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Mini review: Advances and challenges in CAR-T cell therapy: from early chimeric antigen receptors to future frontiers in oncology

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Cell therapy utilizing chimeric antigen receptors (CARs) in conjunction with immune cells, primarily T lymphocytes, is known as CAR-T cell therapy. This innovative approach is revolutionizing the landscape of oncohaematology by precisely targeting specific antigens for elimination. However, despite its promising prospects, CAR-T therapy presents several challenges, including a notable rate of disease relapse, intricate pathologies impeding widespread adoption, prolonged manufacturing timelines, and substantial costs. Looking forward, ongoing research and progress aim to address these challenges to mitigate these constraints, underlining the continuous efforts to enhance the efficacy and accessibility of this transformative therapy

KEYWORDS

car t therapy, CAR T development, FDA approval, limitations and barriers, immunohematology

1 Introduction

CAR-T therapy has emerged as a promising strategy in oncohematology, precisely steering immune responses toward specific targets for effective treatment. However, despite its immense potential, several challenges, such as disease recurrence rates, intricate pathophysiology of target neoplasms, prolonged manufacturing times, and high costs, underscore the imperative to persevere in research and innovation within this transformative field.

2 CAR-T development: early steps and progress

The origins of CAR-T cell therapy can be traced back to 1987 when Kuwana et al. first introduced the concept of HLA-independent antigen recognition through a chimeric receptor (1, 2). In the early 1990s, Arthur Weiss of the University of California (San Francisco) reported that chimeric receptors containing the intracellular signaling domain of CD3ζ, activated T-cell signaling. This work resulted in the binding of the intracellular domains of CD3ζ to the chimeric receptors to transfer the activation signaling to the T cell (3).

Until then, the chimeric receptor (TCR) was composed of an antibody's variable region domain and the TCR's constant region domain. However, transduction exhibited low efficiency due to the necessity of introducing two genes into the same lymphocyte using retroviral vectors to encode the chimeric receptor. In response to this issue, in 1993, Zelig Eshhar, Gideon Gross, and colleagues published the structure of a novel chimeric receptor composed of a single-stranded variable fragment (scFv) derived from an antibody bound to the CD3ζ intracellular complex (4). Both immunologists redirected this research towards the field of oncology (5, 6).

In the mid-1990s, significant progress was made by developing the first clinical trial based on CD8+ T cells modified to express a chimeric receptor structure against a surface antigen present in HIV-infected cells. This work demonstrated that the modified CD8 + T cells effectively identified and eliminated infected cells, showing a lytic capacity of 50-60%. Also, it was described that these ScFv-based receptors had a Major Histocompatibility Complex (MHC)-independent recognition, in contrast to initial cTCRs, suggesting the potential development of universal cell therapy (7). Thus, was born the first T cell designed with the chimeric receptor structure of the products currently marketed, called "T body" (8).

The hypothesis of linking the chimeric receptor to other cells of the immune system such as Natural Killer (NK) lymphocytes was proposed in 1993 to explore its HLA-independent lytic activity (4). This response is mediated by the killer immunoglobulin receptor (KIR) and triggered by detecting reduced inhibitory markers or stress markers on the surface of abnormal cells (9). However, currently, compared to CAR-T cells, CAR-NK cell-based adoptive immunotherapy is generally limited by substantial restrictions prior to large-scale practical implementation. These limitations encompass inadequate proliferation and *in vivo* activation capacity or limited persistence (10, 11).

3 Enhancing CAR-T cell therapy: second and third generations

In response to the non-sustained activation observed in first-generation CAR-T cells, research from 1995 to 1998 delved into the role of co-stimulatory molecules to enhance lymphocyte activation and CAR-T cell persistence. This concept is grounded in the extrapolation of the theory of dual activation signals observed in T-cell antigenic recognition.

During this period, the research published by Sadelain et al. in 1998 showed evidence that CD28 signaling had a substantial impact on the enhanced survival and proliferation of CAR-T cells and an increase of pro-inflammatory cytokines production when they recognized the target. These findings had significant implications for the potential effectiveness of T-cell therapies (12).

Margo Roberts and Helene Finney were the first to develop a 2nd generation CAR-T therapy (CD28-CARs) and achieved a patent in 1997 (Margo Robert's patent was filed in February 1995 and Helene Finney's patent was filed in December 1996) (13–15).

