WHITEPAPER

CAR T Therapy:

Overcoming Limitations and Reshaping Treatment of Hematologic Malignancies

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Introduction

Adoptive cell therapy (ACT) is a rapidly advancing form of targeted cancer immunotherapy that has emerged as a transformative strategy in the field. It involves generating or expanding tumor-specific immune cells—primarily T lymphocytes— outside the body (ex vivo) before reinfusing them into the patient. This approach typically includes the intravenous transfer of either tumor-infiltrating or genetically modified immune cells derived from peripheral blood, aimed at eliciting a potent anti-tumor response.

ACT encompasses several distinct approaches, each defined by the source and method of T-cell modification. Chimeric antigen receptor (CAR) T-cells are genetically engineered to express a synthetic receptor that targets a tumor-specific cell-surface protein. Tumor-infiltrating lymphocytes (TILs), on the other hand, are naturally occurring T-cells extracted and expanded ex vivo from a resected tumor. T-cell receptor (TCR) T-cells are modified to express a natural TCR capable of recognizing intracellular tumor antigens presented on major histocompatibility complex (MHC) molecules.

One of the most transformative breakthroughs in recent years has been CAR T-Cell therapy. Since receiving its first FDA approval in 2017, CAR T-cell therapy has demonstrated promising efficacy against specific hematologic malignancies, targeting CD19 and B cell maturation antigen (BCMA) and offering a potentially curative treatment option for patients with advanced B-cell acute lymphoblastic leukemia (B-ALL), various forms of B-cell lymphoma including diffuse large B-cell lymphoma (DLCBL), and multiple myeloma (MM). This immunotherapeutic strategy based on customizing therapy to individual tumor biology and immune profiles, represents a substantial advancement in precision oncology, offering a promising approach for targeting malignancies that have exhibited resistance to standard therapies.

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Current Landscape of CAR T Therapy in Hematologic Malignancies

Mechanism of CAR T-Cell Therapy

Chimeric antigen receptor (CAR) is a synthetic transmembrane protein expressed on the surface of genetically engineered T lymphocytes that binds to a target cell surface antigen on malignant cells independently of MHC and redirects cytotoxic immune cells to target cells expressing that antigen.

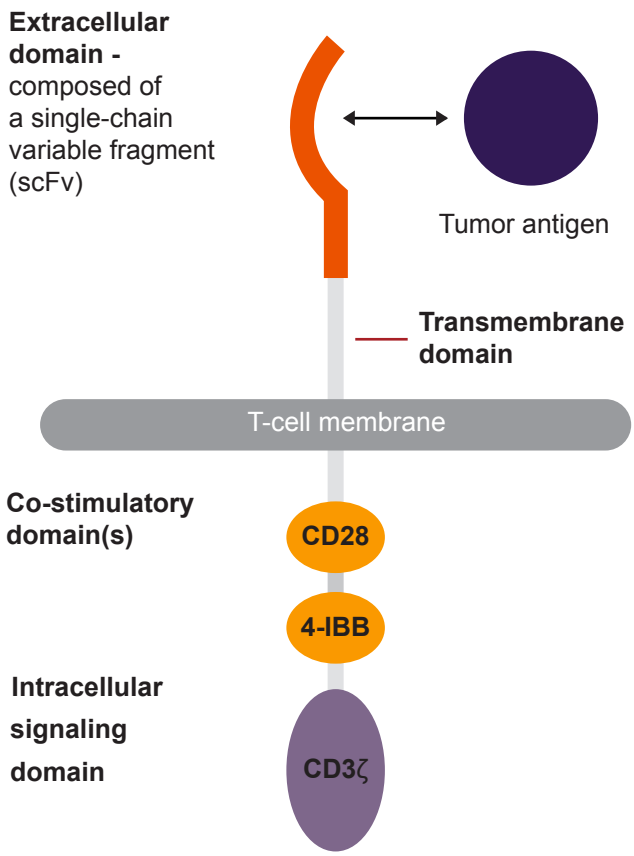
CAR consists of several functional domains:

- **Extracellular domain:** A single-chain variable fragment (ScFV) of immunoglobulin (antibody) that recognizes and binds the tumor antigen
- **Transmembrane domain:** Anchors the CAR in the T-cell membrane and connects extracellular and intracellular regions
- **Costimulatory domain(s):** Usually CD28, 4–1BB, OX40, etc. which enhance T-cell activation and persistence
- **Intracellular signaling domain:** Usually a CD3ζ T-cell activation domain which triggers signaling when the CAR engages the targeted ligand (the tumor antigen) that activates the T-cell

CAR T-cell therapy is an example of remarkable potential of synthetic biology in clinical oncology. The functional behavior of CAR T-cells in vivo is fundamentally determined by how these cells are engineered ex vivo. Because CAR T-cells are living therapeutics, the design of the chimeric receptor—specifically, the signaling domains and antigen-targeting elements—influences key cellular attributes such as cytotoxicity, persistence, and differentiation into specific T-cell phenotypes.

The products that are currently approved and used in clinical practice are autologous CAR T-cells. Therapy begins with leukapheresis to collect patient's own peripheral blood mononuclear cells. T-cells then are separated, stimulated, transduced with viral or non-viral vectors to express the CAR molecule, and expanded with or without cytokines over a period of 7–10 days before reinfusion into the patient. Prior to administration of the CAR T-cells, patients receive a low dose lymphodepleting chemotherapy regimen to create a homeostatic environment for the adoptively transfused CAR T-cells.

Structure of a Chimeric Antigen Receptor (CAR): Domains Involved in T-Cell Activation



The regimen typically consists of cyclophosphamide and fludarabine with the aim of depleting immunosuppressive cell populations, such as regulatory T-cells and myeloid-derived suppressor cells, which may interfere with the activity of the infused cells.

Additionally, lymphodepletion enhances the systemic availability of key homeostatic cytokines, including interleukin-7 (IL-7) and interleukin-15 (IL-15), which support the proliferation, persistence, and functional activity of infused CAR T-cells.

Compared to conventional chemotherapy, T-cell–based immunotherapies offer the advantages of high antigen specificity and potentially durable clinical responses. The functional specificity of T-cells is governed by their antigen-recognition receptors and the nature of the target antigen. In the context of CAR T-cell therapies, the selection of an appropriate target antigen is a critical determinant of both safety and therapeutic efficacy.

As outlined by Morris et al., an ideal tumor-associated antigen for CAR-T targeting should meet the following criteria:

- Expression on the extracellular surface of tumor cells (readily accessible)
- Uniform and consistent expression across all malignant cells
- Resistance to antigen loss or downregulation (to minimize the risk of immune escape)
- Expression on malignant stem cells (to prevent relapse)
- Absence from normal tissues, or limited expression restricted to nonessential tissues (to minimize off-tumor toxicity)

Careful antigen selection remains central in the development of safe and effective CAR T-cell therapies. This principle applies across both hematologic malignancies and solid tumors.

CAR T Therapy Successes in Blood Cancer Treatments

The unprecedented success in clinical trials that led to regulatory approvals of CAR T-cell therapy was observed in studies investigating CD19-directed CAR T-cells (CAR T19) in B-cell malignancies. This success was attributable not only due to the widespread expression of CD19 across various B-cell neoplasms—including chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and mantle cell lymphoma (MCL)—but also to the biological properties of CD19 as a target. CD19 expression is largely confined to mature B lymphocytes and their precursors, while notably absent on hematopoietic stem cells.

This lineage-restricted expression profile minimizes off-tumor toxicity and supports hematologic recovery post-therapy, making CD19 an ideal target antigen for CAR T-cell design.

Currently, six CAR T-cell products are approved by the US Food and Drug Administration (FDA) and commercially available:

- **Tisagenlecleucel** (for pediatric young adult patients with R/R B-cell precursor ALL, adult patients with R/R DLBCL and R/R FL)
- **Axicabtagene Ciloleucel** (for adult patients with R/R large B-cell Lymphoma (LBCL), DLBCL and R/R FL)
- **Brexucabtagene Autoleucel** (for adults with R/R MCL or R/R B-cell precursor ALL)
- **Lisocabtagene Maraleucel** (for adults with R/R LBCL, high grade B-cell Lymphoma)
- **Idecabtagene Vicleucel** (for R/R MM)
- **Ciltacabtagene Autoleucel** (for R/R MM).

