Systematic Review of CAR T-Cell Therapy for Cancer Treatment in Leukemia and Beyond: Applications, Limitations, and Promise

Alexandre Gervaud

Abstract

Chimeric antigen receptor (CAR) T-cell therapy is a new and groundbreaking immunotherapy that has advanced treatment for hematologic cancers like leukemia and has the potential to shape the future of cancer research. CAR T-cell therapy involves the extraction of patient's T-cells, their genetical modification to include the chimeric antigen receptors recognizing the targeted cancer cells, their reproduction, and their reinjection into the patient. Applying this type of therapy has been especially impactful for leukemia, a prevalent and virulent cancer. A systematic literature review was conducted using targeted PubMed searches to synthesize peer-reviewed studies on CAR T-cell therapy, encompassing biological, translational, and clinical advances. This literature review explores the origins, molecular design, and mechanisms of action of CAR T-cells, detailing their evolution across multiple generations of engineering. The review also examines the therapy's clinical efficacy on both solid and hematological malignancies, associated toxicities such as the cytokine release syndrome and the immune effector cell-associated neurotoxicity syndrome, and the current limitations of its solid tumor application. Additionally, considerations pertaining to cost, accessibility, and regulatory frameworks are discussed. Finally, this review highlights the future of CAR T-cell therapy, including allogeneic therapies and optimizations of autologous treatments. Although this therapy is primarily effective against blood cancers, it represents a consequential opportunity for improvement in varied cancer therapies. Further insights into this field will not only deepen our understanding of immunotherapy; they will also underscore the potential for innovative treatment developments to recognize and combat other types of lethal malignancies.

Keywords

CAR T-cell therapy, immunotherapy, leukemia, hematologic cancer, solid malignancy, therapeutic development, treatment efficacy, clinical application, systematic review

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1. Introduction

Cancer is the second greatest worldwide cause of death, following cardiovascular complications. [1] One form thereof is leukemia, a blood cancer that arises from the uncontrolled production of dysfunctioning myeloid or lymphoid blood cells produced in the bone marrow, which freely circulate in the bloodstream rather than forming solid tumors. This overproduction is caused by mutation and reduces space for healthy and functioning thrombocytes, erythrocytes, and lymphocytes to function, reducing the affected body's ability to supply oxygen to tissues and fight infections properly, and causing symptoms such as fatigue, fever, and unexplained weight loss. [2] The affected cells are characterized by the innate capacity to migrate into and invade other regions of the body, being connected with blood circulation. [3] Several types of leukemias are categorized based on the rapidity of their progression and the type of cells impacted, as acute or chronic, and myelogenous or lymphocytic, respectively. [2]

According to estimations, in 2025, there will be 66,890 new cases of leukemia in the United States. [4] Leukemia is predicted to account for 3.3% of novel cancer cases in the United States, placing it as the eleventh most commonly diagnosed cancer in the country. [4] Nationally, it is estimated that 23,540 persons will decease in 2025 due to leukemia, although the annual leukemic death rate has decreased throughout the last decades. [4] Globally, leukemia accounted for approximately 2.5% of new cancer cases and 3.1% of cancer deaths in 2020, reflecting its continued worldwide impact, despite temporal and regional differences. [5]

Current treatments against this blood cancer include chemotherapy, radiation therapy, targeted therapy, stem cell transplant, and chimeric antigen receptor T-cell therapy. [6] While chemotherapy is most widely used to kill or neutralize cancer cells through intake or injection of drugs [7], it can lead to substantial side effects such as nausea, bleeding, hair loss, and fatigue. [8] In addition, this method can increase the risk of developing secondary cancers such as acute lymphoblastic leukemia, and hamper the growth of non-cancerous cells, implying varying costs, depending on the type and length of treatment. [9] Similarly, radiation therapy—employing energy beams, X-rays, or brachytherapy (solid implants)—can inadvertently harm healthy cells near the cancerous ones and is often expensive. [10] Targeted therapy utilizes drugs or monoclonal antibodies to limit the growth of and eradicate cancer, coming with side effects such as fatigue, skin dryness, and high blood pressure. Cancer cells may even develop resistance to these treatments. [11] Finally, stem cell transplants aim to rehabilitate the patient's capacity to produce ordinary stem cells after damaging radiation therapy or chemotherapy. However, it can cause bleeding, exhaustion, muscle weakness, organ damage, and other complications such as graft-versus-host disease (GVHD). [12]

Applying chimeric antigen receptor (CAR) T-cell therapy for leukemia offers a more targeted approach with significant efficacy and reduced toxicity compared to other treatments. Furthermore, CAR T-cell therapy is capable of combating relapsed and refractory leukemia cases, when other common treatments fail to work effectively. [13] Leukemia is a favorable target due to the accessibility of circulating blood cells and its

overexpression of tumor-specific antigens such as CD19. CAR T-cells constitute a type of immunotherapy, an emerging spectrum of treatments entailing the control and modification of a patient's immune cells to combat certain antigens or malignant cells. [14] CARs are engineered proteins that target specific antigens on malignant cells, engendering an immune response against the latter. [15] Their conception necessitates the isolation of T-cells extracted from the patient's blood, the engineering of CARs specifically adapted to the antigens present of the patient's cancerous cells, and the reinjection of the multiplicated T-cells into the patient. [13] Propitious clinical trials on hematological diseases are auspicious for further application of the therapy in cancer management. [13,16]

This literature review provides a comprehensive analysis of CAR T-cell therapy, a trailblazing advancement in the preeminent field of cancer treatment, delving into the historical formation and underlying mechanisms of this innovative therapy, its transformative impact on leukemia patients, and its clinical and public health applications in non-leukemic contexts—including solid cancers and other blood cancers such as lymphoma and multiple myeloma. It endeavors to address salient questions regarding the manner whereby this original cellular immunotherapy has evolved, the identification of barriers currently impeding its broader clinical integration—particularly in solid tumors—and the selection of strategic improvements that may most effectively overcome these limitations. In accordance, a targeted, organized, methodical examination of preponderant peer-reviewed literature publications, retrieved from leading medical databases, was employed. By compiling and synthesizing a breadth of seminal studies, unveiling recent evidence, this review clarifies the promise and current constraints of CAR T-cell therapy, thereby assisting in the guidance of future scientific and biomedical engineering efforts on the most promising avenues for enhancing efficacy and expanding applicability. In doing so, it underscores the significance of CAR T-cell therapy as a pivotal step forward in oncology and as a foundation for future innovations in immunotherapy.

2. Methods

A literature search was conducted using the PubMed database in December 2024 to identify relevant studies addressing diverse facets of CAR T-cell therapy, including but not limited to preclinical and clinical applications, leukemia versus solid tumor efficacy, receptor designs, structural and mechanistic insights, toxicities, historical milestones, costs, and emerging innovations. Multiple keyword combinations were employed—including terms such as "CAR T-cell therapy," "CAR-T," "chimeric antigen receptor," "clinical trial," "toxicity," "leukemia," "solid tumor," "cost," "allogeneic," and "dual CAR"—in conjunction with Boolean operators (mainly AND & OR) in order to refine the search and ensure coverage of distinct precise subtopics related to CAR T-cell therapy. Filters regarding keyword field location (e.g., in articles' "Title/Abstract") and publication date range (primarily 2015–2025) were applied to prioritize recent, relevant, and impactful studies. Additionally, titles and abstracts were systematically reviewed to assess each publication's thematic alignment with the research objectives, based on the presence of desirable keywords; when relevance remained uncertain, full

text components were examined to identify discussions of specific topics of interest—such as CAR structure, clinical outcomes, toxicities, molecular targets, or advanced therapies—before inclusion, especially the methods and results sections in original research or sections titled with CAR- or cancer-related terms in literature reviews. Furthermore, authoritative medical websites—including the American Cancer Society—were consulted in the introductory section to provide clinically grounded definitions, summarize current standard treatments for leukemia, and present nationally reported epidemiological data, thereby offering background and contextualization to complement the peer-reviewed literature.

Given the breadth of available literature, a representative subset of sources was selected to balance disciplinary comprehensiveness with analytical depth, thereby sufficiently capturing the context, limitations, and advances of CAR T-cell therapy in a single review paper. In fact, 3500 search results were initially identified, and 350 were ultimately incorporated herein, approximately. Studies and reviews were included if they directly addressed CAR T-cell therapy's design, mechanism, preclinical or clinical outcomes, safety implications, economic considerations, or innovative variations—such as CAR NK, CAR macrophages, and off-the-shelf CARs. Both clinical and preclinical research articles, as well as narrative and systematic reviews, were considered to capture a broad perspective on therapeutic development, application, and limitations. Publication dates primarily spanned the last decade to reflect recent advances, though seminal earlier works describing the evolution of CAR composition were also included for historical context and progress evaluation; studies focusing exclusively on unrelated immunotherapies or non-CAR adoptive cell transfer methods were generally excluded. Exclusion criteria also eliminated papers unrelated to hematologic or solid malignancies, as well as non–peer-reviewed material.

During the source selection process, for each publication, key details—such as authorship, title, DOI, publication date, study type (e.g., clinical trial, preclinical study, or review), and principal findings—were systematically recorded to permit a structured and traceable synthesis process. The study designs, patient populations or experimental models, target antigens, therapeutic outcomes, observed toxicities, and discussions of significance were extracted from the selected original research studies. On the other hand, reviews contained information about historical advancement of cancer therapeutic strategies, the conception and success of CAR T-cell therapies, and their clinical implementation. Overall, introduction sections contributed definitions and context; methods and results provided quantitative data and detailed findings; and discussions and conclusions captured scientific interpretations and comprehensive implications.

Extracted data were organized thematically to reflect the principal domains of CAR T-cell therapy covered in this review: mechanism of action and structure, clinical applications and outcomes, toxicity profiles, economic considerations, and innovative approaches. Different findings were explained to present different aspects of CAR T-cell therapy, draw appropriate connections in the context of cancer therapy, and highlight evolving strategies that aim to mitigate encountered limitations. Figures were referenced

to precisely illustrate historical milestones, structural elements, molecular processes, applications, and limitations of the therapy.

Although no formal risk-of-bias tool or standardized appraisal checklist (e.g., PRISMA) was employed, the review prioritized peer-reviewed articles from recognized, reputable journals, applied publication date filters to focus on recent advances, and excluded lower-quality or editorial sources to preserve scientific credibility. In the PubMed database, filters that were utilized to favor scholarly, fact-based research included those that enable the consideration of the “Clinical Trial,” “Randomized Controlled Trial,” “Observational Study,” “Journal Article,” “Review,” “Systematic Review,” and “Meta-Analysis” article types.

3. Mechanism of Therapy

The mechanism of CAR T-cell therapy encompasses the conceptual development, molecular structure, and clinical procedure underlying this form of adoptive immunotherapy, which evolved since its first conception.

3.1 Development

The development of CAR T-cell therapy has evolved through progressive refinements in immunoengineering, culminating in clinically viable therapeutic platforms.

3.1.1 Precursor Therapies

Over the past century, immunological strategies have been progressively harnessed for the treatment of malignant neoplasms, encompassing modalities such as tumor vaccines, tumor-infiltrating lymphocytes (TILs), immune checkpoint inhibitors, monoclonal antibodies, bispecific antibodies, cytokine-induced killer (CIK) cells, and, more recently, CAR T-cell therapy. [17] This advancement finds its origins in foundational work aimed at engineering hybrid—chimeric—receptors, marking a pivotal moment in the evolution of cancer immunotherapy.

The development of the predecessors of CAR T-cell therapy emerged in the 21st century, involving therapies such as imatinib (Gleevec) and trastuzumab (Herceptin)—targeted treatments that, like CAR T-cells, specifically attack cancer cells by recognizing unique molecular markers. [18] In fact, imatinib is a tyrosine kinase inhibitor that targets the BCR-ABL fusion protein, blocking tumorous aberrant signaling, while trastuzumab is a monoclonal antibody that binds to the human epidermal growth factor receptor-2 (HER2) receptor, inhibiting proliferation in cancers displaying it and facilitating immune-mediated cell destruction. [18] Immunotherapy innovations over the past decade, reinvigorating patients’ immune systems and enabling them to target malignant cells, include immune checkpoint inhibitors—drugs blocking checkpoint proteins, which reduce immune responses and potentially prevent T-cell action against tumor cells—and CAR T-cell therapy—which are not currently employed to the same magnitude as the former. [18]

3.1.2 Hematological Research Endorsement

As early as 2016, 92 clinical trials had tested CAR T-cells across the world, in regions ranging from the United States to China, passing by the European Union, and the number of trials continued to escalate thereafter. [19] This expansion of CAR T-cell therapy research is reflected by the approval of six different CAR T-cell therapies by the Food and Drug Administration (FDA) since 2017, especially for the treatment of blood cancers, including lymphomas, leukemia, and multiple myeloma (MM). [18] They offered solutions to "virtually untreatable" conditions, as stated by James Kochenderfer, M.D.; for example, the first FDA-approved CAR T-cell therapy—tisagenlecleucel—allowed 60% of children with relapsed acute lymphoblastic leukemia (ALL) receiving the treatment to survive and remain cancer negative 5 years later. [18] In fact, although more than 80% of children diagnosed with ALL in B-cells can be cured with intensive chemotherapy, treatment against cancer relapse had been limited before the arrival of this new type of therapy. [18]

3.1.3 Solid Tumor Adversities

Moreover, CAR T-cells have been experimented on solid tumors. Nonetheless, differentiating antigens present on the surface of solid cancer cells from those that are on the surface of healthy cells has represented an obstacle to the treatment of solid tumors. [18] In addition, physical and chemical barriers in the body have also hindered previous endeavors, due to the distance of certain bodily tumors from their point of injection and the presence of immunosuppressive molecules produced by malignant or immune cells. [18] Furthermore, tumor heterogeneity—the phenomenon whereby solid tumors do not have exactly the same targetable antigens for every patient—contributes to the complexity of the development of general therapies. [18] Despite these problems, however, advancement in armored CAR T-cells, facilitating navigation in microenvironments by secreting cytokines, CAR-engineering technologies targeting specific surface antigens on cancer cells such as GD2 or B7-H3, show the potential of improvement in the future. [18]

3.1.4 Major Chronological Developments

The historical trajectory of CAR T-cell therapy comprises critical milestones in gene transfer, structural design, and clinical approval that have shaped its current therapeutic relevance and implementation. Historically, Gideon Gross and Zelig Eshhar, along with colleagues including Tova Waks, Guy Gorochov, and Daniel Schindler, are regarded as pioneers of CAR T-cell therapy, having established its foundational principles and demonstrated the genetic redirection of cytotoxic T-lymphocytes to target tumor cells. [14] The evolution of CAR T-cell therapy began in 1989 with the generation of effector T-cells, which eliminate infected or cancer cells, expressing chimeric T-cell receptors. [14] In 1993, the first generation of CAR T-cells was introduced, although it demonstrated limited clinical efficacy. [14]

By 2002, CAR T-cells were being utilized in laboratory experiments targeting prostate cancer. [14] Subsequently, the second generation of CAR T-cells entered clinical

trials against leukemia in 2003, marking a significant milestone. [14] In 2009, CD19 CAR T-cells were employed against refractory leukemia [14], followed by a successful clinical trial in 2011 demonstrating their efficacy in treating chronic lymphoblastic leukemia (CLL), although the therapy's efficacy was lower for CLL than for B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL), due to the exhaustion of the immune system that is concomitant with CLL, known as immunosubversion. [20] By 2013, pediatric acute lymphoblastic leukemia (ALL) was being treated through the utilization of CD19 CAR T-cells, and, during this same year, CAR T-cell therapy was recognized as the "Breakthrough of the Year" by the *Science* magazine. [14]

In 2014, the third generation of CAR T-cells was introduced, incorporating additional characteristic possessions such as the caspase-9 gene system, wherein the iCasp9 suicide gene has been incorporated in T-cells to enable the elimination of maladaptively activated CAR T-cells. [21] The induction of iCasp9 is triggered by the administration of the small molecule dimerizer drug AP1903, leading to dimerization and the rapid initiation of apoptosis in transduced cells (which have been genetically modified), with a preference for killing activated cells that express high levels of the transgene (the introduced gene). [21] The iCasp9 gene has been incorporated into vectors for preclinical studies, demonstrating effective and consistent suicide gene activity in phase I clinical trials. [21] A third-generation CAR containing iCasp9 re-directs T-cells to target the GD2 tumor-associated antigen (TAA), which is overexpressed in melanoma and other cancers of neural crest (originating from the embryonic tissue that gives rise to certain cells in the nervous system and other structures) origin. [21]

The clinical trial of the fourth generation of CAR T-cells against ovarian cancer began in 2015, a year that also featured the conceptualization of CAR natural killer (CAR-NK) cells. [14] Indeed, natural killer (NK) immune cells are capable of identifying and destroying tumorous cells, rendering them ideal for genetic reprogrammation in the context of cell-based cancer immunotherapy. [22] Nevertheless, obstacles remained in gene delivery within these cells, but promising strategies to ameliorate NK cells emerged, including autocrine IL-2 and IL-15 stimulation to improve NK cell persistence *in vivo*, the suppression inhibitory NK cell receptors like NKG2A to improve the precision of the direction of NK cells attack of tumorigenic cells, and the reorientation of NK tumorous cell killing through the implementation of CARs, which was effectuated during the creation of CAR-NK cells. [22]

Significant advancements continued in 2017, with the optimization of CAR placement in T-cells, through the usage of a Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) technology, and the FDA's approval of CAR T-cell therapy for ALL—the first FDA-approved CAR T-cell therapy. [14] CRISPR/Cas9 is a genome-editing tool that uses a single guide RNA to direct the Cas9 enzyme to a target DNA sequence at a PAM site, inducing a double-strand break for precise genetic modifications, promoting advantageous traits in CAR T-cells. [23] In the same year, the FDA further approved a CAR T-cell therapy for treating relapsed diffuse large B-cell lymphoma in adults. [14] In 2018, a prototype of fifth-generation CAR T-cells was developed, incorporating a truncated IL-2 receptor β-chain domain and a STAT3-

binding motif to deliver cytokine-like signaling upon antigen engagement. [24] Finally, in 2019, dual CD19/CD22 CAR T-cell therapy was utilized for treating ALL in both children and adults, solidifying its role in modern cancer treatment. [14] Four CAR T-cell therapies were officially approved by the FDA from 2020 to 2022, for the treatment of various malignancies, such as relapsed or refractory (r/r) mantle cell lymphoma (MCL), B-cell precursor ALL (BCP-ALL), large B-cell lymphoma (LBCL), and multiple myeloma (MM).

Year	Achievement
1989	Generation of effector T-cells expressing chimeric T-cell receptor
1993	Introduction of the first-generation of CAR T-cells, with restrained clinical efficacy
2002	Laboratory utilization of CAR T-cells against prostate cancer
2003	Clinical introduction of second-generation CAR T-cells against leukemia
2009	Utilization of CD19 CAR T-cells against refractory leukemia
2011	Successful clinical trial of CD19 CAR T-cells in patients with CLL
2013	Treatment of pediatric ALL with CD19 CAR T-cell therapy
2013	Recognition of CAR T-cell therapy as “Breakthrough of the year” by *Science*
2014	Introduction of third-generation CAR T-cells, with a caspase-9 gene system
2015	Clinical trial of fourth-generation CAR T-cells against ovarian cancer
2015	Introduction of the concept of CAR-NK cell therapy
2017	Optimization of CAR placement in T-cells by using CRISPR
2017	FDA approval of a CAR T-cell therapy for ALL
2017	FDA approval of a CAR T-cell therapy for relapsed DLBCL
2018	Introduction of fifth-generation CAR T-cells, endowed with cytokine receptor domains
2019	Utilization of dual CD19/CD22 CAR T-cell therapy to treat ALL
2020	FDA approval of a CAR T-cell therapy for r/r MCL and BCP-ALL
2021	FDA approval of a CAR T-cell therapy for r/r LBCL
2021	FDA approval of a CAR T-cell therapy for r/r MM
2022	FDA approval of a CAR T-cell therapy for r/r MM after several prior therapies

Table 1: Timeline of CAR T-Cell Therapy Achievements.

Partially adapted from [14].

3.2 Structure

CARs are modular synthetic receptors composed of four constituents—an antigen-binding domain, a hinge region, a transmembrane domain, and intracellular signaling domains. [25] These components of CARs, listed from the extracellular surface inward, underpin the improved functions of the genetically modified T-cell.

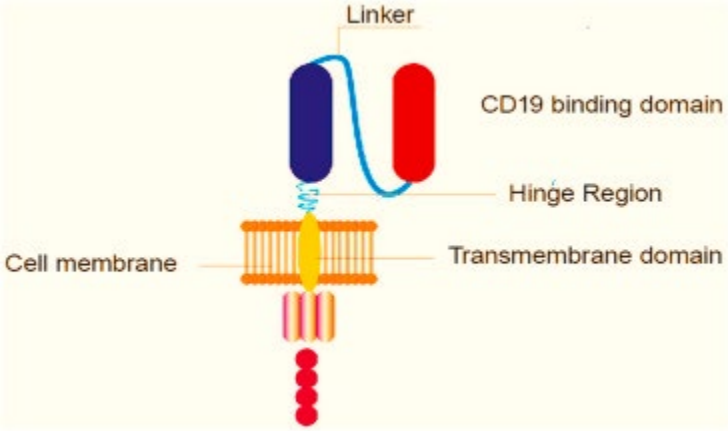

Figure 1: Structure of Chimeric Antigen Receptor (CAR).

Typical CARs are made of a ligand binding domain, linker, hinge region, transmembrane domain, and intracellular signaling/activation domain. CARs recognize surface antigens in a non-MHC manner.

Adapted from [14].

3.2.1 Antigen-Binding Domain

The antigen-binding domain provides target antigen specificity to the CAR. The antigen-binding domain is constituted of a variable heavy (VH) and a variable light (VL) chains of monoclonal antibodies (imitating the immune system's antibodies) connected by a flexible linker, forming a single-chain variable fragment (scFv). [25] The latter target extracellular surface cancer antigens, resulting in major histocompatibility complex-independent T-cell activation. [26] Nonetheless, TCR-like chimeric receptors have been constructed through the junction of the T-cell receptor-mimicking antibody GPA7 with the CD28 and CD3-ζ chain endodomains, which contain the intracellular signaling portion, recognizing successfully intracellular tumorous antigens, with major histocompatibility complex (MHC) dependence. [26] The specificity of the CAR for its target epitope, as well as its affinity, is influenced by the complementarity-determining

regions' positions and the mode of interaction among the VH and VL chains. [27] The binding affinity of the CAR antigens must be sufficiently strong for the recognition of antigens on cancer cells, induction of CAR signaling, and activation of T-cells to occur, without being excessive, else provoking toxicities and activation induced death of the concerned T-cell. [25]

3.2.2 Hinge Region

The hinge region, also called the spacer region, is the extracellular structural region connecting the binding units to the transmembrane domain of the CAR, maintaining flexibility, precluding steric hindrance (and thereby the deceleration of chemical reactions caused by atomic space restriction), affecting epitope recognition, influencing signaling, and providing the necessary length in order to attain the targeted antigen epitope with the paratope of the receptor—without autonomous structural functions. [15,28] An adequate length is crucial in the immunological synapse formation, by establishing intercellular distance; the optimal spacer length must be determined and adapted for every specific antigen-binding domain pair. [29] Short spacer CARs include CD19 and carcinoembryonic antigen (CEA), while long spacer CARs include mucin 1 (MUC1). [25] The most common hinge regions CARs are fabricated with amino acid sequences from CD8, CD28, IgG1, or IgG4. [30] Nevertheless, IgG-derived spacers might engender CAR T-cell depletion and reduced persistence when interacting with Fcγ receptors, although this problem can be solved with engineering structural modifications. [31]

3.2.3 Transmembrane Domain

The transmembrane domain in CAR T-cells is primarily responsible for anchoring the CAR to the T-cell membrane. [32] However, it also plays a role in CAR T-cell function by influencing aspects such as CAR expression, stability, signaling, and synapse formation. [32] Most transmembrane domains are derived from natural proteins, such as CD3ζ, CD4, CD8α, and CD28. [25] The choice of transmembrane domain can impact the CAR's function, with some domains facilitating CAR-mediated T-cell activation, like the CD3ζ transmembrane domain, which promotes CAR dimerization, or pairing, and incorporation into endogenous T-cell receptors (TCRs), already present in the T-cell. [32] This may improve T-cell activation, though it can reduce CAR stability. [25] Conversely, CD28 and CD8α transmembrane domains are often linked with increased CAR stability, but they may not support CAR activation as robustly as CD3ζ. [33] The impact of the transmembrane domain extends to cytokine production and activation-induced cell death (AICD), with variations in domains influencing the levels of cytokines like TNF and IFN-γ, as well as susceptibility to AICD. [34] Overall, the transmembrane domain must be carefully selected to balance CAR T-cell activation, expression, and stability.

3.2.4 Intracellular Signaling Domains

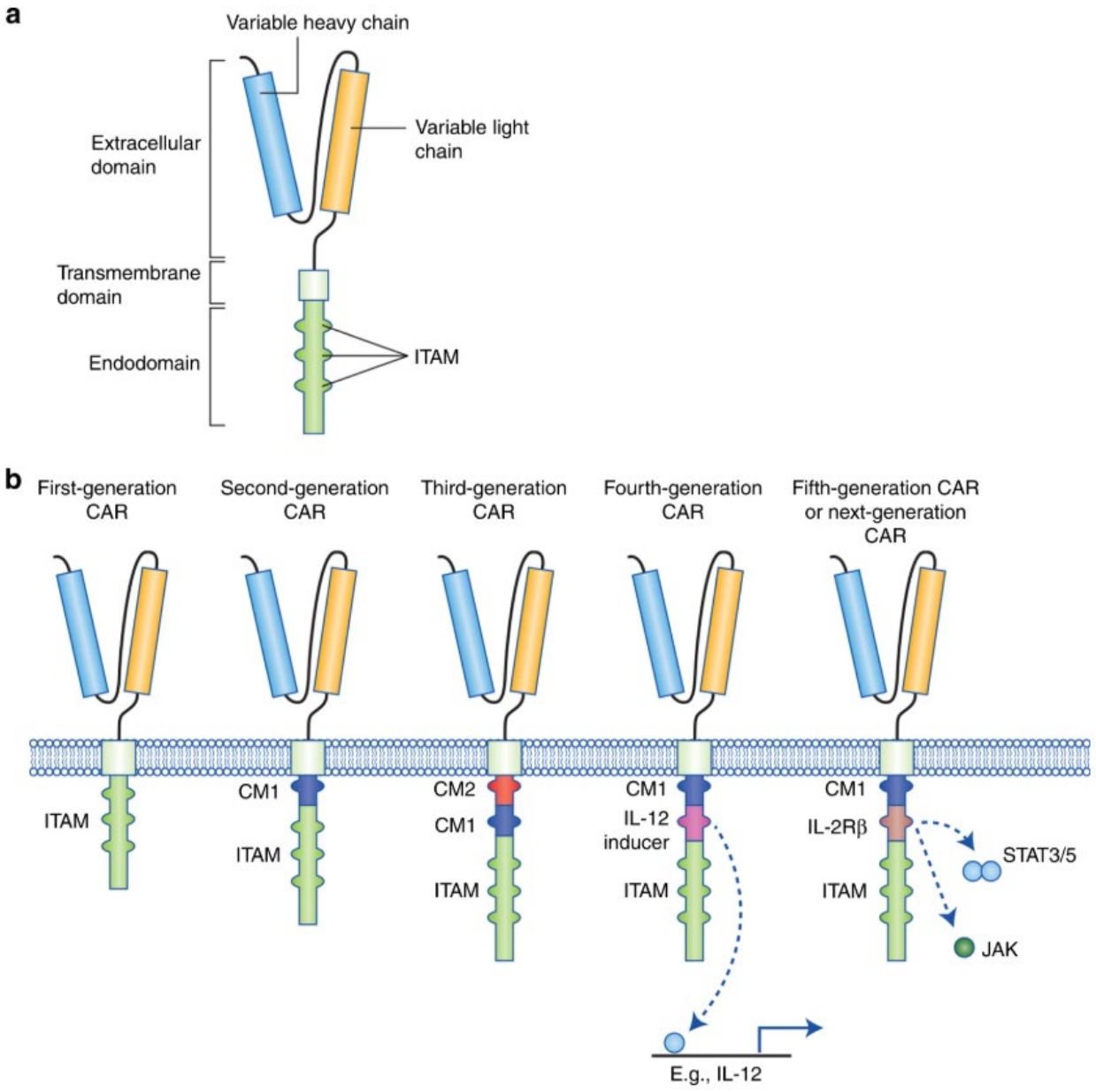

Figure 2: Structure of Different Chimeric Antigen Receptor (CAR) Generations.

a The core structure of a CAR, highlighting the major components of the extracellular domain, the transmembrane domain and the intracellular domain (endodomain). **b** Evolution of the development of CARs from the first generation, which contained only ITAM motifs in the intracellular domain. Second-generation CARs included one costimulatory molecule (CM)1, and third-generation CARs contained a second CM. The fourth generation of CARs was based on second-generation CARs (containing 1–3 ITAMs) paired with a constitutively or inducibly expressed chemokine (e.g., IL-12). These T-cells are also referred to as T-cells redirected for universal cytokine-mediated

killing (TRUCKs). The fifth, or 'next generation,' is also based on the second generation of CARs, with the addition of intracellular domains of cytokine receptors (e.g. IL-2Rβ chain fragment). Abbreviations: ITAM – immunoreceptor tyrosine-based activation motif; CD – costimulatory domain; IL-12 – activation of interleukin-12 transcription; IL-2Rβ – truncated intracellular interleukin-2β-chain receptor with a STAT3/5 binding motif.