In the 2000s, several research was led to study different costimulatory molecules to improve their antileukemic effect. CAR constructs began to be developed with the 4-1BB molecule (CD137), which appeared to provide CAR-T cells with great persistence in the body (16, 17). These studies with second-generation CAR-T cells (CD137 and CD28) focused on targeting the CD19 antigen, which is specific to the B-lymphoid lineage, especially CLL (18) and ALL (16, 17).

In 2005, considering the studies published on both co-stimulatory molecules, Brenner et al. studied third-generation CAR-T consisting of two costimulatory molecules, CD28 and OX40 to improve antileukemic efficiency (19). However, these studies did not demonstrate a therapeutic benefit, but warnings regarding its use have emerged (20) (Figure 1).

4 Bridging the lab to clinical practice: FDA approvals and clinical application

In 2010, a pivotal moment arrived when Steven Rosenberg at the NCI and Carl June and David Porter at the University of Pennsylvania administered CART-19 to a patient with chronic lymphocytic leukemia (CLL), marking a turning point in CAR-T therapy's trajectory (22).

This therapy underwent a revolution with the case of Emily Whitehead, a 7-year-old diagnosed with acute lymphoblastic leukemia (ALL) refractory to standard treatments. She participated in a phase 1 clinical trial (NCT01626495), receiving an infusion of autologous CTL019, a CAR-T therapy with 4-1BB as a co-stimulatory molecule, similar to the previous studies published in CLL (23). An example of the influence of this case is the foundation created by Emily Whitehead's family to encourage donations to support oncology research for children's treatment (24).

Between 2013 and 2014, the first clinical trial results were published and it was not until 2017 that the Food and Drug Administration (FDA) approved Kymriah (tisagenlecleucel) (25) for use in relapsed or refractory ALL after two prior lines for children and young adults up to 25 years old, and Yescarta (axicabtagene ciloleucel) for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of treatment. The latter study included DLBCL, primary mediastinal lymphoma, high-grade B lymphoma, and DLBCL arising from follicular lymphoma. Axicabtagene ciloleucel is an anti-CD19 CAR-T like tisagenlecleucel but differs in the co-stimulatory molecule as it is made up of CD28 rather than 4-1BB (26, 27).

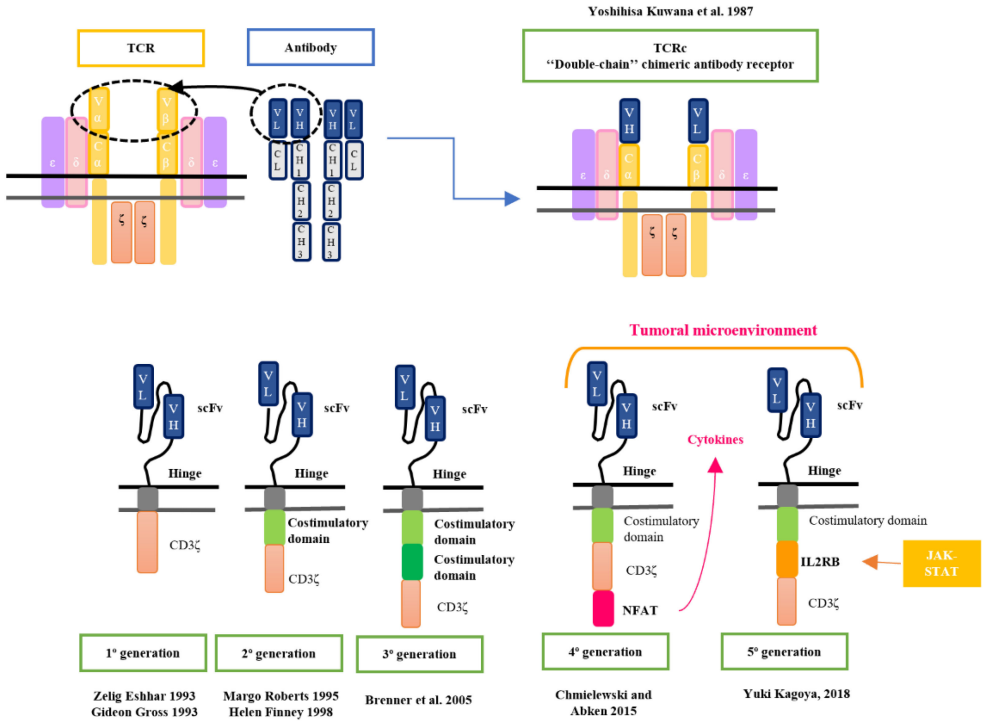
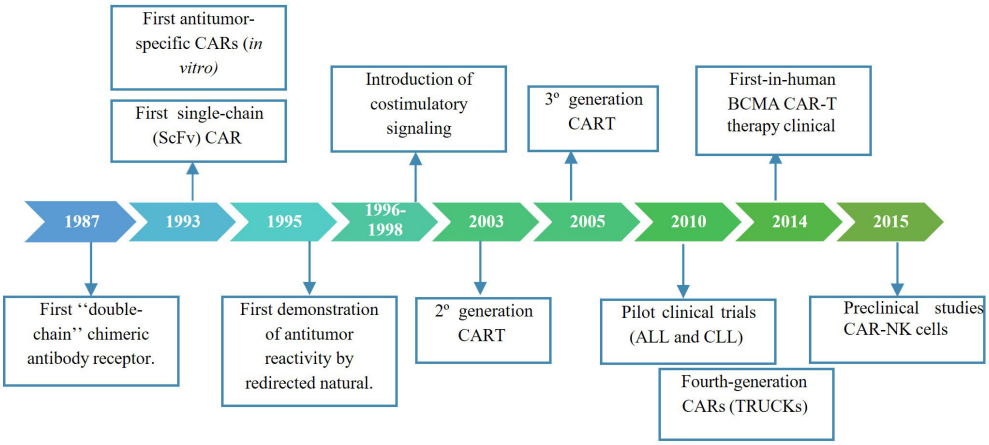


