New Strategies in CAR T-Cell Immunotherapy for Patients with Acute Lymphoblastic Leukemia: Investigating the Rise of the Therapeutic Approach

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Novas Estratégias em Imunoterapia com Células CAR-T em Pacientes com Leucemia Linfoblástica Aguda: Investigando a Ascensão da Terapêutica

Nuevas Estrategias en Inmunoterapia con Células CAR-T en Pacientes con Leucemia Linfoblástica Aguda: Investigando el Ascenso de la Terapéutica

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ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) is a hematologic malignancy characterized by uncontrolled proliferation of mutated B- and/or T-cell lymphoblasts, which severely compromises the human body and exhibits high mortality rates. This condition subjects patients to an exhaustive clinical journey, further aggravated by the adverse effects of conventional therapies. In this context, genetically modified T-cells expressing chimeric antigen receptors (CAR) demonstrate significant efficacy in overcoming the challenges of this aggressive disease. **Objective:** To analyze the clinical implications identified in key studies investigating CAR T-cell therapy for ALL treatment. **Method:** Integrative literature review involving the collection of scientific articles from databases including PubMed, SciELO, Periódicos, Scopus, Web of Science, and J-STAGE, from 2000 onward. Our approach focused on investigating, analyzing, and highlighting the impacts of CAR T-cell therapy on patients with ALL. **Results:** The data demonstrate that, despite challenges posed by adverse effects and tumor resistance, CAR T-cell therapy is a critical therapeutic approach against ALL, showing high rates of remission and overall survival in clinical trials. However, significant limitations persist, including high costs, challenges in ensuring quality control, and elevated recurrence rates, which hinder definitive validation of its efficacy and safety. **Conclusion:** Further research is imperative to optimize CAR T-cell design and identify more precise biomarkers.

Key words: Receptors, Chimeric Antigen; Immunotherapy; Immunologic Techniques; Precursor Cell Lymphoblastic Leukemia-Lymphoma; Genetic Engineering.

RESUMO

Introdução: A leucemia linfoide aguda (LLA) é uma neoplasia hematológica identificada pela proliferação descontrolada de linfoblastos mutados de linhagem B e/ou T, que compromete gravemente o organismo humano e apresenta alta taxa de letalidade. Esse quadro conduz os pacientes a um percurso clínico extenuante, agravado pelos efeitos adversos das terapias convencionais. Nesse contexto, a estratégia de tratamento com células T modificadas geneticamente para expressar o receptor de antígeno quimérico (CAR) demonstra eficácia significativa, superando as adversidades dessa patologia agressiva. **Objetivo:** Analisar as implicações clínicas identificadas nos principais estudos sobre tratamento com células CAR-T no tratamento da LLA. **Método:** Revisão bibliográfica integrativa que envolve coleta de artigos científicos de bases de dados como PubMed, SciELO, Periódicos, Scopus, *Web of Science* e J-STAGE a partir do ano 2000, com foco em investigar, analisar e destacar os impactos da terapia com células CAR-T nos pacientes com LLA. **Resultados:** Os dados evidenciam que, apesar dos obstáculos decorrentes dos efeitos adversos e da resistência tumoral, o uso de células CAR-T é uma abordagem terapêutica essencial no combate à LLA, apresentando altos índices de remissão e sobrevida global, observados nos testes clínicos. No entanto, a terapia apresenta empecilhos consideráveis, incluindo custos elevados, desafios na garantia de qualidade de produção e altas taxas de recidiva, comprometendo a validação definitiva da eficácia e segurança. **Conclusão:** É imprescindível a realização de novas pesquisas para aprimorar a construção do CAR-T e a identificação de biomarcadores mais precisos.

Palavras-chave: Receptores de Antígenos Quiméricos; Imunoterapia; Técnicas Imunológicas; Leucemia-Linfoma Linfoblástico de Células Precursoras; Engenharia Genética.

