ADVANCES IN CAR-T CELL THERAPY: A PROMISING TREATMENT MODALITY

Abstract

This chapter provides a comprehensive exploration of the evolution and significance of chimeric antigen receptor (CAR)-T cell therapy in cancer treatment. Beginning with an overview of CAR-T therapy's historical development and its profound impact, the chapter delves into its core principles, including CAR structure, genetic engineering, and mechanisms of action. The clinical successes of FDA-approved therapies in hematological malignancies are examined, along with considerations for expanding into solid tumors. The chapter discusses target antigen selection, engineering strategies, overcoming tumor microenvironment challenges, and safety management, addressing cytokine release syndrome and neurological toxicities. Next-generation CAR-T therapies, manufacturing complexities, commercialization challenges, global access, future directions, emerging technologies, and ethical considerations are all analyzed. Ultimately, the chapter offers a comprehensive understanding of CAR-T cell therapy's potential, challenges, and transformative impact on cancer treatment.

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I. INTRODUCTION

In recent decades, substantial advancements have been made in immunotherapy to enhance human well-being. Despite notable progress in cancer treatment in recent years, addressing cancer remains a complex task. Conventional approaches like chemotherapy, radiotherapy, and surgery exhibit limited effectiveness, underscoring the importance of exploring novel therapies. A promising newcomer in this field is chimeric antigen receptor (CAR) T-cell therapy, which boasts multiple advantages such as precise targeting of cancer cells, high rates of symptom alleviation, swift tumor elimination, and enduring anti-tumor immunity. This technique has opened up new avenues for tackling tumors.

The pivotal milestones reached in 2017 with the approval of two CAR-T therapies marked the culmination of over six decades of dedicated research[1]. CAR-T cell therapy stands as a revolutionary achievement in cancer care, traceable back to the FDA's authorization of tisagenlecleucel in 2017 to treat relapsed or refractory acute lymphocytic leukemia in pediatric and young adult patients. By April 2023, a total of six CAR-T cell therapies have gained approval , showcasing unparalleled efficacy in B-cell malignancies and multiple myeloma cases. Nevertheless, the presence of challenges like cytokine release syndrome and immune effector cell-associated neurotoxicity adds complexity to CAR-T cell therapy.[1]

In 2017, a significant turning point was reached in the field of medical research, symbolizing the culmination of more than six decades of unwavering dedication. This milestone pertained to the approval of two CAR-T therapies, representing a groundbreaking achievement in cancer treatment. This achievement can be traced to the FDA's green light for tisagenlecleucel in 2017[1], specifically for the management of relapsed or refractory acute lymphocytic leukemia in pediatric and young adult patients. As of April 2023, a total of six CAR-T cell therapies[1] have secured regulatory approval, underscoring their exceptional effectiveness in addressing B-cell malignancies and instances of multiple myeloma. However, it is important to note that CAR-T cell therapy is not without its challenges, as complications such as cytokine release syndrome and immune effector cell-associated neurotoxicity contribute an additional layer of complexity to this innovative approach in cancer care.

This chapter centers on charting the progression of this innovative treatment approach, starting from its underlying principles to its potential to reshape the future of medical care.

II. PRINCIPLES OF CAR-T CELL THERAPY

CAR-T cell technology, which entails expressing antigen receptors on T cells, was first developed by Eshhar et al. [2] This innovative approach has prompted the creation of five generations of CAR structures (CARs).

1. The parts of the Chimeric Antigen Receptor (CAR)

The CARs are composed of three main regions:
- Extracellular antigen-binding region
- Transmembrane region

- Intracellular activation region, which includes co-stimulatory and signaling areas to promote T-cell proliferation and differentiation. [3]

  The detailed structure of CAR is described below: (**Figure 1a**)

- Extracellular Region: It contains an antigen-binding domain, connecting the CAR to its target antigen.

- **Single-Chain Variable Fragments (scFv)**

  ➤ Within the CAR structure, there are two chains that are linked through repeated glycine and serine residues. These chains consist of the variable heavy (VH) and variable light (VL) chains, collectively termed single-chain variable fragments (scFv).
  ➤ These scFv components are responsible for recognizing specific tumor-associated antigens (TAAs), enabling CAR-T cells to directly stimulate T cells without relying on MHC antigen presentation.
  ➤ A recent development involves the replacement of scFv with a single variable domain of a heavy chain (VHH), often referred to as nanobodies.
  ➤ VHH originates from heavy chain-only antibodies (HcAbs) found in Camelidae and sharks.
  ➤ The efficacy of VHH-based CAR-T cells might parallel that of their scFv-based counterparts. [4]

- **Hinge Region:** Also known as the spacer region, it links the extracellular and transmembrane domains.

- **Transmembrane Region:** Provides structural support and facilitates CAR signaling.

  CARs can be designed with different transmembrane domains, such as CD28 or CD8, affecting T-cell expansion and persistence. [4]

- **Co-stimulatory Region**
  ➤ Co-stimulatory domains hold significant importance in the behavior of CAR-T cells, where CD28 and 4-1BB stand out as the key players.
  ➤ CAR-T cells incorporating CD28 demonstrate swift proliferation but reduced persistence, in contrast to T cells incorporating 4-1BB, which display heightened mitochondrial activities and transition into T cells resembling central memory cells.
  ➤ Additional frequently encountered co-stimulatory domains encompass CD27, OX40, and ICOS.
  ➤ Distinct co-stimulatory domains have led to diverse behaviors observed in CAR-T cells. [4]

