized cancer immunotherapy [24, 41].

The grading of cytokine release syndrome (CRS), a common adverse effect of CAR-T therapy, has been refined through grading scales like the Penn grading scale, which categorizes CRS based on clinical features such as fever and organ dysfunction, providing a framework for standardized management and improving patient safety [52].

Recent advancements in CAR-T and TCR-T cell engineering illustrate the dynamic nature of the field, driving the development of more effective and personalized gene-modified immune cell therapies. Innovative strategies, including advanced biomaterials, personalized biomarker profiles, and novel drug delivery systems such as nanoparticles and T cell-mediated therapies, are emerging to tackle significant challenges like unpredictable treatment efficacy, better biomarker identification, and managing adverse effects like autoimmunity and inflammation. By enhancing the precision and effectiveness of immunotherapies, these developments hold the potential to significantly improve patient outcomes across various cancer types and facilitate the integration of immunotherapy into clinical practice [2, 20, 10, 21, 55].

7 Clinical Trials and Regulatory Aspects

7.1 Current Landscape of Clinical Trials

The clinical trial landscape for CAR-T and TCR-T cell therapies is rapidly advancing, with numerous studies evaluating their safety and efficacy across various malignancies. These trials are crucial for understanding the complex immune interactions within the tumor microenvironment that affect clinical outcomes [10]. TCR-T therapies are being investigated for solid tumors and cancer-germline antigens (CGAs) like NY-ESO-1, aiming to expand their application beyond hematological cancers [24]. Successful clinical-scale manufacturing has shown high yields and functional performance, essential for clinical transition.

CAR-T therapies are under examination for multiple cancer types, including lung cancer [21]. Ongoing trials are vital for validating new CAR-T products' safety and efficacy, supported by comprehensive surveys [56]. The Penn grading scale for cytokine release syndrome (CRS) offers a standardized assessment method, enhancing therapeutic decision-making in clinical trials [52].

Clinical trials also explore CAR NK cells targeting malignancies like CD19 in B-cell cancers and CD33 in acute myeloid leukemia (AML) [38]. These studies contribute to refining CAR-T cell toxicity management and improving patient outcomes [65]. Innovative delivery technologies are assessed for their potential to enhance gene-modified immune cell therapies [2], while simulations in controlled environments offer insights into adoptive cell transfer dynamics, informing trial design [3].

These diverse studies aim to enhance CAR-T and TCR-T therapies' efficacy and applicability, addressing challenges like high costs and manufacturing complexities associated with traditional autologous therapies [31, 68, 6]. Insights from these trials are crucial for improving gene-modified immune cell therapies' safety and effectiveness, ultimately enhancing patient outcomes.

7.2 Regulatory Milestones and Approvals

The regulatory landscape for CAR-T and TCR-T cell therapies has evolved significantly, marked by key milestones and approvals that have facilitated their clinical use. Initial approvals by the FDA and EMA set a precedent for commercializing gene-modified immune cell therapies, based on robust clinical trial data demonstrating efficacy and safety, particularly for hematological malignancies [56].

A pivotal milestone was the approval of CAR-T therapies targeting CD19, achieving remarkable success in inducing complete remission in acute lymphoblastic leukemia (ALL) and other B-cell malignancies. The regulatory pathway involved extensive evaluation of clinical trial data focusing on efficacy, safety, and manufacturing consistency. Standardized grading systems for CRS and other toxicities have been integral to the approval process, providing frameworks for assessing and managing adverse effects [56].

Despite these advancements, challenges persist, including ethical concerns, recruitment difficulties, and diversity issues, complicating the regulatory process. High trial failure rates and operational complexities in multinational studies further challenge regulatory approval [56]. Continued collaboration among regulatory bodies, researchers, and industry stakeholders is crucial for developing and approving safe and effective gene-modified immune cell therapies.

These regulatory milestones underscore the significant progress in CAR-T and TCR-T therapies, highlighting their transformative potential in cancer treatment by harnessing the immune system against malignancies [24, 53, 11, 6]. Continued research and refined regulatory frameworks are essential for expanding these innovative therapies' availability and accessibility, ultimately improving patient outcomes across diverse cancer landscapes.

7.3 Manufacturing and Regulatory Challenges

CAR-T and TCR-T cell therapies face substantial manufacturing and regulatory challenges affecting scalability and accessibility. The complex production process involves isolating, genetically modifying, and expanding patient-specific T cells, requiring stringent quality control to ensure safety and efficacy [24].

High manufacturing costs limit widespread adoption, with the individualized nature of autologous therapies prompting alternative approaches like universal CAR-T (UCAR-T) cells to reduce costs and improve efficiency using allogeneic cells [6]. However, these approaches introduce additional regulatory hurdles, particularly concerning graft-versus-host disease (GVHD) and immune rejection risks, requiring rigorous safety assessments [6].

Regulatory compliance involves navigating complex frameworks by agencies like the FDA and EMA, mandating comprehensive data on safety, efficacy, and manufacturing consistency, alongside standardized grading systems for therapy-related toxicities such as CRS [52]. The regulatory pathway is further complicated by the need for international harmonization of standards, given the global nature of clinical trials and varied regulatory environments [56].