RESUMEN

Introducción: La leucemia linfoblástica aguda (LLA) es una neoplasia hematológica caracterizada por la proliferación descontrolada de linfoblastos mutados de linaje B y/o T, que compromete gravemente al organismo humano y presenta una alta tasa de letalidad. Este cuadro conduce a los pacientes a un curso clínico extenuante, agravado por los efectos adversos de las terapias convencionales. En este contexto, la estrategia de tratamiento con células T modificadas genéticamente para expresar el receptor de antígeno quimérico (CAR) demuestra eficacia significativa, superando las adversidades de esta patología agresiva. **Objetivo:** Analizar las implicaciones clínicas identificadas en los principales estudios sobre el tratamiento con células CAR-T para el tratamiento de la LLA. **Método:** Revisión bibliográfica integradora que incluyó la recopilación de artículos científicos de bases de datos como PubMed, SciELO, Periódicos, Scopus, *Web of Science* y J-STAGE a partir del año 2000, con el fin de investigar, analizar y destacar los impactos de la terapia con células CAR-T en pacientes con LLA. **Resultados:** Los datos evidencian que, a pesar de los obstáculos derivados de los efectos adversos y la resistencia tumoral, el uso de células CAR-T es un enfoque terapéutico esencial en el tratamiento de la LLA, con altas tasas de remisión y supervivencia global observadas en ensayos clínicos. Sin embargo, la terapia presenta desafíos considerables, incluyendo altos costos, retos en la garantía de calidad de producción y elevadas tasas de recidiva, lo que compromete la validación definitiva de su eficacia y seguridad. **Conclusión:** Se considera imprescindible la realización de nuevas investigaciones para optimizar la construcción de las células CAR-T y la identificación de biomarcadores más precisos.

Palabras clave: Receptores Quiméricos de Antígenos; Inmunoterapia; Técnicas Inmunológicas; Leucemia-Linfoma Linfoblástico de Células Precursoras; Ingeniería Genética.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a serious hematological neoplasm, frequently associated with a deadly prognosis[1,2]. A deregulation in one of the steps of lymph cell maturation compromises the differentiation and proliferation of B- and/or T-cells[3-6]. This uncontrolled expansion of immature and anomalous cells compromises hematological homeostasis, resulting in the formation of hematopoietic cancers, such as ALL[3-7]. The ALL diagnosis is established after molecular and histopathological analysis of mutated cells[3,7]. Its classification consists of two main categories: B-ALL and T-ALL[1,8,9]. ALL manifests in the main form of acute leukemia in children, with a predominance of B-ALL cases in pediatric and young adult patients, representing more than half of lymphoblastic leukemia cases[1,3,5,6]. Although expressive in children, there are concerning records in adults as well[7,10-14].

In contrast, T-ALL results from mutations associated with the development of T-lymphocytes, leading to the uncontrolled proliferation of those cells[1,4,11,15,16]. This pathological form is more prevalent in adults and is associated with an unfavorable prognosis, due to its aggressive physiopathology and refractoriness to conventional treatments[1,3,11,12,17-21]. ALL tumorigenesis results from multiple factors[13-18]. However, genetic mutations and chromosomal alterations play essential roles in the origin of this anomaly, which can happen spontaneously or in response to exposure to mutagenic agents, like pesticides, radioactive material, and other chemical or physical agents capable of damaging the genetic machinery, resulting in changes to the normal lymphoid cell cycle[2,11,14,15,18].

ALL is highly heterogeneous, with subtypes classified according to phenotypical and genotypical characteristics. This multifaceted nature contributes to a progressive clinical course, imposing significant challenges to treatments, such as therapeutic resistance and high recurrence rates, which impair remission and reduce chances of cure[1,3,6,13].

There was a reduction in the mortality of children and adolescents with leukemia between 1980 and 2015 in Brazil; however, the survival rate of patients with ALL remains relatively low, around 64%[21]. This index is considerably lower than the ones observed in developed countries, where survival rates surpass 80%, reflecting the therapeutic challenges still faced in Brazil, especially in refractory or recurrent cases[21-27]. Comparatively, countries with greater investments in medical research on immunotherapy present superior survival rates, evidencing the need for improvements and/or new strategic approaches to treatment in Brazil[21,22,27-29].