- **Intracellular Signaling Component**

> The most distal intracellular component of CARs is the CD3-ζ (CD247) signal area with three immunoreceptor tyrosine-based activation motifs (ITAMs).
> Tyrosine kinase-related protein of 70 kDa (ZAP70) phosphorylates ITAMs, triggering signal transduction and activating T cell responses, including proliferation and cytokine release [4].
> CAR-T cell technology has evolved through five generations of CAR structures, each with its unique components. Co-stimulatory regions and transmembrane domains influence the behavior of CAR-T cells, affecting factors like persistence and cytokine release. Understanding these elements is vital for optimizing CAR-T therapy and harnessing its potential for treating various diseases.
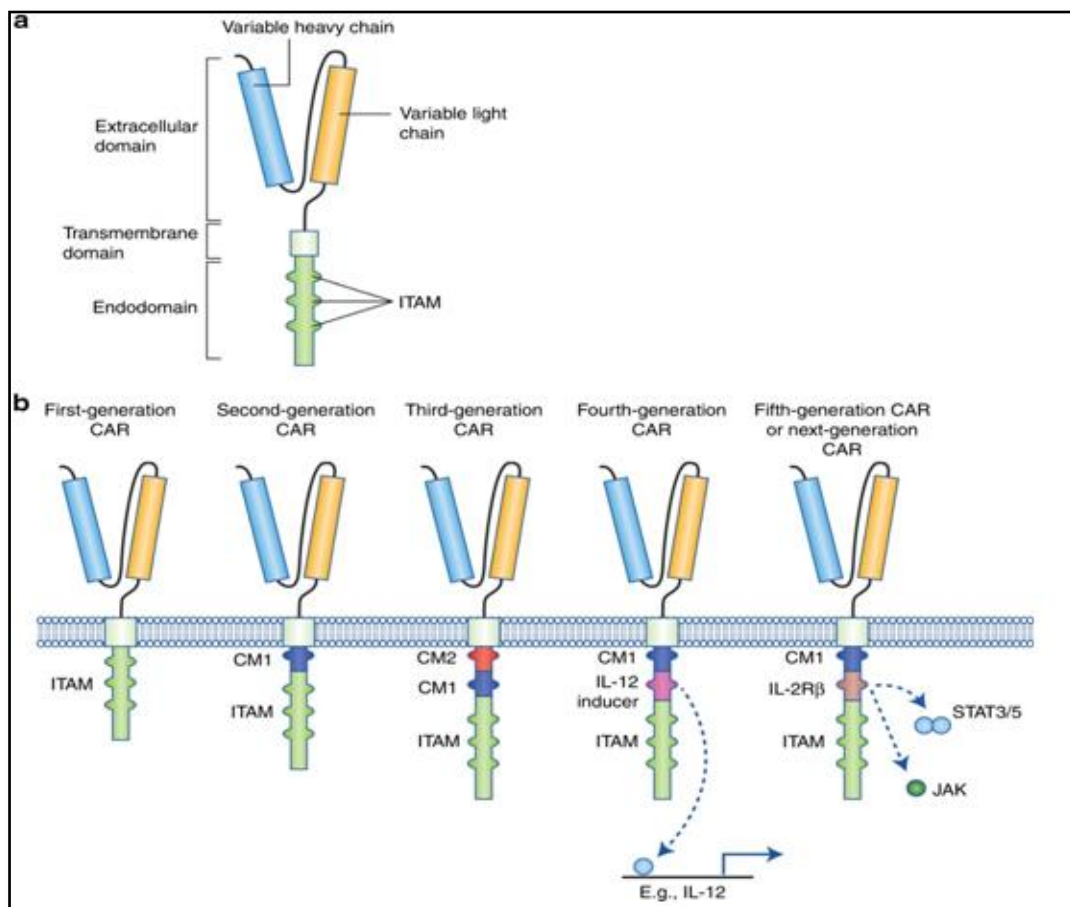
Figure 1: Structure of Different Chimeric Antigen Receptor (CAR) Generations: **(a)** The fundamental CAR structure, highlighting key elements within the extracellular, transmembrane, and intracellular (endodomain) domains; **(b)** The progression of CAR development through generations, starting with the initial generation containing solely ITAM motifs in the intracellular domain. The second generation introduced a single co-stimulatory molecule (CM1), while the third generation incorporated an additional CM. The fourth CAR generation was an extension of second-generation CARs (comprising 1–3 ITAMs) coupled with a constitutively or inducibly expressed chemokine like IL-12, leading to T cells often termed as T cell redirected for universal cytokine-mediated killing (TRUCKs). The 'next generation', denoted as the fifth generation, built upon the

second generation of CARs, introducing intracellular domains from cytokine receptors (e.g., IL-2Rβ chain fragment). Key abbreviations used include ITAM for immunoreceptor tyrosine-based activation motifs, CD for co-stimulatory domain, IL-12 for interleukin 12, and IL-2Rβ for truncated intracellular interleukin 2β chain receptor with a STAT3/5 binding motif.

[Source: (5) Tokarew N, Ogonek J, Endres S, von Bergwelt-Baildon M, Kobold S. Teaching an old dog new tricks: next-generation CAR T cells. Br J Cancer. 2019 Jan;120(1):26-37. doi: 10.1038/s41416-018-0325-1. Epub 2018 Nov 9. PMID: 30413825; PMCID: PMC6325111]

2. **Development of the CARs:** The CARs have evolved through five generations due to advancements in genetic engineering and immunotherapy. (Figure 1b)

- **First-Generation CARs**

  - Initial CARs contained only the CD3 intracellular signaling molecule.
  - Showed success in enhancing T cell anti-tumor activity and recognizing tumor antigens.
  - Nonetheless, their therapeutic effects were limited due to the absence of co-stimulatory molecules (CD27, CD28, CD134, 4-1BB) and cytokine signaling (interleukin-2, IL-2).
  - The proliferative powers of first-generation CARs were poor. [4]

- **Second-Generation CARs**

  - Combining CD3 with co-stimulatory molecules (CD28 or 4-1BB) significantly increased cell proliferation and cytotoxicity. [4]

- **Third-Generation CARs**

  - Introduced two distinct costimulatory domains (CD28-4-1BB and ICOS-4-1BB), building on second-generation CARs.
  - Examples include CAR 22–19. [4]

- **Fourth Generation CARs (TRUCKs or Armored CARs)**
  - Included an IL-12 expression system to strengthen the capacity to attack tumor cells.
  - Increased tumor cytotoxicity by defeating the immunosuppressive networks in the tumor microenvironment (TME) and releasing significant chemicals or cytokines.
  - TRUCKs can trigger IL-12 downstream of the nuclear factor-activated T cell (NFAT) transcription factor, attracting additional immune cells like dendritic cells (DC), phagocytes, and natural killer cells (NK). [4]

- **Fifth Generation CARs**
  - Expanding upon the framework of the second generation, fifth-generation CARs integrate a segment that binds to STAT3 and a truncated cytoplasmic

domain of the IL-2 receptor chain (IL-2R).

> Stimulation of the fifth-generation CAR triggers both T cell receptor (TCR) and cytokine-mediated JAK-STAT signaling pathways, fostering escalated proliferation and heightened activation of engineered T cells. [4]

Despite rapid evolution, there have been no direct comparisons between CAR designs, leaving the question of which design offers the best clinical efficacy unanswered. Further research is needed to address this.

3. Mechanisms Of CAR-T Cell Therapy

- CAR-T cells, engineered with antigen specificity and T-cell cytotoxicity, are crucial for targeted cancer treatment.
- The primary targets of hematological malignancies are CD19, BCMA, CD20, and CD22. Additional targets include CD23, CD30, CD33, SLAMF, ROR1, GRP78, and CD138.
- CD19 is widely expressed in most B-cell malignancies, making it a popular target. CD30 is typically expressed in Hodgkin's lymphoma tumor cells. CD33 is a favorable target for leukemia, particularly acute myeloid leukemia (AML).
- SLAMF, BCMA, and CD138 are potential targets for multiple myeloma (MM), with BCMA being particularly significant.
- ITAMs activation: CAR-T cells detect specific TAAs, triggering ITAMs phosphorylation. This leads to proliferation, cytokine production, and enhanced immune responses [4]

4. Challenges

- Tumor resistance can occur due to loss of TAA expression, like CD19 or CD20.
- Additionally, TAAs differ from tumor-specific antigens (TSAs) in that TAAs have low selectivity, are overexpressed in tumors, but are also expressed in healthy organs and tissues, leading to off-target effects and generating safety concerns [4].