Ethical considerations, recruitment challenges, and the need for diversity in trials add complexity to the regulatory landscape. High trial failure rates and operational complexities in multinational studies highlight the need for streamlined regulatory processes and enhanced collaboration among stakeholders to facilitate these innovative therapies' development and approval [56].

Addressing CAR-T and TCR-T therapies' manufacturing and regulatory challenges is vital for successful clinical translation and broader adoption. By tackling significant issues related to gene-modified immune cell therapies—such as controlled immune response modulation, tumor-specific T cell receptor optimization, and counteracting immunosuppressive tumor microenvironments—these therapies can fulfill their potential, offering renewed hope for patients with resistant or difficult-to-treat malignancies, leading to more effective, personalized treatment options that improve long-term outcomes and reduce adverse effects [53, 49, 2, 20, 22].

8 Future Directions and Challenges

Exploring the future directions and challenges of gene-modified immune cell therapies requires a detailed understanding of the factors influencing their development and application. This section delves into promising research avenues and strategies poised to transform cancer treatment, enhance therapeutic efficacy, and address existing limitations.

8.1 Future Prospects and Research Directions

The advancement of gene-modified immune cell therapies, particularly CAR-T and TCR-T therapies, is propelled by ongoing research and technological innovations. A significant area of focus is the development of universal CAR-T (UCAR-T) cells, which aim to streamline manufacturing and reduce costs, thereby improving patient accessibility and outcomes [6]. Future research should aim to enhance UCAR-T cell persistence and safety, explore novel targets for solid tumors, and develop strategies to minimize GVHD risks [6].

For TCR-T therapies, refining neoantigen prediction algorithms and optimizing TCR specificity are crucial for broadening their applicability across various cancer types [8]. Improving TCR-T cell persistence, optimizing dosing strategies, and exploring combination therapies with immune checkpoint inhibitors are vital to enhance efficacy within the tumor microenvironment [39]. Innovative engineering approaches and modifications to the tumor microenvironment (TME) are essential for promoting effective CAR-T responses in solid tumors [39]. Multi-antigen targeting strategies and optimized CAR-T constructs are critical for overcoming resistance mechanisms and improving therapeutic outcomes [39].

Single-cell technologies are expected to elucidate the spatial and functional dynamics of immune cells in the TME, providing insights into interactions that influence therapeutic outcomes [9]. Coupled with multi-scale models that consider intracellular signaling and toxicity, these technologies could significantly enhance translational applications and foster collaborations between modelers and clinicians [25].

Advanced imaging techniques, such as molecular MRI, are anticipated to improve treatment monitoring and assessment. Future research should characterize the temporal dynamics of IFN signaling and explore combination therapies that mitigate adverse effects while enhancing antitumor properties [45]. Investigating metabolic pathways involved in immune cell activation and differentiation, along with new metabolic targets for immunotherapy, remains critical [8]. The sustained influx of B-cells is essential for CAR-T cell activation and tumor eradication, providing a theoretical basis for future immunotherapy research [39]. Additionally, developing responsive delivery platforms tailored to the TME for improved targeting of solid tumors is a key area for exploration [9].

Future prospects in gene-modified immune cell therapies promise to transform cancer treatment. By leveraging advanced delivery technologies and innovative combination strategies, such as nanoparticles and immune checkpoint inhibitors, these therapies can effectively enhance immune responses while minimizing adverse effects, thus addressing existing challenges and improving patient outcomes across various cancer types [2, 22].

8.2 Safety and Efficacy Optimization

Optimizing the safety and efficacy of CAR-T and TCR-T cell therapies is crucial for maximizing their therapeutic potential and ensuring successful clinical application. A primary strategy involves early identification and management of adverse reactions, like cytokine release syndrome (CRS), to minimize associated risks. Implementing standardized grading systems, such as the Penn grading scale, facilitates consistent assessment and management of CRS across clinical settings [52].

The interplay between CAR-T cell proliferation and tumor immunosuppression significantly influences therapy outcomes. Therapy failure often results from the interaction of insufficient CAR-T cell proliferation and an immunosuppressive tumor environment [69]. Understanding these dynamics is crucial for developing individualized treatment plans that optimize efficacy while mitigating adverse effects. Identifying a therapeutic window for TCR T cell dosages based on initial tumor size offers valuable insights into optimizing treatment regimens [32].

Glycodesign offers a promising approach to enhance therapeutic effectiveness while minimizing adverse effects by tailoring glycan structures, thereby improving the safety and efficacy profiles of CAR-T therapies [7]. Strategies to prevent epitope masking, which can significantly impact CAR-T efficacy, are also essential for optimizing therapeutic outcomes [67].

Future research should integrate automated manufacturing solutions, novel gene delivery methods, and address regulatory challenges to scale production and enhance the safety and efficacy of personalized therapies [37]. The adoption of new technologies and regulatory reforms is necessary to improve the clinical trial process and address ethical and recruitment challenges [56].