Adapted from [35].

The endodomain, composed of one or more intracellular signaling domains, is critical to CAR T-cell activation and function. Early CAR designs relied on the CD3ζ signaling domain, but this alone was insufficient for generating durable T-cell responses, as first-generation CARs lacked effective costimulation and failed to persist over the long-term. [36]

The introduction of a costimulatory domain in second-generation CARs addressed this limitation, with costimulatory domains, like the FDA-approved CD28 and 4-1BB (CD137), that enhance T-cell persistence and proliferation. [37] These costimulatory domains influence the CAR T-cell's metabolic profile, with CD28-based CARs tending to use aerobic glycolysis, while 4-1BB-based CARs rely more on oxidative metabolism, utilizing oxygen to extract energy from carbohydrates; present heightened mitochondrial biogenesis, augmenting the number or mass of mitochondria; and tend to differentiate into central memory T-cells. [37] Second-generation CAR T-cells have been highly effective in treating hematological malignancies (including diffuse large B-cell lymphoma, B-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma), and their use in solid tumors is under investigation. [25] Moreover, alternative costimulatory domains—including ICOS, CD27, MYD88-CD40, and OX40 (CD134)—have shown promising efficacy in preclinical models, although their clinical evaluation remains forthcoming. [25]

Despite this success, the development of third-generation CARs, which incorporate two costimulatory domains, aims to further enhance CAR T-cell activity through complete activation. [38] While preclinical studies have shown promising results in some models (e.g., lymphoma), the efficacy of third-generation CARs has been contentious, with some cancers (e.g., leukemia and pancreatic neoplasm) showing no improvement over second-generation constructs, suggesting that optimal CAR design might require more than one costimulatory domain, although the exact configurations still require further investigation. [25]

Fourth-generation CAR T-cells, also referred to as T-cells redirected for universal cytokine-mediated killing (TRUCKs), represent an advancement derived from second-generation CAR T-cells. [39] In fact, these constructs, building upon the core signaling architecture of second-generation CAR T-cells, incorporate genes that encode pro-inflammatory cytokines—most commonly interleukin-12 (IL-12)—which are either constantly produced at a baseline level (constitutively expressed) or triggered to be produced only in response to antigen recognition by the CAR (inducibly activated upon CAR engagement). [39] Upon antigen recognition, TRUCKs initiate localized cytokine release within the tumor microenvironment, thereby enhancing cytotoxicity not only

through conventional mechanisms such as perforin- and granzyme-mediated lysis or apoptosis via Fas–Fas ligand (Fas–FasL) and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) pathways, but also by recruiting and modulating other immune effector cells—such as macrophages, natural killer cells, and dendritic cells. [39] This multifaceted immune orchestration extends the therapeutic reach of CAR T-cells in solid tumors.

Fifth-generation CARs represent a further refinement of the second-generation design. In addition to incorporating CD3ζ and costimulatory domains (e.g., CD28), these constructs—exemplified by the 28-ΔIL2RB-z(YXXQ) prototype—include a truncated cytoplasmic segment of the IL-2 receptor β-chain—a signaling subunit normally involved in T-cell growth responses—containing a STAT3-binding motif—a site for the transcription factor STAT3, which regulates genes involved in proliferation and survival. [24] Upon antigen-specific activation, this configuration concurrently initiates TCR signaling, co-stimulation, and cytokine-driven JAK–STAT3/5 pathways. [24] JAK–STAT3/5 pathways constitute a signaling cascade in which Janus kinases (JAKs) phosphorylate and activate STAT proteins, leading to the transcription of essential genes in immune cell function. [24] The integrated activation of these three synergistic signals closely mimics the natural sequence of intracellular events required for robust T-cell activation, proliferation, and persistence, offering a more comprehensive and autonomous immune response. [24]

Emerging constructs—including dual CARs, split CARs, and inducible-split CARs—further refine the precision and tunability of adoptively transferred T-cells. For instance, dual CARs co-express two structurally identical antigen-targeting receptors, except for the targeted antigen, enhancing T-cell efficacy through simultaneous recognition of both tumor-associated antigens. [40] In addition, split CARs are engineered receptors in which the costimulatory domain (e.g., CD28 or 4-1BB) and the CD3ζ signaling domain are divided between two separate CAR constructs, necessitating the concurrent recognition of two distinct antigens to achieve full T-cell activation. [41] Moreover, inducible-split CARs require both dual antigen recognition by the separate domains and a controllable external signal to reconstitute full T-cell activation, allowing for fine-tuned and safer immunotherapy. [42]

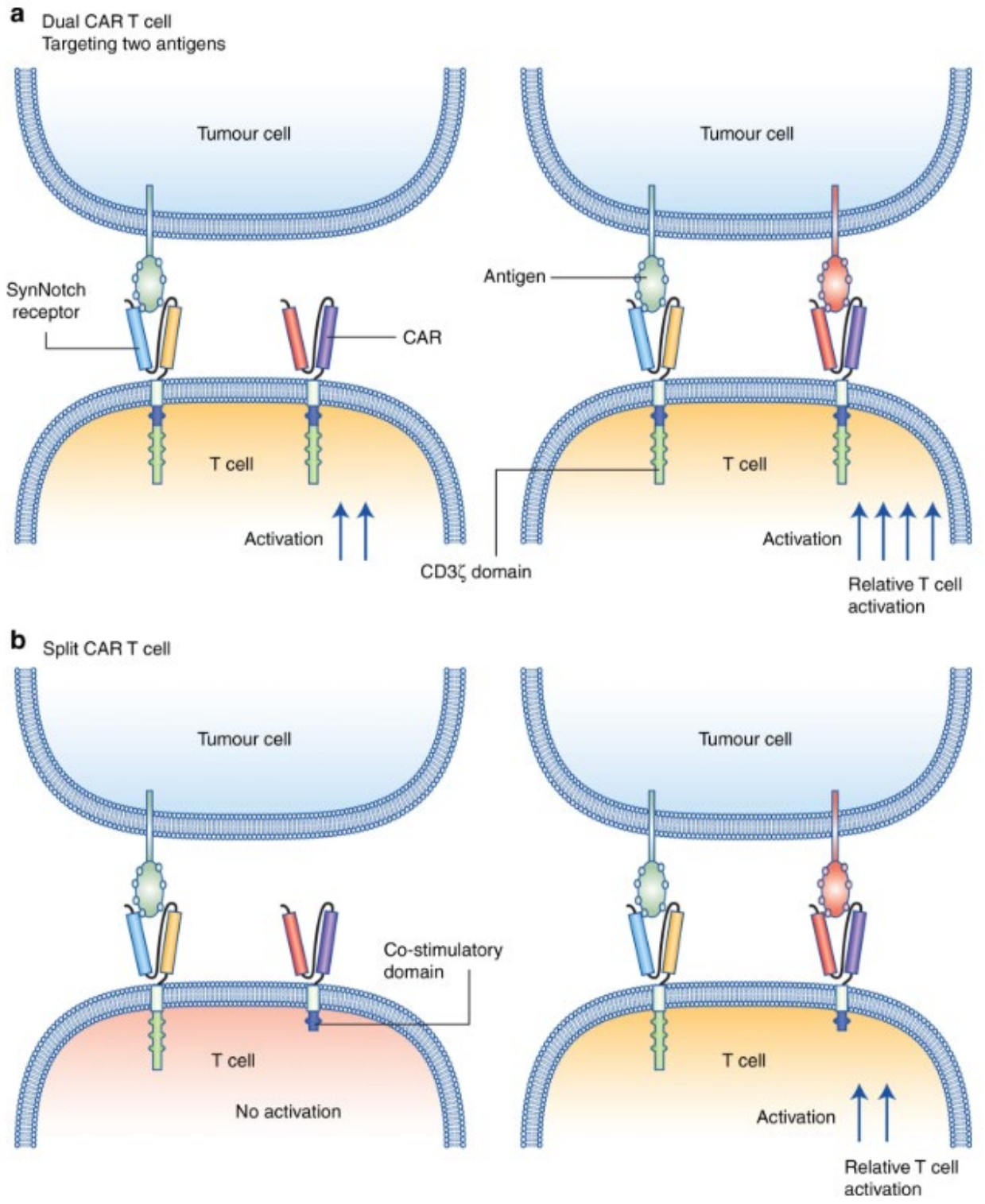

Figure 3: Schematic Representation of Selected Strategies to Enhance CAR T-Cell Recognition and Activation.

Recent strategies to enhance the activation of CAR T-cells include the use of dual CARs, targeting two surface antigens (**a**) and split CARs (**b**).

Adapted from [35].

3.3 Procedure

The clinical administration of CAR T-cell therapy entails a multi-step process centered on the collection, engineering, and reinfusion of the patient's own T-cells. T-cells are constituents of the immune system, functioning by binding to specific antigens on the surface of cells possessing them, allowing them to activate, killing the targeted cancer cell through cytotoxic mechanisms. [43] T-cells have receptors that have a relationship similar to that of a lock-and-key with cell antigens: only one specific TCR can attach to a body's antigen. [43] Foreign or malfunctioning cells such as cancer cells can therefore be destroyed by T-cells displaying the appropriate receptor for their anomalous antigens—a common one whereof is the CD19 antigen. [43]

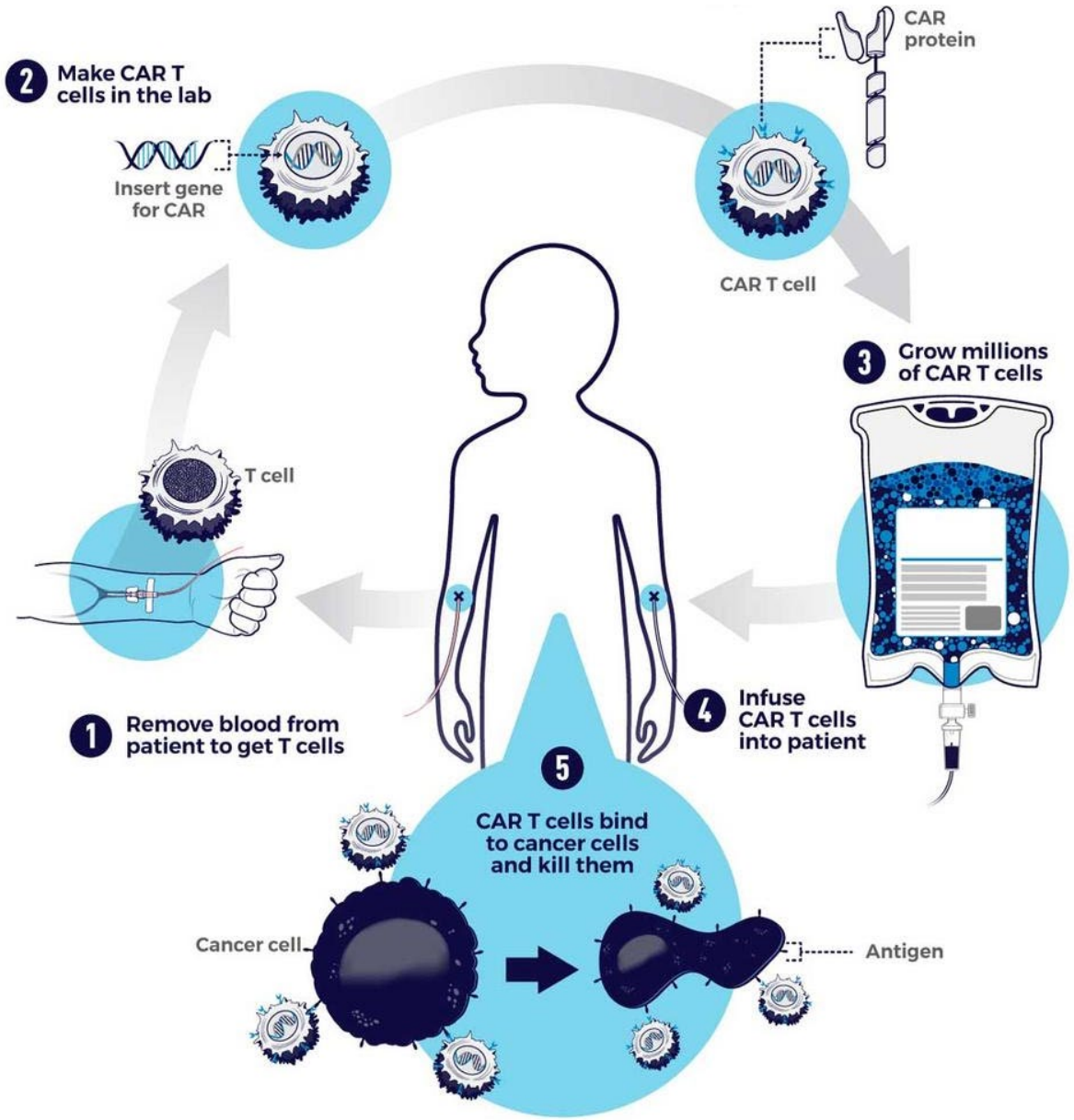

Figure 4: CAR T-Cell Therapy.

CAR T-cell therapy is a type of treatment in which a patient's T-cells are genetically engineered in the laboratory so they will bind to specific proteins (antigens) on cancer cells and kill them. (**1**) A patient's T-cells are removed from their blood. Then, (**2**) the gene for a special receptor called a chimeric antigen receptor (CAR) is inserted into the T-cells in the laboratory. The gene encodes the engineered CAR protein that is expressed on the surface of the patient's T-cells, creating a CAR T-cell. (**3**) Millions of CAR T-cells are grown in the laboratory. (**4**) They are then given to the patient by intravenous infusion (**5**) The CAR T-cells bind to antigens on the cancer cells and kill them.

Adapted from [44].

3.3.1 Leukapheresis

In CAR T-cell therapy, T-cells are collected during the leukapheresis procedure. Two intravenous lines are required; in one line, blood is drawn to obtain its white blood cells, which include T-cells, through centrifugal separation; in the other, unutilized blood is returned to the patient's veins. [43] Subsequently, T-cells are separated and expedited into a laboratory, where the gene coding for the specific CAR of the patient's cancer cells is implemented. [43]

3.3.2 Genetic Modification

After being extracted from the patient and cultured *in vitro*, T-cells are improved to target specific malignant cells, through the stable transduction of a CAR gene. These targets are independent of peptide processing or human leukocyte antigen (HLA) expression, potentially making CARs broadly applicable to diverse patient populations. [45] CARs can be engineered with activation domains and costimulatory components (e.g., CD28, 4-1BB) to enhance T-cell activation, expansion, and persistence. [45] Additional features, such as cytokine secretion or costimulatory ligands, may be incorporated to ameliorate T-cell function and alter the tumor microenvironment (TME). [45]

However, CAR T-cell design has not evinced constancy; it evolved over time. In first-generation cells, an extracellular antigen-binding domain is present (such as an scFv), as well as an intracellular signaling domain (typically the CD3-ζ chain), despite lacking costimulatory signals, which are needed for sustained T-cell expansion, cytokine production (crucial for signaling and immune activation), and long-term persistence. [45] Second-generation CAR T-cells, however, combine the CD3-ζ chain with a costimulatory domain (usually CD28, 4-1BB, OX40, or ICOS), enabling further efficiency in dual-signaling receptors, and the cell in general. [45] Third-generation CAR T-cells combine two costimulatory domains along with the CD3-ζ chain in the cytoplasmic domain, aiming to maximize T-cell activation and persistence in tumor microenvironments, as demonstrated in murine models, although additional clinical trials are necessary, due in part to their increased toxicity and manufacturing complexity. [46] The ability to recognize alternative structures (e.g., carbohydrates), cytokine secretion, chimeric costimulatory receptors appearance, and the coexpression of costimulatory ligands, could further potentiate the antitumor response. [45]

3.3.3 Infusion of CAR T-Cells

Afterward, T-cells with this recombinant genetic material are multiplied, sometimes during several weeks, and injected back into the patient, where they will fight the cancer; receivers might be administered a lymphodepleting chemotherapy regimen before the injection to reduce the presence of other immune cells, which could prevent T-cell action. [43]

3.3.4 Tumor-Associated Antigen Attachment

Once a CAR T-cell is engineered to express the appropriate receptor, it can bind to a specific tumor-associated antigen (TAA), such as epidermal growth factor receptor (EGFR), HER2, or mesothelin. [47] The TAA is a protein or molecule that is either exclusively or overexpressed on the surface of cancer cells compared to normal cells. [47] This binding does not require the antigen to be presented in the context of MHC, allowing CAR T-cells to target a broader range of cancer cells. [48] In effect, CAR T-cells, unlike regular T-cells, engage tumor antigens on the cell surface directly, bypassing antigen processing or the recognition of HLA—a group of human proteins allowing the distinction of autologous cells from foreign ones. [48] This enables recognition of tumors that evade natural immunity through downregulation of HLA or antigen-processing machinery. [47,48] EGFRvIII, a mutated form of the EGFR found in glioblastomas, and HER2, often overexpressed in breast and ovarian cancers, are common targets for CAR T-cell therapy [49,50]; in some cases, mesothelin, a glycoprotein implicated in various cancers like mesothelioma and ovarian cancer, is also a key target. [47]

3.3.5 Intracellular Signaling

Upon recognition of a TAA, the extracellular scFv of the CAR binds to the antigen on the tumor cell surface, which activates the intracellular signaling pathways. [47] The engagement of the CAR with the TAA causes the phosphorylation of immunoreceptor tyrosine-based activation motifs ITAMs—signaling amino acid sequences within immune receptor domains that initiate intracellular activation cascades. [47] This entails the addition of phosphate groups to ITAMs by kinases, the activation of cytokine signaling pathways for immune cell responses in the intracellular domain of the receptor, and the initiation of a cascade of signaling events within the T-cell. [47] This cascade results in the activation of key molecules like nuclear factor kB (NF-kB) and activator protein 1 (AP-1), which promote T-cell activation, survival, and proliferation. [51,52] In response to this activation, the CAR T-cell undergoes clonal expansion, rapidly dividing to generate many copies of itself, ensuring that enough T-cells are present to attack the tumor. [47]

3.3.6 Cytotoxic Response

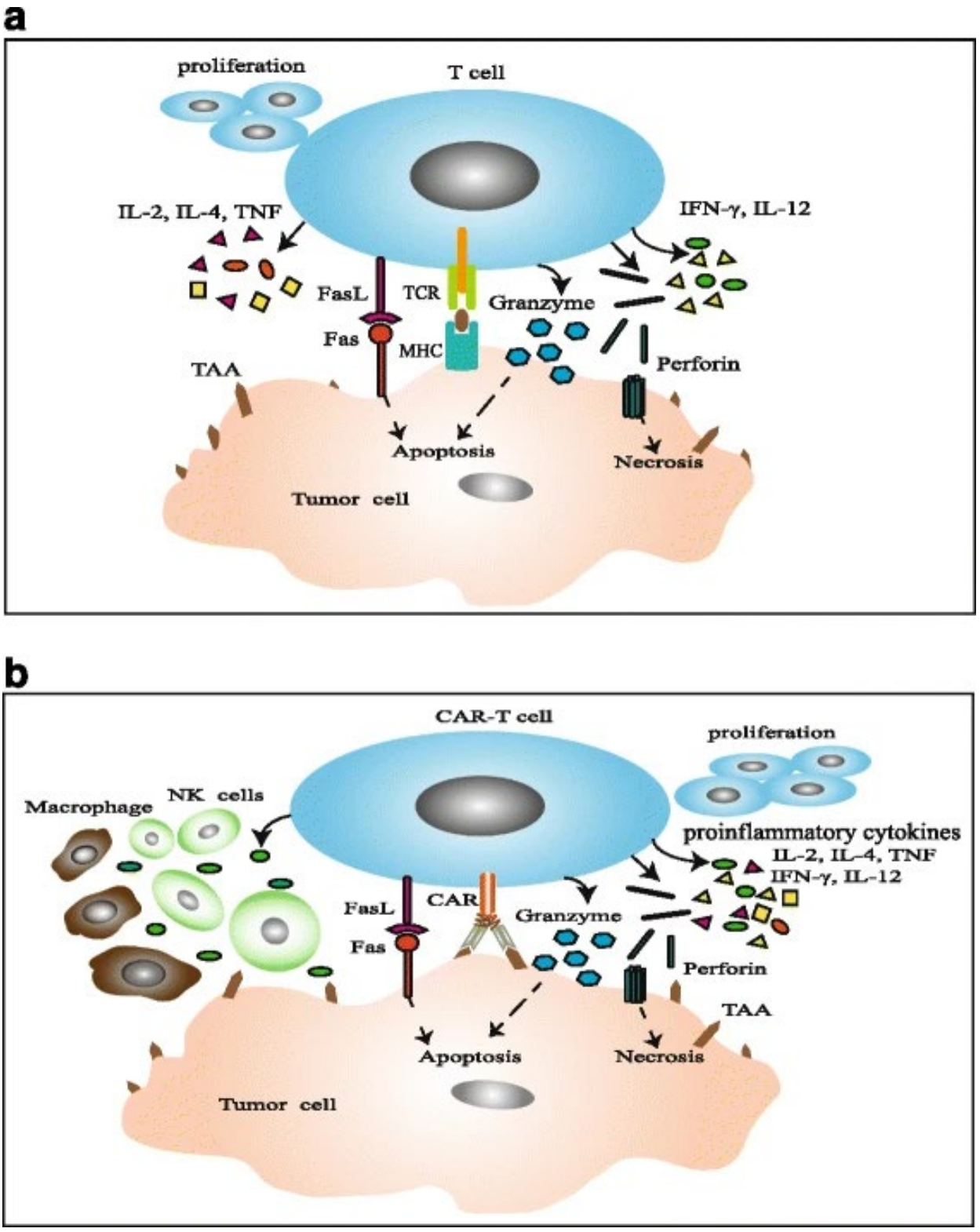

Figure 5: Antitumor Mechanism of CAR T-Cells.

a TCR recognizes TAAs depending on the MHC presentation. The advantage is that TCR could recognize intracellular and extracellular antigens. While tumor cells often downregulate MHC expression to escape the killer T-cells, **b** CAR T-cells can specifically recognize the tumor antigens in a MHC-independent manner. And then, the T-cells were activated through the phosphorylation of ITAMs followed by enhanced cytokine (including IL-2, IL-4, IFN-γ, IL-12, and TNF) secretion, T-cell proliferation, and cytotoxicity. IL-12 could recruit and reinforce the functions of innate immune cells such as NK cells and macrophages. Activated T- and CAR T-cells perform cytotoxicity mainly through secretion of perforin and granzyme granules, and through the death receptor pathway such as Fas/Fas-L. Due to added costimulatory signals to the endodomain, the antitumor activity mediated by CARs is stronger than that of TCRs.

Adapted from [47].

As the CAR T-cells proliferate, they begin to secrete cytokines such as IFN-γ, IL-2, and TNF-α, which play crucial roles in amplifying the immune response. [47] These cytokines not only help in the recruitment and activation of other immune cells (such as macrophages and dendritic cells) to the tumor site but also intensify the cytotoxic activity of the CAR T-cells. [47] The secretion of granzyme and perforin by activated CAR T-cells is another crucial aspect of their mechanism. [53] These molecules induce apoptosis (programmed cell death that eliminates damaged or unneeded cells without causing inflammation, through cell shrinkage, blebbing formation, chromatin condensation, cytoskeleton collapse, nuclear envelope disintegration, and phagocytosis) in the target tumor cells by disrupting their membranes and initiating the intrinsic apoptotic pathway. [47] Additionally, CAR T-cells can engage death receptor pathways (provoking apoptosis) like Fas/FasL and TNF/TNF-R to further instigate tumor cell death. [54]

As a matter of fact, the ability of CAR T-cells to effectively eliminate tumor cells is largely due to their cytotoxic activity, which can manifest through several mechanisms, encompassing two major ones. [55] First, when CAR T-cells are activated, they release perforin, a protein that forms pores in the membrane of the tumor cell, and granzyme, an enzyme that enters the tumor cell through these pores to trigger cell death. [47] Second, the Fas/FasL gene interaction is another pathway by which CAR T-cells kill cancer cells. [53] In this process, the Fas ligand (FasL) expressed on the surface of CAR T-cells attaches to the Fas receptor on tumor cells, leading to a cascade of signals resulting in apoptosis. [47]

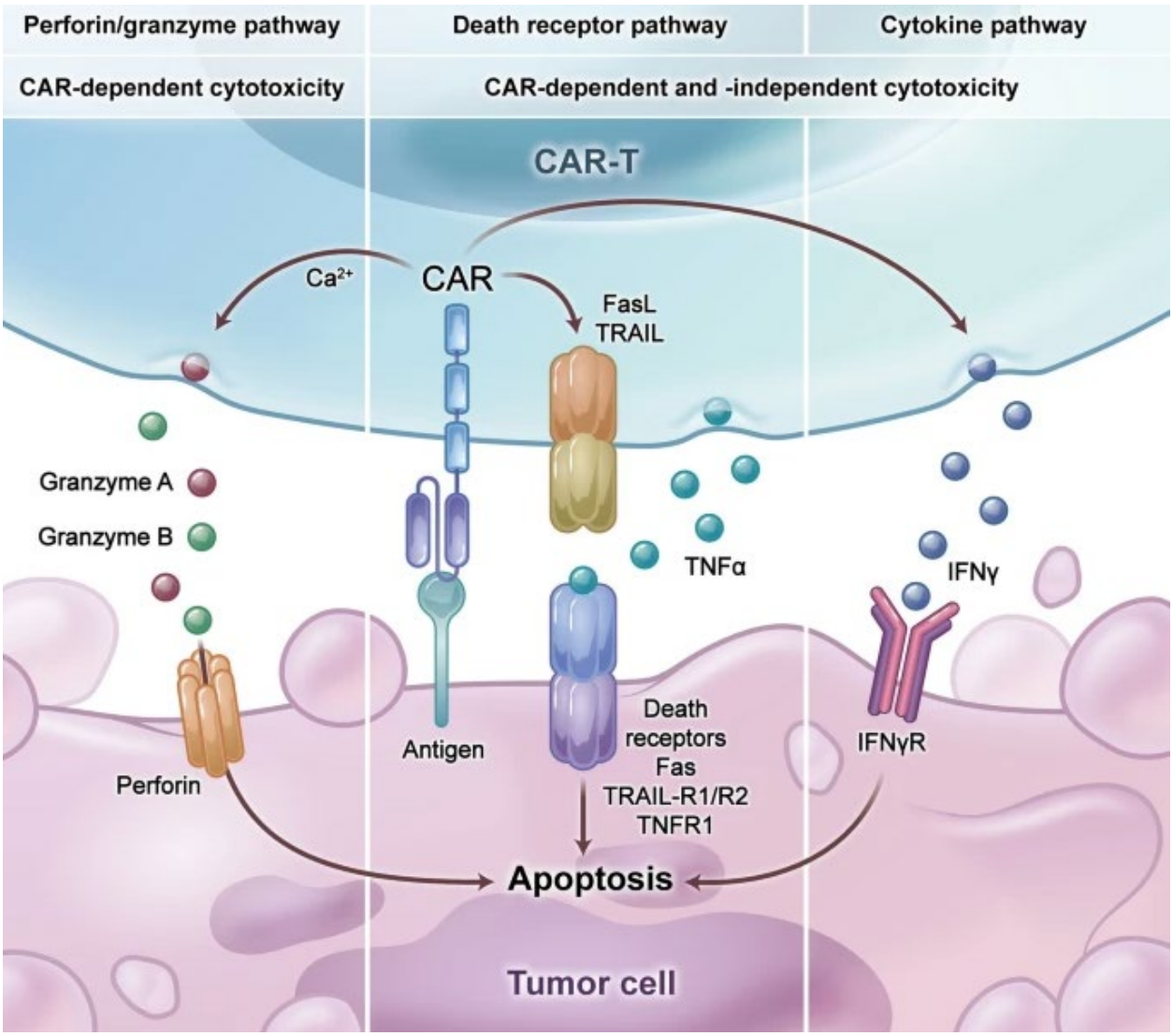

Figure 6: Killing Mechanisms of CAR T-Cells.