CAR-T cells utilize these mechanisms to target specific antigens, offering promising prospects for cancer treatment. However, addressing challenges like TAA loss and off-target effects remains critical for improving the therapy's safety and efficacy.

III. CLINICAL SUCCESS AND APPROVED INDICATIONS

As of April 2023, a total of six CAR-T cell therapies had gained approval, showcasing unparalleled effectiveness among patients diagnosed with B-cell malignancies and multiple myeloma, as indicated in **Table 1**.

Table 1: An overview of approved CAR-T cell therapies

[Source: (6) Chen YJ, Abila B, Mostafa Kamel Y. CAR-T: What Is Next? Cancers. 2023 Jan 21;15(3):663.]

CAR-T Cell Product Name	FDA-Approved Date	Indication(s)	Antige n
Kymriah® (tisagenlecleucel)	2017	• Individuals under the age of 25 who are affected by refractory or second or subsequent relapse B-cell precursor acute lymphoblastic leukemia (ALL). • Adults diagnosed with large B-cell lymphoma (LBCL), including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high-grade B-cell lymphoma, and DLBCL originating from follicular lymphoma, who have experienced relapse or refractory (r/r) disease after undergoing two or more rounds of systemic treatment. • Adults with relapsed or refractory follicular lymphoma (FL) following two or more courses of systemic therapy.	CD-19
Yescarta® (axicabtagene ciloleucel)	2020	• Adult patients with relapsed or refractory mantle cell lymphoma (MCL). • Adult patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL).	CD-19
Tecartus® (brexucabtagene autoleucal)	2020	• Adult patients with relapsed or refractory mantle cell lymphoma (MCL). • Adult patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL).	CD-19
Breyanzi® (lisocabtagene maraleucel)	2021	• Adult patients diagnosed with various forms of large B-cell lymphoma (LBCL), including diffuse large B-cell lymphoma (DLBCL) that is not specifically categorized (including cases arising from indolent lymphoma), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B, provided that they meet the following criteria: • refractory disease to first-line chemoimmunotherapy or relapse within 12 months of first-line chemoimmunotherapy; or • refractory disease to first-line chemoimmunotherapy or relapse after first-line chemoimmunotherapy and are	CD-19

		not eligible for hematopoietic stem cell transplantation (HSCT) due to comorbidities or age; or • relapsed or refractory disease after two or more lines of systemic therapy.	
Abecma® (idecabtagene vicleucel)	2021	Adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody.	BCMA
Carvykti® (ciltacabtagene autoleucel)	2022	Adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody.	BCMA

CAR-T therapy has displayed significant promise in treating hematological malignancies, attributed to its ability to achieve high rates of remission, swiftly eliminate tumors, and elicit enduring responses. [1] Nonetheless, the administration of CAR-T cells carries inherent risks, and it is imperative to carefully deliberate over specific hurdles. Despite ongoing endeavors, the results of using cellular therapy for solid tumors are notably suboptimal in comparison to their hematological counterparts. These constraints primarily arise from factors such as evasion of antigens, undesirable consequences of CAR-T cell therapy, inconsistencies in antigen presentation, challenges related to the migration and infiltration of CAR-T cells within tumors, concerns regarding stability, the inhibitory character of the tumor microenvironment, and inefficacies in combatting malignancies associated with B cells[7].

IV. TARGET ANTIGENS AND ENGINEERING STRATEGIES

1. Target Antigens

Table 2. An overview of Target Antigens for CAR-T cell therapy

[Source: (8) Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. Nat Rev Cancer. 2021 Mar;21(3):145-161. doi: 10.1038/s41568-020-00323-z. Epub 2021 Jan 22. PMID: 33483715; PMCID: PMC8353572]
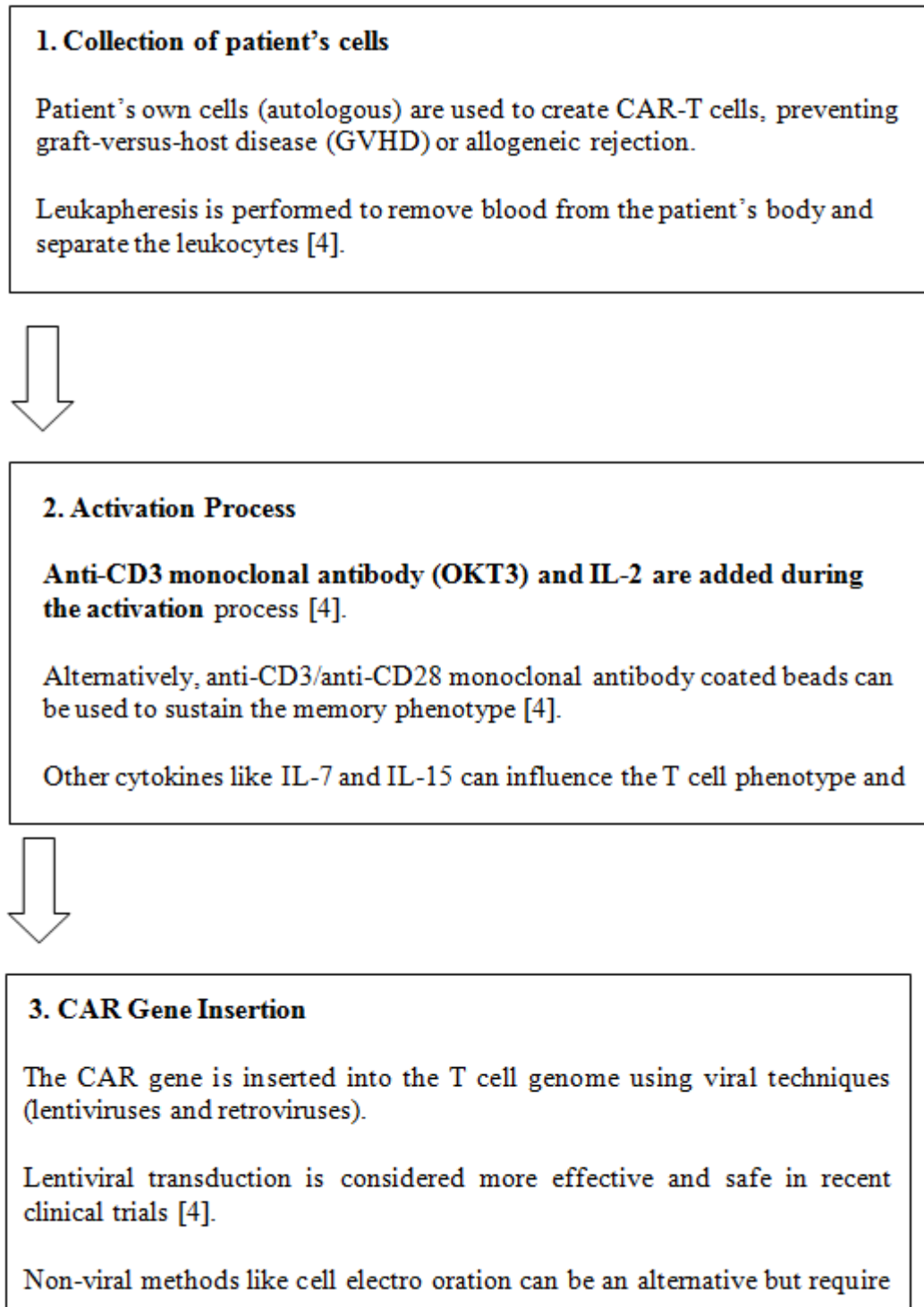
B-ALL signifies B cell acute lymphoblastic leukemia; BCMA represents B cell maturation antigen; CAR stands for chimeric antigen receptor; CRS denotes cytokine release syndrome; DLBCL pertains to diffuse large B cell lymphoma; MSKCC abbreviates Memorial Sloan Kettering Cancer Center; NCI stands for National Cancer Institute; and UPenn refers to the University of Pennsylvania. These designations are associated with an approval by the US Food and Drug Administration (FDA)[8].