Given the lethality of ALL, it is clear that its implications generate profound psychosocial impacts, affecting both patients and their support network, reinforcing the need for investments in epidemiological control strategies and the availability of more efficient therapies in the public health system[3,21,22,29-32].

Over the last years, the treatment of hematological neoplasms has shown expressive advancements, especially with the incorporation of immunotherapies, which use elements from the immunological system itself to combat cancer[3,22-29,31,33,34]. This therapy is highlighted due to its ability to specifically target malignant cells, minimizing collateral damage from conventional treatments, such as chemotherapy (cytarabine, methotrexate, cyclophosphamide, for instance), radiotherapy, and surgical interventions[1,3,22-27,30-35]. These traditional methods, although effective, often entail high levels of toxicity and provoke a broad scope of adverse reactions (AR) in patients, highlighting the need for less aggressive and more selective alternatives[1,3,5,11,21,22,30-40].

The application of T-lymphocytes with chimeric antigen receptor (CAR; CAR-T lymphocytes application – CAR-T) represents an advanced therapeutic strategy based on immunotherapy, in which patients' T-cells are genetically modified to express specific receptors capable of identifying and cooperating for precise elimination of leukemic blasts[1,4,34,35-38]. The more recent generations of CAR-T cells show an improved ability for selective and effective action, offering better safety and optimization of therapeutic results[1,3,31,34-37]. Given the benefits observed in pre-clinical and clinical trials, the North American Food and Drug Administration (FDA) regulatory agency approved, in 2017, the use of CAR-T cells for treating ALL and large B-cell lymphoma[27-29,34,35,38]. This approval has expanded the therapeutic options available and contributed to increasing survival rates in patients affected by these illnesses[3,27-29,32-35].

Currently, there are six FDA-approved immunotherapies based on CAR-T cells, which are developed from second-generation CAR-T technology[18,39-41]. These cells have demonstrated a relevant clinical efficacy in inducing tumoral remission in several hematological neoplasms[10,18,29,34,35,40,41]. Considering the evidences of CAR-T cell clinical efficacy, the Brazilian Health Surveillance Agency (Anvisa) has approved the following four therapies: KYMRIAH (tisagenlecleucel), CARVYKTI (ciltacabtagene autoleucel), YESCARTA (axicabtagene ciloleucel), and TECARTUS (brexucabtagene autoleucel), predominantly indicated for leukemias[22-26,41]. However, some obstacles currently impair the use of this biopharmaceutical as the first line of treatment for patients with ALL[1,18,42,43]. Among these limitations, tumoral

immunosuppression mechanisms are highlighted, such as immunological escape, loss of *in vivo* target-antigen, and critical AR, like cytokine release syndrome (CRS) and, in some cases, graft versus host disease (GVHD) [1,3,18,36,42-44].

Considering this challenging scenario, new approaches have been proposed to overcome these obstacles, which are broadly attributed to the tumor immunosuppressive microenvironment (TIM)[4,31,32,44,45]. Recent studies indicate that it is possible to optimize CAR-T cells to increase specificity, making them more effective for combating ALL[1,5,7,17,18,31,37,44-55]. A promising strategy is cell reprogramming using CRISPR-Cas9 technology, a high-precision tool for genetic editing. Moreover, innovative techniques, such as using induced pluripotent stem cells (iPSC), enable cellular remodeling, facilitating the study and modification of interest cells[34,37,44,48-50,56-58]. Another proposal is to employ molecular engineering combined with nanotechnology to optimize efficacy and expand the use of CAR-T cells[37,42,53]. With these techniques, it is possible to combine attributes from different immune cells to develop chimeric cells that are more effective in primary recognition of leukemic cells, generating CAR-T cells that are more adapted and capable of attacking multiple targets[12,46-48,57,59].