Clinical trials continue to demonstrate the efficacy of CAR T therapy across various subtypes of blood cancers, and its integration into earlier lines of treatment is being actively explored. While initial approvals focused on relapsed/refractory settings, newer studies are pushing the boundaries into frontline therapy and even consolidation after stem cell transplantation.

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Limitations of Current CAR T Therapies in Hematologic Malignancies

Despite promising initial response rates achieved with currently approved CAR T-cell therapies, follow-up studies have demonstrated high rates of post-treatment relapse, as well as a continued incidence of significant treatment-related toxicities. In both pediatric and adult patients with elapsed/refractory ALL, treatment with CAR T19 cell therapy has achieved initial complete response rates of up to 80–90%. Nevertheless, nearly 50% of patients experience relapse after the first year post-treatment. The durability of response is even lower in patients with lymphoma or CLL.

Reduced Treatment Efficacy and Durability

Tumor cells develop strategies to evade CAR T-cell therapy, often with the support of a highly immunosuppressive and protective tumor microenvironment.

Antigen loss represents a sophisticated mechanism by which cancer cells evade the selective pressure imposed by targeted immunotherapy. Mechanisms of immune escape and CAR T-cell resistance include antigen downregulation or dim expression, complete target loss, point mutations in CD19, and persistent CD19 loss without identifiable genomic alterations—suggesting a role for epigenetic mechanisms in antigen loss.

Apart from antigen escape, immune exhaustion and tumor microenvironment (TME)-mediated upregulation of anti-apoptotic pathways have been identified as major mechanisms of tumor escape from CAR T-cell therapy.

CAR T-cell exhaustion: Chronic viral infection is a well-established cause of T-cell exhaustion which leads to reduced cytokine production (e.g. IFN-γ, IL-2, TNF-α), impaired proliferation and memory, upregulation of inhibitory receptors (e.g., PD-1, CTLA-4, TIM-3, LAG-3) and reduced cytotoxicity. This phenomenon is similar to what we observe in CAR T-cell exhaustion, where prolonged antigen exposure and immunosuppressive environments drive T-cells into a hypo-functional state.

TME-induced T-cell inhibition: The TME is composed of endothelial cells, extracellular matrix (ECM), myeloid-derived suppressor cells (MDSCs, tumor-associated macrophages (TAMs), regulatory T-cells (Tregs), and cancer-associated fibroblasts (CAFs), which interact closely with tumor cells and contribute to tumorigensis. The TME can greatly limit the efficacy of CAR T-cell therapy by creating multiple barriers to effective immune responses. These include metabolic competition, physical obstacles to T-cell infiltration, and the presence of immunosuppressive cytokines and cell populations. Metabolic reprogramming within the TME is now recognized as a hallmark of tumor progression. In particular, competition for key nutrients, such as glucose, between CAR T-cells and tumor cells impairs TCR/CAR signaling and effector functions, ultimately compromising the therapeutic activity of CAR T-cells.

Acute and Long-Term Toxicities

After administration, CAR T-cells can proliferate in the recipient up to 1000-fold causing elevated cytokine levels such as IL-6, IFN-γ, TNF-α, IL-1β and GM-CSF among others. High levels of these potent proinflammatory immune mediators in circulation can cause **cytokine release syndrome (CRS)** which manifests as fever, hypotension or hypoxia and can be life threatening when not diagnosed and treated promptly.

CAR T-cells can cross the blood-brain barrier, particularly in hematologic malignancies involving the CNS. This has important therapeutic implications but also contributes to developing reversible **immune effector cell-associated neurotoxicity syndrome (ICANS)** – a serious complication, sometimes requiring intensive care support.