FIGURE 1
Evolution of the chimeric receptor structure (1, 8, 21).



YEAR	PRODUCT	PATHOLOGY	CLINICAL TRIAL
2017	Kymriah®	ALL	ELIANA
	Yescarta®	NHL	Zuma-1
2018	Kymriah®	NHL	JULIET
2020	Tecartus®	MCL	Zuma-2
2021	Breyanzi®	NHL	Transcend
	Abecma®	MM	KarMMa
	Yescarta®	FL	Zuma-5
2022	Carvykti®	MM	Cartitude-1
	Kymriah®	FL	ELARA

FIGURE 2
(A) Key preclinical events in the history of the development of CAR-T therapy. **(B)** The First FDA Approvals for CAR-T Cell Therapies. ALL, acute lymphoblastic leukaemia; NHL, non-Hodgkin lymphoma; ML, Mantle lymphoma; MM, multiple myeloma; FL, follicular lymphoma.

Since that time until now, various regulatory agencies such as the FDA or the European Medicine Agency (EMA) have approved different products for oncohematological pathologies. For instance, Tecartus (brexucabtagene autoleucel) has been approved for mantle cell lymphoma treatment (28), Breyanzi (lisocabtagene maraleucel) for DLBCL treatment (29) or Abecma (Idecabtagene vicleucel) (30) and Carvykti (ciltacabtagene autoleucel) (31) for multiple myeloma treatment. Additionally, tisagenlecleucel and axicabtagene ciloleucel are seeking to expand their indications to follicular lymphoma treatment through the ELARA (32) and ZUMA-5 (33) clinical trials, respectively. These products have been approved for these pathologies in the relapsed or refractory setting after two or more lines of treatment (Figure 2).

Considering the promising results, several pivotal trials of CAR-T products with extensive experience have been published, aiming to administer them in earlier lines of therapy. The ZUMA-7 (34) and ZUMA-12 (35) trials have reported results of administering axicabtagene ciloleucel in the 2nd and 1st line of treatment, respectively.

However, currently, several limitations related to this therapy have been described.

5 Limitations of CAR-T therapy: an in-depth analysis and exploring solutions

5.1 "On-target/Off tumor" side effect

In the first place, the coexistence of the target antigen in the tumor and healthy cells results in an "on-target/off-tumor" activity. In the case of B cell lymphoid pathologies, CART anti-CD19 cells cause an aplasia in this line, leading to a break in the humoral immune system and, consequently, hypogammaglobulinemia. This condition increases the risk of infection due to reduced levels of antibodies but can be resolved through intravenous immunoglobulin supplementation and antiviral, antibacterial, and antifungal prophylaxis (36, 37), although an increase in fungal infection has not been documented in these patients (38).