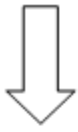| Target antigen	Disease	CAR	Clinical trial identifier	Sponsor	Number of patient s analyze d	Median age (yes)	Response	Patient s with CRS (%)	Patients with neurotoxicity (%)
CD19	B-ALL (pediatric)	Tisagenlecleuc el; 4-1BB co-stimulation; CTL019	NCT0243584 9	Novartis Pharmac eutic als	75	11	6-month relapse free survival rate of 80%	77	40

CD19	Relapsed or refractory DLBCL	Axicabtagene ciloleucel;CD2 8 co-stimulation; KTE-X19	NCT0234821 6 ZUMA-1	Kite Pharma (aGilead Sciences company	101	58	83% objective response; 58% complete response	93	67
CD19	Refractor y B cell lymphom as	Tisagenlecleuc el; 4-1BB co-stimulation; CTL019	NCT0203083 4	UPenn	28	58.5	64% overall response; 43% complete remission	57	39
CD19	Mantle cell lymphom a	Axicabtagene ciloleucel;CD2 8 co-stimulation; KTE-X19	NCT0260131 3 ZUMA-2	Kite Pharma	68	65	93% objective response rate; 67% complete response	91	63
CD19	B-ALL	CD28 co-stimulation	NCT0104406 9	MSKCC	53	44	83% complete remission; median overall survival 12.9mont hs	85	44

CD22	Relapsed or refractory pre-B-ALL	4-1BB co-stimulation	NCT0231561 2	NCI	21	19	73% complete remission treated with higher dose	76	Unreported
BCMA	Relapsed or refractory multiple myeloma	Idecaptagene Cicleucel; 4-1BB co-stimulation; bb2121	NCT0265892 9	Celgene	33	60	85% objective response rate; 45% complete response rate	76	42
BCMA	Multiple myeloma	4-1BB co-stimulation	NCT0254616 7	UPenn	25	58	48% overall response rate	88	32

2. **Engineering Approaches:** The following flowchart depicts the engineering approach to the CAR-T cell therapy

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1. Collection of patient's cells

Patient's own cells (autologous) are used to create CAR-T cells, preventing graft-versus-host disease (GVHD) or allogeneic rejection.

Leukapheresis is performed to remove blood from the patient's body and separate the leukocytes [4].

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2. Activation Process

Anti-CD3 monoclonal antibody (OKT3) and IL-2 are added during the activation process [4].

Alternatively, anti-CD3/anti-CD28 monoclonal antibody coated beads can be used to sustain the memory phenotype [4].

Other cytokines like IL-7 and IL-15 can influence the T cell phenotype and

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3. CAR Gene Insertion

The CAR gene is inserted into the T cell genome using viral techniques (lentiviruses and retroviruses).

Lentiviral transduction is considered more effective and safe in recent clinical trials [4].

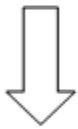Non-viral methods like cell electro oration can be an alternative but require

4. Expansion of CAR-T Cells

Three methods for expansion: plates/T-flasks, static culture bags, and rocking motion bioreactors [4].

Rocking motion bioreactor is popular, using cutting-edge technology and suitable for larger patient cohorts [4].

5. Elimination of Regulatory T Cells

Regulatory T cells (Treg) and immunosuppressive elements must be eliminated using chemotherapeutic methods (lymphodepleting conditioning regimen) [4].

Antimetabolites like cyclophosphamide and fludarabine are used for pretreatment.
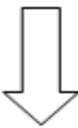
6. Efficacy Enhancement

Lymphodepleting conditioning regimen improves therapy efficacy through various mechanisms [4].

It upregulates indoleamine 2,3-dioxygenase on the tumor and induces costimulatory molecules.

Increases homeostatic cytokines (IL-2, IL-7, and IL-15) and enhances

3. Challenges and Advancements

- CAR-T cell therapy has limitations due to lengthy preparation, complexity, and patient customization.
- Multifunctional Alginate Scaffold for T Cell Engineering and Release (MASTER) is a recent advancement.
- MASTER's integration of T cell activation, reprogramming, and in vivo amplification shortens manufacturing to just one day [4].

4. Potential Improvements

- Non-activated CAR-T cells created quickly (within 24 hours) showed superioranti-leukemic in vivo activity per cell than conventionally activated CAR-T cells [4].
- Traditional manufacturing may lead to gradual differentiation and loss of anti-leukemic action.
- Faster and more practical methods could expand the application of CAR-T cell therapy
- The manufacturing process is subject to ongoing research and advancements to further optimize CAR-T cell therapy.

V. OVERCOMING TUMOR MICRO-ENVIRONMENT CHALLENGES (TME)

A formidable obstacle encountered in CAR-T cell therapy revolved around overcoming the challenging Tumor Microenvironment (TME). After CAR-T cells breach the endothelial barrier, their ability to persist within the hostile TME becomes crucial, given the multitude of obstacles it presents, including suppressive elements like tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and inhospitable hypoxic conditions. Substantial research has demonstrated that enhancing therapeutic effectiveness can be accomplished by addressing the immune checkpoint known as programmed cell death protein-1 (PD-1). This can be achieved by administering PD-1-targeting monoclonal antibodies concurrently with CAR-T cell infusion or by employing PD-1 gene editing techniques on CAR-T cells, both in preclinical and clinical settings. It's worth noting that a clinical trial involving MUC1-targeted CAR-T cells with PD-1 knockout (KO) via CRISPR-Cas9 showed safety, although outcomes varied among patients[9]. Given diverse clinical responses following PD-1 blockade, alternative strategies are pursued to conquer the TME, including interventions targeting transforming growth factor β (TGF-β), a pivotal multifunctional peptide impeding T cell function via various avenues like hindering T helper cell maturation. [10] Another avenue involved CRISPR/Cas9-mediated knockout of TGF-β receptor II (TGFβR2) in CAR-T cells, revealing that TGFβR2 inhibition counteracted CAR-T exhaustion in preclinical hepatic and pancreatic tumor models. [9]