In the longer term, patients may experience **prolonged myelosuppression** – defined as high grade neutropenia, anemia or thrombocytopenia lasting more than 30 days. According to different sources, it affects up to 22% - 55% of patients and contributes to severe infections.

In terms of B-cell malignancies, anti-CD19 CAR T-cells may cause **B-cell aplasia with resulting hypogammaglobulinemia** leading to an increased risk of infections necessitating close monitoring and supportive care.

Limited Access to Therapy

The complex, individualized manufacturing process of autologous CAR T-cell products, combined with high production costs and the need for specialized infrastructure, significantly restricts access. This is particularly challenging in low- and middle-income countries and even in certain high-resource settings due to logistical delays and reimbursement limitations.

Rare Risk of Therapy-Related Myeloid Malignancies

Although rare, there have been emerging reports of secondary myeloid malignancies affecting up to 2%-10% of patients within 5 years of CAR T-cell therapy. These include therapy-related myelodysplastic syndrome or acute myeloid leukemia. The etiology remains unclear but may be related to prior cytotoxic therapies, underlying genomic instability, or insertional mutagenesis during cell manufacturing.

Overcoming Limitations of CAR T Therapy: Innovative Solutions

To address the challenges, the field is advancing rapidly in multiple directions.

Advances in Engineering and Targeting

Several strategies are currently being explored to enhance the efficacy of CAR T-cell therapies in hematological malignancies. One major area of focus involves **optimizing costimulatory domains beyond the traditional CD28 or 4-1BB.** For example, inducible systems such as MyD88/CD40 are being investigated to optimize CAR T-cell activation and persistence.

Another promising approach includes **the use of "TRUCKs" (T-cells Redirected for Universal Cytokine-mediated Killing)**, which are engineered to express cytokines such as IL-15 or IL-18. These cytokines may help prevent CAR T-cell exhaustion and improve their infiltration into tumor tissue - a concept especially relevant in solid tumors but potentially beneficial in lymphomas as well.

On the manufacturing front, there is growing interest **in allogeneic, or "off-the-shelf," CAR T-cell products.** These include gene-edited T-cells engineered to resist graft-versus-host disease (GvHD) while maintaining anti-tumor potency. Additionally, faster manufacturing protocols have been shown to enrich for less differentiated T-cell subsets, such as T stem-cell memory and central memory phenotypes, which are associated with improved in vivo persistence and expansion. In contrast, manufacturing processes that yield terminally differentiated effector T-cells may result in high initial efficacy but reduced durability.

Innovations Addressing CAR T Limitations

- **Costimulatory Enhancements**
  Exploring inducible domains (e.g., MyD88/CD40) to improve persistence
- **Cytokine-Enhanced Car Ts (TRUCKS)**
  Engineered to secrete IL-15 or IL-18 to reduce exhaustion and boost infiltration
- **Off-The-Shelf Car T Products**
  Gene-edited allogeneic cells and iPCS-derived CAR Ts aim to improve scalability
- **In Vivo Car T Generation**
  Direct delivery via mRNA or viral vectors—promising but still early-stage
- **Expanded Antigen Targets**
  New targets like CD70, CD123, FCRL5 under investigation to improve specificity
- **Safety & Durability Focus**
  Improved manufacturing and longterm follow-up to manage toxicity and enhance persistence

Efforts are also underway in the pluripotent stem cell (iPSC) space to generate allogeneic CAR T-cells. Furthermore, **in vivo CAR T-cell generation**, such as through mRNA-based or viral vector delivery, represents a promising and seemingly cost-effective frontier, although this field remains in early stages compared to established ex-vivo platforms. There are a few potential challenges that come with this innovative approach to CAR T-cell generation. First, engineering highly-specific vectors that can selectively deliver CAR constructs to T-cells in the patient remains technically demanding. The CAR must be delivered selectively into the T lymphocytes of the patient.

Second, unlike conventional CAR T-cell therapy, in vivo CAR T-cell approaches cannot rely on lymphodepletion—a step that typically creates space for CAR T-cells to expand. Using lymphodepletion here would deplete the very T-cells required for transduction. It remains to be seen whether sufficient CAR T-cell

expansion can occur in the absence of conditioning to achieve therapeutic efficacy.