Adapted from [56].

3.3.7 Immediate Solid Tumor Limitations

Through the agency of the tumor microenvironment (TME), solid tumors present challenges for CAR T-cell therapy, despite powerful mechanisms of the latter. This environment is typically immunosuppressive, for it can inhibit the effective action of CAR T-cells, embodying one limitation to the treatment's effectuality. Indeed, factors such as hypoxia, tumor-induced cell death, and the presence of immune checkpoints such as PD-1 or CTLA-4 contribute to T-cell exhaustion. [57] In this state, CAR T-cells become less effective when killing tumor cells and may even cease functioning altogether. [57] Consequently, researchers are investigating strategies to block immune checkpoint pathways and to modify the CAR T-cells themselves to render them more resistant to exhaustion. [47]

Recent advancements have focused on improving the trafficking of CAR T-cells to the tumor site and enhancing their persistence within the hostile TME. [47] Approaches such as cytokine modulation, which enhances T-cells' ability to fight the tumor, as well as the usage of bispecific antibodies, like αHER2/CD3, that target both the tumor and immune cells, are under exploration. [58] Additionally, dual-targeting strategies, where CAR T-cells are engineered to target more than one antigen (e.g., GPC3 and ASGR1 in hepatocellular carcinoma), have shown promise in increasing tumor cell recognition and reducing the chance of antigen escape. [59]

3.3.8 Effects of Adoptive Cell Transfer

Overall, as a principal modality within adoptive cell transfer (ACT), CAR T-cell therapy exemplifies the manner whereby genetically engineered lymphocytes can be harnessed to elicit antitumor responses. CAR T-cell therapy is a type of cancer immunotherapy; more specifically, it is one of the subcategories of the technology of adoptive cell transfer (ACT), along with that of tumor-infiltrating lymphocytes (TILs)—which involves the isolation of tumor-specific T-cells from a resected tumor, expansion *in vitro*, and reinfusion for therapy without genetical engineering of T-cells, whereas CAR T-cell therapy entails modification of peripheral blood T-cells to express a TCR that targets a specific cancer antigen. [35] Although TIL therapy offers notable advantages, including a diverse TCR clonality, enhanced tumor-homing capability, and minimal off-target toxicity, making it a promising approach for treating solid tumors, its current clinical application remains restricted to a limited range of tumor types, hindering its more comprehensive implementation. [60]

In immunotherapy, ACT is employed in therapies that entail the collection, *ex vivo* amplification and potential modification, and reinfusion of a patient's T-cells to combat cancer or infected cells. [61] This approach can involve expanding tumor-reactive T-cells, introducing engineered receptors such as CARs, or modulating intracellular signaling to improve T-cell function. [61] ACT aims to improve T-cell efficacy through methods like retroviral transfer of genes, wherein specific genes are introduced into target cells through retroviruses possessing the natural ability to insert their genetic material into the DNA of host cells; downregulation of inhibitory proteins such as Cbl-b; or overexpression of activating molecules such as NKG2D, which strengthens TCR signaling—the process whereby T-cells recognize and respond to antigens. [61] Safety features, such as suicide genes, have been incorporated into genetically modified cells to mitigate potential risks like autoimmunity or oncogenesis. [61] These advancements allow ACT to tailor T-cell responses for maximum therapeutic efficacy and safety in treating diseases such as melanoma, leukemia, and viral infections, fostering progress in CAR T-cell research. [61]

4. Repercussions on Patients

Despite the innovation of CAR T-cell therapy, their application entails certain drawbacks—various toxicities, cardiovascular and pulmonary complications, on-target off-tumor effects, antigen escape, inhibited trafficking and tumor infiltration, and immunosuppressive microenvironment constraints.

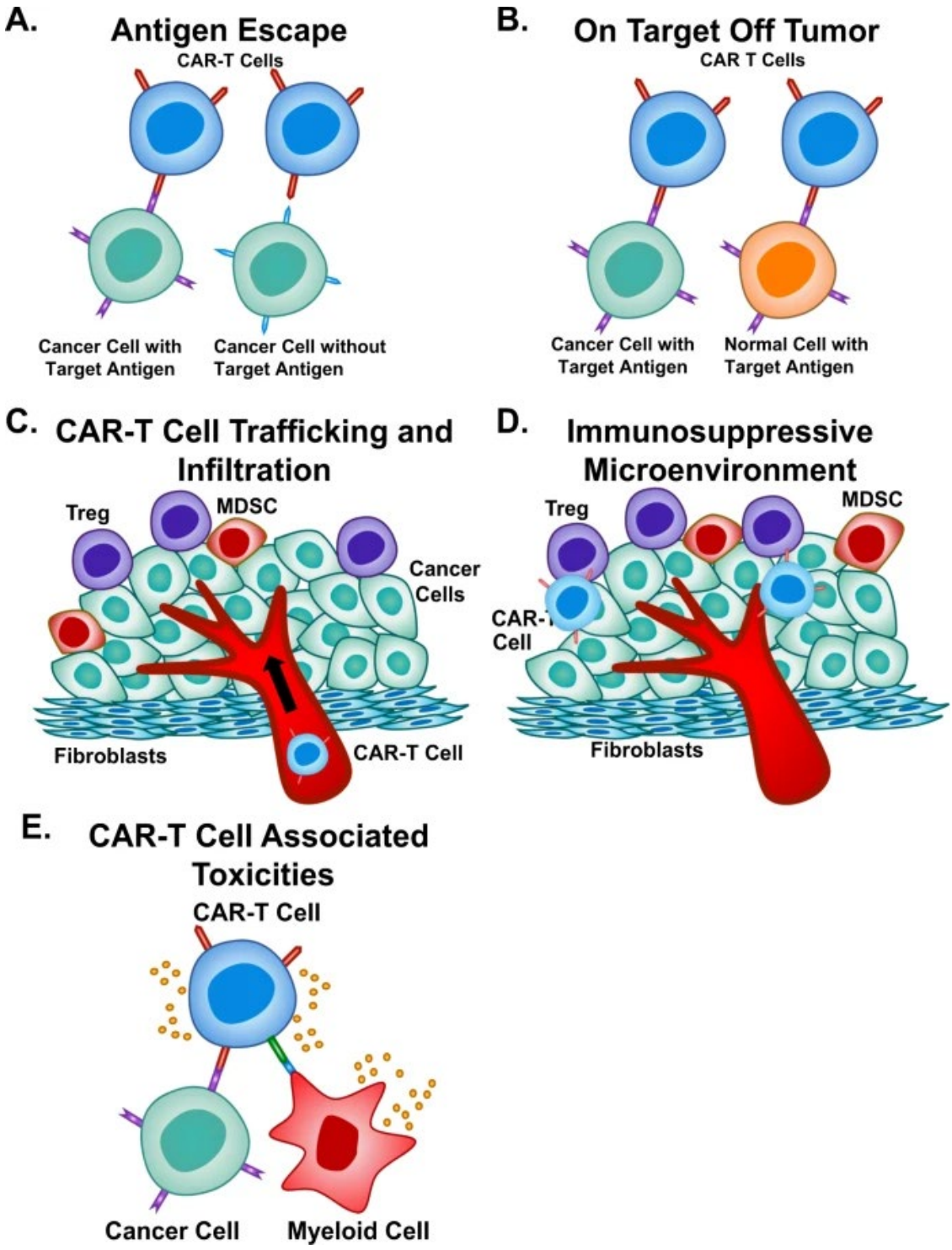

Figure 7: Limitations of CAR T-Cell Therapy.

Current challenges in CAR T-cell therapy include (**A**) antigen escape, (**B**) on-target off-tumor effects, (**C**) trafficking and infiltration of tumors, (**D**) the immunosuppressive tumor microenvironment, and (**E**) CAR T-cell–associated toxicities.

Adapted from [25].

4.1 Associated Toxicities

While CAR T-cell therapy has exhibited burgeoning aptitudes, it comprehends notable side effects, such as cytokine release syndrome (CRS), hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), and immune effector cell-associated neurotoxicity syndrome (ICANS), which are toxicities that are linked to excessive cytokine production and excessive T-cell activation. [25] The manifestations of CRS, for example, may vary in severity, ranging from mild symptoms such as fever and fatigue to more severe manifestations, including hypotension and organ failure. [25] Specifically, the severity of toxicities is graded on a scale ranging from 1 to 4, where 1 symbolizes the minimal gravity of the condition and 4 represents the most critical one before death. [62] IL-6 is a key mediator, promoting inflammation, potentially inducing CRS, and management typically involves IL-6 receptor blockade (e.g., tocilizumab) and corticosteroids, but despite treatment, severe CRS and fatalities still occur. [25] Moreover, HLH/MAS is a hyperinflammatory syndrome characterized by features like elevated ferritin (protein storing iron) and organ dysfunction, which may require chemotherapy, rather than IL-6 inhibitors. [25] In addition, ICANS results in neurological toxicity, which manifests as confusion, encephalopathy, seizures, or cerebral edema, the management whereof focuses on corticosteroids, as IL-6 inhibitors are often ineffective. [25] The incidence of these toxicities varies, with CRS occurring in 77–93% of leukemia patients and severe neurotoxicity in up to 67%. [25] Optimizing CAR design and developing strategies to reduce toxicity are crucial to improving patient safety and expanding clinical applications.

Virtually every patient had some toxicity manifestations after receiving CAR T-cell therapy for acute lymphoblastic leukemia/lymphoma (ALL/LBL), and 23-46% underwent massive *in vivo* T-cell expansion and severe supraphysiologic cytokine production, with abnormally elevated interleukin 6 (IL-6) cytokine levels, manifested by fever and malaise, *inter alia*. [63] This is associated with cytokine-release syndrome (CRS), hemophagocytic lymphohistiocytosis and/or macrophage activation syndrome (HLH/MAS), and ICANS. [25] Such toxicities have especially been recorded in relation to the first FDA approved CAR T-cell therapy, CD19-directed CARs. [25] Furthermore, among patients treated with the CAR T-cell tisagenlecleucel treatment for relapsed or refractory B-ALL, 46% experienced grade 3 or higher cytokine release syndrome (grade ≥3 CRS), while 13–18% of patients receiving axicabtagene ciloleucel and tisagenlecleucel, respectively, for diffuse large B-cell lymphoma (DLBCL), also presented with grade ≥3 CRS. [64] Grade ≥3 adverse events observed during axicabtagene ciloleucel CAR T-Cell therapy included neutropenia (in 78% of patients), anemia (in 43%), and thrombocytopenia (in 38%). [64]

4.1.1 Cytokine Release Syndrome

Cytokine release syndrome (CRS) is the most recurrent toxicity caused by CAR T-cell therapy. [65] CRS is a severe inflammatory condition triggered by the excessive secretion of cytokines, such as IL-6, IL-10, and IFN-γ, into the bloodstream during CAR T-cell therapy, following T-cell engagement with target antigens. [66] It results from the release of cytokines by activated CAR T-cells, stimulating a chain reaction of immune cell activation, particularly T-cells. [65] Symptoms of CRS include high fever, fatigue, joint and muscle pain, low blood pressure, nausea, and neurological effects like seizures and hallucinations. [67] Laboratory findings that confirm CRS generally report elevated levels of nitrogenous compounds, D-dimers, transaminases, and bilirubin—which are associated with health conditions such as impaired kidney function, active clot formation and breakdown, liver cell damage, and dysfunction in hemoglobin breakdown, respectively. [68] If untreated, CRS can be life-threatening, necessitating immediate intervention. [14] Indeed, evidence from studies on patients undergoing haploidentical hematopoietic cell transplantation (haplo-HCT)—wherein stem cells from a donor, following treatments such as radiation therapy or chemotherapy—indicates that severe CRS, though less frequent than mild forms, significantly exacerbates patient outcomes. [69] Among 75 individuals receiving T-cell–replete haplo-HCT, 87% exhibited CRS symptoms, with 12% experiencing severe cases (grades 3 or 4), which was associated with markedly reduced median survival (2.6 months versus 13.1 months in mild cases) and increased transplantation-related mortality, with a hazard ratio (HR) of 4.59. [69] Additionally, these patients faced delayed neutrophil engraftment, further complicating recovery, as well as elevated serum IL-6 levels, which underscore the inflammatory nature of CRS. [69] Treatments include FDA-approved monoclonal antibodies such as Tocilizumab, Sarilumab, and Siltuximab, with corticosteroids also proving effective in clinical trials. [70]

Current management strategies are guided by severity grading. In fact, grade 1 CRS, characterized by fever and mild constitutional symptoms, is managed with supportive care, including antipyretics, reducing fever by acting on the hypothalamus, and fluid administration, alongside infection work-up and empiric antibiotics. [71] Grade 2 CRS, involving mild hypotension or hypoxia, requires intravenous fluids, such as crystalloids and albumin, and low-flow oxygen, delivered through nasal cannula, with careful monitoring of fluid balance due to the risk of capillary leak syndrome—the excessive leakage of plasma and proteins from capillaries into surrounding tissues. [72] While crystalloids are frequently administered to critically ill patients, albumin solutions have been proposed as a more advantageous alternative for individuals with CRS, as they mitigate the risk of capillary leak and pulmonary edema while also improving endothelial function. [73]

Moreover, tocilizumab, an IL-6 receptor antagonist, is recommended for CRS of grade 2 or higher, though its prophylactic use remains unadvised due to its neurotoxicity risk—caused by peripheral IL-6 levels—and potential effects on CAR T-cell proliferation—due to disruption of the cytokine microenvironment. [74–76] For patients with persistent CRS despite tocilizumab, or those at high risk of severe toxicity, corticosteroids such as dexamethasone or methylprednisolone may be administered,

exerting anti-inflammatory and immunosuppressive effects. [77] Grade 3 CRS, marked by hypotension unresponsive to fluids or high oxygen demand, necessitates ICU-level care, vasopressors such as norepinephrine, and corticosteroids. [77] In grade 4 CRS, characterized by life-threatening hypotension requiring mechanical ventilation, intensive management continues, with high-dose methylprednisolone as the preferred corticosteroid. [72,77]

4.1.2 Immune Effector Cell-Associated Neurotoxicity Syndrome

Immune effector cell-associated neurotoxicity syndrome (ICANS) is a neurotoxic complication associated with CAR T-cell therapy, particularly in leukemia and lymphoma patients, wherein incidence rates reach 67% and 62%, respectively. [78] ICANS symptoms often appear within 1 to 3 weeks post-therapy and include dysgraphia (writing problems), tremors, lethargy, and aphasia (speech problems), potentially progressing to seizures, coma, or cerebral edema if untreated. [79] Key cytokines implicated in its pathophysiology include IL-1, IL-6, IL-15, and GM-CSF, which may disrupt the neurovascular unit through interactions with endothelial cells, pericytes, and astrocytes—components of the neurovascular unit (NVU). [79] Microglia—macrophages of the nervous system—and neurons are also affected, contributing to central nervous system (CNS) inflammation. [79] Moreover, while ICANS and CRS can occur independently, they are sometimes correlated. [80] In fact, approximately one-third of patients experience severe toxicities directly linked to the robust activation of immune effector responses triggered by CAR T-cell therapy. [80] Proper management with supportive care and corticosteroids like dexamethasone or methylprednisolone can mitigate symptoms, often resolving them within 1 week. [14,77] Severe cases may still pose significant risks, such as fatal cerebral complications like intracerebral hemorrhage and malignant cerebral edema, heretofore counterbalancing the efficacy of CAR T-cell therapy in B-cell cancers such as non-Hodgkin lymphomas (NHL) and ALL to a certain extent. [81]

The management of ICANS depends on severity, corticosteroids being the first-line therapy thereof, while tocilizumab is ineffective due to poor blood-brain barrier penetration and should only be used when ICANS occurs alongside CRS. [72,82] Grade 1 ICANS is managed with supportive care and close monitoring, with diagnostic testing, including electroencephalography (EEG) and lumbar puncture, to exclude alternative causes such as stroke, malignancy, infection, or hemorrhage. [72,83,84] Furthermore, for grade 2 ICANS, corticosteroids should be considered, and they are required for grade 3 ICANS, the standard treatment whereof consists of dexamethasone 10 mg intravenous injections (IV) every 6-8 hours. [72] On the other hand, high-grade ICANS requires intensive care unit admission, and in grade 4, severe cases, methylprednisolone 1000 mg IV for 3 days is recommended. [72] In cases of cerebral edema, additional interventions such as head elevation, hyperosmolar therapy with mannitol or hypertonic saline, and hyperventilation may be necessary. [85,86] Against seizures, levetiracetam is the preferred agent due to its favorable safety profile, without cytokine level variances, although cases of seizure have been reported despite its utilization. [87] Whereas routine prophylaxis is generally not recommended, active seizures should be managed with benzodiazepines and additional anti-epileptics like levetiracetam or phenobarbital. [77]

4.1.3 Cytopenia

Severe cytopenias (grades ≥3) represent frequent complications following CAR T-cell therapy, with febrile neutropenia of this severity observed in 17% and 31% of patients in the JULIET and ZUMA-1 trials, respectively, dangerously increasing susceptibility to infections. [72,78,88] Among patients receiving CD19-directed CAR T-cells, prolonged cytopenias persisting beyond 30 days occur in approximately 30% of those treated with axi-cel or tisa-cel, often manifesting in a biphasic pattern. [72] In fact, hematologic toxicities such as neutropenia, thrombocytopenia, and anemia affect 94%, 80%, and 51% of patients, respectively. [89] Notably, 93% of cases of hematologic toxicity arise after 21 days post-infusion; late-onset cytopenias more frequently observed in individuals who experienced high-grade CRS or had undergone recent stem cell transplantation. [89] As these events occur after the resolution of CRS and lymphodepleting chemotherapy, disturbances in SDF-1 levels, perturbing hematopoiesis, have been implicated in late neutropenia, possibly linked to B-cell recovery. [89]

4.1.4 B-Cell Aplasia and Hypogammaglobulinemia

As a possible on-target, off-tumor effect of CD19-directed CAR T-cell therapy, B-cell aplasia leads to sustained hypogammaglobulinemia, posing risks of recurrent infections and necessitating long-term immunoglobulin replacement therapy. [90] Cases of prolonged B-cell aplasia extending up to 5 years have been reported in ALL, with longer durations observed in patients receiving CAR T-cells with a 4-1BB costimulatory domain, thereby permitting B-cell levels to serve as pharmacodynamic biomarkers for assessing CAR T-cell persistence. [91] In ALL, the duration of B-cell aplasia correlates with remission duration, whereas in lymphoma, B-cell recovery may occur despite ongoing remission following CAR T-cell therapy. [72]

B-cell aplasia can precipitate hypogammaglobulinemia—which augments the risk of infections due to the decrease in immunoglobulin levels. [92] In pediatric patients, empiric immunoglobulin (Ig) replacement is routinely administered during B-cell aplasia; in contrast, adults may retain humoral immune function through antibody-secreting CD19-negative memory plasma cells, despite undergoing CAR T-cell therapy. [72] Consequently, various Ig replacement strategies have been proposed for adult patients, including prophylactic intravenous immunoglobulin (IVIG) and vaccination, although no standardized approach has been established and further testing is necessitated. [72,93,94]

4.1.5 Infections and Anti-Infective Prophylaxis

CAR T-cell recipients are particularly vulnerable to infections, prompting stringent anti-infective prophylactic strategies. Infections may occur both early and late following CAR T-cell infusion, formal risk factors whereof include ALL as the underlying malignancy, extensive prior chemotherapy (≥4 regimens), baseline absolute neutrophil count (ANC) below 500 cells/mm³, higher doses of infused CAR T-cells, and greater severity of CRS. [95,96] Although bacterial and viral infections are the most frequently observed [97], invasive fungal infections and reactivation of latent DNA viruses such as

cytomegalovirus (CMV), Epstein-Barr virus (EBV), and hepatitis B virus (HBV) have also been reported. [72] Despite the absence of standardized evidence-based guidelines for infection prophylaxis, current recommendations advocate for herpes simplex virus (HSV) and varicella-zoster virus (VZV) prophylaxis, as well as Pneumocystis jirovecii prophylaxis for up to 1 year post-treatment or until $CD4^+$ T-cell counts exceed 200/μl, or even fungal and bacterial prophylaxis for neutropenic patients, given their heightened susceptibility to opportunistic infections, even though the practice is not universally implemented. [72]

4.1.6 Tumor Lysis Syndrome

Tumor lysis syndrome (TLS) constitutes a potential life-threatening complication of CAR T-cell therapy, arising either from lymphodepleting chemotherapy or direct CAR T-cell-mediated destruction of malignant cells, which can precipitate severe outcomes such as fatal arrhythmias and renal failure. [98,99] However, TLS has also been documented in patients who have not undergone prior lymphodepleting chemotherapy. [100] Given the risks associated with high tumor burden, prophylactic measures should be implemented in accordance with standard medical guidelines—including adequate hydration and the administration of hypouricemic agents such as allopurinol, rasburicase, or febuxostat—before initiating lymphodepletion or CAR T-cell infusion, diminishing uric acid levels and the consequent risk of renal failure. [101,102]

4.1.7 Macrophage Activation Syndrome

Hemophagocytic lymphohistiocytosis (HLH), when associated with autoimmunity, is named macrophage activation syndrome (MAS)—a severe systemic inflammatory condition that often engenders tissue damage. [103] Both HLH and MAS have been observed in patients treated with CAR T-cells; notably, patients who meet the criteria for grade 3 cytokine-release syndrome (CRS) often also fulfill the diagnostic criteria for HLH, leading to ambiguity as to whether HLH/MAS represents an extreme manifestation of CRS hyperinflammation or constitutes a distinct toxic entity, despite the fact that separate diagnostic criteria for CAR T-cell-related HLH/MAS have been defined by the American Society for Transplantation and Cellular Therapy (ASTCT). [72,85] The exact incidence of HLH/MAS following CAR T-cell therapy remains unclear, with reports suggesting an occurrence rate of approximately 1%, albeit with substantial underdiagnosis suspicion, given the significant overlap between high-grade CRS and HLH/MAS. [72] In most cases, CAR T-cell-related HLH/MAS resolves with CRS treatments like corticosteroids and tocilizumab. [104,105] For refractory cases, etoposide with intrathecal cytarabine or methotrexate has been suggested, though controversially; similarly, anakinra, an anti-IL-1 therapy, is considered, while remaining unverified. [72]

4.1.8 Genotoxicity and Secondary Malignancies

Although the genetic engineering involved in CAR T-cell therapy raises theoretical concerns regarding DNA mutation possibilities and oncogenic transformation, clinical evidence suggests that such genotoxic events remain rare.

Specifically, even though viral vectors used for CAR T-cell manufacturing pose a risk of insertional mutagenesis (IM), no genotoxicity has been reported in differentiated cells, including T-cells, although IM has yet been observed in hematopoietic stem cells transduced via retroviral and lentiviral vectors. [72] Furthermore, no retrovirus-related transformational events have occurred in over 500 follow-up years of patients treated with engineered T-cells, and neither have replication-competent lentivirus infections. [106,107] However, unintentional transduction of a leukemic B-cell during CD10-targeted CAR T-cell manufacturing led to relapse, progressive leukemia, and eventual death in a patient with ALL. [108] Secondary malignancies, potentially originating from genotoxicity mutations, such as myelodysplastic syndrome, bladder cancer, or non-melanoma skin cancer, have been observed post-CAR T-cell therapy, though prior treatments in this heavily pre-treated population may have contributed. [72]

4.1.9 Infusion Reactions

Infusion reactions are immune-mediated adverse responses that may occur during or shortly after the intravenous administration of certain treatments, such as monoclonal antibodies or T-cell therapies (including CAR T-cell therapy), typically involving symptoms such as fever, chills, hypotension, rash, or respiratory distress. [109] These reactions can be allergic (IgE-mediated) or non-allergic (e.g., cytokine-driven), yet both forms present with similar clinical manifestations and necessitate prompt assessment and management to prevent serious complications. [109]

The FDA recommends at least 4 hours of post-infusion monitoring for early detection of T-cell infusion–related adverse events (AEs), particularly in initial studies of novel biologic agents, although its necessity for well-established T-cell therapies remains uncertain. [110] A retrospective review of 381 *ex vivo*–expanded T-cell infusions across one hundred and eighty patients over 10 years, targeting malignancies or post-transplant viral infections, revealed no grade 3–4 infusion reactions during initial monitoring or within 24 hours post-infusion. [110] Mild AEs (grades 1–2) occurred in 21 infusions, predominantly comprising nausea and vomiting (41.6%), likely due to dimethyl sulfoxide cryoprotectant, as well as hypotension (20.8%), attributed to diphenhydramine premedication. [110] An additional 22 non-severe AEs were reported within 24 hours, most commonly fever, chills, and nausea. [110] Older age was associated with a slightly increased risk of AEs, while patients with allergies had a higher incidence of immediate infusion-related events; sex, disease type, and T-cell source had no significant impact. [110] The study culminated in the conclusion that a 1-hour monitoring period may suffice and that a reduction of diphenhydramine premedication to 0.25 mg/kg could mitigate related AEs. [110] The conditions that T-cell therapies cause contribute to the toxicities that CAR T-cell therapy engenders, the realization whereof fosters the development of symptomatic treatment for CAR T-cell therapies.