Furthermore, novel strategies aimed at countering T-cell fatigue within the formidable Tumor Microenvironment (TME) involve modifications at the epigenetic and transcriptional levels. In an effort to leverage the crucial role of DNA methylation in enhancing T cell differentiation and potency, CAR-T cells were engineered with the deletion of DNA methyltransferase 3a (DNMT3A). These modified cells displayed sustained functional effectiveness against persistent tumors. Notably, experiments conducted in vitro and within NSG murine solid tumor models underscored their ability to manage secondary tumor challenges, thereby preserving their memory functions and providing protection against

tumor recurrence. Importantly, this research pinpointed the DNMT3A exhaustion signature as a promising potential biomarker for future investigations. [11]

Efforts to tackle the hypoxic TME led to the development of hypoxia-inducible CARs (HiCARs) with incorporated hypoxia response elements (HRE) and an oxygen-dependent degradation domain (ODD), maintaining stability under low-oxygen conditions. ODD fusion enabled oxygen-dependent CAR-T cell activation and sustained effector T cell functionality. [12] Furthermore, disparities in the TME composition between adult and pediatric solid tumors underscore the need for tailored approaches in therapeutic development. [13]

VI. SAFETY CONSIDERATIONS AND MANAGEMENT OF TOXICITIES

While CAR-T cell therapy stands as a pioneering approach to cancer treatment, its application as a primary treatment option has been limited by notable instances of toxicities, some of which have resulted in fatalities. These adverse effects include:

Cytokine Release Syndrome (CRS): Despite the FDA-approved treatment for severe CRS, tocilizumab, severe CRS and mortality still happen. Linked to substantial in vivo T cell expansion and excessive cytokine production, the mechanism behind CAR-T cell therapy involves the infusion of CAR-T cells that subsequently become hyperactivated, leading to the release of substantial cytokine quantities and the resultant cytokine release syndrome (CRS). The manifestation of mild CRS is marked by fever accompanied by fatigue, diarrhea, headache, rashes, arthralgia, and myalgia. In more critical scenarios, patients can develop hypotension, cardiac dysfunction, circulatory collapse, respiratory and renal failure, multiorgan system breakdown, with potential progression to fatal outcomes[14]. On a pathophysiological level, IL-6 is believed to play a pivotal role in triggering CRS. Consequently, therapeutic interventions utilizing tocilizumab and corticosteroids are employed to target the IL-6 receptor and mitigate its effects[14]. Despite the approved usage of tocilizumab as a treatment for severe CRS by the FDA, instances of severe CRS and resultant mortality persist.

1. **Hemophagocytic Lymphohistiocytosis (HLH) and/or Macrophage Activation Syndrome (MAS):** Characterized as an intense hyperinflammatory state accompanied by cytokine release syndrome (CRS), elevated serum ferritin levels, hemophagocytosis, renal dysfunction, elevated liver enzyme counts, splenomegaly, pulmonary edema, and/or diminished natural killer (NK) cell activity, it is noteworthy that CAR-T cell therapy-triggered hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) can sometimes exhibit resistance to IL-6 inhibition and necessitate chemotherapy instead. Intriguingly, the occurrence of HLH/MAS as a result of CAR-T cell therapy remains uncertain due to its overlap with severe CRS, although records indicate its incidence in approximately 1% of treated patients[14]

2. **Immune Effectors Cell-Associated Neurotoxicity Syndrome (ICANS)**: Marked by disruption of the blood-brain barrier and heightened cytokine levels within the cerebrospinal fluid, the etiology and fundamental mechanisms underlying neurotoxicity associated with CAR-T cell therapy remain incompletely elucidated[14]. Clinical symptoms of immune effectors cell-associated neurotoxicity syndrome (ICANS) encompass a range of manifestations, including confusion, headache, diminished

attention span, language difficulties, focal neurological irregularities, encephalopathy, life-threatening cerebral edema, transient coma, or seizures[14]. Despite the frequent inefficacy of IL-6 inhibitors in managing CAR-T therapy-linked neurotoxicity, the focus of neurotoxicity management primarily centers on corticosteroid interventions[14]

3. **Long Term Safety Monitoring and Management Strategies**

   Changing the CAR's structure to reduce toxicity:
- Lowering the antigen-binding domain's affinities to micromolar levels [14].
- Cytokine release can be controlled by altering the CAR hinge and transmembrane sections[14].

- Customizing the costimulatory domain involves considering factors such as the specific tumor type, tumor burden, density of antigens, the pairing of the target antigen with the antigen-binding domain, and addressing potential toxicity concerns..[14]
- By using human/humanized antibody fragments rather than CARs generated from murine cells, CAR immunogenicity can be reduced[14].
- "Off-switches" for CAR
- CAR constructions that are tailored to express CD20 make it easier to remove CAR-T cells using rituximab[14].
- Because dasatinib treatment temporarily inhibits CAR-T cell function and may enable rescue therapy when toxicities have passed, it offers interesting promise[14].

VII. NEXT-GENERATION CAR-T CELL THERAPIES

1. **Armored CAR-T Cells and Dual-Targeting Strategies:** Enhancing the potency of CAR-T therapy, immune checkpoint modulation has emerged as a strategy to counteract inhibitory signals within the tumor microenvironment. In hematological settings, ongoing clinical trials investigating this avenue predominantly focus on disrupting the programmed cell death protein 1 (PD-1) pathway, showcasing distinctive methods to dysregulate PD-1 signaling[15].

   One approach involves the conversion of the suppressive PD-1 signal into a stimulating one. This design incorporates the extracellular PD-1 domain with the intracellular CD28 domain. By modifying the interaction between PD-1 and PD-L1 within the tumor microenvironment, this configuration enhances the activating signals, thus strengthening the CAR-T cell response. b.Alternatively, another tactic entails the secretion of PD-1 Fc to block the inhibitory PD-L1 signaling. Engineered CAR-T cells produce and release a protein composed of the PD-1 domain and the fragment crystallizable region (Fc) of an antibody. This secreted protein hinders PD-L1 molecules on cancerous cells, making them susceptible to attack by innate immune cells.

   c.A different strategy involves the reduction of PD-1 expression, achieved by transfecting CAR-T cells with short hairpin RNA (shRNA), which subsequently suppresses PD-1 expression through RNA interference.

Armored CAR-T cells exhibit promising potential in addressing CD19-positive malignancies. Across diverse trials, complete response rates range from 41.2% to an impressive 78%[16].

However, given the limited patient enrollment in these studies, larger-scale investigations are warranted to thoroughly assess the feasibility of these constructs.