Finally, key safety concerns—such as potential immune responses to vector components and the theoretical risk of insertional oncogenesis from genome-integrating vectors—will require long-term follow-up to fully assess and characterize their impact.

Beyond delivery strategies, **antigen targeting** remains another major focus of innovation. To address the challenge of a limited number of novel target antigens, new candidates such as CD70, CD123, and Fc receptor-like protein 5 (FCRL5) are currently under investigation. CD70 has been tested in AML and mantle cell lymphoma (MCL), CD123 and CD33 in AML, and FCRL5 in multiple myeloma (MM), often in combination with BCMA-targeted approaches.

Altogether, these innovations aim to improve the durability, safety, and applicability of CAR T-cell therapies in hematologic cancers and potentially solid tumors. In AML however, due to biological heterogeneity of the disease and the presence of target antigens on normal hematopoietic cells, toxicity remains a significant concern.

Improving Patient Selection

Biomarkers are becoming pivotal in determining which patients will benefit most from CAR T therapy. Genetic and immune profiling help identify responders, predict toxicities, and guide personalized treatment decisions. Precision medicine tools can stratify patients based on tumor antigen expression, immune cell fitness, and microenvironment characteristics. This biomarker-driven approach helps maximize efficacy while minimizing unnecessary risks.

Reducing Toxicity

Numerous improved CAR T-cell designs and treatment strategies have been proposed to mitigate CRS/neurotoxicity by avoiding excessive cytokine release. Pharmacologic strategies to mitigate acute toxicities associated with CAR T-cell therapy are actively evolving. While agents such as anticytokine therapies (e.g., siltuximab, anakinra, emapalumab) and small molecule inhibitors (e.g., ibrutinib, dasatinib, itacitinib) have been explored, their efficacy in managing CAR T-cell– related toxicities remains unproven.

Strategies to mitigate toxicities include:

- Early intervention with agents like tocilizumab and corticosteroids
- Modified CAR constructs with safety switches or “on/off” controls
- Supportive care protocols tailored to each patient’s risk profile
- Some innovations involve incorporating suicide genes or dose-modulating switches in CAR T-cells, allowing physicians to deactivate the therapy if toxicities emerge.

The Future of CAR T Therapy in Hematologic Malignancies

In the immuno-oncology landscape, CAR T-cell therapy is an example of the future of cancer care: targeted, adaptive, and deeply personalized. With ongoing optimization of

manufacturing protocols and advances in off-the-shelf CAR T-cell platforms, improvements in precision, manageable toxicity, and cost-effectiveness are anticipated. These developments are expected to enhance accessiblity and reduce logistical barriers, ultimately broadening patient access to CAR T-cell therapies.

Earlier intervention, possibly even as frontline therapy in high-risk patients, is under investigation. Combination strategies with immune checkpoint inhibitors, monoclonal antibodies, or kinase inhibitors may overcome resistance and enhance efficacy.

CAR T-cell therapy is increasingly establishing itself as a central component of modern immuno-oncology, complementing other emerging modalities such as bispecific antibodies, tumor vaccines, and natural killer (NK) cell-based therapies. The incorporation of artificial intelligence and machine learning into CAR T-cell development and patient stratification brings another promise for advancing personalized treatment approaches and optimizing clinical outcomes.

ABOUT THE AUTHOR

With over 16 years of experience in hematology, oncology, and stem cell transplantation, Dr. Oksana Fabri is a recognized expert in the development of advanced cell therapies. She brings deep clinical and strategic insight into CAR T-cell therapy, with a particular focus on overcoming treatment limitations and accelerating innovation. Dr. Fabri's extensive understanding of current treatment modalities, cutting-edge therapies, and evolving regulatory landscapes further strengthens her ability to guide development strategies. Her leadership spans early-phase trial design through execution, where she provides high-level medical oversight and ensures scientific rigor, regulatory alignment, and patient-centered outcomes.

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