Optimizing cancer immunotherapy protocols, as suggested by recent studies, can achieve more effective treatment outcomes with reduced medication side effects [70]. By focusing on critical parameters influencing treatment outcomes, researchers can develop strategies to enhance the safety and efficacy of CAR-T therapies [28]. Continuous refinement of these strategies is vital for advancing gene-modified immune cell therapies and improving patient outcomes.

8.3 Overcoming Tumor Microenvironment Barriers

The tumor microenvironment (TME) poses significant barriers to the effectiveness of adoptive T cell immunotherapy, including CAR-T and TCR-T therapies. Characterized by a complex network of cellular and molecular interactions, the TME creates a hostile environment for therapeutic immune cells, imposing physical and functional constraints that limit T cell infiltration, survival, and cytotoxic activity [25].

The TME's immunosuppressive characteristics, driven by regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), hinder antitumor immune responses and facilitate tumor progression and immune evasion. Understanding the complex interactions among these cells is vital for developing novel therapeutic strategies to enhance the efficacy of cancer immunotherapies [71, 72, 2, 10, 73]. These immune cells secrete immunosuppressive cytokines and express inhibitory ligands that dampen T cell activity. Additionally, the TME is often hypoxic and nutrient-deprived, further impairing T cell function and persistence.

To overcome these barriers, several strategies are being explored. Genetically modifying T cells to enhance their resistance to immunosuppressive signals in the TME can improve their functionality and persistence against solid tumors. This approach leverages advancements in technologies such as CARs and genome editing tools like CRISPR-Cas9, aiming to optimize T cell responses by overcoming TME inhibitory effects [34, 5, 72]. This may include incorporating cytokine receptors or co-stimulatory domains that boost T cell activation and survival. Combination therapies targeting multiple TME components, such as immune checkpoint inhibitors and agents modulating the tumor's metabolic landscape, are also being investigated.

Advanced engineering techniques, including TCR-engineered T cells and innovative drug delivery systems like nanoparticles, are employed to enhance T cell infiltration into tumors. These approaches aim to improve cancer immunotherapy effectiveness by enabling T cells to better recognize and attack tumor cells while minimizing adverse effects associated with traditional treatments. Responsive delivery technologies that release therapeutic agents at T cell activation sites are being developed to optimize T cell therapeutic potential within the TME [30, 24, 2, 10, 34]. For instance, chemokine receptor engineering can enhance T cell homing to tumors, while modifications to T cell cytoskeletal components can improve navigation through the dense extracellular matrix of the TME.

Three-dimensional (3D) culture models of the TME are being utilized to gain insights into T cell interactions within the tumor microenvironment. These models provide a more accurate representation of *in vivo* conditions and facilitate testing of novel therapeutic strategies in a controlled setting [25].

Addressing TME challenges is essential for improving adoptive T cell therapy effectiveness, as these barriers can hinder T cell function and persistence, ultimately impacting patient outcomes. Recent advancements in gene engineering and manufacturing protocols aim to enhance T cell specificity and resilience, but further innovations are necessary to optimize tumor-specific T cell receptors and develop strategies that enable T cells to overcome TME immunosuppressive effects [34, 24, 53]. By integrating innovative genetic engineering approaches, combination therapies, and advanced modeling techniques, researchers strive to develop more effective strategies to surmount TME barriers and improve patient outcomes.

9 Conclusion

The exploration of gene-modified immune cell therapies, particularly CAR-T and TCR-T cell therapies, underscores their pivotal role in revolutionizing cancer treatment. These therapies have demonstrated significant success in hematological cancers, achieving sustained remissions in conditions that were previously difficult to treat. However, the complexity of the tumor microenvironment and the inherent challenges associated with targeting solid tumors necessitate continuous research and the development of innovative engineering solutions. A comprehensive understanding of the tumor microenvironment is essential for enhancing the effectiveness of immunotherapies, with combination therapies showing promise in overcoming immunosuppressive barriers. Additionally, the advancement of humanized CAR constructs is vital to reduce immunogenicity, alongside vigilant monitoring of immune responses in clinical applications.

The integration of combination strategies to enhance the efficacy of immunotherapies, coupled with a deeper insight into resistance mechanisms, is crucial for improving patient outcomes. Employing

mathematical models to tackle challenges in immunotherapy and developing personalized treatment models tailored to individual patient profiles are critical for optimizing therapeutic outcomes. Furthermore, targeting metabolic pathways within the tumor microenvironment could potentially enhance the efficacy of immunotherapies, highlighting the need for further investigation into these metabolic interactions.

Ongoing advancements in the research and development of gene-modified immune cell therapies are essential for overcoming existing limitations and expanding their application across a broader spectrum of cancer types. The continued dedication to these therapies promises to transform the landscape of cancer treatment, offering renewed hope to patients worldwide. By leveraging cutting-edge strategies and technologies, these therapies have the potential to significantly improve patient outcomes and redefine the paradigms of cancer treatment.

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