2. **Off-The-Shelf CAR-T Cell Therapies:** Given the wide range of CAR therapy configurations available, the development of "off-the-shelf" universal CAR T cells (UCART) is emerging as a potentially safer, more efficient, and cost-effective alternative to traditional CAR T cells. The UCART approach allows for **the creation** of a quality-controlled product with multiple genetic modifications in a shorter time frame through batch production. The production of off-the-shelf CAR-T therapies, sourced from unrelated healthy donors and employing gene editing technology to ensure the safety of the final product for use in recipients with HLA-mismatched profiles, provides a means to overcome the challenges associated with manufacturing autologous CAR T products. These challenges include unpredictable pharmacology due to the heterogeneity of individual products and the assurance of drug product availability when needed[17].

3. **CAR-T Cells And Combination Therapies:** Traditionally, human antibodies are known for their monospecificity, targeting a single antigen. In contrast, bispecific antibodies, exemplified by Bispecific T cell engagers (BiTEs), possess the ability to simultaneously target multiple antigens. BiTEs are dual-specific antibodies explicitly designed to recruit T cells into the tumor microenvironment, thereby augmenting their effectiveness in combating tumors. These BiTEs have the capacity to engage both T cells and tumor cells by binding to CD3 on CAR or bystander T cells (via an anti-CD3 monoclonal antibody) and simultaneously binding to a target antigen on tumor cells (via an anti-tumor-associated antigen monoclonal antibody). This mechanistic interaction establishes a connection between CAR-T cells and distinct tumor cells, resulting in efficient eradication of the tumor cells.

In a complementary approach, CAR-T cells can be administered concurrently or sequentially with bispecific adapters that carry conjugated antibody segments referred to as "tags" (e.g., fluorescein isothiocyanate, biotin, etc.). The precise targeting of antigens is accomplished through the collaboration between these bispecific adapters (tags), which target tumor antigens, and anti-tag CAR-T cells. This cooperative synergy between BiTEs and CAR-T cells reinforces robust anti-tumor responses, offering a promising avenue for combination therapy. Thus, the adoption of such a combined strategy involving CAR-T cells has the potential to address challenges like antigen loss, antigen heterogeneity, and the limited persistence and efficacy often associated with CAR-T cell therapy.

Similarly, the integration of CAR-T cell therapy with other therapeutic modalities, such as chemotherapy, radiotherapy, oncolytic viruses, cancer vaccines, cytokines, immunomodulatory agents, Hematopoietic Stem Cell Transplantation (HSCT), and metabolic inhibitors, represents a multifaceted approach that enhances the safety and efficacy of CAR-T cells. This comprehensive strategy underscores the potential for synergistic effects in augmenting the success of CAR-T cell therapy. These combined treatment strategies modify the tumor microenvironment, optimize CAR structure,

establish connections between CAR-T cells and tumor cells, potentially target multiple antigens, counteract tumor-immune escape mechanisms, and mitigate the toxicity of CAR-T cell therapy[18].

4. **CAR-NK Cell Therapy And Other Novel Approaches:** The heterogeneous population of natural killer (NK) cells comprises a potent group of immune cells renowned for their robust cytotoxic capabilities, which make distinctive contributions to both innate and adaptive immune responses. However, the **efficacy of these cells** can be compromised within the tumor microenvironment. Engineered NK cells, equipped with Chimeric Antigen Receptors (CARs), referred to as CAR-NK cells, have emerged as a groundbreaking advancement in anti-tumor immunotherapy.

These cells differentiate between healthy and cancerous tissues by recognizing germline-encoded cell surface receptors. Unlike NK cells and CAR-T cells, genetically modified CAR-NK cells offer the advantage of enhancing specificity and cytotoxicity without the associated adverse effects like Graft-versus-Host Disease (GvHD), Cytokine Release Syndrome (CRS), or Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). Consequently, CAR-NK cells, known for their potent cytotoxicity, limited lifespan, and cost-effective manufacturing, hold promise as viable alternatives to engineered CAR-T cells.

Despite the promising advancements in CAR-NK cell-based immunotherapy, challenges persist in achieving a satisfactory balance between treatment efficacy and minimizing toxicity, as well as expanding the scope of treatable conditions. Similar to CAR-T cells, the broad application of CAR-NK cell-based therapy faces limitations, such as restricted in vivo proliferation, activation, and durability. Complications also arise during CAR-NK cell preparation due to issues like low genetic transfection efficiency, a low proportion of NK cells in the bloodstream, and limited amplification efficiency. On the positive side, CAR-NK cells offer distinct advantages due to their unique biological characteristics. They can be sourced more conveniently from a wider range of autologous and allogeneic sources without eliciting severe adverse reactions like acute GvHD or CRS, which are often associated with CAR-T cell transplantation. Additionally, CAR-NK cells can engage in immunosurveillance without prior sensitization, demonstrating versatile killing abilities against both hematologic and solid tumor cells. This capability stems from both CAR-dependent mechanisms and CAR-independent intrinsic mechanisms, offering alternative strategies to counteract tumor escape and mitigate various adverse events, including the "on-target, off-tumor toxicity" frequently observed in CAR-T cell therapy.

In summary, pioneering preclinical and clinical investigations underscore the multifaceted opportunities and challenges inherent to allogeneic CAR-NK cells. These cells are poised to become vital "off-the-shelf" products in the next-generation of cellular immunotherapies, specifically targeting recurring and resistant malignancies[19].

VIII. MANUFACTURING AND COMMERCIALISATION CHALLENGES

CAR T-cell therapy stands as an impressive demonstration of how foundational immunology can be harnessed to treat individuals grappling with hematological malignancies. Notwithstanding the current strides, this domain confronts several challenges. From a financial and logistical perspective, numerous constraints hinder the widespread provision of this therapy to eligible patients. Accessibility remains limited to those possessing sufficient insurance coverage and proximity to CAR T-cell therapy centers. Significantly expanding access demands substantial infrastructure development.

As more commercial entities and academic institutions venture into CAR T-cell production, quality control and regulatory intricacies will undoubtedly arise[20]. It's evident that a considerable number of patients experience relapse following CAR T-cell therapy, and research into treatment options for these recurrences is ongoing. Despite the obstacles, the ongoing commitment to advancing CAR T-cell therapy holds the promise of even greater achievements in the future.

1. **Scalability and Manufacturing Complexities:** To ensure the successful delivery of CAR-T therapy, a series of intricate steps in the manufacturing process must be meticulously coordinated and executed. The optimization of manufacturing timelines, especially considering the short turnaround times required, hinges on the availability of essential raw materials, particularly viral vectors which often involve extended lead periods. This task becomes challenging due to uncertain demand and resource scarcity. Many decisions regarding the manufacturing process need to be settled prior to seeking regulatory approval, as they significantly impact therapy quality and efficacy.