The primary objective of this study was to investigate the main clinical implications currently discussed in therapeutic studies that employ CAR-T cells in the treatment of patients with ALL. This study analyzed the tumoral mechanisms of ALL that compromised CAR-T therapy success, focusing on evaluating results from the main clinical trials that described its therapeutic efficiency, limitations of use, adverse events (AE), and perspectives to broaden its application. The specific objectives of this article aim to research the main emerging CAR-T strategies and its variations that are being implemented to overcome obstacles such as immunosuppression, toxicity, and tumoral escape; analyze the characteristics of CAR-T cells' actions, and the suppressor mechanisms imposed by ALL; and highlight, considering current studies, the results of CAR-T treatment in patients with ALL.

METHOD

Integrative literature review to investigate, evaluate, and describe the clinical implications of immunotherapy with CAR-T cells in the treatment of ALL. The research process included critical analysis of current aspects involving the research topic, definition of the central theme, identification of the most relevant questions, search and selection of studies for sampling, detailed analysis and delimitation of the studies included for sample composition, categorization of the selected studies, in-depth interpretation of the results, presentation of the results, discussion and promotion of the evidence obtained. The study is a mixed-methods research, combining qualitative and quantitative aspects for data analysis. The qualitative review involved searching, assessing, and selecting articles about the clinical implications and tumoral mechanisms of using CAR-T cells for the treatment of ALL.

Additionally, we collected and analyzed quantitative data related to complete remission (CR) rates, AE, and overall survival (OS) indexes, to assess the therapeutic efficacy of CAR-T cells. The methodology emphasized clinical studies that assessed effectiveness, side effects, immunosuppression mechanisms, and future perspectives on the use of CAR-T cells for the treatment of ALL. The study followed Whittemore and Knafl's[60] guidelines, updated in 2021, which provide the basis for structuring and integrating evidence from different types of studies, including qualitative and quantitative[61]. This methodology offers a comprehensive analysis of CAR-T cells therapy[60,61].

The inclusion criteria focused on clinical trials, prioritizing this study method to verify the implications of the treatment, checking CR rates, AE incidences, OS rates, and event-free survival (EFS) in patients diagnosed with ALL submitted to CAR-T cell treatment. The studies selected were those that evaluated the viability and performance of CAR-T, detailing the mechanisms that contributed to its effectiveness or ineffectiveness, and that reported CR, AE, OS, and EFS, published between 2000 and 2024.

The investigation and selection of articles occurred from January to September 2024. Data acquisition was conducted using a collection instrument previously structured by the author to answer the following research questions: "What are the consequences of treatment with CAR-T cells in patients with ALL?"; "What are the most severe side effects of therapy with CAR-T cells?"; and "Is the use of CAR-T cells in individuals with ALL capable of causing remission with low adverse reaction rates?".

The exclusion criteria were private studies, nontransparent, and interrupted for adverse reasons, or those that did not detail CR, AE, EFS, and/or OS. The article selection considered the patients' sample, their clinical characteristics, and the applied therapy's study method.

The articles were searched using descriptors in English and Portuguese only, on electronic databases like PubMed, SciELO, *Periódicos Capes Scopus*, Web of Science, J-STAGE, China National Knowledge Infrastructure (CNKI), ClinicalTrials.gov, China Clinical-Trial Registry (ChiCTR), in addition to data obtained from Anvisa, FDA, and the National Cancer Institute of the United States (NCI)

and Brazil (INCA). The source for descriptor selection was the *Descritores em Ciências da Saúde* (DeCS) system, with the following terms selected: "Leucemia-Linfoma Linfoblástico de Células-T Precursoras", "Leucemia-Linfoma Linfoblástico de Células Precursoras B", "Imunoterapia Adotiva", "Terapia por Receptor Antigênico Quimérico", "Técnicas Imunológicas", "Imunoterapia", "Exaustão das Células T", "Senescência de Células T", "Leucemia de Células-T", "Leucemia de Células-B".