4.2 Cardiovascular Conditions

CAR T-cell therapy has been associated with cardiovascular complications in certain cases. For instance, some patients with refractory DLBCL have developed CRS,

leading to acute pericardial effusion—the rapid accumulation of fluid in the membrane surrounding the heart—and cardiac tamponade following CAR T-cell infusion. [111,112] In fact, among fatal cases, CRS was reported in 63%, with overlapping encephalopathy in 54%, while additional contributors included cancer progression (46.8%), sepsis (27.7%), hemorrhage (13.8%), and specific cardiac conditions (15.4%), underlining the significant cardiovascular risks associated with CAR T-cell therapy, which are primarily mediated through CRS. [113]

In one case, a 65-year-old man with refractory DLBCL and a history of dilated cardiomyopathy and transient atrial fibrillation, a temporary irregular heart rhythm, developed grade 1 CRS with hypotension 1 day after CAR T-cell therapy. [111] He was treated with tocilizumab and dexamethasone, a corticosteroid used to reduce inflammation, and transferred to the ICU, where echocardiography revealed pericardial effusion, an accumulation of fluid around the heart, causing cardiac tamponade, a life-threatening condition compressing the heart. [111] Despite severe thrombocytopenia, a critically low platelet count, pericardiocentesis—a fluid drainage procedure—was performed, stabilizing his hemodynamic status, referring to blood circulation. [111] Though initially responding well, the patient died 50 days later from a DLBCL relapse, highlighting the need for vigilance and risk management in patients with pericardial DLBCL undergoing CAR T-cell therapy, especially for complications linked to CAR T-cell expansion. [111]

In another case, a 59-year-old man with refractory diffuse large DLBCL developed grade 2 CRS on the day of CAR T-cell infusion, which progressed to grade 4 by day 7, presenting with fever exceeding 39°C, hypoxia requiring intubation, hypotension treated with vasopressors, and supraventricular tachycardia. [112] Echocardiography revealed significant pericardial effusion, fluid around the heart compressing the right side, though cardiac function remained intact. [112] Due to severe myelosuppression, a condition of decreased bone marrow activity, pericardiocentesis was deemed too risky. [112] Instead, CRS was managed with tocilizumab, an interleukin-6 (IL-6) inhibitor, and high-dose methylprednisolone, a corticosteroid. [112] By day 8, pericardial effusion reduced, hemodynamic stability improved, and CRS did not worsen as steroid doses were tapered; the lymphoma also responded to therapy, with no regrowth observed 3 months post-infusion. [112]

Furthermore, a 42-year-old woman with a history of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and anti-CD19 CAR T-cell therapy for B-cell lymphoblastic lymphoma/acute lymphoblastic leukemia developed a cardiac mass and myocardial infiltration, having previously experienced massive pericardial effusion, which was successfully managed with ultrasound-guided pericardiocentesis. [114] Analysis revealed elevated cytokine levels and an increased copy number of CAR DNA in both the pericardial fluid and serum. [114] Upon discovery of the cardiac mass and myocardial infiltration, treatment with tocilizumab stabilized serum cytokine levels, while reduced-intensity chemotherapy with vindesine, cyclophosphamide, and prednisolone was administered; nonetheless, the patient succumbed to multiple organ failure, underscoring the importance of early detection and prompt treatment of cardiac

involvement in such contexts, as well as cardiac risks connected to CAR T-cell therapy. [114]

Another study highlighted the occurrence of acute cardiovascular events during CAR T-cell infusion in a 76-year-old DLBCL patient, who, while receiving CD19-directed autologous CAR T-cell therapy, experienced coronary vasospasm, suggesting a cause distinct from CRS, such as an anaphylactic response or cardiotoxic effects induced by the cell therapy agent. [115] Furthermore, a study identified cardiovascular events in 12% of 137 CAR T-cell recipients, all associated with grade ≥2 CRS, including six fatalities (heart failure and arrhythmias). [116] Other analyzes, covering 1,921 safety reports for axicabtagene ciloleucel and tisagenlecleucel-T, revealed cardiovascular toxicities in 13.3% of cases (e.g., arrhythmias, pericardial effusion, stress cardiomyopathy, left ventricular dysfunction, and cardiac arrest), with 25.5% of these being fatal. [113]

4.3 Pulmonary Complications

Pulmonary complications have emerged as clinically significant adverse effects of CAR T-cell therapy, as demonstrated by recent clinical investigations. In a phase I dose-escalation study investigating CAR T-cell therapy targeting mesothelin (MSLN), a cell surface protein involved in cell adhesion and overexpressed in various solid tumors, two cases of severe pulmonary toxicity were reported in the high-dose cohort. [117] Both patients developed progressive hypoxemia within 48 hours of infusion, accompanied by clinical and laboratory evidence consistent with CRS, and one case progressed to fatal grade 5 respiratory failure. [117] Autopsy findings revealed acute lung injury with extensive T-cell infiltration and CAR T-cell accumulation in the lungs, and further analysis confirmed low-level MSLN expression on pulmonary pneumocytes—specialized lung cells responsible for gas exchange—and not pleural mesothelial cells, which line the lung's outer surface and pleural cavity, implicating pneumocytes as contributors to dose-limiting toxicity. [117] These findings highlight the potential for off-target effects of CAR T-cell therapy, wherein the targeting of mesothelin inadvertently impacts normal pulmonary cells, leading to severe respiratory complications.

4.4 On-Target Off-Tumor Effects

The variability of tumor antigens and the possibility of metastasis complicate CAR T-cell therapy by making precise targeting difficult. [14] CAR T-cells targeting solid tumors face the challenge of the concurrent presence of cancer-associated antigens on normal tissues, leading to "on-target off-tumor" toxicity; careful antigen selection is therefore critical to balance therapeutic efficacy and toxicity minimization. [25]

One promising approach involves targeting tumor-restricted post-translational modifications, such as overexpressed truncated O-glycans, incomplete sugar molecules attached to specific amino acids, e.g., Tn and sialyl-Tn antigens; in fact, investigated targets include TAG72, B7-H3, MUC1, and MUC16. [25] Although early-generation TAG72-targeted CAR T-cells showed no efficacy in colorectal cancer, second-generation CAR T-cells targeting tumor-specific modifications are under development to

reduce antigen escape while optimizing antigen selection to improve antitumor efficacy and limit toxicity, especially for solid tumors. [25] Dual CAR systems, selectively targeting tumor cells that express shared antigens, can reduce off-target effects while maintaining anti-tumor activity. [14]

Another approach consists in the implementation of iCARs (inhibitory CARs) are engineered receptors that recognize antigens on healthy cells and deliver inhibitory signals through domains like PD-1 or CTLA-4, suppressing T-cell activation even when the activating CAR (with CD3ζ and CD28 or 4-1BB) binds its tumor antigen, thereby reducing possibilities of on-target off-tumor effects. This is an example of a NOT-gate circuit system. [118]

4.5 Antigen Escape

Antigens are molecules—typically proteins or polysaccharides—that can be recognized by the immune system, triggering immune responses, as their presentation on the surface of pathogens and abnormal cells permits the binding of specific immune cell receptors, which are synthetically present on CAR T-cells with the design of targeting and eliminating cancerous cells. Antigen escape is a significant limitation in CAR T-cell therapy, wherein tumor cells evade immune destruction by losing the expression of the targeted antigen. [25] Indeed, despite initial elevated response rates, many patients experience partial or complete loss of the antigen, leading to relapse. For example, while 70–90% of relapsed/refractory (r/r) ALL patients initially respond to CD19-targeted CAR T-cell therapy, 30–70% of relapsed cases present CD19 downregulation or loss. [25] Similar patterns are observed in multiple myeloma (MM)—arising from plasma cell malignancy in the bone marrow—with B-cell maturation antigen (BCMA)-targeted CAR T-cells and in solid tumors, such as glioblastoma (brain cancer originating from the glial neuronal cells), where the IL13Ra2 antigen expression diminishes during recurrence. [25]

To mitigate this issue, strategies focusing on targeting multiple antigens have been developed. These include dual CAR constructs (targeting two antigens with separate CARs) and tandem CARs (a single CAR with two scFvs targeting multiple antigens). [25] Preliminary clinical trials of CD19/CD22 or CD19/BCMA-targeted CAR T-cells have shown promising results in prolonging remission in hematological cancers. [25] For example, CD19/CD22 CAR T-cell therapy has been effective in treating ALL and DLBCL, while BCMA/CD19 CARs have demonstrated high efficacy and safety in MM. [25] Preclinical models of solid tumors, such as HER2/IL13Ra2-targeted CARs in glioblastoma and HER2/MUC1-targeted CARs in breast cancer, also show enhanced antitumor activity and reduced antigen escape compared to single-antigen therapies. [25]

4.6 Inhibition of Trafficking and Tumor Infiltration

In solid tumors, the efficacy CAR T-cell therapy is impeded by limited infiltration and poor trafficking, characterized by hampered movement from the bloodstream to the tumor site, due to physical barriers like the tumor stroma and the immunosuppressive

microenvironment. [25] In effect, tumors create dense stromal barriers using fibroblast and myeloid cells, permitting their proliferation and obstructing effective CAR T-cell penetration and tumor necrosis. [119] Strategies to overcome these obstacles include localized delivery methods, such as intraventricular or intrapleural injections, which enhance CAR T-cell efficacy by directly targeting the tumor and reducing off-tumor effects. [25] For instance, intraventricular injection of CAR T-cells targeting receptor proteins HER2 and IL13Ra2 has shown promising preclinical results in glioblastoma and brain metastases, leading to ongoing clinical trials; similarly, intrapleural injection has shown efficacy in treating malignant pleural mesothelioma. [25] Additionally, oncolytic viruses such as adenovirus and lentivirus have been employed to infect tumor cells, replicate, and lyse them, before provoking the liberation of tumor-associated antigens (TAA) that CAR T-cells can target, thereby enhancing the latter's cytolytic effects. [14]

Furthermore, limited infiltration of CAR T-cells into solid tumors is partly attributed to the absence of appropriate chemokine-chemokine receptor interactions. [19] To overcome this limitation, CAR T-cells have been genetically modified to coexpress chemokine receptors such as CXCR2 or CCR4, thereby enhancing their ability to migrate into tumor tissues. [120,121] Chemokine receptors enable this migration by recognizing and responding to specific chemokine gradients within the TME, guiding T-cells toward tumor sites. [120] Moreover, recent preclinical data indicate that macrophages residing outside the TME critically regulate T-cell infiltration, particularly in pancreatic cancer models. [122] This observation suggests that strategies aimed at modulating the activity of both intratumoral and extratumoral macrophages may further facilitate T-cell access to tumor sites, ultimately improving the clinical efficacy of CAR T-cell therapy in solid tumors.

Similarly, CAR T-cells can be engineered to express chemokine receptors like CXCR1, which respond to tumor-derived chemokines (signaling molecules recruiting immunosuppressive cells), enhancing trafficking and antitumor activity. [25] The principal component having to be degraded in the stroma, composed of the extracellular matrix, is heparin sulfate proteoglycan (HSPG); consequently, in order to address physical barriers, CAR T-cells have been modified to express enzymes like heparanase, which degrades extracellular matrix components, improving tumor penetration and impeding cancer progression. [25] Targeting tumor-associated fibroblasts (forming the extracellular matrix and collagen) with fibroblast activation protein (FAP)-specific CAR T-cells also enhances tumor infiltration and cytotoxicity. [25]

Heparanase (HPSE), the sole mammalian enzyme capable of cleaving heparan sulfate chains within the extracellular matrix, facilitates tissue remodeling by releasing matrix-bound growth factors and cytokines. [123] Through both enzymatic degradation and non-enzymatic signaling roles, it contributes to processes such as angiogenesis, inflammation, immune cell trafficking, and metastasis. [123] Notably, its overexpression is a common feature in cancers, correlating with tumor progression and poor prognosis by promoting multiple cancer hallmarks; this has positioned heparanase as a target for anti-cancer therapies aimed at modifying the TME, as demonstrated in previous studies, despite some emerging evidence suggesting that heparanase may also support anti-tumor immunity. [123]

While tumor-mediated immunosuppression plays a role in the limitation of the achievements of CAR T-cells, functional alterations arising from *ex vivo* expansion also contribute to the impaired ability of cultured CAR T-cells to infiltrate these tumors. [124] Specifically, *in vitro*-expanded CAR T-cells exhibit downregulated HPSE mRNA expression, probably due to TP53 binding at the HPSE gene promoter, which engenders a loss of heparanase enzyme production. [124] For heparanase degrades heparan sulfate proteoglycans, major constituents of the extracellular matrix (ECM), its absence diminishes CAR T-cells' capacity to remodel the ECM and penetrate the tumor stroma. [124] Genetic engineering to restore HPSE expression in CAR T-cells has been found to significantly enhance their ECM-degrading activity, promoting improved tumor infiltration and antitumor efficacy within solid tumors characterized by dense stromal barriers. [124]

4.7 Immunosuppressive Microenvironment

The tumor microenvironment (TME), the network surrounding and supporting tumorigenic cells, often contains immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T-cells (Tregs), as well as inhibitory molecules like PD-1/PD-L1 and CTLA-4. [25] These factors suppress CAR T-cell function, causing poor T-cell expansion, exhaustion, and reduced persistence. [25]

Combination therapies, such as CAR T-cells with immune checkpoint inhibitors, aim to overcome these limitations. For instance, combining PD-1 blockade with CD19 CAR T-cells in heavily pretreated ALL patients improved T-cell persistence and outcomes. [25] In solid tumors, combining CAR T-cells with checkpoint inhibitors, which stimulate immune reactions against cancer cells, has shown promising results, as exemplified by the 72% response rate in mesothelioma patients treated with mesothelin-targeted CAR T-cells and anti-PD-1 therapy. [25] Engineering CAR T-cells to resist immunosuppressive signals is another promising strategy. For example, CARs have been designed to resist TGF-β-mediated immune inhibition or secrete immunostimulatory cytokines such as IL-12 and IL-15, enhancing survival, proliferation, and antitumor activity. [25] Nonetheless, further research is needed to optimize combination strategies and develop innovative approaches to overcome the TME's inhibitory effects, as this problem persists.

One strategy to increase the therapeutic response consists in counteracting the suppressive effects of transforming growth factor-beta (TGFβ), a cytokine abundantly produced by stromal cells within solid tumors. [19] TGFβ promotes the differentiation and activity of regulatory T-cells, thereby inhibiting effector T-cell responses, in conjunction with IL10 and indoleamine-2,3-dioxygenase. [125] To neutralize this inhibitory signaling, CAR T-cells have been engineered to express a dominant-negative TGFβ receptor II (DNRII), which renders them resistant to TGFβ-mediated suppression. [126,127] Preclinical investigations have demonstrated that T-cells modified with this receptor exhibit superior survival, augmented functionality, and improved antitumor activity compared to unmodified T-cells, suggesting the therapeutic value of this approach. [126,127]

Moreover, tumor cells frequently express programmed death-ligand 1 (PD-L1), which, upon engagement with programmed cell death protein 1 (PD-1) on T-cells, induces T-cell exhaustion and attenuates cytotoxic activity. [128] Given that conventional CAR T-cells are typically limited to recognizing one or two tumor-associated surface antigens, combining CAR T-cell therapy with PD-1/PD-L1 immune checkpoint blockade could be advantageous. [125] Whereas CAR T-cells alone exhibit restricted antigen specificity, checkpoint inhibitors mobilize endogenous T-cells capable of recognizing a wider spectrum of neoantigens derived from tumor-specific mutations. [129] The clinical approval of PD-1/PD-L1 inhibitors by the FDA for various solid tumors underscores the potential of this combinatorial strategy to further potentiate CAR T-cell therapy. [125]

Equipping CAR T-cells with the capacity to secrete IL-12, a proinflammatory cytokine known for its role in enhancing innate and adaptive immune responses, led to the generation of T-cells redirected for universal cytokine-mediated killing (TRUCKs). [130] IL-12 not only amplifies innate immune responses but also suppresses the immunosuppressive functions of regulatory T-cells and myeloid-derived suppressor cells, both of which are commonly enriched in the TME. [131,132] Despite these promising attributes, previous clinical studies using recombinant IL-12 have revealed that excessive IL-12 levels may result in severe toxicities, particularly when endogenous T-cell receptors (TCRs) are concurrently activated during inflammatory episodes. [133] Consequently, this approach requires careful modulation to ensure therapeutic efficacy without inducing deleterious systemic effects.

Additionally, researchers have engineered a “cell reservoir” delivery system designed for localized and sustained therapeutic release. [56] This platform incorporates a biocompatible hydrogel scaffold embedded with CAR T-cells, IL-15–loaded nanoparticles, and platelets conjugated with anti-PD-L1 antibodies. [134] The hydrogel matrix permits controlled, prolonged release of CAR T-cells while simultaneously maintaining their viability and functionality through IL-15–mediated metabolic support. [134] Inflammatory stimuli at the tumor site activate the engineered platelets, which then secrete anti-PD-L1 antibodies, thereby inhibiting checkpoint pathways and enhancing CAR T-cell cytotoxicity to suppress tumor recurrence. [134]

CAR T-cell therapy's efficacy is challenged in the TME due to immunosuppressive elements like immune checkpoints (e.g., PD-1, CTLA-4, TIM-3), suppressive cells, and inhibitory cytokines, hindering CAR T-cell activity. [14] Gene-editing technologies such as CRISPR offer solutions by incapacitating checkpoint proteins (e.g., PD-L1), thereby enhancing CAR T-cell efficacy; additional strategies include targeting protein kinase A, which prevents connections between TCRs and tumor-MHC binding sites, to strengthen TCR-tumor interactions. [14]

5. Advantages of Therapy

CAR T-cell therapy confers numerous therapeutic advantages, including its robust efficacy against hematologic malignancies, its antigen-specific adaptability to a range of solid tumors, and its potential to markedly enhance patients' quality of life.

5.1 Blood Cancer Efficacy

One of the most compelling outcomes of CAR T-cell therapy is its pronounced success in addressing malignancies of hematopoietic origin, setting a precedent for precision-targeted immunotherapies in oncology.

5.1.1 Therapeutic Effectiveness in Leukemia

CAR T-cell therapy has demonstrated remarkable efficacy in treating hematological malignancies, especially leukemias. For example, CAR T-cell therapy exhibited efficacy in acute myeloid leukemia (AML), possessing a complete response rate (with total disease disappearance) of 49.5% and an overall response rate (with significant response) of 65.2%. [135] This proves particularly advantageous in light of the fact that AML is among the most aggressive hematological malignancies in adults, with a 5-year survival rate of only 30.5%. [136] Moreover, cure rates have only ranged from 5-15% in individuals over 60 years of age and 35-40% in those younger than 60. [137] On the other hand, in a study, CAR T-cell therapy was employed as a treatment for B-cell acute lymphoblastic leukemia (B-ALL), demonstrating an impressive combined CR rate of 92%, with a 12-month PFS of 65.0% and an OS of 73.0%. [138] The fruitfulness of the therapy against leukemia was further exemplified when CD22-targeting CAR T-cell therapies for relapsed (returning)/refractory (resistant) B-cell malignancies demonstrated a complete response (CR) rate of 68% in ALL, even in patients previously treated with anti-CD19 CAR T-cells. [139] By comparison, in this study, a lower but still notable CR rate of 64% was observed in non-Hodgkin lymphoma (NHL). [139] Moreover, dual CD19/CD22 CAR T-cells exhibited even greater efficacy, achieving a CR rate of 90% in ALL. [139] These findings eloquently underscore the transformative impact of CAR T-cell therapy on leukemia treatment, as well as its broader promise across hematologic malignancies.

5.1.2 Therapeutic Effectiveness in Lymphoma

Beyond leukemia, CAR T-cell therapy has shown substantial therapeutic benefit against other hematologic malignancies such as lymphomas, notably including B-cell non-Hodgkin lymphoma (B-NHL). In effect, a pooled best overall response rate (ORR) of 77% and a complete response (CR) rate of 52% in patients with B-cell non-Hodgkin lymphoma (B-NHL), alongside a 12-month progression-free survival (PFS) rate of 54.0% and an overall survival (OS) rate of 66.0% have been reported. [138] Moreover, a novel strategy utilizing CRISPR-Cas9 to engineer gene-specific targeted CAR T-cells has shown significant efficacy, achieving high rates of complete remission and durable responses in patients with aggressive B-cell non-Hodgkin lymphoma (B-NHL)—a

subtype of NHL arising from malignant B-lymphocytes. [140] In another investigation, the therapy resulted in a 4-year OS rate of 54.6% in large B-cell lymphoma (LBCL) patients treated with axicabtagene ciloleucel (axi-cel). [141] Moreover, the median PFS in the axi-cel group was notably longer at 14.7 months, compared to 3.7 months in the standard-care cohort, with no additional treatment-related fatalities observed. [141] In fact, with a median follow-up of 47.2 months, axi-cel demonstrated significantly improved overall survival compared to standard care when used as a second-line treatment for patients with early r/r LBCL—a form of B-NHL. [141]

5.1.3 Therapeutic Effectiveness in Myeloma

Furthermore, studies have provided evidence of the potency of CAR T-cell therapy in the treatment of myelomas, the most prominent form whereof is multiple myeloma. For instance, in the context of relapsed or refractory multiple myeloma (r/r MM), a pooled ORR of 77%, a CR rate of 37%, and a notably high pooled survival rate of 87% was observed. [142] Additionally, CAR T-cell therapy has a pooled ORR of 85.2% and a CR rate of 47.0% when used in the context for r/r MM. [143] Furthermore, the minimal residual disease (MRD) negativity rate was reported to be 97.8%, with a pooled incidence of grades 3–4 CRS of 6.6%, while the occurrence of neurotoxicity was 2.2%; the median progression-free survival (PFS) was observed at 14.0 months, with a median overall survival (OS) of 24.0 months. [143] Dual epitope-binding CAR T-cells demonstrated the most favorable therapeutic outcomes, whereas humanized CAR T-cells exhibited the highest safety profile, thereby demonstrating the possibility of balance between efficacy and toxicity control. [143] Similarly, cabtagene autoleucel (cilta-cel)—another CAR T-cell therapy—yielded impressive outcomes, demonstrating a 12-month PFS of 75.9% versus 48.6% in the standard-care group. [144] Additionally, the cilta-cel group exhibited higher rates of overall response, complete remission (CR), and minimal residual disease (MRD) negativity. [140] In a separate study, anti-GPRC5D CAR T-cell therapy achieved an extraordinary overall response rate (ORR) of 91%, including stringent CRs, even in patients who had previously not responded to anti-BCMA CAR T-cell therapies, highlighting the potential of anti-GPRC5D CAR T-cell therapy to serve as an alternative of the anti-BCMA one, advancing the efficacy of such treatments. [145]

5.2 Solid Tumor Potency

CAR T-cell therapies have proven efficient in the eradication of certain leukemic conditions, but solid tumor success has been revealed to constitute a more complex objective, although some success has already been achieved.

In studies involving CAR T-cell therapies for triple-negative breast cancer (TNBC) and melanoma, patients experienced manageable (grades 1 and 2) toxicities, with some resulting in stable disease post-treatment, prompting the conclusion that the intravenous delivery of CAR T-cells, modified via RNA electroporation (a process using electrical pulses to introduce RNA into cells) and designed to target cMET (a receptor often overexpressed in various cancers), is both innocuous and possible. [146] For heavily pretreated children with neuroblastoma undergoing GD2-CART01 therapy, mild CRS

was observed in 74% of the cases, and 95% of those diagnosed with the toxicity experienced mild symptoms. [147] However, the ORR was 63%, with a significant portion achieving complete remission. [147] Among patients receiving the recommended dose, the 3-year OS rate was 60%, and event-free survival (measure of the length of patient remission without the experience of significant setbacks) was 36%, highlighting the potential of GD2-CART01 therapy in treating neuroblastoma—an immature nerve tissue solid tumor. [147]

CAR T-cell therapy afamitresgene autoleucel (afami-cel) showed an ORR of 24% in patients with relapsed/refractory (r/r) metastatic solid tumors, including durable responses in those with synovial sarcoma (a soft tissue cancer). [148] While grade ≥3 hematologic toxicities were omnipresent and CRS was present in 55% of patients (with 90% of those impacted experiencing grade ≤2 CRS), these events were manageable. [148] The therapy demonstrated tumor infiltration, activation of adaptive immune responses, and an acceptable benefit-risk profile, encouraging further investigation in larger trials. [148]

CLDN18.2-targeted CAR T-cells (CT041) were evaluated in digestive system cancers, with the entirety of patients experiencing hematologic toxicity and 94.6% of patients enduring grades 1–2 CRS, although no severe CRS, neurotoxicities, treatment-related deaths, or dose-limiting toxicities occurred. [149] The therapy achieved an ORR of 48.6% and a disease control rate (DCR) of 73.0%, with particularly notable results in gastric cancer patients, who had an ORR of 57.1% and a 6-month OS rate of 81.2%. [149]

In castration-resistant prostate cancer, CAR T-cells equipped with a dominant-negative TGF-β receptor (inhibiting TGF-β signaling, which contributes to the maintenance of an immunosuppressive TME) were tested, meeting safety and feasibility endpoints. [150] 38.5% of patients experienced grade ≥2 CRS, including one death, due to grade 4 CRS with concurrent sepsis (causing widespread inflammation due to an infection), despite marked CAR T-cell expansion and a significant reduction in prostate-specific antigen (PSA). [150] In addition, ≥30% PSA reductions were observed in three of the thirteen cases, although the patients conjointly demonstrated CAR T-cell failure, likely due to the immunosuppressive TME molecules, indicating the potentiality of further refinements in CAR T-cell therapy for solid tumors. [150] AMG 119, the first CAR T-cell therapy targeting Delta-like ligand 3 (DLL3), an inhibitory ligand for the Notch signaling pathway and thereby a catalyst of the evasion of tumorous cells from normal growth regulation, has shown promising results in patients with relapsed/refractory (r/r) small cell lung cancer (SCLC). [151] Demonstrating robust cellular expansion and long-lasting cell persistence, AMG 119 indicates its potential efficacy in treating SCLC; the therapy was well-tolerated at the tested doses, with no dose-limiting toxicities (DLTs) reported, highlighting its favorable safety profile. [151] The encouraging cellular kinetics observed further support the potential for CAR T-cell therapy in the solid tumor space, where no CAR T-cell therapies have yet been approved. [151]

5.3 Target Specificity for Other Cancers

CAR T-cells have the ability to specifically target a variety of antigens that are frequently present or overexpressed on the surface of various types of both hematological and solid cancers, enabling the attack of tumor cells, while reducing normal cell damage. [152] Numerous clinical trials, both ongoing and completed, have explored the use of CAR T-cell therapy for various solid tumors, including glioblastoma, lung cancer, liver cancer, gastric cancer, renal cancer, breast cancer, ovarian cancer, prostate cancer, osteosarcoma, peritoneal carcinomatosis, pleural cancer, central nervous system tumors, neuroblastoma, and lymphoma, in addition to leukemia. [152]

5.3.1 CD19-Targeted CAR T-Cell Therapy for Acute Lymphoblastic Leukemia and Non-Hodgkin Lymphoma

Targeting the CD19 antigen has emerged as a cornerstone of CAR T-cell therapy, demonstrating profound clinical efficacy in treating both acute lymphoblastic leukemia (ALL) and various subtypes of non-Hodgkin lymphoma (NHL), for example. CD19, a transmembrane coreceptor protein consistently expressed on B-cells, amplifies B-cell receptor signaling to promote activation, proliferation, and antibody production, rendering it essential in B-cell malignancy. [153] Tisagenlecleucel, a CD19-directed CAR T-cell therapy, is approved for the treatment of pediatric and young adult patients with r/r ALL and adults with NHL—a category of lymphoid malignancies, arising from cells of the lymphatic system, including B-cells, T-cells, and NK cells. [154] In a real-world analysis based on a cellular therapy registry, data from five hundred and eleven patients demonstrated outcomes consistent with pivotal clinical trials while showcasing improved safety. [154] Among patients with ALL, an initial CR rate of 85.5% was observed, with 12-month rates of duration of response (DOR), event-free survival (EFS), and OS reaching 60.9%, 52.4%, and 77.2%, respectively. [154] In adults with NHL, the best ORR was 61.8%, including an initial CR rate of 39.5%, while 6-month DOR, progression-free survival (PFS), and OS rates were 55.3%, 38.7%, and 70.7%, respectively. [154] Adverse events of grade ≥3 CRS and neurotoxicity occurred in 11.6% and 7.5% of patients, respectively. [154] Comparable efficacy and safety outcomes were documented for patients treated with in-specification (biologics meeting the pre-defined quality control criteria) and out-of-specification products, the latter characterized by cell viability ranging from 61% to 79%. [154]

In a phase II study, tisagenlecleucel demonstrated high remission rates in pediatric and young adult patients with r/r CD19+ B-ALL. [155] The overall remission rate within 3 months was 81%, with all responders negative for MRD, as assessed by flow cytometry. [155] At 6 months, EFS and OS were 73% and 90%, respectively; at 12 months, these rates were 50% and 76%. [155] Tisagenlecleucel persisted in the blood for a maximum duration of 20 months. [155] Although 73% of patients experienced grades 3–4 adverse events, these were mainly transient; CRS was observed in 77% of patients, with 48% receiving tocilizumab for management. [155] Neurologic events occurred in 40%, and were addressed with supportive care, with no reports of cerebral edema. [155] These findings underscore the potential of a single infusion of tisagenlecleucel to achieve

durable remission and long-term persistence, despite transient high-grade toxicities in pediatric and young adult patients with r/r B-cell ALL.