   Such decisions encompass several aspects, including (a) the condition of therapy transfer (fresh or cryopreserved); (b) the choice of vector type (lenti- or retro-vector); and (c) procedural design. Moreover, choices pertaining to outsourcing and/or in-house processing of specific manufacturing components (such as quality control, storage, etc.) must be determined well in advance, as these choices directly influence the business model and supply chain. The patient-specific nature of CAR T cell manufacturing contrasts with conventional pharmaceutical products, presenting challenges in scaling up production volume and complicating the path to commercialization[21].

   Efforts to streamline CAR-T production timelines are a future imperative. The current duration of three to four weeks for patients with relapsed or refractory disease is often too protracted, leading to patients missing out on treatment due to disease progression, death, or production constraints. Potential strategies to cut down on production time include novel manufacturing techniques, decentralized production models, and exploration of alternative non-viral vector approaches to genetic engineering with quicker turnaround times[22]. For instance, Jackson et al. have demonstrated the feasibility of producing CAR-T cells in a hospital environment using a closed system compliant with Good Manufacturing Practices (GMP)[23]. By employing such an approach, the authors were able to shorten the cell manufacturing time to just eight days, thus enhancing cost-effectiveness and expediting patient care. Localized manufacturing offers the advantage of rapid turnaround times compared to centralized methods.

The evolution of allogeneic or commercially available CAR-T therapies, derived from healthy donor cells, is an ongoing research priority[22]. Such "off-the-shelf" therapies hold the potential to treat a large number of patients per batch and offer the advantage of immediate availability without risk of delays or production failures. Although this approach is in its nascent stages, researchers are already considering its potential efficiency in delivering autologous CAR-T therapy. Sources for healthy donor cells include peripheral blood mononuclear cells, umbilical cord blood, induced pluripotent stem cells, embryonic stem cells, and HLA-silenced CD34+ progenitor stem cells[22]. One specific drug under study, UCART19, demonstrates a manageable safety profile in extensively treated pediatric and adult patients with relapsed or refractory B-cell Acute Lymphoblastic Leukemia (B-ALL). However, allogeneic cells carry the risk of immune rejection by host T cells and potential allo-reactivation by CAR-T cells via the TCR receptor, potentially leading to graft versus host disease (GVHD)[22]. Ongoing clinical trials explore commercially available therapies, some of which involve gene editing procedures to eliminate the surface TRAC molecule, aiming to prevent GVHD[22].

2. **Cost Considerations and Reimbursement Challenges:** The existing price of commercially available medications is notably high, posing challenges in establishing suitable reimbursement policies. The evaluation and payment methods for these treatments are currently under discussion, addressing several **fundamental aspects.** These include (a) assessing the value of a cure while considering societal preferences, (b) determining appropriate standards for evaluating curative treatments, (c) accurately characterizing uncertainty, and (d) applying appropriate discount rates [21]. The broad adoption of CAR T cell therapies in clinical settings will ultimately depend on the treatments' effectiveness and their ability to reduce adverse effects. Moreover, ensuring clinical accountability and maintaining a clear chain of custody are additional crucial considerations.

   Following the FDA approval of the first CAR-T cell therapies in 2017, these one-time treatments have demonstrated remarkable response rates among patients with relapsed or refractory lymphoid malignancies. However, these therapies come with substantial price tags, often around $373,000 for a single infusion.

   Italy's hospitals introduced a tariff calculation system in the late 1980s based on Diagnosis-Related Groups (DRGs) [22]. DRGs are also employed in North America [24]. They are determined by a "grouper" algorithm that considers International Classification of Diseases diagnoses, patient attributes (age, gender), procedures, the presence of complications or comorbidities, and discharge status. The assigned DRG dictates the hospital bill, considering the length of the hospital stay. Since patients within a given category are expected to utilize similar hospital resources (fixed pricing), DRGs encompass all necessary procedures for each treatment and diagnosis.

   Within the DRG framework, the average cost of CAR-T cell therapy in recognized Italian facilities is reported at $59,806. However, this cost does not accurately reflect the repayment within the DRG system, nor does it encompass the production cost of the therapy or potential long-term side effects management. The model's complexity arises from the omission of post-relapse alternative therapies and related expenses. This complexity suggests that cell therapy might warrant a distinct payment model[25].

One method aimed at enhancing efficiency and cost-effectiveness is Activity-Based Costing (ABC). This approach revolves around the concept that producing a product or providing a service involves resource-intensive activities [22]. ABC endeavors to allocate costs to each of these activities and resources, thus providing a clearer overview of total expenses. Some pharmaceutical companies have explored an outcome-based pricing model, where patients would not be charged if the therapy proves ineffective. However, the vagueness in defining "ineffectiveness" poses one of several challenges to this intriguing concept [22].

3. **Supply Chain Logistics And Global Access To CAR-T Cell Therapy:** The current supply chain model for CAR T cell therapy manufacturing faces significant challenges related to scalability, prompt delivery, and pricing. To establish an effective supply chain network, key factors like sample tracking, packaging, shipping, storage validation, and chain-of-custody documentation are crucial. However, decisions concerning these factors must align with the unique characteristics of CAR T cell products to ensure success. CAR T cells, like other cell-based products, are susceptible to damage from mishandling, which can lead to contamination, loss of functionality, or loss of identity. Handling during transportation requires expertise due to their sensitivity to stress and temperature fluctuations. Mitigating these risks often demands skilled personnel throughout the process, which may not always be feasible. Moreover, the limited shelf-life of CAR T cell therapies must be considered, limiting waiting and storage periods[21].

Patient scheduling plays a pivotal role in autologous CAR T cell therapy, as patients are their own donors. Minimizing intervals between product release and administration and between collection and manufacturing is crucial. The one-to-one nature of patient-specific therapies means any lost samples or therapies cannot be replaced from stock. Developing the CAR T cell supply chain and commercial strategy is constrained by time due to the short shelf-life of these therapies. Storage, production, and transportation durations need stringent control to ensure product quality. Sample tracking, involving bidirectional tracking, patient identification, and cloud-based platforms, is vital to preventing errors [21].

Storage and equipment validation, packaging, shipment, and chain-of-custody documentation are also significant risk factors. Delicate CAR T cell products require proper storage and transportation conditions to mitigate loss risks. Shipping solutions ensuring temperature maintenance and appropriate duration are essential, as is validating equipment for proper storage conditions[21]. Digital tools and software platforms have emerged to streamline operations and facilitate sample tracking, maintaining patient privacy[26, 27]. While digitalization raises concerns about automation and job loss, it can enhance processes, free up human operators for more strategic roles, and improve overall efficiency [28, 29].

IX. FUTURE PROSPECTIVE

Genetically modified T cell-based adoptive immunotherapies represent a groundbreaking approach to addressing resistant tumors, showing significant promise in specific cancer types. However, their application encounters obstacles such as the complexities of manufacturing, the occurrence of severe adverse events, regulatory hurdles, and high costs. Diverse strategies are being employed to address these

constraints. The implementation of automated and streamlined manufacturing procedures has the potential to impact both the quality of the end product and its cost-effectiveness. The utilization of allogeneic donor T cells holds the promise of increasing availability and enabling the development of readily accessible therapies, thus significantly reducing expenses.