Terms in English were also searched, following standardized descriptors from *Medical Subject Headings* (MeSH) for research in international databases, including: "Immunotherapy, Adoptive", "Acute Lymphoblastic Leukemia", "Immunologic Techniques", "Precursor B-Cell Lymphoblastic Leukemia-Lymphoma", "T-Cell Senescence", "Receptors, Immunologic", "Receptors, Chimeric Antigen", "Receptors, Antigen, B-Cell", "Receptors, Antigen, T-Cell", "Immunomodulation", "Therapy, CAR-T-Cell", "Antigens, CD19", "Genetic Engineering". The analyzed variables included: CR rates, AR incidence and severity, OS data, and recurrence-free survival (RFS). The selected clinical studies were qualitatively analyzed according to the methodological index for non-randomized studies (MINORS), a tool validated for assessment of non-randomized experimental designs[62,63]. MINORS was applied to each study, with nine items from the scale adapted and assessed, with scores ranging from 0 (not met), 1 (partially met), and 2 (totally met) for each criterion, totaling a maximum score of 16 for non-comparative studies and 24 for comparative studies[62,63]. The methodological quality of the studies was categorized according to criteria used in the literature, as follows: ≥12 good quality, 9–11 acceptable limitations, and ≤8 risk of bias/limited utility[62,63].

The analysis was conducted exclusively by the author, who independently assessed all the methodological criteria (Table 1). To ensure rigor, the results were reviewed by an external specialist, who had no direct participation in the scoring.

RESULTS

From the 30 initial articles, 17 met the inclusion criteria. Of those, five clinical studies analyzed the use of CAR-T cells in patients with B-ALL (Table 2), carefully detailing the clinical condition and response to therapy. The other six studies focused on the use of CAR-T in T-ALL (Table 3), while six more designated articles investigated variations in the CAR cells approach for different antigens (Table 4), being considered relevant for review. In the end, 17 articles of applied research were selected.

The clinical trial of phases 1 and 2, performed by Celgene[64], investigated the safety and effectiveness of Lisocabtagene Maraleucel in pediatric patients aged ≤25 years with B-ALL or B-NHL(r/r), positive for CD19-antigen. The study focused on investigating AR rates and tolerability to the treatment with CAR-T in three dosage levels (0.05; 0.15, and 0.50 x 10^6 CAR-T cells+/kg), verifying the viability of each stipulated dose[64]. In total, 21 patients were included, who received chemotherapy of lymphodepletion with fludarabine and cyclophosphamide to prepare for CAR-T cells infusion[64]. Of the 9 patients who received the first dose (0.05 x 10^6 CAR-T cells+/kg), 7 continued treatment[64]. The second dose (0.15 x 10^6 CAR-T cells+/kg) was administered to 6 patients, and only 1 patient received the third dose (0.50 x 10^6 CAR-T cells+/kg)[64]. Conjointly, one patient was submitted to treatment with only leukapheresis, and their death was later verified[64]. The average response rate (CR/CRi) on the 28th and 56th day was 50%, with average OS of 7.7 months, demonstrating stability in disease remission over that period[64]. The study also reported that the average treatment duration of response (DOR) was 13.7 months, indicating a sustained response[64].

AR varied according to dose[64]. The following reactions were observed for the first dose: pneumonia, sepsis, viremia, neurotoxicity, common in therapies with a high tumoral load[64]. The second dose was associated with CRS and neurotoxicity, while the third dose presented cases of brain edema and AR on the central nervous system (CNS)[64]. In the study by Liu et al.[10], 27 patients received CAR-T-CD19 infusion in the first round of intervention, with CR in 85%. A second round of CAR-T-CD22 infusion was applied to 21 of the 27 initial patients, in a period of ≅1 month after the first, with the infusion of CAR-T-CD19/CD22. Continuous remission was confirmed in 14 of the 21 patients who received both infusions, with an average follow-up period of ≅19 months. AE like GVHD, CRS, and neurotoxicity were also reported[10]. The study aiming KTE-X19 analyzed 55 patients in phase 2 of the clinical trial, demonstrating CR in 70.9% and 12.8 months DOR[65]. The AR included cardiac disturbances and neurotoxicity[65]. Additional clinical trials in 21 patients with CAR-T anti-ROR1 (tyrosine kinase-like orphan receptor 1) and another with CAR-T-CD5 administered to 16 participants with ALL, also reported CRS, neurological disturbances, and critical infections[50,66]. The research aimed at ROR1-antigen identified an OS increase in estimated 45% of participants, based on an analysis conducted after ≅1 year of administering the medication[66]. Whereas in the study by Zhang et al.[54], aimed at CD19/CD22 in 52 patients with B-ALL, OS was measured around 87%.