5.3.2 CD22-Targeted CAR T-Cell Therapy for Acute Lymphoblastic Leukemia

CD22 has emerged as a promising alternative antigen for CAR T-cell therapy, particularly in acute lymphoblastic leukemia. The B-cell surface glycoprotein CD22 regulates B-cell receptor signaling by modulating activation thresholds and promoting immune tolerance, instating it as a vital element upon which to direct immune attacks against B-cell leukemia. In a phase I trial, CD22-targeted CAR T-cells were tested in twenty-one patients, including seventeen previously treated with CD19-targeted immunotherapy for r/r pre-B-ALL. [156] The trial demonstrated dose-dependent antileukemic activity, with 73% of patients achieving CR, including all five patients with CD19dim (which downregulated the expression of CD19) or CD19 B-ALL. [156] The median duration of remission was 6 months. [156] Relapses were linked to reduced CD22 site density, allowing CD22+ cells to evade destruction by the CD22 CAR T-cells. [156] These results represent the first clinical evidence of CD22 CAR effectiveness in B-ALL, including cases resistant to anti-CD19 immunotherapy, showing similar potency to CD19 CAR therapy at biologically effective doses. [156]

Furthermore, in a phase I trial of sequential CD19/CD22 CAR T-cell therapy, twenty pediatric patients with relapsed or refractory B-cell acute lymphoblastic leukemia (r/r B-ALL) were enrolled, with 70% having hematologic relapse and 30% having refractory disease. [157] All patients underwent lymphodepleting chemotherapy before receiving CD19 CAR T-cells, followed by CD22 CAR T-cell infusion upon the detection of undetectable CD19 CAR T-cells in peripheral blood. [157] Both CD19 and CD22 CAR T-cells expanded efficiently in peripheral blood, and no significant correlation was found between CAR T-cell expansion, viability, and transduction efficiency. [157] The overall remission rate was 100%, with all patients achieving CR and MRD negativity by day 30 after CD19 CAR T-cell infusion. [157] CRS occurred in 90% of patients, being primarily mild to moderate. [157] Moreover, grade 1 neurotoxicity was observed in three patients, while grade 3 was observed in one. [157]

At the study's endpoint, with no patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT), the 1-year leukemia-free survival (LFS) and OS rates were 79.5% and 92.3%, respectively. [157] Patients with early immunoglobulin recovery (<12 months) had a significantly higher relapse rate, suggesting a correlation between the recovery of this type of antibody and the loss of CAR T-cell activity. [157] Antigen escape was reduced, as only two patients exhibited loss of CD19 expression upon relapse, and CD22 downregulation was seen in one patient. [157] The sequential CAR T-cell strategy, consisting of the injection of two subsequent CAR T-cell therapies that target different antigens, led to improved long-term efficacy compared to single-agent CD19 or CD22 CAR therapies previously administered in the hospital. [157]

Additionally, in a phase I dose-escalation study of a novel murine stem cell virus (MSCV)-CD19/CD22-4-1BB bivalent CAR T-cell, CD19.22.BBζ CAR T-cells exhibited potency in heavily pretreated patients, while eradicating leukemic cells in humanized

mice, further demonstrating the plausibility of co-expression of CD19 and CD22 receptors. [158] Approximately 40-60% of patients with B-NHL treated with CAR T-cell therapy benefitted from either durable remission or survival, and this proportion increased to 80-90% in patients diagnosed with B-ALL, showing the extent of the effectiveness of this technology. [158,159]

5.3.3 BCMA-Targeted CAR T-Cell Therapy for Multiple Myeloma

The B-cell maturation antigen (BCMA), a membrane protein of the TNF receptor superfamily, members whereof regulate gene expression for cell survival and differentiation by binding specific cytokines [160], can be targeted to combat multiple myeloma—a plasma cell malignancy. This is supported by a study that aimed to compare the safety, efficacy, and pharmacokinetics of anti-BCMA CAR T-cell therapy in patients with extramedullary multiple myeloma (EMM) and non-EMM. [161] Published data showed that the objective response rate (ORR) for EMM patients ranged from 57% to 100%, with CR rates between 29% and 60%. [161] Sixty-one subjects (twenty-five with EMM and thirty-six with non-EMM) were treated with anti-BCMA CAR T-cell therapy, and no significant differences were observed in adverse events between the two groups. [161] The median PFS, however, was significantly shorter for EMM patients (121 days) than for non-EMM patients (361 days). [161] Similarly, OS was lower for the EMM group (248 days) than the non-EMM group (1024 days). [161] Additionally, pharmacokinetic analysis revealed lower Cmax and AUC0-28d in the EMM group, indicating lower bodily exposure to CAR T-cells. [161]

Moreover, in a study assessing BCMA-CD38 bispecific CAR T-cell therapy in patients with r/r MM, promising results were observed. CD38 is an enzyme and receptor found on hematopoietic cells, including cells infected with MM, regulating immune cell differentiation, inflammation, and NAD metabolism—a prominent coenzyme in cellular metabolism. [162] CMA-CD38 CAR T-cells exhibited enhanced killing of BCMA+CD38+ cells *in vitro*, surpassing the effectiveness of BCMA-only or CD38-only CAR T-cells. [163] Among the sixteen enrolled patients, 87.5% responded to the treatment, including 13 who achieved stringent complete response (sCR) and 1 who had partial response (PR). [163] The median follow-up period was 11.5 months, during which 76.9% of patients with sCR remained relapse-free. [163] However, three patients relapsed, and four died, with one death attributed to hemophagocytic lymphohistiocytosis syndrome (HLH), which is characterized by excessive activation of the immune system, due to severe CRS. [163] The 1-year PFS rate was 68.8%, and the 1-year OS rate was 75.0%; in addition, extramedullary lesions were eliminated in 62.5% of patients. [163] Common post-infusion symptoms included cytopenia (100%), fever (62.5%), fatigue (50%), and myalgias (50%), and CRS was observed in 75% of patients, with 31.3% experiencing severe CRS (grade ≥3). [163] CAR+ cell expansion correlated with CRS severity, and transient clonal isotype (class of antibody production) switching, occurred after infusion. [163]

5.3.4 EGFR-Targeted CAR T-Cell Therapy for Small-Cell Lung Carcinoma and Glioblastoma

Epidermal growth factor receptor (EGFR), a key regulator in tumor progression, has been targeted in CAR T-cell therapies for various cancers. In a phase I clinical trial involving a CAR T-cell therapy targeting this antigen, no severe toxicity was observed in patients with r/r on-small-cell lung carcinoma (NSCLC), characterized by EGFR overexpression in more than 50% of cancer cells. [164] Among eleven evaluable participants, two exhibited partial responses, and five experienced disease stabilization lasting between 2 and 8 months. [164] Tumor biopsies demonstrated the eradication of EGFR-positive tumor cells following therapy, and CAR-EGFR transgenes were detected in tumor-infiltrating T-cells in all four biopsied cases, confirming the safety and effectiveness of EGFR-targeted CAR T-cell therapy for treating advanced, relapsed, or refractory EGFR-positive NSCLC. [164]

Similarly, a phase I clinical trial evaluated the safety and feasibility of EGFR CAR T-cells generated using the piggyBac transposon system—a alternative CAR transgene introduction method into T-cells, simpler than viral techniques—in nine patients with advanced r/r EGFR-positive NSCLC, illuminating its potential efficacy and safety. [165] The treatment was well tolerated, with fever of grades 1 to 3 representing the most frequent adverse events, and no grade 4 or severe CRS was reported. [165] Post-treatment, eight patients still had detectable EGFR-CAR T-cells in peripheral blood. [165] Clinical outcomes included the achievement of a partial response (PR) lasting over 13 months in one patient, six patients with stable disease, and two with disease progression. The median PFS was 7.13 months, while the median overall survival reached 15.63 months. [165]

Moreover, in a first-in-human study, autologous T-cells engineered with a CAR targeting the epidermal growth factor receptor variant III (EGFRvIII) mutation were intravenously delivered to ten patients with recurrent glioblastoma (GBM). [166] The manufacturing and infusion of these CAR-modified T-cells (CART-EGFRvIII) were found to be feasible and safe, with no signs of off-tumor toxicity or CRS. [166] One patient exhibited stable residual disease for over 18 months. [166] Transient expansion of CART-EGFRvIII cells in peripheral blood was detected in all patients, and tissue-specific analysis from seven patients who underwent post-treatment surgical intervention confirmed trafficking of these cells to GBM tumors. [166] Notably, EGFRvIII expression decreased in five of these cases, while the TME exhibited increased inhibitory molecule expression and infiltration by regulatory T-cells after infusion, highlighting the on-target activity of CART-EGFRvIII cells in the brain but underscore the challenges posed by antigen heterogeneity and adaptive immune resistance in recurrent GBM. [166]

5.3.5 MSLN-Targeted CAR T-Cell Therapy for Mesothelioma, Ovarian Carcinoma, Pancreatic Cancer, Lung Cancer, and Breast Cancer

Mesothelin (MSLN), a tumor-associated antigen that is mostly overexpressed in ovarian cancers and otherwise generally restricted to mesothelial cells [167], has been broadly explored in CAR T-cell trials. [168] A study combining MSLN-targeted CAR T-cells

with checkpoint inhibitors and angiogenesis inhibitors achieved partial remission in an ovarian cancer patient, with survival exceeding 17 months. [169] Specifically, the patient, who had relapsed after multiple lines of chemotherapy, received autologous αPD-1-mesoCAR T-cells engineered to target MSLN and secrete PD-1 antibodies, which prevent the immune suppression of T-cells that occurs at protein 1 receptors. [169] Post-infusion, the modified CAR T-cells demonstrated increased copy numbers and PD-1 antibody secretion in the blood. [169] MRI revealed a significant reduction in the size of metastatic liver nodules, with average diameters narrowing from 71.3 mm to 39.1 mm within 2 months. [169] The therapy, combined with apatinib—which prevents the formation of blood vessels, angiogenesis, that supply oxygen to tumors—to enhance cytotoxic CD8+ T-cell infiltration, resulted in only mild side effects, including grade 1 hypertension and fatigue [169], underscoring the therapeutic potential of αPD-1-mesoCAR T-cell therapy in addressing advanced refractory ovarian cancer, which ranks as the second most frequent global cause of gynecologic cancer-related deaths [170], even within its immunosuppressive and proangiogenic TME.

Moreover, a phase I study assessed the safety and activity of lentiviral-transduced CAR-modified T-cells, introducing the CAR gene via a lentivirus, targeting mesothelin (CART-meso) in patients with chemotherapy-refractory mesothelioma, ovarian carcinoma, and pancreatic cancer, wherein fifteen patients received a single infusion of CART-meso cells, engineered with a construct targeting mesothelin. [171] The therapy was well tolerated, except in one patient, which presented a grade 4 toxicity—sepsis. [171] The best response was stable disease in eleven of the fifteen patients, but although CART-meso cells expanded in the blood, they only showed transient persistence. [171] Despite human anti-chimeric antibodies detection in eighteen of fourteen patients, tumor biopsies revealed CART-meso DNA presence in seven of ten patients, suggesting limited clinical efficacy. [171]

Furthermore, a phase I study of regionally delivered, autologous mesothelin-targeted CAR T-cell therapy in patients with malignant pleural diseases, including metastatic lung and breast cancers and malignant pleural mesothelioma (MPM), demonstrated safety and tolerability, with CAR T-cells detectable in peripheral blood for over 100 days in 39% of twenty-seven patients. [172] In eighteen MPM patients receiving additional pembrolizumab, an immune checkpoint inhibitor that blocks PD-1 proteins, the median OS was 23.9 months, with an 83% 1-year survival rate. [173] Eight patients maintained stable disease for at least 6 months, and two achieved complete metabolic response. [173] These findings support the further evaluation of CAR T-cell therapy combined with PD-1 blockade in solid tumors, forestalling the suppression of immune T-cell activity. [173]

5.3.6 HER2-Targeted CAR T-Cell Therapy for HER2-Positive Sarcomas

Targeting HER2 with CAR T-cells has shown encouraging therapeutic activity, particularly in HER2-overexpressing solid tumors, offering new potential for malignancies traditionally resistant to immunotherapy. A phase I/II clinical trial evaluating CAR T-cell therapy targeting the human epidermal growth factor receptor-2 (HER2), which are proteins allowing cell growth particularly expressed on tumor cells,

demonstrated promising results in nineteen patients with HER2-positive sarcomas, including osteosarcomas, primitive neuroectodermal tumors, and Ewing sarcomas. [174] Indeed, among seventeen evaluable patients, four exhibited stable disease for 3 to 14 months, with three undergoing tumor resection and one achieving ≥90% tumor necrosis; the median OS was 10.3 months, with no severe adverse events reported except for a transient fever in one patient. [174] Similarly, a phase I study on HER2-specific CAR T-cells for glioblastoma revealed favorable tolerance, a median OS of 11.1 months post-treatment, and prolonged survival in three patients without disease progression during the 24–29 months of investigation. [175] Another phase I trial in pancreatic and biliary tract cancers demonstrated a median OS of 4.8 months with mild-to-moderate toxicity, except in two cases, which presented a grade 3 acute febrile syndrome and an abnormal elevation of transaminase. [176] Furthermore, HER2-targeted CAR T-cell therapy in pediatric central nervous system tumors, including diffuse midline gliomas, virulent brain tumors, increased CXCL10 and CCL2 chemokine secretion in cerebrospinal fluid without noted dose-dependent toxicity, suggesting the potential for engineering CAR T-cells with enhanced chemokine receptor expression for improved tumor targeting by the immune system. [177]

5.3.7 CD133-Targeted CAR T-Cell Therapy for Hepatocellular Carcinoma

CD133, a marker of cancer stem cells, has been effectively targeted by CAR T-cell therapy in hepatocellular carcinoma, demonstrating antitumor activity. CD133, also known as Prominin-1, is a pentaspan transmembrane glycoprotein (having five membrane-spanning domains to anchor it) that serves as a key biomarker when isolating cancer stem cells (CSCs), as it is implicated in tumorigenesis, metastasis, chemoresistance, and cellular differentiation. [178] CD133, expressed by cancer stem cells of various epithelial origins, as well as hepatocellular carcinoma (HCC) [179], is an attractive therapeutic target, for the cancer is the second global leading cause of cancer-related deaths. [180] CD133 is also highly expressed in endothelial progenitor cells (EPCs), which circulate in increased numbers in the blood of patients with highly vascularized cancers, contributing to angiogenesis and vasculogenesis of HCC. [181] A phase II study evaluated CD133-targeted CAR T-cells (CART-133) in 21 adults with advanced HCC. [182] One patient achieved PR, 14 showed stable disease for 2 to 16 months, and 6 had disease progression. [182] The most common high-grade adverse event was hyperbilirubinemia, which is associated with impaired liver function. [182] The study found that changes in EPC counts, vascular endothelial growth factor (VEGF), soluble VEGF receptor 2 (sVEGFR2), stromal cell-derived factor 1 (SDF-1), and interferon γ (IFN-γ) after infusion were associated with survival, since they are key regulators of angiogenesis, immune response, and tumor microenvironment dynamics, playing critical roles in promoting or modulating tumor growth and vascularization. [182] CART-133 therapy showed promising antitumor activity with a manageable safety profile, and the potential of these markers as predictive biomarkers might be beneficial in the future. [182]

5.3.8 Claudin 18.2-Targeted CAR T-Cell Therapy for Metastatic Gastric and Pancreatic Adenocarcinoma

Claudin 18.2 has been successfully targeted by CAR T-cell therapies, offering promising clinical outcomes in metastatic gastric and pancreatic adenocarcinomas. Claudin 18.2 is a tetraspan tight junction protein isoform, aberrantly exposed in gastric malignancies, that belongs to the family of claudins—transmembrane proteins that regulate epithelial barrier function and paracellular permeability (regarding molecular passage between these cells). [183] Claudin 18.2, a variant of Claudin-18 specific to the stomach, is present in 70% of primary gastric adenocarcinomas and their metastatic sites. [184] A phase I trial, concerning twelve patients with metastatic gastric or pancreatic adenocarcinoma, demonstrated proper toleration of the treatment with no severe neurotoxicity, treatment-related deaths, or grade 4 adverse events, except for temporary decreases in lymphocytes, neutrophils, and white blood cells; cytokine release syndromes were mild (grades 1–2). [185] Among eleven evaluable patients, one achieved complete remission (gastric adenocarcinoma), three had partial responses (two gastric, one pancreatic adenocarcinoma), five had stable disease, and two experienced disease progression. [185] The overall objective response rate was 33.3%, with a median PFS of 130 days, supporting conclusions that the therapy is a safe and potentially effective treatment option for advanced gastric and pancreatic adenocarcinomas. [185]

5.3.9 IL-13Rα2-Targeted CAR T-Cell Therapy for Glioblastoma

CAR T-cell therapy directed against IL-13Rα2 has demonstrated potential in glioblastoma, exploiting the receptor's selective tumor expression to enhance specificity and minimize off-tumor effects. Interleukin 13 receptor alpha 2 (IL13Rα2) is a high-affinity receptor for interleukin 13 (IL-13) that mediates IL-13 signaling, which is linked to tumorigenic signaling cascades; its overexpression is frequently associated with invasion, metastasis, and poor prognosis in malignancies such as glioblastoma, colorectal, and breast cancer. [186] IL-13 receptor alpha 2 (IL13Rα2), a receptor overexpressed in glioblastoma but rarely present in normal brain cells, has shown potential as a therapeutic target in a study wherein repeated infusions of IL-13Rα2-targeted CAR T-cells, induced complete tumor regression for approximately 8 months in a patient with disseminated glioblastoma, as well as augmentations in cytokine and cerebrospinal fluid immune cell levels, without toxicity above grade 2. [187] Additionally, another clinical trial, with CAR T-cells endowed with a CAR expressing IL13(E13Y)-zetakine, whereby a receptor IL-13Rα2 enhances tumor targeting and alters immunosuppressive cytokines, involving three patients with recurrent glioblastoma (GBM) observed controlled brain inflammation, as well as an augmentation in tumor necrotic volume in one case, and transient remission in two cases, potentially limited by antigen loss. [188]

5.3.10 GD2-Targeted CAR T-Cell Therapy for Glioma and Neuroblastoma

GD2 ganglioside is a tumor-associated glycosphingolipid normally limited to the central nervous system but overexpressed in neuroectodermal cancers, where it enhances tumor growth and immune suppression through membrane organization and

signaling. [189] Disialoganglioside GD2, as also called, has been investigated as a CAR T-cell therapy target; as a matter of fact, a phase I trial in patients with H3K27M-mutated diffuse intrinsic pontine gliomas or spinal cord diffuse midline gliomas, aggressive tumors affecting critical CNS structures following histone function alteration, revealed clinical and radiographic improvements in three of four patients, with reversible adverse effects and insignificant off-target toxicity. [190] Additionally, GD2-targeted CAR T-cell therapy demonstrated CR in three of eleven neuroblastoma patients, persisting durably in two of them, while showing proportionality between the length of persistence and the amount of CD4+ cells—helper T-cells, which activate other immune cells via cytokine production—and central memory cells in the injection. [191] There were maximum persistencies of 192 weeks for CAR–activated T-cells (ATCs) and 96 weeks for CAR–cytotoxic T-lymphocytes (CTLs). [191]

5.3.11 ROR1-Targeted CAR T-Cell Therapy for Lung and Breast Cancers

The receptor tyrosine kinase-like orphan receptor 1 (ROR1), implicated in embryonic development and tumor cell survival, is a cell-surface protein that has been targeted in CAR T-cell therapies for epithelial and lymphatic malignancies, as well as chronic lymphocytic leukemia (CLL). [192,193] This approach is particularly promising since ROR1 is expressed on the surface of various tumors but is absent in most adult tissues, except for B-cell precursors and some low-expression sites such as the adipocytes, pancreas, and lung. [192] In a phase I trial involving patients with lung and breast cancers, four of five participants exhibited PRs, characterized by diminished tumor mass at specific metastatic sites. [194] Additionally, ROR1-targeted CAR T-cells have shown safety in preclinical studies involving nonhuman primates, indicating that low-level ROR1 expression in normal tissues did not result in significant toxicity, and ROR1 CAR T-cells successfully accumulated in bone marrow and lymph nodes, where they targeted ROR1-positive B-cells, demonstrating the potential of this strategy for treating cancers while minimizing adverse effects. [194]

5.3.12 CEA-Targeted CAR T-Cell Therapy for Metastatic Colorectal Cancer and Liver Metastasis

Carcinoembryonic antigen (CEA), a fetal development glycoprotein frequently overexpressed in malignancies such as colorectal, gastric, medullary thyroid, breast, and ovarian cancers, has been utilized as a CAR T-cell target. [195–197] A dose-escalation trial in metastatic colorectal cancer showed stable disease in seven of ten patients for as long as 30 weeks, with two experiencing tumor reduction for more than 30 weeks, two experiencing tumor size reduction, and most patients presenting a decline in serum CEA level in the long term. [198] Additionally, intra-arterial CEA-targeted CAR T-cells combined with radiation therapy demonstrated safety and efficacy in treating liver metastases (LM), with a mean transduction efficiency of 60.4%, a median survival time of 8 months, and no instances of grades 4–5 toxicities, including neurotoxicity and CRS. [199]

5.3.13 MUC1-Targeted CAR T-Cell Therapy for Ovarian Cancer and Esophageal, Colorectal, Breast, and Pancreatic Carcinomas

Mucin 1 (MUC1) has been investigated as a viable target for CAR T-cell therapy, causing the revelation of antitumor activity across multiple malignancies by early studies. MUC1, a transmembrane glycoprotein belonging to the mucin family, is linked to tumor progression and metastasis [200], particularly in gastric cancer, the third most common cancer at the international level. [180] In normal cells, it provides lubrication and hydration, but in cancerous ones, including multiple epithelial adenocarcinomas, it promotes growth via aberrant glycosylation and intracellular signaling pathways. [201] A phase I clinical trial utilizing the CAR T-cell therapy P-MUC1C-ALLO1 exhibited significant infiltration and activity in triple-negative breast cancer (TNBC) and ovarian cancer xenografts, with CAR T-cells comprising over 90% of the tumor mass by day 10 and achieving complete tumor eradication within 2 weeks. [202] Four patients with esophageal, colorectal, breast, or pancreatic carcinoma received infusions of P-MUC1C-ALLO1, without reported concomitant toxicities, and early efficacy was observed, with one PR. [202] In fact, the MUC1-C epitope is abundantly expressed in many common epithelial cancers, while its expression in normal tissues is limited to the apical surface, the outward-facing side of epithelial cells. [202]

5.3.14 CD70-Targeted CAR T-Cell Therapy for Renal Cell Carcinoma

CD70 is an immune checkpoint molecule that, along with its receptor CD27, is dysregulated in multiple malignancies, contributing to tumor progression and immunosuppression. [203] An allogeneic CD70-targeting CAR T-cell product, CTX130, was developed for advanced or refractory clear cell renal cell carcinoma (ccRCC). [204] Preclinical studies demonstrated favorable proliferation and cytotoxicity profiles of CTX130, with complete regression of RCC xenograft tumors. [204] In a phase I, multicenter, first-in-human clinical trial involving sixteen patients with r/r ccRCC, CTX130 was well-tolerated, with no dose-limiting toxicities, and achieved disease control in 81.3% of patients; notably, one patient remained in a durable complete response for 3 years. [204] Furthermore, the next-generation CAR T construct, CTX131, showed enhanced expansion and efficacy in preclinical models, demonstrating the potential of CD70-targeted allogeneic CAR T-cells for treating ccRCC and other CD70+ malignancies. [204]

5.3.15 PSMA-Targeted CAR T-Cell Therapy for Prostate Cancer

Similar to pancreatic cancer, PSMA is a frequently occurring transmembrane glycoprotein in aggressive prostate cancer, positioning it as a viable overexpressed antigen target for CAR T-cell therapy targeting. [205] During a Phase I clinical trial evaluating CAR-modified "designer" T-cells (dTc) targeting PSMA for prostate cancer treatment, after genetic engineering of T-cells, patients underwent chemotherapy conditioning followed by dTc infusion with low-dose IL-2. [173] The trial observed a significant expansion of infused T-cells, with a 20- to 560-fold increase over 2 weeks, and engraftment—successful integration and persistence—ranging from 5% to 56%. [173] Despite these high engraftments, IL-2 levels were unexpectedly depleted up to 20-fold,

showing an inverse correlation with T-cell engraftment. [173] Notably, there were no observed anti-PSMA toxicities or anti-CAR reactivities; two of the five treated patients achieved PRs, with PSA reductions of 50% and 70%, and significant delays in PSA progression of 78 and 150 days, respectively. [173] The clinical responses were inversely correlated with T-cell engraftment and directly correlated with plasma IL-2 levels, remarkably indicating that higher plasma IL-2 concentrations may enhance anti-tumor efficacy. [173] Moreover, CAR T-cell therapy holds promise for integration alongside other treatment modalities, such as androgen deprivation therapy, radiotherapy, or chemotherapy, and may be applied as focal, localized CAR T-cell therapy for prostate cancer, potentially enhancing the therapeutic impact thereof. [206]

5.4 Quality of Life Measurements

CAR T-cell therapy has markedly influenced patient-reported outcomes, particularly in relation to psychological well-being and physical symptom burden. A longitudinal study of one hundred and three patients who underwent CAR T-cell therapy communicates that their quality of life (QOL) and depression worsened within the first week but showed notable improvement by 6 months. [207] After this recovery, only 18% of patients experienced significant depression; 22% reported anxiety; and 22% exhibited PTSD symptoms 6 months after CAR T-cell therapy. [207] Nevertheless, although severe physical symptoms were displayed by 52% of patients after 1 week, they decreased to 28% within 6 months, as substantiated by the utilization of the Edmonton symptom assessment system—which evaluates pain, fatigue, drowsiness, nausea, appetite, dyspnea, and well-being, with additional consideration of insomnia and dysphagia due to their prevalence in cancer patients. [207] Factors associated with a higher QOL trajectory included poorer baseline performance status (with functional abilities prior to treatment, using the Eastern Cooperative Oncology Group scale) and the utilization of tocilizumab or corticosteroids to manage CAR T-cell therapy–related CRS or neurotoxicity. [207] These findings underscore both the early disturbance occasioned by the the therapy and the potential for subsequent long-term recovery in physical and psychological domains.

6. Clinical Utilization and Public Health Implications

The clinical implementation of CAR T-cell therapy has expanded significantly following regulatory approval for various hematologic malignancies, prompting critical considerations regarding its cost-effectiveness, accessibility, and integration within existing therapeutic frameworks. These factors bear substantial public health implications, particularly in balancing innovative cancer treatment with economic sustainability and equitable patient access.

6.1 Approved Therapies

Patient trials permitted the clinic approval of six CAR T-cell therapies for blood cancers—i.e., leukemia, lymphoma, and myeloma—by the food and drug administration (FDA) between 2017 and 2022, representing significant advancements in cancer immunotherapy. [208] Kymriah® (tisagenlecleucel), approved in 2017, targets CD19 and is indicated for patients up to 25 years of age with relapsed or refractory (r/r) B-cell precursor ALL and adult patients with r/r LBCL or follicular lymphoma (FL). [209] Similarly, Yescarta® (axicabtagene ciloleucel), also approved in 2017, targets CD19 and is indicated for adult patients with r/r LBCL, including DLBCL and FL. [210] Tecartus® (brexucabtagene autoleucel), approved in 2020, is another CD19-directed therapy used for adult patients with r/r mantle cell lymphoma (MCL) and B-cell precursor ALL. [211]

Breyanzi® (lisocabtagene maraleucel), approved in 2021, is a CD19-targeted therapy indicated for adult patients with various forms of r/r LBCL, including DLBCL, high-grade B-cell lymphoma (BCL), and grade 3B FL, particularly in cases refractory to or relapsing after chemoimmunotherapy or hematopoietic stem cell transplantation (HSCT). [212] Shifting to another antigen, Abecma® (idecabtagene vicleucel), approved in 2021, targets BCMA and is indicated for adult patients with r/r multiple myeloma (MM) after four or more prior therapies. [213] Lastly, Carvykti® (ciltacabtagene autoleucel), approved in 2022, also targets BCMA and is used for adult patients with r/r MM after multiple prior therapies, including proteasome inhibitors, immunomodulatory agents, and anti-CD38 monoclonal antibodies. [214] These therapies demonstrate unique applications for r/r hematologic malignancies, highlighting the clinical importance of targeting antigens like CD19 in lymphomas and leukemias, and BCMA in MM.