An obstacle in CAR-T cell development for myeloid and T-cell malignancies is the concern surrounding "on-target/off-tumor" toxicity. This toxicity in the case of these pathologies produces aplasia in granulocytes and T-cell lineage. However, managing this aplasia presents a significant challenge as effective strategies to mitigate infectious risks remain unidentified. Allogeneic transplantation following CAR-T cell therapy stands as the most explored option (39, 40).

One of the barriers to overcome in T-lymphoid neoplasms is the shared presence of tumor T cells and CAR-T cells, which could lead to CAR-T fratricide at the time of expansion and reduce T cell activity (41, 42). To avoid this, Li et al. designed an anti-CD7 CART, with a built-in command to retain its own CD7 in the endoplasmic reticulum so that it is not expressed on the membrane (39).

5.2 Manufacturing limitations

Currently, the manufacturing process of an autologous CAR-T product takes approximately 15 days (43, 44), although the timeline we handle with the industry from the patient's apheresis to administration is around 30 days (45).

Many groups are studying methods to minimize manufacturing times without compromising anti-tumor activity (46, 47) by reducing the *ex-vivo* phase or developing a universal CAR-T therapy (48, 49).

The development of an allogeneic CAR-T or a universal CAR-T would allow administering the product with greater flexibility, as it would not rely on the patient's apheresis process, and its manufacturing could be initiated in advance.

5.3 Relapse after CAR-T therapy

After CAR-T therapy, disease relapse in the case of ALL or lymphoma can occur in 40-60% of patients at 12 months (50) and the overall survival and event-free survival at 5 years is around 35-45% (51). Among these relapses, 60% still express the target antigen and it is considered that the issue lies in a low persistence or suboptimal functionality of the infused product (25).

5.3.1 Loss of therapeutic efficacy

A decreased functionality or persistence may be caused by exhausted CAR-T cells, defined as more differentiated lymphocytes that exhibit increased expression of cell exhaustion molecules (PD-1, TIM3, LAG-3, TRAIL), making them more susceptible to antigen-induced cell death (AICD) following antigen exposure. Consequently, exhausted T cells experience reduced activity and proliferative capacity, potentially functioning as a detrimental mechanism in the functionality of the CAR-T product (52, 53).

A retrospective study conducted on patients who received CART-19 (both tisagenlecleucel and axicabtagene ciloleucel) reveals that a lower presence of Treg (54) and CAR-T cells predominantly composed of memory T lymphocytes is indicative of a favourable treatment response (55).

Another cause of reduced functionality resulting from an increase in AICD is the high density of CAR on the membrane. This can lead to an increase in tonic signalling and facilitate its elimination (56).

To avoid this kind of relapse there are several projects aimed at selecting less differentiated CAR-T lymphocyte populations (Naïve, Stem Central Memory, and Central Memory) to improve CAR-T persistence (57–59). In addition, allogeneic post-CAR-T transplantation is being considered as a consolidation therapy for patients at higher risk of relapse following this treatment.

5.3.2 Immune evasion

However, around 30-40% of disease relapses present malignant cells that do not express the target antigen and may occur despite detecting the presence of CAR-T cells in peripheral blood. In these cases, there are various hypotheses to explain this mechanism of treatment resistance while being an immune escape from the CART-CD19 attack (41).

One of them is the negative selection that occurred after the CART-CD19 administration. This selection can promote the

survival of cellular subclones exhibiting diminished CD19 expression. These subclones acquire a competitive advantage for survival and proliferation in the presence of CAR-T cells, leading to relapse (60).

Another hypothesis is that the loss of CD19 antigen expression may also be associated with changes in the epigenetic regulation of genes. Alterations in DNA methylation or histone modifications could influence the expression of CD19 and contribute to its reduction or changes in the isoform (61).

To address this issue, dual-targeting CAR-T cells are being developed, capable of recognizing different antigens in the tumor, thus preventing cancer cells from escaping therapy by eliminating one of the target antigens. A well-known approach in this field is the use of CAR-T cells targeting both CD19 and CD22 antigens, providing a more effective strategy for treating malignant diseases and reducing the possibility of treatment resistance or evasion (42, 48).