The accurate prediction of potential on-target off-tumor toxicity is of paramount importance, particularly concerning TCR-T cells, given their heightened sensitivity to antigens, especially when utilizing affinity-enhanced TCRs. Innovative preclinical models are essential to anticipate adverse events like Cytokine Release Syndrome (CRS) and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). These models play a critical role in facilitating the development of strategies to mitigate severe effects before advancing to clinical trials. The rapid pace of innovation in this field is evident through the multitude of clinical studies exploring novel approaches to address these challenges.

However, regulating these intricate therapies poses a challenge, emphasizing the need for standardization to ensure patient safety and product comparability. Overcoming resistance is a major hurdle, necessitating personalized combinatorial strategies to target multiple antigens and counter the immunosuppressive tumor microenvironment, applicable to both solid tumors and resistant hematological malignancies. While genetically engineered T cell therapy primarily focuses on cancer, its potential for treating other diseases is being recognized. Initial results of CD19 CAR-T cell therapy in refractory systemic lupus erythematosus patients indicate tolerability and high effectiveness[30]. Exploring its application for autoimmune and genetic diseases holds potential for innovative treatment options in the future.

X. ETHICAL CONSIDERATIONS AND PATIENT PERSPECTIVES

1. Ethical Implications Of CAR-T Cell Therapy

- **Accessibility and Cost-Effectiveness:** CAR-T cell therapy is a sophisticated and pricey medical procedure. Many patients, especially those from low-income families or in developing nations, may not be able to afford this therapy due to the exorbitant expenses. This raises questions about equity and the possibility for inequities in healthcare.

- **Obtaining Informed Consent:** CAR-T cell therapy is a sophisticated procedure that entails altering a patient's own cells genetically. Before giving informed permission, patients and their families must have a thorough understanding of the risks, advantages, and potential long-term repercussions of this therapy. It is essential to ensure effective instruction and treatment knowledge.

- **Patient Selection:** CAR-T cell therapy is now authorized for a limited range of cancers, and patients are chosen in accordance with predetermined standards. The screening approach raises ethical issues because it may disqualify certain individuals who might otherwise match the criteria but could still benefit from the therapy.

- **Long-term Consequences:** CAR-T cell therapy's long-term effects are still being investigated, and there may be unidentified hazards involved with this course of action. It's critical to keep an eye on individuals receiving this therapy to verify their safety and collect information on any potential long-term impacts.

- **Genetic Engineering and Germline Editing:** CAR-T cell therapy includes genetically modifying a patient's T cells. Even though this modification is unique to the patient, there remain worries about the moral ramifications of genetic modification in general and the dangers that could arise from germline editing.

- **Resource distribution:** The high price and restricted availability of CAR-T cell treatment raise concerns regarding resource distribution in healthcare systems. The decision of who receives this specialist therapy may be impacted by variables like financial resources or social position, diverting money from other healthcare sectors.

   While CAR-T cell therapy appears to be a viable alternative for treating some cancers, there are still some ethical issues that need to be carefully considered, including those involving patient selection, informed consent, long-term effects, genetic modification, and resource allocation.

2. **Informed Consent and Shared Decision Making:** CAR-T cell therapy relies heavily on informed consent and group decision-making. Patients and their families must have a thorough understanding of the operation, potential advantages, and potential adverse effects before making an informed choice given the intricacy and potential hazards associated with this treatment.

   In order for patients to provide their consent to a therapy, they must be given complete and unbiased information about it, including information about its goals, potential drawbacks, advantages, and alternatives as well as the likely results. So that they can make a choice that is consistent with their values and interests, patients are better able to comprehend the ramifications of CAR-T cell therapy. The time and assistance patients need to ask questions, get clarification, and talk about concerns should be provided by healthcare personnel.

   In a procedure known as "shared decision making," the patient and the healthcare professional work together to determine the course of treatment. The complexity of CAR-T cell therapy and its potential to affect the patient's quality of life make collaborative decision making particularly crucial in this situation. Healthcare professionals should tell patients about all available options, explain them to them, and involve them in the decision-making process.

   In addition to respecting patients' autonomy and their right to make decisions about their own healthcare, shared decision making recognizes the significance of patient autonomy. It acknowledges that each patient has particular beliefs, preferences, and goals and that their involvement is important in choosing the best course of action.

   In CAR-T cell treatment, shared decision-making and informed consent are not just confined to the patient. The patient's family or carers may also be involved in the decision-making process since they can offer support and aid in understanding the

treatment's intricacies.

Informed consent and shared decision-making are crucial in CAR-T cell therapy in order to protect patient autonomy, guarantee comprehension of the procedure, and advance a patient-centered approach to treatment. Healthcare professionals can guarantee that the patient's values, preferences, and goals are taken into consideration when making decisions about their care by actively involving patients in the process. This will ultimately result in more individualized and efficient care.

3. **Patient Experiences and Quality of Life Considerations:** Patient experiences and considerations for quality of life are pivotal in CAR-T cell treatment. Although this treatment offers a potential cure for certain cancers, it presents challenges and potential drawbacks that can impact a patient's overall well-being. Patients undergoing CAR-T cell therapy may encounter various mental and emotional side effects, including infections, neurotoxicity, cytokine release syndrome (CRS), and long-term effects of genetic alterations. The severity and duration of these effects can vary, necessitating close monitoring, therapeutic interventions, and supportive care. The physical symptoms such as fatigue, pain, and difficulties with daily activities can significantly impair a patient's functionality and daily life. Emotional and psychological consequences such as depression, anxiety, and fear of recurrence can also have a profound negative impact on a patient's health.

Addressing these concerns and providing comprehensive supportive care throughout the CAR-T cell therapy journey is essential. Multidisciplinary teams comprising oncologists, nurses, psychologists, social workers, and other professionals who offer both medical and psychosocial support can be instrumental. Involving patients in shared decision-making and providing them with accurate, understandable information about the treatment can help manage their expectations and anxieties. Open and consistent communication between patients and healthcare professionals is crucial to addressing any concerns, offering timely assistance, and ensuring that patients feel valued and engaged in their care. Long-term monitoring and survivorship care are also crucial factors to consider. As CAR-T cell therapy is relatively new, there is much to learn about its long-term effects on quality of life. Regular monitoring, screening for late effects, and delivering appropriate survivorship care are vital to optimizing long-term outcomes and quality of life for CAR-T cell therapy recipients.

In conclusion, prioritizing patient experiences and quality of life considerations in CAR-T cell therapy is paramount. Healthcare professionals can enhance the well-being of patients undergoing this transformative treatment by addressing their physical, emotional, and psychosocial needs, providing comprehensive supportive care, involving them in decision-making, and ensuring ongoing follow-up care.

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