6.2 Clinical Implications

Despite the clinical promise of CAR T-cell therapy, its real-world implementation remains constrained by significant challenges related to elevated treatment costs and optimizable insurance coverage.

6.2.1 Treatment Costs

Cost still represents a significant impediment of CAR T-cell therapy. Indeed, the two first FDA-approved CAR T-cell therapies in the U.S., Kymriah (Novartis) and Yescarta (Gilead Pharmaceuticals), are priced at $475,000 and $373,000, respectively.

[215] These figures exclude additional costs such as extended hospital stays, follow-up care, and management of complications like CRS, which are estimated to attain more than US$547,000. [216] In the case of the most recently approved CAR T-cell therapy, the cost amounts to more than $450,000. [18] While increased production might lower costs, this process is slow and raises concerns about maintaining quality standards. [216] Furthermore, the lack of a specific billing code for CAR T-cell therapies exacerbates payment delays, straining hospital finances and limiting patient access. [216]

A study of two hundred and seventy-one patients with predominantly DLBCL revealed a median total cost of $608,100 (interquartile range: $534,100–$732,800), with 8.5% of patients incurring costs exceeding $1 million. [217] The therapy product itself accounted for a substantial portion, with a median cost of $402,500, while out-of-pocket copayments were relatively modest at $510. [217] This study pinpoints the variability of costs for CAR T-cell therapies, and the extent of their currently elevated prices.

For certain MM patients, CAR T-cell therapy expenses can reach $528,020 to $565,534 per patient, driven largely by drug acquisition expenses exceeding $400,000. [218] These therapies are administered as a one-time treatment but involve additional costs for administration and adverse event management. [218] In comparison, MM, an incurable malignancy with a median life expectancy of 7–10 years, incurs annual chemotherapy costs of around $300,000 for standard three-drug regimens, with anticipated quadruplet therapy escalating the annual costs to over $500,000, although chemotherapy does not always request such treatment charges. [219]

Moreover, when examining chemotherapy costs across various regimens for different cancers, a substantial cost variation is evident, since the 6-month mean cost of chemotherapy was $26,989 in certain cases, with significant cost differentials between regimens for curative and metastatic therapies, such as $35,315 for metastatic cancer versus $18,107 for curative treatments. [218] Furthermore, chemotherapy regimens incorporating biologics significantly increase costs, with the mean cost for biologic-inclusive regimens reaching $77,278 compared to $13,646 for those without biologics. [218] In certain cases, chemotherapy costs can vary by as much as $90,843, reflecting the differences in treatment types and cancer stages.

6.2.2 Insurance Coverage

Insurance benefits that cover CAR T-cell therapy remain inconsistent, as Medicare and private insurers evaluate treatments on a case-by-case basis. [216] In 2018, around half of the patients undergoing CAR T-cell therapy were covered by Medicare, but stringent guidelines and the absence of billing codes create delays, often rendering treatment inaccessible for critically ill patients. [216] Additionally, only 15 hospitals were entitled with the permission to deliver CAR T-cell treatments in 2018, 1 year after the first FDA approval of a CAR T-cell therapy. [216]

Alternative payment models, such as outcomes-based approaches where costs are incurred only if the treatment succeeds, have been proposed to address financial challenges. [216] Some countries have explored payment systems tied to the number of

additional years a patient benefits from treatment. [216] Despite these upfront costs, CAR T-cell therapy offers potential long-term cost-effectiveness by aiming for curative outcomes, unlike traditional therapies requiring prolonged treatment.

As of 2022, Medicaid was federally mandated to cover nearly all FDA-approved therapies if the manufacturer participates in the National Drug Rebate Agreement; however, individual state Medicaid programs impose varying restrictions on coverage for CAR T-cell therapies. [220] A 2019 review found that only 24 states had publicly available policies regarding CAR T-cell therapy, with approximately 75% of them implementing more restrictive criteria than the FDA-approved indications. [220] These restrictions often include requiring patients to be free of active infections such as HIV, hepatitis B, or hepatitis C, which are not present in the FDA label. [220] Additionally, nearly half of state Medicaid programs deny coverage to patients who have previously undergone CAR T-cell therapy; many states further restrict access by limiting coverage to populations that match clinical trial eligibility criteria rather than the broader FDA-approved population. [220]

At the federal level, the Centers for Medicare & Medicaid Services (CMS) provides national coverage for autologous CAR T-cell therapy when administered at healthcare facilities enrolled in the FDA's Risk Evaluation and Mitigation Strategies (REMS) program and used for a medically accepted indication; this includes FDA-approved indications, as well as off-label uses supported by at least one CMS-approved compendium. [221] Additionally, routine costs for clinical trials involving CAR T-cell therapies are covered if they meet the requirements outlined in National Coverage Determination (NCD). [221] Nevertheless, non-FDA-approved CAR T-cell therapies are categorically excluded from coverage. [221]

6.3 Treatments Comparison

The therapeutic outcomes of CAR T-cells have been compared with those of other cancer treatments. For instance, ciltacabtagene autoleucel (cilta-cel), an anti-B-cell maturation antigen CAR T-cell therapy, demonstrated superior efficacy to non–CAR T-cell therapies for triple-class exposed patients with relapsed/refractory multiple myeloma (MM), previously administered immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), and anti-CD38 monoclonal antibodies. [222] In the CARTITUDE-1 trial, cilta-cel achieved a notably higher ORR of 84%, instead of 28%, and significantly prolonged progression-free survival (PFS) and overall survival (OS) compared to real-world treatment regimens analyzed in the MAMMOTH clinical dataset, which comprehends results of chemotherapy, targeted therapies, biologics, and supportive treatments. [222] Propensity score-matched analyses confirmed these results, with cilta-cel showing ORR of 96% versus 30% in treated populations and substantial reductions in PFS and OS hazard ratios, underscoring cilta-cel's efficacy advantage in heavily pretreated MM populations, in which standard treatments failed. [222]

A study compared anti-CD19 CAR T-cell therapy and blinatumomab—a bispecific T-cell engager (BiTE) immunotherapy that attaches simultaneously to CD19 and CD3, activating patient T-cells to target CD19-positive cancer cells—in patients with

refractory or relapsed acute lymphocytic leukemia (r/r ALL). [223] CAR T-cell therapy demonstrated superior efficacy, achieving higher CR (86% vs. 48%) and MRD-negative rates (80% vs. 31%). [223] Additionally, it was associated with significantly prolonged OS (55% vs. 25%) and relapse-free survival (RFS) (40% vs. 22%) at 2 years. [223] CAR T-cell therapy was also more effective for bridging to allogeneic stem cell transplantation (allo-SCT), with a 2 year OS of 75%, versus 57% for the other immunotherapy. [223] Nonetheless, blinatumomab, while less effective overall, showed promise as a pre-SCT bridging agent, stabilizing a patient's condition before alloSCT, particularly for patients achieving MRD-negative status, with lower risk of relapse. [223] Moreover, regarding adverse effects, blinatumomab had a lower incidence of severe hematological toxicity, CRS, and neurological events compared to CAR T-cell therapy. [223]

Despite differences in treatments, several can be administered to improve patient survival chances, as reflected by the approval of CAR T-cells targeting LBCL as a second-line therapy after chemotherapy failure. [224] In fact, high-dose chemotherapy followed by autologous stem cell transplantation (ASCT), which constitutes the standard second-line therapy for r/r LBCL, demonstrated inferior efficacy to CAR T-cell therapy—particularly in patients whose disease recurs or progresses within 12 months. [224] Anti-CD19 CAR T-cells achieved elevated OS, event-free survival (EFS), and objective response rates (ORR), along with a lower hazard ratio (0.57 vs. 0.77) and no additional toxicity. [224] This underscores the complementarity of diverse technologies in the treatment of leukemia, as well as the advantage of CAR T-cell development to compensate for the shortcomings of alternative approaches.

7. Future Treatments

In addition to aforementioned antigen-targeting CAR T-cell autologous therapies, other types of treatments have been experimented to counteract solid tumors.

7.1 Off-The-Shelf Therapies

Off-the-shelf CAR therapies, which often involve cell types beyond conventional T-cells, can offer logistical and manufacturing advantages over traditional autologous approaches. Autologous, personalized CAR T-cell therapies, albeit revolutionary in hematological malignancy treatment, entail significant impediments. These include the necessity of specialized facilities for leukapheresis, the lengthy production time of approximately 3 weeks for personalized T-cells, elevated costs, complex manufacturing processes, and logistical issues regarding material transportation. [225,226] These barriers have led to the development of universal allogeneic CAR T-cells ("off-the-shelf" CARs) and alternative CAR-based approaches. Off-the-shelf CARs—derived from healthy donors and non-individually manufactured in large batches—offer advantages such as improved T-cell quality, immediate availability, and reduced costs through large-scale production. [227] Moreover, the quality and quantity of patient and donor T-cells is an important factor determining the efficacy of CAR T-cell therapies. [96] The treatment history of the donor is equally important, since the reception of chemotherapy prior to the infusion could result in a reduction of proper T-cells to harvest for the sake of the CAR T-cell therapy. [228] However, these therapies risk complications like graft-versus-host disease (GVHD) due to MHC mismatches, potentially reducing antitumor efficacy. [208] Strategies to overcome GVHD include using virus-specific T-cells (targeting viral antigens), genetically modified T-cells (engineered to eliminate endogenous molecules like TCRs and MHC to prevent immune rejection), and non-conventional T-cells (leveraging MHC-independent mechanisms for antitumor activity). [229] Other types of allogeneic CAR therapies involve memory T-cells, NK cells, and iNKT-cells. [226]

7.1.1 Virus-Specific T-Cells

Virus-specific T-cells (VSTs) are engineered to target viral antigens and are primarily used to treat viral infections, particularly in immunocompromised patients, whereas traditional CAR T-cells are designed to recognize tumor-associated antigens. One approach to developing off-the-shelf VST therapies involves the creation of VST banks from healthy donors, allowing for rapid access to virus-specific immune cells without the requirement of patient-specific manufacturing. A virus-specific T-cell (VST) bank of 32 lines was created from individuals with common human leukocyte antigen (HLA) polymorphisms who were immune to Epstein-Barr virus (EBV), cytomegalovirus, or adenovirus. [230] HLA polymorphisms are genetic variations that influence immune recognition and transplant compatibility in HLA genes—which encode cell-surface proteins that regulate immune responses by distinguishing self from non-self. [230] The bank aimed to avoid generating separate lines for each patient, particularly in emergency situations. [230]

To evaluate the feasibility and safety of this approach, a study was conducted using the banked VSTs. Eighteen of these lines were administered to fifty patients with severe, refractory infections following hematopoietic stem cell transplantation (HSCT), a procedure wherein a patient's impaired blood-forming stem cells are replaced with healthy donor-derived cells, often leading to immune suppression and increased vulnerability to viral infections. [230] The cumulative rates of complete or partial responses at 6 weeks post-infusion were 74.0% overall, with 73.9% for cytomegalovirus, 77.8% for adenovirus, and 66.7% for EBV. [230] Only four responders experienced recurrence or progression, and no immediate infusion-related adverse events were observed; *de novo* graft-versus-host disease (GVHD) only occurred in two patients. [230] Following infusion, the frequency of VSTs increased significantly, with corresponding reductions in viral DNA and clinical symptom resolution. [230] The use of banked third-party VSTs was shown to be a feasible and safe approach for treating severe viral infections post-transplantation.

Beyond their application in viral infection treatment, virus-specific T-cells have been explored as a platform for CAR T-cell therapies, particularly in the context of CD19-targeted immunotherapy. In fact, although autologous T-cells expressing a CD19-specific CAR are effective against B-cell malignancies [231], the safety and efficacy of allogeneic CD19 CAR T-cells remained unclear. [232] In a study, after allogeneic HSCT, donor-derived virus-specific T-cells (VSTs) expanded *in vivo*, persisted in the long-term, and demonstrated antiviral activity without causing GVHD. [233] To assess their therapeutic potential, eight patients were treated with donor-derived CD19 CAR-VSTs, ranging from 3 months to 13 years after HSCT, and no infusion-related toxicities occurred, while VSTs persisted in blood for a median of 8 weeks and up to 9 weeks at disease sites. [233] Objective antitumor activity was observed in two of six patients with relapsed disease, while two patients in remission remained disease-free. [233] In two of three patients with viral reactivation, donor CD19 CAR-VSTs expanded alongside VSTs, suggesting that CD19 CAR-VSTs have antitumor activity and may be enhanced by viral stimuli. [233] These findings attest that early treatment post-HSCT or vaccination with viral antigens could further enhance disease control, evincing the potential of CD19 CAR-VSTs as a dual-function immunotherapy.

7.1.2 Genetically Modified αβ Conventional T-Cells

Genetically modified αβ conventional T-cells are autologous or allogeneic T-cells of the αβ lineage that have been engineered to express synthetic receptors or undergo targeted gene editing to enhance antitumor efficacy while mitigating adverse effects such as graft-versus-host disease (GVHD). [234] Autologous T-cells engineered to express a CAR targeting the CD19 antigen (CAR19) have shown significant potential in achieving leukemic remissions in early-phase trials [235]; however, the manufacturing process can be challenging, particularly for infants or heavily pretreated patients, due to low T-cell availability, T-cell dysfunction, and variability in product quality. [236] To address this issue, researchers developed universal CAR19 T-cells (UCART19) by transducing non–human leukocyte antigen (HLA) mismatched donor cells with lentivirus, though predisposing to complications such as GVHD. [237] They subsequently utilized transcription activator-like effector nuclease (TALEN) technology, involving DNA cutting

through the attachment of TAL effector proteins in the presence of nuclease enzymes, to edit the T-cell receptor (TCR) α chain and CD52 gene loci. [237] Following these advancements, UCART19 was evaluated in clinical settings to assess its therapeutic potential. In one examination, two infants with relapsed, refractory CD19+ B-ALL received a single infusion of UCART19 cells after undergoing lymphodepleting chemotherapy and anti-CD52 serotherapy. [237] Both infants achieved molecular remissions within 28 days, and UCART19 cells persisted until allogeneic stem cell transplantation (allo-SCT). [237] This innovative approach bypasses patient-specific cell production, offering a promising bridge-to-transplantation strategy. It substantiates the therapeutic potential of gene-editing technologies in CAR T-cell therapy.

Universal CAR T-cell therapy, or UCAR T-cell therapy, is an advanced immunotherapy that utilizes gene-edited allogeneic T-cells from healthy donors to create off-the-shelf CAR T-cell products, eliminating the need for patient-specific cell manufacturing while enhancing accessibility, consistency, and scalability in the treatment of hematologic malignancies and other cancers. [238] In UCAR-T therapy, donor-derived αβ T-cells undergo gene editing to remove their endogenous αβ TCR, which prevents GVHD and allows for universal application across multiple patients. Advances in genome-editing technologies, such as ZFN, TALEN, and CRISPR-Cas9, are enabling the generation of these universal third-party T-cells. [239] UCART019, a CRISPR/Cas9-engineered UCAR-T product targeting CD19, demonstrated clinical efficacy and safety, as exemplified in a phase I study wherein CRISPR/Cas9-engineered universal CD19/CD22-targeting CAR T-cells (CTA101) demonstrated high gene-editing efficiency without genotoxicity or chromosomal abnormalities. [240] Indeed, among six patients with r/r ALL treated with CTA101, the CR rate was 83.3% at day 28 post-infusion, with three of five CR/CRi patients maintaining MRD negativity at a median follow-up of 4.3 months. [240] Despite the occurrence of CRS in all patients, no dose-limiting toxicity, GVHD, neurotoxicity, or gene-editing-related adverse events were observed, underscoring the therapy's potential. [240]

Moreover, UCART19, designed for non-HLA-matched patients with r/r B-ALL, incorporates TRAC and CD52 gene knockouts to reduce alloreactivity and enable safe administration. [241] Data pooled from the ongoing CALM (adult) and PALL (pediatric) studies demonstrates a manageable safety profile and robust anti-leukemic efficacy. [241] CRS was observed in 94% of patients, with most cases being mild to moderate (grades 1–2) and only 17% experiencing severe CRS (grades 3–4). [241] Neurotoxicity was mild and self-limiting, and acute cutaneous GVHD occurred in two of eighteen patients, resolving with steroids. [241] UCART19 expansion, detected in 72% of patients, was associated with anti-leukemic activity, with 88% achieving CR or complete remission with incomplete recovery (CRi) by days 28–42, and 86% of these becoming minimal residual disease-negative (MRD–), and eleven patients proceeded to allo-SCT. [241] These findings highlight UCART19's potential as a safe and effective therapy for heavily pre-treated B-ALL patients.

Similarly, UCART7, designed for CD7-positive T-cell malignancies, incorporates TRAC and CD7 knockouts to prevent GVHD and fratricide, thereby enhancing persistence and reducing immune-related toxicity. [242] In parallel, alternative approaches

such as CYAD-101, an NKG2D-based UCAR-T product, have shown success in mitigating GVHD without requiring extensive gene editing, as evidenced by preliminary phase I trials in metastatic colorectal cancer. [243] Beyond genetic modifications, the SUPRA CAR system has introduced a modular approach, allowing for precise, adaptable, and fine-tuned tumor targeting by incorporating switchable recognition domains and synergistic molecules to enhance efficacy. [244] This system addresses key limitations of conventional CAR T-cell therapy, such as antigen escape and poor T-cell expansion, by facilitating multi-antigen targeting and dynamic control of CAR activation. [244] Ongoing clinical trials are investigating its efficacy in CD19/CD20 and CD123 malignancies, endeavoring to determine its advantages over standard treatments and its potential role in earlier disease intervention. [244]

Moreover, bispecific CAR T-cell constructs employing an 'AND' gating mechanism—a genetic design strategy whereby T-cell activation requires the simultaneous recognition of two distinct antigens—such as prostate cancer-targeting CAR T-cells, designed to recognize both PSMA and PSCA, have been developed to enhance specificity and minimize off-target effects. [245] This approach aligns with the broader efforts to improve the clinical outcomes of CAR T-cell therapies by optimizing the effector cells' ability to engraft, proliferate, and selectively target tumor cells. [245] Notably, the development of multidrug-resistant TCR αβ-deficient CAR T-cells is a significant advancement in this domain, as these engineered T-cells demonstrate efficient antitumor activity and resistance to lymphodepleting regimens, which are commonly used as preconditioning for CAR T-cell therapy. [245] Such modifications not only reduce the risk of graft-versus-host and host-versus-graft reactions but also enhance the compatibility of CAR T-cells for allogeneic infusion, thereby supporting the broader applicability and efficacy of these immunotherapies in clinical settings. [245]

Gene editing of the TCR, specifically targeting the TCR of αβ T-cells, a predominant subtype of T-cells in circulation, to reduce the risk of alloreactivity, constitutes an approach to forestall GVHD through reduction in TCR-mediated MHC recognition. This is often achieved by disrupting either the T-cell receptor alpha chain (TRAC) or T-cell receptor beta chain (TRBC) genes, which has been shown to maintain the cytotoxic capabilities of T-cells, as in the case of CD19-CAR T-cells. [229] This strategy of targeted gene editing, specifically directing the CAR to the T-cell receptor α constant (TRAC) locus, not only ensures uniform CAR expression in human peripheral blood T-cells but also significantly enhances T-cell potency. [246] Such edited CAR T-cells demonstrate superior efficacy in preclinical models of ALL compared to conventionally generated CAR T-cells. [246] Furthermore, this approach circumvents tonic CAR signaling, which can lead to exhaustion, and facilitates the internalization and re-expression of the CAR upon repeated antigen exposure. [246] However, despite these promising results, one concern is that the persistence of gene-edited T-cells *in vivo* may be reduced, as knockout of the endogenous TCR, while reducing alloreactivity and enhancing antileukemic activity, led to shorter T-cell persistence compared to when the TCR was coexpressed with the CAR, highlighting a potential limitation of this strategy. [247]

In addition to targeting the TCR, strategies to reduce immunogenicity focus on disrupting the expression of β-2 microglobulin (B2M), which is a key component of HLA class I molecules that mediate the immune system's recognition of foreign cells [248]; by repressing B2M and PD1—an immune checkpoint receptor—in CAR T-cells, researchers observed a reduction of immune system recognition and rejection of allogeneic cells *in vivo*, as well as improved antitumor activity. [249] Furthermore, the combination of TCR knockout with lymphodepleting chemotherapy (such as alemtuzumab) and the suppression of CD52, which is a target for alemtuzumab, has been tested to prevent depletion of the CAR T-cells post-infusion, offering an additional layer of protection against immune-mediated clearance. [250] While these approaches have shown promise, the risk of viral reactivation in patients receiving alemtuzumab remains a concern, underscoring the need for close monitoring in clinical trials. [251]

Furthermore, TruUCAR™ GC027, a first-in-human universal CAR T-cell therapy for r/r T-cell acute lymphoblastic leukemia (r/r T-ALL), was evaluated for safety and efficacy in a prospective study. [252] The therapy employs CRISPR/Cas9 to disrupt TCRα and CD7, preventing GVHD and fratricide. [252] Among five heavily pre-treated patients (median age 24), GC027 achieved MRD-negative complete responses (MRD-CR) in four patients by day 28, with three maintaining MRD-negative status without requiring HSCT. [252] Peak CAR T-cell expansion occurred within 2 weeks, and persistence was observed in both cerebrospinal fluid and bone marrow in one patient with central nervous system involvement. [252] Adverse events included manageable grades 3–4 CRS in all patients, with no neurotoxicity or GVHD.

7.1.3 Non-Conventional γδ T-Cells

Gamma delta T (γδT) lymphocytes are inherently equipped for rapid activation and cytotoxic responses against cancer cells, contributing to immediate stress responses. [253] Upon activation, they can also serve as professional antigen-presenting cells. [253] CARs, which enhance T-cell functionality by targeting specific tumor antigens and providing costimulatory signals, were hypothesized to augment the natural tumor tropism of γδ T-cells, improving recognition and cytotoxicity while preserving their migratory abilities and antigen-presenting functions. [253] Using GD2-specific CARs as a model, both Vδ1 and Vδ2 γδ T-cell subsets were successfully expanded and engineered to achieve clinically viable numbers. [253] The CAR modification increased GD2-targeted cytotoxicity without impairing tumor-directed migration; furthermore, CAR-transduced Vδ2 cells maintained the capacity to process and present tumor antigens, stimulating responder αβ T-cells. [253] These findings support the potential of γδ CAR T-cell products of killing *in vitro*, and for clinical applications in solid tumor therapy.

Animal studies demonstrate that γδ T-cells play a pivotal role in tissue homeostasis and cancer immunosurveillance. [254] Following lymphodepleting chemotherapy, allogeneic γδ T-cells have been administered to cancer patients, where they expanded *in vivo* without inducing GVHD. [255] In this study, donor cell proliferation persisted for up to 28 days, leading to CR in three out of four previously refractory patients—lasting 8 months in one with plasma cell leukemia—while one patient succumbed to infection 6 weeks post-treatment. [255] Unlike αβ T-cells, γδ T-cells

recognize cancer through diverse receptors rather than clonal expansion, possessing varied cell functions and immunobiology, potentially reducing the risk of tumor evasion due to antigen loss. [256] Their abundance in tissues and MHC-independent target recognition further minimize alloreactivity and GVHD, enhancing their potential in CAR T-cell therapies for solid tumors. [226,254]

Polyclonal γδ T-cells, engineered with a CD19-specific CAR, have demonstrated significant expansion and antitumor effects in both laboratory and animal models. [257] These cells were generated by electroporating peripheral blood mononuclear cells (PBMCs) with the Sleeping Beauty (SB) transposon system, a method that facilitates the insertion of genetic material into cells, thereby enabling the expression of the CD19-CAR in multiple γδ T-cell subsets, including Vδ1, Vδ2, and Vδ3. [257] The engineered cells were expanded using CD19+ artificial antigen-presenting cells (aAPCs), resulting in the production of over a billion CAR+ γδ T-cells from just a small starting number. [257] The engineered γδ T-cells, which exhibit a broad spectrum of TCRs such as Vγ2, Vγ7, Vγ8, Vγ9, and Vγ10, were functionally enhanced when both the TCRγδ and CAR were activated, showing superior killing of CD19+ tumor cell lines compared to non-engineered γδ T-cells. [257] *In vivo*, these CAR+ γδ T-cells effectively reduced CD19+ leukemia xenografts in mouse models. [257] With the integration of the SB system and aAPC technology for human application, clinical trials are now poised to explore the therapeutic potential of polyclonal γδ T-cells in cancer treatment. [257] Several companies, including Adicet Bio, Cytomed Therapeutics, GammaDelta Therapeutics, and TC BioPharm, are advancing clinical trials for allogeneic CAR γδ T-cells. [258]

7.1.4 Natural Killer Cells

Natural killer (NK) cells are a subset of lymphocytes in the innate immune system, primarily responsible for the rapid detection and destruction of infected, stressed, or malignant cells. NK cells are of considerable interest in cancer treatment due to their role in graft-versus-tumor (GVT) effects, as they contribute to eliminating tumor cells without causing GVHD. This unique property of NK cells is further supported by studies showing that they suppress GVHD, inhibiting the activation and proliferation of alloreactive donor T-cells, while avoiding its development themselves, mediating this suppression through mechanisms such as perforin- and Fas ligand (FasL)-induced T-cell apoptosis, and directly lysing activated donor T-cells *in vitro*. [259] NK cells, which are part of the innate immune system, can specifically target cancer cells that downregulate HLA class I molecules, a common escape mechanism used by tumors to avoid detection by T-cells. [260] Tumor cells that downregulate HLA molecules to escape T-lymphocytes become more susceptible to NK cell-mediated cytotoxicity, which affects cells that do not express MHC class I molecules. [261]

Clinically, non-CAR engineered allogeneic NK cells have been shown to be safe when adoptively transferred to cancer patients, and recent studies suggest that CAR-engineered NK cells, such as CD33-CAR NK cells tested in patients with relapsed and refractory acute myeloid leukemia (AML), exhibit a promising safety profile even at high doses of up to 5 billion cells per patient, with no significant adverse effects observed. [262] Furthermore, CAR NK cells, such as the NK-92 cell line, offer a more cost-effective

production process compared to CAR T-cells, highlighting their potential accessibility and utility in cancer therapy after further optimization. [262] Preclinical research has highlighted the efficacy of CAR-engineered NK cells in targeting both solid tumors and hematologic malignancies.

In the context of neuroblastoma, a challenging pediatric cancer, GD2-specific CAR NK-92 cells were engineered to combine antibody-mediated recognition of GD2 with potent NK-cell cytotoxicity. [263] These modified NK cells effectively eliminated GD2-expressing neuroblastoma cells, including primary tumor cells and those resistant to unmodified NK-92 cells, demonstrating antigen-specific cytotoxicity. [263] Similarly, CD20-specific CAR NK-92 cells showed enhanced cytotoxic activity against CD20-expressing lymphoma and leukemia cells, overcoming resistance mechanisms that limit natural NK-cell activity and antibody-dependent cytotoxicity. [264] These findings thereby suggest the clinical potential of CAR NK cells in treating both GD2-positive solid tumors and CD20-expressing hematologic malignancies.