Table 1. Methodological quality assessment following the Methodological Index for Non-Randomized Studies

Study (ID/reference)	Clear objective	Consecutive inclusion	Prospective data	Adequate outcome criteria	Unbiased assessment	Adequate follow-up time	Reported losses	Calculated sample size	Equivalent groups (comparative)	Total/max. score
JCAR017 (NCT03743246)	2	1	2	2	1	2	2	1	1	14/24
Liu et al., 2021[10]	2	1	1	2	1	2	2	1	N/A	12/16
NCT02614066	2	2	2	2	1	2	1	1	N/A	13/16
NCT02706392	2	1	2	2	1	1	2	0	1	12/24
Zhang et al.[54], 2020	2	2	1	2	1	2	2	1	1	14/24
ISRCTN15323014	2	2	2	2	1	2	2	0	N/A	13/16
Tan et al., 2023[20]	2	2	1	2	1	2	2	0	N/A	12/16
NCT06326463*	2	0	1	0	1	2	N/A	1	2	9/24
NCT05745181*	2	0	2	2	1	2	N/A	0	N/A	9/16
NCT06420076*	2	0	1	2	1	1	N/A	2	N/A	9/16
NCT05032599	2	1	1	2	1	1	2	1	N/A	11/16
NCT03056339	2	2	2	2	1	1	2	1	1	14/24
NCT05528887*	2	0	2	2	1	1	N/A	1	N/A	9/16
NCT05887167 *	2	0	2	2	2	2	N/A	1	N/A	11/16
NCT02028455*	2	1	2	2	1	2	2	2	1	15/24
NCT05110742*	2	0	2	2	1	1	N/A	1	N/A	9/16
NCT03389035	2	1	2	2	1	1	1	1	N/A	11/16

Source: Adapted from Slim et al.62.
Captions: * = Trials in recruitment or initial phase (preliminary data); N/A = Not applicable.

In parallel, Table 3 summarizes the published data from the clinical trials that employed CAR-T cells in patients with T-ALL.

In the studies by Chiesa et al.[67], CAR-T cells were developed to recognize CD7-antigen in leukemic blasts of pediatric patients with T-ALL (r/r). Of the three treated patients, one achieved CR on the 27th day, with no signs of minimal residual disease (MRD), and maintained cellular remission for 9 months after HPSCT (hematopoietic stem cell transplantation)[67]. The second patient presented partial remission on the 28th day; however, AR were recorded, such as CRS and a serious infection, with the patient dying on the 33rd day[67]. The third had complications on the CNS, but with CR confirmed, allowing for treatment continuity[67].

In the study by Tan et al.[20], 17 of the 20 patients with T-ALL (r/r) treated achieved CR one month after treatment with CAR-T-CD7. After 27 months, the OS rate was 42%, with 25% of patients presenting serious infectious complications[20]. In 67% of patients with recurrence, there was loss of CD7 expression, indicating resistance to treatment[20].