5.4 Early side effects after administration

Rather than these limitations, there are two commonly described side effects related to this therapy in the literature: cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

The previously described case of Emily Whitehead not only marked a milestone in demonstrating CAR-T efficacy but also represented the first instance of using tocilizumab in the treatment of CRS. This patient experienced severe side effects, primarily a persistent fever with hemodynamic instability. These inflammatory effects were accompanied by significantly elevated IL-6 levels. The patient's condition improved after the administration of tocilizumab (62), an anti-IL6 monoclonal antibody recently approved by the FDA for rheumatoid arthritis (63).

CRS is the most common side effect (6, 64, 65) and affects approximately 80% of patients, with roughly 20% experiencing severe cases (45, 51). This condition results from the release of effector cytokines (IFN-g, TNF-a, IL-2) that can trigger the release of proinflammatory cytokines (IL-1, IL-6, IFN-g, IL-10, and monocyte chemoattractant protein-1). Clinical features are fever, hemodynamic instability, and hypoxemia. The treatment for this clinical condition is protocolized and severity-dependent, with tocilizumab (anti-IL-6 monoclonal antibody) proving to be the most effective option.

The most severe cases of CRS, which can even lead to multiorgan failure, are often confused with Macrophage Activation Syndrome (MAS) due to shared clinical features stemming from hyperinflammation. Unlike CRS, these patients do not respond to Tocilizumab administration and require other treatments such as Anakinra (anti-IL1) or corticosteroids. Therefore, it represents a significant diagnostic challenge, and an early diagnosis of this condition has a notable impact on prognosis.

Its pathophysiology is akin to that described in Hemophagocytic Lymphohistiocytosis, characterized by hemophagocytosis-related features such as hepatomegaly, cytopenia, coagulopathy, and multiorgan failure. In response to this challenge, a proposed name change to "Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis-Like Syndrome" (IEC-HS) has been suggested, acknowledging its resemblance to hemophagocytic lymphohistiocytosis (HLH) (66).

ICANS affects 20-60% of patients and is characterized by neurological symptoms of varying intensity, including confusion, altered speech, dysarthria, emotional lability, seizures, delirium, or changes in consciousness level. The pathophysiology of ICANS is likely due to the release of inflammatory cytokines that increase vascular permeability and endothelial activation, potentially leading to disruption of the blood-brain barrier, resulting in elevated cytokines in the cerebrospinal fluid and even cerebral edema (36).

Several factors that might contribute to the manifestation of clinical conditions associated with CAR-T cell therapy have been identified. One of the most studied factors is the type of CAR-T product infused. Studies on products like Yescarta, Kymriah, and lisocabtagene maraleucel have revealed incidence rates of cytokine release syndrome (CRS) around 92% (26), 60% (67), and 42% (29), respectively. Furthermore, researchers are actively investigating ways to improve the effectiveness of CAR-T therapy and reduce its side effects after the manufacturing process. These studies cover a range of strategies, such as optimizing the dosage of fludarabine or cyclophosphamide (68) for lymphodepletion therapy and exploring the use of CAR-T administered in divided doses, among other innovative approaches. For instance, at the Hospital Clinic in Barcelona (Spain) they've introduced a protocol for administering their academic CAR-T therapy (ARI-002) in two or three divided doses to minimize the risk of side effects (69).

5.5 Solid tumors: microenvironment's role

Developing CAR-T cells for certain diseases like T-ALL, AML, and solid tumors is posing the greatest challenge. The tumor microenvironment has been identified as a significant factor contributing to tumor escape and resistance in solid organ neoplasms. Strategies involving CAR-T cells that can effectively interact with the tumor microenvironment are being explored. These strategies include the development of CAR-T cells capable of producing cytokines (such as IL12 or IL18) or enhancing their proliferation and activation through specific cytokines present in the tumor stroma. These advanced CAR-T cell designs are often referred to as TRUCKs (T cells Redirected for Universal Cytokine Killing) or "fourth-generation CART cells" and "fifth-generation or next-generation CAR-T", respectively (70–72).

Initiated in the 1980s, research in this field gained momentum with promising results from pivotal clinical trials of various CAR-T

cell therapy products in oncohematology. These scientific advancements represent a significant milestone in cancer treatment, potentially leading to more effective and less side effect therapies in the future. However, it's crucial to emphasize that there is still much to explore and investigate to address the current limitations.

Author contributions

The article has been written by CC and reviewed by MCV, CP, JRR-M, and FP. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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