To address limitations in CAR T-cell therapy for solid tumors, next-generation CAR gene–receiving cells are being developed using innate immune cells like natural killer (NK) cells and macrophages. In fact, NK cells recognize tumor cells independently of MHC, reducing the risk of GVHD, circumventing antigen escape, and allowing them to serve off-the-shelf functions; their activity is influenced by activating and inhibitory receptors, as well as cytokines, including IL-15, IL-12, and IL-18. [265] Preclinical and early clinical studies have demonstrated their safety and efficacy against both hematological and solid tumors, with the ambition to overcome challenges such as angiogenesis, tumor invasion, and immunosuppression. [266] Recruiting clinical studies involve therapies such as anti-CD33/CLL1 CAR-NK (for AML), anti-BCMA CAR-NK (for r/r MM), anti-PSMA CAR-NK (for metastatic castration-resistant prostate cancer), anti-CD19 CAR-NK (for r/r ALL), DLL3-CAR-NK (for extensive stage small cell lung cancer), and CD70-CAR-NK (for r/r T-cell lymphoma), targeting an extensive spectrum of malignancies. [267]

One study evaluated the safety and efficacy of anti-CD19 CAR-modified natural killer (CAR-NK) cells derived from cord blood in eleven patients with r/r CD19-positive cancers, including NHL and CLL. [268] Administered as a single infusion following lymphodepleting chemotherapy, CAR-NK cells were engineered to express interleukin-15 (IL-15) and an inducible caspase 9 safety switch. [268] Notably, no cases of CRS, neurotoxicity, or GVHD were observed, and inflammatory cytokine levels, such as IL-6, did not increase. [268] Among the patients, 73% achieved a response, with 7 attaining CR (4 with lymphoma and 3 with CLL). [268] Responses were rapid, occurring within 30 days across all dose levels, and CAR-NK cells demonstrated sustained persistence at low levels for up to 12 months, underscoring the potential of CAR-NK cell therapy to deliver clinical benefits with minimal toxicity. [268]

Allogeneic NK cells can be derived from multiple sources, including peripheral blood mononuclear cells (PBMCs), pluripotent stem cells (iPSCs), and umbilical cord blood. [269] Current strategies for generating clinical-grade NK cells from peripheral blood mononuclear cells (PBMCs) or cord blood rely on the use of irradiated feeder cells,

which are inactivated cells that support NK-cell proliferation without dividing themselves. [270] A commonly used feeder cell line is the leukemia cell line K562, genetically modified to express membrane-bound interleukin-15 (mbIL-15) and 4-1BB ligand (4-1BBL), which significantly enhances NK-cell expansion and cytotoxicity compared to cytokine stimulation alone; this approach has demonstrated the ability to produce highly functional NK cells, with a median 21.6-fold expansion in CD56+CD3− NK cells and robust activity against AML cells, providing a practical platform for large-scale clinical applications. [270] Recent advancements also include the use of exosomes or plasma membrane particles derived from K562 cells expressing membrane-bound IL-21 for the expansion of NK cells, fostering *in vivo* expansion. [271]

Furthermore, induced pluripotent stem cells (iPSCs), which are artificially reprogrammed adult somatic cells capable of differentiating into almost any cell type, have been developed as a robust and renewable source for generating high-quality NK cells, addressing the limitations in cell number and quality associated with donor-derived NK cells. [272] iPSC-derived NK (iNK) cells exhibit strong cytotoxicity against both hematologic and solid tumors, produce inflammatory cytokines, and enhance antitumor responses by recruiting T-cells and cooperating with anti–PD-1 (immune system inhibitor) antibody therapies. [272] This scalable manufacturing process enables the production of large, “off-the-shelf” doses of iNK cells for immunotherapy, offering a promising approach to augment checkpoint inhibitor therapies and improve tumor targeting. [272]

7.1.5 iNKT-Cells

Invariant natural killer T (iNKT) cells are a specialized T-cell population that recognizes lipid antigens presented by CD1d, rapidly activating through TCR interactions and cytokine production, bridging innate and adaptive immunity. [273] iNKT-cells, expressing the invariant Vα24invt TCR, recognize the CD1d molecule, a lipid-presenting glycoprotein on professional antigen-presenting cells such as B-cells, thymocytes, and monocytes. [274] This specific interaction enables iNKT-cells to carry out immunoregulatory functions, while the invariant nature of their TCR ensures a uniform response to self-ligands, thereby reducing the risk of GVHD, which is often triggered by the diverse TCR repertoire seen in other T-cell therapies, making iNKT-cells a promising therapeutic alternative. [274] These cells are often reduced in number and functionally impaired in cancer patients, with circulating iNKT-cell numbers approximately 50% lower than in age- and gender-matched healthy controls, regardless of tumor type or load; this reduction, accompanied by diminished absolute numbers of IFN-γ-secreting iNKT-cells despite normal percentages, may impair tumor immunosurveillance and contribute to tumor development. [275] Indeed, severe deficiencies in circulating iNKT-cells are significantly associated with decreased 3-year OS rates (39% compared to 75% and 92%), disease-specific survival rates (43% compared to 87% and 92%), and locoregional control rates (31% compared to 74% and 92%) in head and neck squamous cell carcinoma (HNSCC) patients, underscoring their critical role in antitumor immune responses. [276]

Invariant natural killer T (iNKT) cells have revealed remarkable efficacy when engineered with CARs targeting GD2, a ganglioside, or complex lipid group, highly expressed in neuroblastoma. [277] In preclinical studies, primary human iNKT-cells were isolated and activated before being transduced with CAR-GD2 constructs, achieving stable expression in 50%–70% of cells. [277] Following retroviral transduction, the engineered cells were expanded *ex vivo*, whereafter iNKT-cells comprised over 98% of the cultured population, ensuring purity and therapeutic consistency. [277] CAR-GD2 iNKT-cells demonstrated robust antitumor activity, efficiently targeting neuroblastoma cells while maintaining CD1d-dependent reactivity and avoiding GVHD. [277] Additionally, the inclusion of specific signaling domains, such as CD28 and 4-1BB, significantly enhanced persistence, tumor localization, and antitumor efficacy *in vivo*, underscoring the scalability, safety, and effectiveness of CAR-GD2 iNKT-cells, which offer a promising platform for the treatment of GD2-expressing malignancies. [277]

In addition, in a phase I dose-escalation trial, autologous Vα24-invariant natural killer T (iNKT) cells engineered to co-express a GD2-specific CAR and IL-15 were investigated for treating children with relapsed or resistant neuroblastoma. [278] Despite the recognized scarcity of iNKT-cells in humans, the approach demonstrated feasibility, as highly purified NKT-cells were expanded *ex vivo* and infused following lymphodepleting conditioning with cyclophosphamide/fludarabine (Cy/Flu), preparing a favorable environment for the infusion. [278] No dose-limiting toxicities were observed, although grades 3–4 hematologic adverse events were probably related to the conditioning regimen, since they occurred prior to CAR-NKT infusion. [278] The CAR–NKT-cells expanded *in vivo*, localized to tumor sites, and induced an objective response in one patient, with regression of bone metastatic lesions, emphasizing the safety and potential efficacy of this novel CAR-NKT approach in pediatric neuroblastoma treatment. [278]

Invariant natural killer T (iNKT) cells, engineered to express anti-CD19 chimeric antigen receptors (CAR19), demonstrate superior efficacy compared to CAR19–T-cells in targeting CD19+ B-cell lymphomas, particularly those expressing CD1d molecules. [279] CAR19–iNKT-cells exploit dual activation mechanisms—CD1d-restricted and CAR19–CD19–dependent—resulting in enhanced cytotoxicity. [279] *In vivo*, these cells exhibit faster and more robust anti-lymphoma activity, including the eradication of brain lymphomas, which significantly improves tumor-free and overall survival rates. [279] This efficacy is further amplified by transcriptional de-repression of CD1D via all-trans retinoic acid, derived from vitamin A, which stimulates antigen presentation that is critical for iNKT-cell activation. [279]

Moreover, optimization of engineering protocols revealed that upfront lentiviral transduction, performed prior to iNKT-cell expansion, results in higher transduction efficiencies and greater cell proliferation over 3 weeks compared to CAR19–T-cells. [279] This process, incorporating CD3/CD28-mediated activation and IL-15, also preserves the CD4– iNKT subset, which exhibits a stronger cytotoxic profile characterized by elevated levels of interferon-γ (IFNγ), perforin, and granzyme B. [279] Notably, 40% of CAR19–iNKT-cells are tri-functional, simultaneously co-expressing IFNγ, perforin, and granzyme B, versus <5% of CAR19–T-cells, reflecting superior cytotoxic potential.

Additionally, CAR19–iNKT-cells secrete higher levels of Th1 and Th2 cytokines during activation, further enhancing their antitumor efficacy, underlining the significant advantages of CAR19–iNKT-cells over CAR19–T-cells in treating CD19+ BCL. [279]

Furthermore, CD4+ natural killer T (NKT) cells, alongside CD4+CD25+ regulatory T-cells (Tregs), are pivotal in regulating aberrant immune responses. [280] In a murine model of allogeneic hematopoietic cell transplantation (HCT), adoptive transfer of highly purified CD4+ NKT-cells (>95% purity), comprising both invariant (iNKT) and non-invariant NKT-cell populations, effectively mitigated GVHD without inducing significant morbidity or mortality. [280] These cells demonstrated migration and proliferation patterns akin to conventional T-cells (Tcons), initially localizing in secondary lymphoid organs before infiltrating GVHD-affected tissues, where they persisted for over 100 days. [280] GVHD suppression was instigated via interleukin-4 (IL-4)–dependent mechanism and characterized by reduced interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) production by Tcons, as well as diminished pathology in skin, spleen, and gastrointestinal tissues. [280] Importantly, NKT-cells minimally impacted Tcon proliferation and preserved their graft-versus-tumor (GVT) activity against B-cell lymphoma-1 (BCL-1) tumors, reflecting the potency of such therapies. [280]

7.1.6 Memory T-Cells

Memory T-cells, formed after an initial infection by differentiating from activated T-cells, provide long-term protection by "remembering" the pathogen and responding more rapidly upon re-infection, with several subsets—effector memory (TEM), central memory (TCM), and tissue-resident memory (TRM)—serving distinct roles. [281] In the development of allogeneic cellular therapies, utilizing memory T-cell subsets as effector cells may reduce the risk of GVHD; in fact, memory T-cells are more mature, and thus less alloreactive and likely to cause GVHD in HLA-mismatched settings. [282] Moreover, the persistence and efficacy of CAR T-cells are influenced by the differentiation status of the T-cell subsets, as seen in autologous CAR T-cell platforms, with distinct roles attributed to CD4+ and CD8+ subsets, including their memory and effector functions, which play a pivotal role in CAR T-cell immunotherapy. [283] CD4+ subsets, such as Th1, Th2, Th9, Th17, Th22, regulatory T-cells (Tregs), and follicular helper T-cells (Tfh), and CD8+ memory and effector T-cells, differ significantly in their extracellular markers (e.g., CD25, CD45RO, CD45RA, CCR7, and CD62L), intracellular markers (e.g., FOXP3), epigenetic and genetic programming, as well as metabolic pathways, allowing enhancement of CAR T-cell therapy efficacy via modulation of specific functional and phenotypic distinctions. [283]

Memory T-cell subsets can be distinguished by surface markers, such as CD45RO, CD45RA, CD62L, CCR7, and CD27. [284] Research indicates that central memory (CD45RO+/CD62L+ or CCR7+) T-cells and memory stem cells (Tscm) confer enhanced CAR T-cell effector functions, with Tscm cells, characterized as long-lived, self-renewing, and multipotent, demonstrating the capacity to induce profound and sustained tumor regression. [285] Despite their rarity, recent advancements have enabled the generation of large numbers of clinical-grade tumor-redirected Tscm cells from naive CD8+CD62L+CD45RA+ T-cell precursors through a process involving CD3/CD28

activation, which stimulates T-cell activation and proliferation, in the presence of interleukin-7 (IL-7), interleukin-21 (IL-21), and the glycogen synthase-3β inhibitor TWS119—which assists in the generation of Tscm by supporting metabolic fitness and multipotency. [285] These Tscm cells, genetically engineered to express CD19-CAR, exhibit enhanced metabolic fitness, robust antitumor responses, and phenotypic and functional equivalence to their naturally occurring counterparts, thus offering a promising avenue for therapeutic applications, including the treatment of B-cell malignancies refractory to prior allogeneic hematopoietic stem cell transplantation. [285]

The use of CD45RA-negative T-cells, encompassing central and effector memory subsets, has also demonstrated cancer management and reduced GVHD incidence, with recall performance *in vitro* and *in vivo*. [286] In preclinical animal models and clinical settings, CD45RA-depletion has demonstrated a decreased risk of GVHD when applied to both primary graft manipulation and post-transplant donor lymphocyte infusion (DLI). [287] Notably, in a clinical study involving haploidentical hematopoietic cell transplantation (HCT) for children with relapsed or refractory solid tumors, CD45RA-depletion resulted in a profound reduction of naïve T-cells—exceeding 4.5 log (i.e., >31,000-fold) depletion of CD3+CD45RA+ cells—while maintaining sufficient T-cell doses for engraftment. [287] This approach facilitated rapid engraftment within 14 days, achieving 100% donor chimerism without acute GVHD or secondary graft failure, thereby underscoring its potential to minimize GVHD risk while preserving engraftment efficiency. [287]

Preclinical studies also suggest that CD27-negative T-cells (effector and terminal effector memory) expressing CD19-CARs could be a potential strategy, as CD27-depleted cell fractions are enriched for effector memory (helper) CD4+ T-cells, terminal effector memory (cytotoxic) CD8+ T-cells, and natural killer (NK) cells, which collectively exhibit strong immunologic responses against common pathogens. [288] Furthermore, CD27-depleted cells demonstrate significantly reduced GVHD potential, as evidenced by *in vitro* lymphocyte proliferation assays and *in vivo* studies in immunodeficient, NOD scid gamma (NSG) mice. [288] When transduced with a CD19-CAR vector produced by stable cell lines, CD27-depleted cells retain robust anticancer activity, as shown in cytotoxicity assays and murine leukemia models. [288] These findings underscore the potential of CD27-depletion as a dual-purpose approach, enabling both infection control and effective antitumor immunity while minimizing the risk of alloreactivity in adoptive cell therapy (ACT).

7.1.7 HCT-Derived CAR T-Cells in Post-Transplantation Treatment

HCT-derived CAR T-cells represent a clinically relevant form of allogeneic therapy, as they originate from transplant donors and are administered post-hematopoietic cell transplantation (HCT) to enhance immune-mediated antitumor responses. Indeed, while off-the-shelf allogeneic CAR T-cell therapies offer a broad, pre-manufactured solution for various cancers, donor lymphocyte infusion (DLI) following allogeneic hematopoietic cell transplantation (HCT) remains a standard practice that relies on T-cells from the transplant donor. [289] The primary therapeutic purpose of DLI is the correction of mixed chimerism, distinguished by the presence of

two genetically distinct populations, and the confrontation of viral infections, although its effectiveness in combating cancer is limited due to the lack of specificity for tumor-associated antigens (TAAs), rendering it particularly useful in MRD contexts. [289] Early clinical investigations into allogeneic CAR T-cell therapies extended this approach by utilizing HCT-donor-derived CD19-directed CAR T-cells in post-HCT patients with progressive B-cell malignancies, including ALL, CLL, and lymphoma. [290]

One of these trials demonstrated significant antitumor benefits, with eight of twenty patients achieving remission, including six complete remissions (CRs) and two partial remissions, with the highest success observed in ALL, where four of five patients achieved MRD-negative CR [290], which is especially propitious when considering the low rate of CR following alloHSCT that culminates in relapse. [291] Responses were also noted in chronic lymphocytic leukemia and lymphoma, with the longest ongoing CR exceeding 30 months. [290] Notably, no new-onset GVHD was reported following CAR T-cell infusion, underscoring the safety of this approach; although toxicities included fever, tachycardia, and hypotension. [290] Although this method enhances the graft-versus-tumor effect without significantly increasing the risk of GVHD, it faces challenges due to its reliance on available donors and the need for specialized manufacturing facilities. Despite these limitations, this approach retains several advantages over off-the-shelf products, including the use of healthy donor cells, precise leukapheresis timing, and minimized risks of HLA mismatch-related persistence issues. [226] Additionally, it has held promise for prophylactic applications to reduce relapse in high-risk post-HCT populations, while also addressing viral reactivations via native TCR activity. [292]

Allogeneic hematopoietic stem-cell transplantation (alloHSCT) serves as a potentially curative treatment for select patients with advanced B-cell malignancies, particularly within the context of nonmyeloablative (NMA) conditioning regimens, which employ reduced-intensity preparative protocols to facilitate engraftment while minimizing toxicity and fostering an immune-mediated graft-versus-lymphoma effect; however, despite its promise, a substantial proportion of patients fail to achieve CR following alloHSCT, and many who initially achieve CR ultimately relapse. [293] Progressive malignancy remains the primary cause of mortality post-alloHSCT. [294] Among these relapses, patients with ALL have a median survival of only 5.5 months, with estimated 1-year post-relapse survival rate of 30%, 2-year survival in 16%, and 5-year survival in 8%. [295] Those with refractory DLBCL also face complications such as GVHD, in which T-cells from the donated stem cells attack the patient's cells, as well as 4-year estimated nonrelapse mortality chance of 32%, despite undergoing alloHSCT with reduced-intensity transplantation (RIT). [296]

Furthermore, in a study, researchers employed the Sleeping Beauty transposon system to introduce the CD19RCD28 CAR into donor T-cells, which were expanded and infused after HCT. [297] A cohort of twenty-one patients with advanced CD19+ malignancies was treated with HLA-matched or haploidentical donor-derived CAR T-cells. [297] The study found no significant acute or late toxicity, and only three patients developed acute GVHD, consistent with expectations for post-HCT patients. [297] Moreover, CAR T-cell infusion resulted in a reduced reactivation rate of cytomegalovirus (CMV)—an infectious herpesvirus—compared to previous cohorts. [297] With a median

follow-up of 5.2 months, 48% of patients remained in CR, highlighting the efficacy of this preemptive approach, bolstering the viability of CAR T-cell infusion as an adjunct to HCT. [297]

7.2 Optimization Strategies

Despite the promise of CAR T-cell therapy, certain limitations—such as suboptimal T-cell activation and cellular exhaustion—have hindered its efficacy and broader clinical adoption. Consequently, targeted innovations have emerged to mitigate these challenges, illustrating a growing trend toward the addressment of CAR T-cell–related shortcomings through strategies that aim to further refine conventional CAR T-cell platforms, in parallel with previously discussed allogeneic developments.

7.2.1 T-Cell Expansion and Effector Function

To address the challenge of suboptimal CAR T-cell expansion and persistence in the immunosuppressive TME, researchers have developed strategies to enhance T-cell activation and effector function. In standard CARs, costimulation is provided, but the crucial signal 3, required for T-cell expansion, is lacking. [298] To overcome this limitation, researchers have integrated the truncated cytoplasmic domain of IL-2Rβ and a STAT3-binding YXXQ motif into CD28ζ-CARs targeting CD19. [24] Alternatively, other studies have shown that incorporating the Toll/IL-1 receptor domain of Toll-like receptor (TLR) 2—which detects pathogen-associated molecular patterns—into CD28ζ-CARs enhances T-cell effector function, ameliorating cytotoxic activity, cytokine secretion, and immune cell activation. [299] These modifications deliver signals simultaneously, differing from natural T-cell activation, where signals occur in a specific temporal and spatial order, promoting faster and more sustained T-cell activation. [298] Upon activation, T-cells express costimulatory receptor 4-1BB, and studies indicate that presenting 4-1BB ligand (4-1BBL) on the cell surface of CD28ζ-CAR T-cells results in superior effector function compared to directly incorporating the 4-1BB signaling domain into the CAR. [300,301] Additionally, other CAR T-cells expressing tumor necrosis factor) TNF superfamily ligands, like CD40L, enhance antitumor efficacy by counteracting immune escape, activating antigen-presenting cells, and recruiting immune effectors. [302] Furthermore, ongoing research is focused on activating TLR pathways by using inducible costimulatory molecules containing MyD88, the central signaling molecule of TLRs, along with IL-1β and IL-18, and demonstrated certain ameliorations of the cells' effector function in both xenograft and syngeneic murine models. [298]

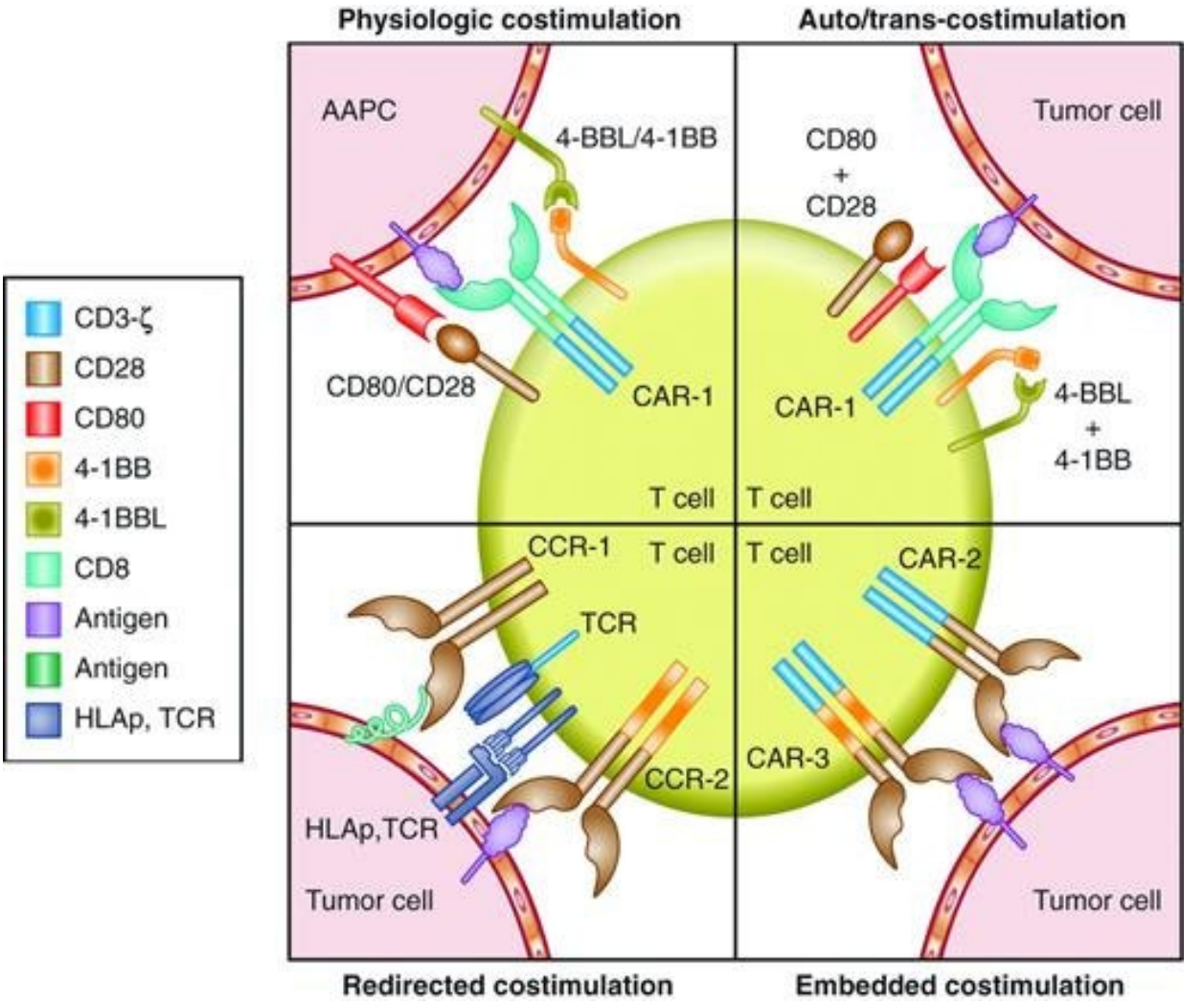

Figure 8: Strategies to Provide Costimulatory Support to CAR-Modified T-Cells.

In the upper left, physiologic costimulatory ligand display is performed by professional or artificial antigen-presenting cells (aAPCs); in the upper right, auto- and trans-costimulation is conducted by T-cells expressing costimulatory ligands; in the lower right, embedded costimulation is provided by second- or third-generation CARs; and in the lower left, redirected costimulation is mediated by an antigen-specific chimeric costimulatory receptor. Abbreviations: CAR-1, CAR-2, CAR-3 – first-, second-, and third-generation CARs; HLAp – HLA-peptide complex.

Adapted from [45].

7.2.2 Expression of Cytokines and Their Receptors

To enhance the persistence and efficacy of CAR T-cells, researchers have investigated the modulation of cytokine signaling pathways, particularly those involving the JAK/STAT cascade, generating transcription factors that regulate immune response gene expression, proliferation, and survival. Common γ-chain cytokines (IL-2, IL-7, IL-

15, IL-21) primarily activate JAK1/JAK2 and STAT5, whereas IL-12 and IL-23 signal through JAK2, TYK2, and STAT3 or STAT4. [298] Transgenic expression of these cytokines has been demonstrated in preclinical studies to improve CAR T-cell expansion and persistence, thereby enhancing antitumor activity. [303]

In addition to secreted cytokines, membrane-bound versions have been explored, as they may increase cytokine activity while restricting their effects to the modified cells, reducing systemic toxicity. [304] To further limit adverse effects, cytokine expression has been placed under the control of the nuclear factor of activated T-cells (NFAT) promoter, which regulates the expression of transgenes depending on the activation of T-cells, minimizing systemic targeting genes expression. [298] Nevertheless, while this strategy successfully mitigated IL-12–related toxicity in preclinical models, clinical data suggests that the NFAT promoter may not entirely restrict cytokine expression to activated T-cells. [305] Alternatively, positioning cytokine genes downstream of an internal ribosomal entry site (IRES), which facilitates the co-expression of multiple genes from a single mRNA, offers another method to regulate secretion via the simultaneous expression of other regulatory or functional proteins—such as safety switches or other modulators. [306] Furthermore, IRESs are particularly advantageous due to their ability to maintain translation under stress conditions, such as those seen in ischemic diseases and cancer. [306] Several clinical trials are currently evaluating CAR T-cell constructs incorporating IL-12 or IL-15, including those targeting neuroblastoma, with additional safety measures such as the inducible caspase-9 (iC9) safety switch. [298]

Alternative methods to activate the JAK/STAT pathways include the use of constitutively active cytokine receptors like the IL-7 receptor α (C7R), which continuously activates cytokine signaling without requiring external cytokine input. [307] This approach enhances T-cell expansion and antitumor activity while preventing bystander activation, improving the efficacy and proliferation of tumor-targeted CAR T-cell therapies. [307] The C7R system has shown promise by stimulating T-cell survival and function in metastatic neuroblastoma and glioblastoma xenograft models, supporting its clinical potential. [307]

7.2.3 Suppression of CAR T-Cell Regulation Molecules

Researchers have investigated the mechanisms whereby T-cells regulate their activation, employing accurate screening methods to identify key inhibitory molecules within this tightly controlled system; indeed, early studies utilizing short hairpin RNA (shRNA) approaches identified the phosphatase PP2R2D as a negative regulator of αβ TCR activation in tumor models. [308] More recent advancements have leveraged CRISPR-Cas9 gene-editing technology, revealing TCEB2, SOCS1, CBLB, and RASA2 as suppressors of αβ TCR activation *in vitro*. [309] Additionally, an *in vivo* screen identified REGNASE1 as a crucial negative regulator, with REGNASE1-deficient CD19-CAR T-cells demonstrating enhanced antitumor efficacy in a syngeneic leukemia model, highlighting the desirability of the identification of regulators of TCR function to suppress their inhibition of CAR functionality. [310]

7.2.4 Modulation of Transcription Factors

"Modulating transcription factor activity has emerged as a promising strategy to enhance CAR T-cell persistence, functionality, and resistance to exhaustion, thereby improving therapeutic outcomes. Transcription factor networks play a pivotal role in regulating T-cell plasticity, since they regulate gene expression, governing the potential of T-cells to differentiate into various functional subtypes—including effector, memory, and exhausted states. [298] For instance, the transcription factor c-Myb facilitates memory formation by activating TCF7 while simultaneously repressing ZEB2, which drives T-cell differentiation. [311] Additionally, TOX has been identified as a key enforcer of T-cell exhaustion, and murine CAR T-cells that were deprived of TOX and TOX2 transcription factors consequently exhibited enhanced tumor suppression, cytokine expression, and survival of tumor-bearing mice. [312] Moreover, chromatin remodeling increases the accessibility of DNA regions that are rich in binding sites for key regulatory transcription factors, such as nuclear factor κB (NFκB) and basic region-leucine zipper (bZIP) proteins, which play essential roles in immune activation, inflammatory signaling, and T-cell function. [312] Other nuclear receptor transcription factors, including NR4A1 (NUR77), NR4A2 (NURR1), and NR4A3 (NOR1), have been implicated in promoting exhaustion-associated transcriptional programs, and their simultaneous deletion in CAR T-cells has been shown to optimize function. [313] Conversely, overexpression of c-Jun (AP-1) mitigates terminal differentiation while augmenting functional capacity, thereby embodying another possibility to ameliorate antitumor efficacy. [314]

7.2.5 CRISPR and Non-Permanent Gene Editing Platforms

Gene editing technologies, particularly those employing CRISPR-Cas9 systems, offer transformative potential in optimizing CAR T-cell functionality. The CRISPR/Cas9 system, derived from a bacterial adaptive immune mechanism, utilizes a single guide RNA (sgRNA)—a fusion of crRNA and tracrRNA—to direct the Cas9 endonuclease to specific DNA loci adjacent to protospacer adjacent motif (PAM) sequences, where it induces double-strand breaks (DSBs). [23] These DSBs are subsequently repaired by cellular machinery, enabling precise genetic modifications. [23] This process has been widely applied to engineer CAR T-cells with enhanced cytotoxicity, resistance to exhaustion, and evasion of immunosuppressive signaling. [23] However, concerns persist regarding the irreversibility of Cas9-induced genomic alterations and their long-term safety profile. [315] In response, researchers devised the MEGA RNA editing platform, a CRISPR-based approach that modifies messenger RNA rather than genomic DNA, thus offering a reversible and potentially safer alternative. [316] Although early experiments revealed no immediate improvement in tumor suppression compared to conventional CAR T-cells, longitudinal studies demonstrated that MEGA-edited T-cells exhibited markedly enhanced antitumor efficacy, characterized by up to a tenfold increase in cell proliferation and functional persistence. [316] This underscores the promise of transient, RNA-targeted editing strategies to hone T-cell behavior without introducing permanent genetic changes, paving the way for safer and more adaptable clinical interventions.

7.2.6 Gut Microbiota Influence

The gut microbiota plays a critical role in preserving intestinal barrier function and regulating systemic immune responses. [317] Moreover, mounting evidence suggests that microbial composition may significantly affect both the efficacy and toxicity of CAR T-cell therapies. [318] A study found that pre-treatment with broad-spectrum antibiotics prior to CD19-targeted T-cell infusion correlated with diminished therapeutic benefit. [319] Similarly, researchers observed increased ICANS neurotoxicity and reduced survival in patients exposed to such antibiotics before CD19 CAR T-cell infusion. [320] Strong associations were identified between microbiota fluctuations and both cytokine release syndrome (CRS) severity and clinical outcomes in recipients of BCMA- or CD19-directed CAR T-cells for hematologic malignancies—including B-NHL, B-ALL, and MM. [321] These findings collectively suggest that gut microbial profiles may serve as non-invasive biomarkers for treatment prognosis; moreover, given their role in immune-related adverse events (irAEs), modulation of commensal bacterial communities offers a promising strategy to mitigate toxicity. [56] Nonetheless, further prospective studies are required to confirm these associations and guide microbiota-based therapeutic approaches. [317]

7.2.7 Organoid Experimentation

Organoids—three-dimensional, miniaturized organ structures cultivated from patient-derived tissues—represent biologically sophisticated *in vitro* systems that recapitulate the physiological and pathological features of native human tissues. [56] Their intricate architecture and cellular heterogeneity provide a critical bridge between conventional two-dimensional culture and *in vivo* modeling. [56] In the development of immunotherapeutics, particularly CAR T-cell therapy, tumor-derived organoids co-cultured with immune cells furnish a dynamic environment for investigating tumor-specific responses, including antigen recognition, immune evasion, and cytolytic efficacy. [56] For instance, researchers have employed organoid models representing basal and luminal tumor subtypes to evaluate the performance of second-generation CAR T-cells targeting tumor-associated antigens such as MUC1, thereby enabling a nuanced understanding of subtype-specific therapeutic vulnerabilities. [322] By faithfully replicating the TME, organoid platforms further support large-scale functional assays and predictive modeling, thus accelerating the translational advancement of personalized CAR T-cell therapies. [56]

7.2.8 Artificial Intelligence

The intrinsic heterogeneity of CAR T-cells and the phenotypic resemblance they share with other hematologic cells pose diagnostic impediments and complicate the timely identification of abnormal cell populations. [56] This limitation, combined with the high cost and prolonged time period required for biochemical analyses and the training of blood morphologists, underscores the value of advanced diagnostic tools. [56] Recent developments in artificial intelligence—particularly deep learning (DL) techniques—offer promising avenues to address these obstacles. [323] For instance, a high-precision DL model, RCMNet, attained a top-1 classification accuracy of 99.63% in identifying CAR

T-cells from cell image datasets. [324] Moreover, DL frameworks have demonstrated utility in predicting patient-specific therapeutic responses and adverse events, such as CRS, thereby contributing to personalized treatment protocols. [325] In a related effort, neural network models were employed to analyze a CAR library comprising over 2,300 synthetic costimulatory domains. [326] This approach facilitated the filtering and clustering of domain combinations in an endeavor to ameliorate neoantigen recognition and improve T-cell receptor design. [326] Therefore, while still in the developmental phase, artificial intelligence holds considerable potential to optimize CAR T-cell therapy through improved target selection, toxicity prediction, and response assessment.

7.2.9 *In Vivo* CAR T-Cell Generation

The direct *in vivo* engineering of CAR T-cells—through systemic administration of genetic vectors inside one's living body—eliminates the need for laborious *ex vivo* manipulation, enabling T-cell programming within the patient in 1 or 2 days only and potentially mitigating the risk of GVHD. [56] Among delivery vectors currently employed, lentiviral vectors (LVs) are predominant in clinical settings, particularly for *ex vivo* transduction of hematopoietic and T-cell populations. [327] However, their widespread application is constrained by limited global manufacturing capacity and high production costs. [328] Furthermore, the application of genome-wide CRISPR screening, in an attempt to optimize producer cell lines, has enhanced vector yield and transduction efficiency. [329]

Adeno-associated viruses (AAVs), the most utilized gene delivery vectors for *in vivo* applications, possess strong tropism for various tissues and demonstrate efficient cell entry. [330] AAV6 was leveraged to integrate anti-CD19 CAR constructs into human T-cells, while simultaneously disrupting endogenous TCR genes—an innovation that enables the development of universally applicable allogeneic CAR T products. [56] Owing to its high transduction efficiency and capacity for stable gene integration, AAV6 facilitates consistent CAR expression and supports the generation of "off-the-shelf" therapeutic cells. [331] Despite these advances, primary T-cell transduction by AAVs remains inefficient; efforts to boost AAV-mediated gene expression through the usage of monoclonal antibody OKT3 and tyrosine kinase inhibitor genistein, which modulate T-cell activation, offer promising avenues for improvement, having multiplied the intensity of gene expression of rAAV6–transduced T-cells by a factor of seven in disease models. [332]

Lipid nanoparticles (LNPs), notable for their non-integrative, RNA-based delivery capabilities, offer an alternative to viral vectors with the advantages of reduced cytotoxicity, lower manufacturing costs, and greater flexibility. [333] Although LNPs typically localize to hepatic tissue upon systemic administration, targeted adaptations have enabled broader tissue tropism, via antibody-conjugated LNPs (Ab-LNPs) with spleen-targeting capabilities that permits *in situ* CAR T-cell generation beyond the liver. [334] Moreover, CD3-targeted LNPs, capable of selectively co-delivering CD19 CAR mRNA and IL-6 shRNA into T-cells, produced functional CAR T-cells that not only targeted leukemic CD19-presenting cells but also attenuated CRS by suppressing IL-6 expression. [335] The LNP-mRNA platform has likewise demonstrated therapeutic efficacy

in a range of cardiac pathologies, including myocardial fibrosis, myocarditis, and infarction. [336] Collectively, these findings establish LNP-mRNA platforms as potent vectors for precise, modular, and clinically scalable immunotherapies.

7.2.10 Nanobody-Based CAR T-Cell Constructs

VHHs, or variable heavy domains of heavy chain antibodies—also referred to as nanobodies—can be extracted from camelids and have recently been incorporated into CAR constructs as alternatives to conventional single-chain variable fragments (scFvs), which are typically composed of both heavy and light antibody chains linked by a flexible peptide. [337] These nanobodies exhibit comparable antigen-binding affinities to full-sized monoclonal antibodies while offering superior properties such as enhanced solubility, thermal stability, and efficient recombinant expression, without the necessity of extensive antibody sequence libraries or complex rounds of affinity maturation. [338] Unlike conventional scFv-based CARs, which consist of both heavy and light chains linked by a flexible peptide, VHH-based CARs require only a single domain, thereby simplifying construct architecture and potentially improving stability.

Empirical studies have shown that CAR T-cells equipped with anti-CD47 VHHs induce potent immune responses, including increased IFN-γ production and cytotoxicity, without compromising cytokine output relative to standard constructs. [339] Encouraged by these preclinical outcomes, several VHH-based CAR T-cell therapies have advanced to clinical trials. [340] Notably, cilta-cel, a VHH-based construct targeting B-cell maturation antigen (BCMA), has received regulatory approval in the United States for treating relapsed or refractory MM, underscoring the clinical viability of VHH-based platforms. [341]

7.2.11 Naive and Stem Cell Memory T-Cells

Naive and stem cell memory T-cell subsets (T N/SCM), early-differentiated T-cells that retain stem-like properties, offer distinct advantages as vehicles for CAR engineering due to their multipotency, proliferative capacity at lower doses, resistance to exhaustion, attenuation of CRS risk, and memory phenotype enhancement. [342] Experimental evidence from humanized mouse models of hematopoietic malignancies demonstrates that CAR T-cells derived from T N/SCM populations exhibit prolonged *in vivo* persistence and enhanced anti-leukemic activity relative to unselected, heterogeneous T-cell populations (T BULK). [342] These attributes translate into improved therapeutic indices, characterized by robust cytotoxic function and attenuated inflammatory responses. [342]

Clinical observations further affirm these findings: T N/SCM–derived CAR T-cells targeting CD19 or co-targeting CD19/CD20 display heightened efficacy and reduced toxicity in adults with r/r B-ALL and NHL. [343] The strategic use of these early-differentiated T-cell subsets thus represents a promising refinement in CAR T-cell therapy, enhancing both durability and safety of the therapeutic response.

7.2.12 $CD8^+CD161^+$ T-Cells

$CD8^+CD161^+$ T-cells, characterized by the co-expression of CD8—a coreceptor marking cytotoxic lymphocytes—and CD161, a C-type lectin-like receptor that is associated with enhanced effector function and memory potential, exhibit enhanced cytotoxic functionality and a proclivity for memory formation, enhancing CAR T-cell therapeutic efficacy. [344] Additionally, a correlation between the secretion of pro-inflammatory cytokine interleukin-17 and the expression of CD161 was manifested. [344] This polyclonal subset, with distinct antigen receptors, demonstrates elevated levels of key effector molecules such as granzyme B and perforin—markers indicating robust immune responsiveness. [345] When genetically engineered to express CARs, these T-cells displayed superior antitumor activity and significantly improved survival outcomes when compared to their $CD161^-$ counterparts, regarding melanoma tumor control in murine models. [345] $CD8^+CD161^+$ CAR T-cells represent a particularly promising immunotherapeutic modality for malignancies necessitating sustained immune surveillance and durable tumor control. [345]

7.3 Other CAR Therapies

Beyond conventional CAR T-cell therapy, emerging approaches such as CAR-engineered macrophages and *in vitro*–transcribed (IVT) mRNA CAR T-cells represent promising innovations in the field of adoptive immunotherapy.

7.3.1 CAR Macrophages

Expanding the scope of CAR-based immunotherapy beyond T-cells, researchers have developed chimeric antigen receptor macrophages (CAR-Ms) to harness the unique tumor-clearing and immunomodulatory functions of macrophages. Macrophages, critical components of the innate immune system, defend against pathogens and cancer through binary M1/M2 polarization, playing central roles in immune responses and TME regulation. [346] They exhibit unique abilities, such as tumor-selective phagocytosis, antigen presentation, and immunomodulation. [266] Researchers engineered human macrophages with CARs to enhance their tumor-targeting ability; a chimeric adenoviral vector was utilized to overcome the resistance of primary macrophages to genetic modification, resulting in a sustained pro-inflammatory (M1) phenotype. [347] *In vitro*, CAR macrophages (CAR-Ms) effectively cleared tumors through antigen-specific phagocytosis. [347] In solid tumor xenograft mouse models, a single infusion of CAR-Ms reduced tumor burden and extended survival. [347] CAR-Ms expressed pro-inflammatory cytokines, reprogrammed anti-inflammatory M2 pro-tumor macrophages to pro-inflammatory M1 anti-tumor immune cells, enhanced antigen presentation, recruited T-cells, and resisted immunosuppressive signals; in humanized mice, CAR-Ms induced a pro-inflammatory tumor environment and augmented T-cell-mediated anti-tumor responses. [347] Additionally, a Phase I clinical study utilizing CAR macrophages (CAR-Ms) to target HER2-overexpressing solid tumors is currently being active. [348]

7.3.2 IVT mRNA CAR T-Cells

A transient *in vitro*–transcribed mRNA-based chimeric antigen receptor T-cell (IVT mRNA CAR T) therapy has been developed to deliver controlled cytotoxicity for a limited duration, minimizing potential adverse effects in patients. [349] Messenger RNA (mRNA) is a type of genetic material that carries instructions from DNA to the ribosome for protein synthesis, and its use in CAR T-cell therapy allows for the temporary expression of the CAR, distinguishing it from traditional infusion delivery methods that rely on the direct administration of engineered cells or proteins. [350] This approach has shown promise in preclinical studies, demonstrating therapeutic efficacy against solid tumors, including melanoma, neuroblastoma, and ovarian cancer, although only a limited number of clinical trials have been conducted to date. [349]

Mesothelioma is a malignant tumor affecting the linings of the lungs, heart, or stomach, where mesothelin and fibroblast activation protein are notable tumor-associated antigens (TAAs). [349] Intratumoral administration of mesothelin-targeting IVT mRNA CAR T-cells significantly reduced mesothelioma tumors in a mouse model. [351] Furthermore, similar protective effects were observed in a mouse model of disseminated intraperitoneal mesothelioma derived from a patient, where autologous T-cells redirected against TAAs utilizing IVT mRNA achieved notable therapeutic outcomes. [351] In colon cancer, which originates in the inner lining of the large intestine, TAAs such as HER2, carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), and human leukocyte antigen (HLA) have been identified as potential targets, offering avenues for the therapeutic application of IVT mRNA CAR T-cell therapies. [352] Additionally, explorations of the cytotoxic potential of IVT mRNA-engineered CAR T-cells targeting the natural killer group 2D receptor (NKG2D) in Ewing's sarcoma family of tumors (ESFT), affecting bones and proximate soft tissues, revealed that the expression of NKG2D receptors encoded by mRNA persisted for only a few hours after transfection and subsequently diminished irreversibly, underscoring the capability of IVT mRNA to provide tightly regulated, transient expression of CARs in T-cells. [353]

Ovarian and breast cancers are prevalent malignancies in older women, with identified TAAs including HER2, c-Met, mesothelin, and folate receptor alpha (FRα) in ovarian cancer, and HER2, c-Met, NKG2D, and ErbB2+MUC1 in breast cancer. [349] In ovarian cancer, a study demonstrated the efficacy of FRα-directed IVT mRNA CAR T-cells, which successfully killed ovarian cancer cell lines *in vitro* and significantly inhibited tumor growth in both localized and disseminated murine models. [354] Furthermore, a recent study utilized a non-integrating RNA platform to engineer human T-cells expressing FRα-specific, CD27 costimulatory CARs, such as C4-27z and its codon-optimized variant, C4opt-27z. [354] These CAR T-cells exhibited strong cytolytic activity, secreting high levels of Th-1 cytokines, and showed significant antitumor effects against human FRα+ cancers *in vitro* and *in vivo*. [354] Notably, C4opt-27z CAR T-cells demonstrated complete regression of fully disseminated ovarian cancer xenografts in mice, emphasizing their potential for clinical translation and offering a promising new approach for targeted ovarian cancer therapy. [354]

Similarly, mesothelin-targeted IVT mRNA CAR T-cells exhibited strong antitumor activity, suppressing tumor progression in humanized ovarian cancer murine models and demonstrating cytotoxicity against mesothelin-expressing cancer cells. [355] In a related study, anti-human mesothelin mRNA CAR transfected peripheral blood lymphocytes (CARMA-hMeso) were developed and demonstrated potent mesothelin-specific cytotoxicity *in vitro*. In a murine ovarian cancer model, a single intraperitoneal injection of CARMA-hMeso resulted in dose-dependent inhibition of tumor growth and enhanced survival. [355] Weekly-repeated intraperitoneal administrations further prolonged disease control and survival, with no significant off-target toxicities observed. [355]

Moreover, for breast cancer, hepatocyte growth factor receptor (c-Met) was identified as a key TAA, and c-Met IVT mRNA CAR T-cells demonstrated significant cytotoxicity in breast cancer cell lines. [356] These T-cells also inhibited tumor growth in murine ovarian cancer models, suggesting their broad applicability across various solid tumors. [356] In a phase 0 clinical trial for metastatic breast cancer, intratumoral administration of mRNA-transfected c-Met-CAR T-cells was evaluated for safety and feasibility; the treatment was well tolerated, with no adverse effects exceeding grade 1. [356] Immunohistochemical analysis of excised tumors revealed extensive tumor necrosis at the injection site, loss of c-Met expression, and an inflammatory response within the tumor, supporting the efficacy and safety of this approach for treating metastatic breast cancer. [356]

Neuroblastoma and glioblastoma multiforme are prevalent malignant tumors originating in the central nervous system, with tumor-associated antigens (TAAs) such as disialoganglioside GD2 and L1-CAM identified in neuroblastoma, and EGFR variant III, HER2, CD133, and B7-H3 in glioblastoma multiforme. [349] GD2-targeted mRNA CAR T-cells have shown significant anti-cancer effects in murine neuroblastoma models, particularly with localized tumors. [357] However, intravenous injection failed to reach the tumor site, highlighting the importance of maintaining CAR expression at the tumor location for effective cytotoxic response. [357] This challenge was also observed in studies comparing permanently modified and transiently modified CAR T-cells targeting GD2 in neuroblastoma. [357] Indeed, while lentivirally-modified GD2 CAR T-cells succeeded in long-term control of disseminated disease, multiple infusions of RNA-modified GD2 CAR T-cells only delayed disease progression and improved survival, without achieving long-term control. [357]

Anti-EGFR mRNA CAR T-cells demonstrated significant cytolytic efficacy against glioblastoma cell lines, highlighting their potential as a targeted therapy with reduced on-target, off-tissue toxicity. [358] T-cells were expanded *ex vivo* using K562-derived activating and propagating cells (AaPC) preloaded with anti-CD3 antibodies to prepare them for genetic modification, with lower AaPC ratios favoring a higher proportion of CD8+ and central memory T-cells. [358] These RNA-modified T-cells, while producing lower cytokine levels than DNA-modified counterparts, maintained comparable cytolytic activity. [358] However, the transient CAR expression, influenced by cytokine and antigen stimulation, necessitates further optimization to sustain their antitumor effects in solid tumor contexts. [358]

In a pilot trial, the efficacy and safety of nonviral RNA-electroporated anti-CD19 CAR T-cells (CART19) were evaluated in five patients with r/r classical Hodgkin lymphoma (cHL). [359] This approach aimed to indirectly target Hodgkin and Reed-Sternberg (HRS) cells, which lack CD19 expression, by eradicating CD19+ B-cells within the tumor microenvironment (TME) and putative circulating CD19+ HRS (Hodgkin and Reed–Sternberg) clonotypic cells. [359] Nonviral RNA CART19 cells were employed to mitigate toxicity, as their CAR expression is transient compared to the more persistent expression that is observed with viral vector-transduced CART19 cells. [359] Manufacturing of RNA CART19 was successful for all five patients, and four received the protocol-specified infusion dose; the treatment was well-tolerated, with no severe toxicities reported. [359] While responses were observed in this first clinical trial utilizing nonviral RNA CART19 in cHL, they were transient, highlighting the limited durability of the therapeutic effect. [359]

Furthermore, this therapy has also been employed in leukemia cases. In a pilot study, seven patients with relapsed/refractory acute myeloid leukemia (r/r-AML) were enrolled to evaluate the safety and feasibility of RNA-electroporated anti-CD123 CAR T-cells (CART123). [360] Among these, manufacturing was successful for six patients, though only 14 of the planned 24 doses were produced, with a median manufacturing time of 50 days. [360] No treatment-related deaths or significant vascular, neurological, or hematological toxicities occurred, although all infusions were accompanied by fever, and CRS was observed in nearly all cases, with severe CRS (grades 3–4) requiring tocilizumab in two patients, but episodes resolved within 24 hours, and a mild increase in IL-6 levels was noted. [360] Despite these biological effects, CART123 cells demonstrated limited bioactivity, with only minimal and transient detection in peripheral blood and no expansion or presence in the bone marrow; consequently, no reduction in CD123-expressing cells was observed, and all treated patients experienced disease progression before day 28. [360] The trial was even terminated early due to lack of efficacy, highlighting challenges related to manufacturing sufficient doses and the poor persistence of RNA-electroporated CAR T-cells. [360] The authors proposed exploring lentivirally transduced CART123 cells derived from healthy donors, combined with CAR T-cell depletion and allogeneic stem cell transplantation, as a potential strategy to address these limitations. [360]

8. Discussion

By systematically synthesizing and critically appraising the experimental evidence surrounding CAR T-cell therapy—while judiciously restraining the corpus of consulted publications to preserve analytical depth, in spite of the vastness of findings associated with CAR T-cell therapy—the present literature survey has elucidated its transformative promise beyond hematological malignancies, noting persistent obstacles to surmount, and thus contributing to the orientation of future research and biomedical innovation toward the circumvention of these impediments in cellular immunotherapy. In effect, beyond its clinical efficacy, CAR T-cell therapy elicits far-reaching implications for both medical practice and patient care, warranting a nuanced appraisal of its extant limitations, ongoing refinements, and place within the oncological research landscape.

8.1 Development Implications

Leukemia treatment has traditionally relied on various therapies—including chemotherapy, radiation therapy, stem cell therapy, and hematopoietic stem cell transplantation—that each present limitations such as toxicity, relapse risk, and donor dependency. CAR T-cell therapy offers a transformative alternative by engineering T-cells to specifically recognize, target, and eliminate malignant cells, leading to remarkable remission rates in certain leukemias, surpassing other medicinal techniques in certain aspects, a fortiori when combined with complementary procedures and/or molecules. Nonetheless, drawbacks to overcome encompass toxicities, on-target off-tumor effects, antigen escape, tumor microenvironment inhibition, manufacturing time, elevated expenditures, and limited efficacy in solid tumors.

8.2 Deficiencies and Ameliorations

Although CAR T-cell therapy presents several limitations, each has become the focus of targeted refinements aimed at overcoming its challenges to advance clinical efficacy.

First, CAR T-cell therapy often entails toxicities, such as CRS, ICANS, or cytopenia, which can be life-threatening in some cases. Their management is based on severity and includes corticosteroids, diagnostic testing, supportive care, monoclonal antibodies, and ICU entrance. [72] Cardiac and pulmonary complications can ensue as well, necessitating additional treatments like pericardiocentesis and tocilizumab to prevent cardiac tamponade, due to acute pericardial effusion. [111,112] IVT mRNA CAR T-cells, on the other hand, provide transient CAR expression after *in vitro* transcription (IVT) of mRNA, limiting prolonged immune activation wherefrom severe toxicities emerge. [349] Furthermore, on-target-off-tumor effects are caused by the attack of normal tissues by CAR T-cells that affect tumors with the same antigens; an approach to preempt this disruption comprised the targeting of tumor-restricted post-translational modifications—such as TAG72, B7-H3, MUC1, and MUC16—that are not found on regular tissues. [25]

Second, antigen escape has represented a barrier to the sustained efficacy of CAR T-cells, involving the loss or downregulation of targeted cancer antigens. Moreover, this factor heightens the risk of relapse in certain cancer conditions. Solutions that have been tested to combat this challenge include dual CAR cell designs—which target two different antigens to enhance chances of presenting the suitable antigen—and tandem CAR configurations, endowed with two antigen-binding domains, permitting precision and variety in their attachment compatibility with cancer cells. [25] For example, CD19/CD22–, CD19/BCMA–, and BCMA/CD19–CAR T-cells revealed certain accomplishments, although laboratory research is required to expand the experimentation and validation of such treatments.

Third, tumor microenvironment presents obstacles to the efficacy of the therapy, due to immunoregulatory factors that impair CAR T-cell function and persistence. They encompass immunosuppressive cells, such as myeloid-derived suppressor cells, tumor-

associated macrophages, and regulatory T-cells; immune checkpoint molecules, like PD-1/PD-L1, CTLA-4, and TIM-3; and inhibitory cytokines, including TGF-β and IL-10. [25] Combination therapies incorporating immune checkpoint inhibitors have therefore been proposed to enhance CAR T-cell proliferation and survival. [18] CAR-transduced natural killer cells, which naturally bypass inhibitory checkpoint signals in the TME, have also been tried. [259] Moreover, physical barriers such as the tumor stroma and the extracellular matrix warranted chemokine receptor–engineered, fibroblast activation protein–targeted, and heparanase-expressing CAR T-cells, *inter alia*, to improve cell migration and penetration into tumors. [120,124,349]

Fourth, the time-intensive and costly manufacturing process of CAR T-cells poses challenges for accessibility and scalability. Indeed, the two first FDA-approved CAR T-cell therapies cost $475,000 and $373,000 per patient, respectively, without accounting for the price of side effects management, which can exceed $547,000. [216] Consequently, allogeneic CAR T-cell therapies have been conceived; they offer protection without patient specificity, thereby embodying a propitious, logistically practical approach, despite remaining compatibility and toxicity impediments. [238] Also referred to as off-the-shelf, they could enable the avoidance of weeks of cell genetic manipulation and multiplication before CAR T-cell transplant and the reduction in costs associated with patient-specific manufacture, as their cells come from healthy donors providing patients with anteriorly prepared treatments. On the other hand, *in vivo* engineering of CAR T-cells and nanobodies-based CAR constructs constitute potential technologies to simplify the fabrication methodology.

Fifth, despite holding promises in hematologic malignancies, CAR T-cells face significant limitations in solid tumors, in part due to the hostile tumor microenvironment, physical barriers to T-cell infiltration, and antigen heterogeneity. Hence, a multitude of trials have been performed to examine the potential of certain antigens—including CD19, CD22, BCMA, EFGR, MSLN, HER2, CD133, Claudin 18.2, IL-13Rα2, GD2, ROR1, CEA, MUC1, CD70, and PSMA—to serve as foundations of CAR T-cell hostility in various cancers, demonstrating the feasibility of the application of certain CAR T-cells in non-hematological tumors. Other CAR constructs, including CAR macrophages, have also been tested, exhibiting the ability to infiltrate dense tumorigenic tissues. [347] The modulation of cytokine signaling pathways and transcription factors have also been investigated to hone activation and differentiation of CAR T-cells, respectively. [298]

8.3 Research Progression

Ongoing advancements endeavor to enhance CAR T-cell persistence, mitigate adverse effects, and extend its leukemic success to the broader spectrum of oncology, as demonstrated by preclinical and clinical efficacy in targeting tumor-associated antigens across various malignancies, including refractory cases. In essence, the encouragement of hematologic potency and safety outcomes of this novel therapy substantiates aspirations to extend its application to numerous cancers, dynamically participating in the multinational campaign to control cancer. In conclusion, CAR T-cell therapy embodies a promising panacea against cancerogenic diseases, meriting continued research and clinical trials to counteract extant deficiencies, optimize

effectiveness, and broaden its spectrum of target-specific application, in an enterprise to potentiate the pervasive and dependable medical deployment thereof.

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