The research by Pan et al.[50] treated 16 patients with T-ALL (r/r) with CAR-T-CD5 cells edited by CRISPR-Cas9. All the patients presented CR after a month, but 5 died from serious infections[50]. Ongoing studies, like NCT06326463 and NCT05745181, explored the CD70 and CD1a targets, respectively, broadly expressed in T-ALL and other hematological malignancies[68,69]. These studies, focused on molecules still little explored in T-cell leukemias, seek to expand the spectrum of therapeutic choice for resistant cases or those with low expression of the target antigens conventionally used in CAR-T cell therapy.[3,4,11,16,49,50,67-70].

The studies demonstrated high CR rates in patients with ALL treated with CAR-T; however, AEs like CRS, GVHD, neurotoxicity, and infections are still significant challenges, especially with the loss of expression of target-antigens, which leads to recurrence in many cases[21,50,54,64-67].

Given the significant obstacles that still impair the therapeutic success of CAR-T cells[3,4,21,54,59,64-67], Table 4 describes the most relevant and recent clinical studies that drive development and implementation of alternative and innovative strategies, involving immune T cells and/or natural killers (NK) – also known as killer cells – genetically modified to exert antitumoral mechanisms reactive to immunosuppressor processes observed in leukemias[8,12,16,40,47,48,57,59,71]. The most common challenges include self-destruction (fratricide), antigenic escape,

Table 2. Summary of studies used to evaluate clinical trials with CAR-T cells in patients with B-ALL

CAR-T cells engineering	Targeted cancer	Polytherapy	Clinical trial phase(s)/ # of participants	Study reference/ identification (ID)	Clinical response index	Critical side effects
CAR-T anti-CD19/4-1BB (JCAR017)	B-ALL(r/r) and LNH-B	CAR-T, fludarabine, cyclophosphamide	Phase 1/2 21participants	NCT03743246	1 - ORR on the 56th day= 5 PR total (1st, 2nd, and 3rd Do) 2 - DOR: 13.7 months in 3 PR with 1st Do. INS on the 2nd and 3rd Do 3 - RFS: 1st Do - 7.7m 2nd Do - 6.9 months 3rd Do - INS 4 - OS: 1st Do - 7.13 months 2nd Do - 7.08 months 3rd Do - 8.9 months	1st Do - Pneumonia, sepsis, viremia, and neurotoxicity 2nd Do - CRS and neurotoxicity 3rd Do - Brain edema and neurotoxicity
Dual-targeting CAR-T	B-ALL(r/r) post-transplant that express CD19/ CD22-antigens	Allo-HSCT, CAR-T cells anti-CD19/CD22, fludarabine, cyclophosphamide	Phase 1/2 27 participants	Liu et al., 2021[10]	1st - Round; 27 Pt used CAR-T CD19: CR in ≅85% (23P) 2nd - Round; 21 Pt treated with CAR-T CD22: CR in ≅95% (20 PR) Average follow-up of 19.7 m after 2 rounds: Lasting CR in 14 PR, 7 recurrences, and 2 deaths	GVHD, CRS, neurotoxicity, pulmonary hemorrhage, and chronic pneumonia
Brexucabtagene autoleucel-KTE-X19 (autologous CAR-T)	B-ALL(r/r)	CAR-T-anti-CD19, cyclophosphamide, and fludarabine	Phase 1/2 125 participants	NCT02614066	1- CR/CRi: 55 Pt, CR, and CRi in 39 PR ≅70.9% 2- DOR: 55 Pt/12.8 m (median) 3-OS: 55 Pt/ 18. 2 m (median) 4- RFS: 55 Pt/11.6 m (median)	Cardiac, gastrointestinal and IS disturbances, in addition to multiple syndromes
CAR-T autologous anti-ROR1	B-ALL	CAR-T anti-ROR1, fludarabine and cyclophosphamide	Phase 1 21 participants	NCT02706392	1-CR: 20 Pt/ 1 PR with CR 2 - EFS: 8 Pt/ 1 PR with EFS	IS disturbances and alterations in CNS and PNS
CAR-T anti-CD19/CD22 combined with allo-HSCT	B-ALL (r/r)	Fludarabine and procedures based on busulfan, cyclophosphamide, allo-HSCT, and CAR-T cells anti-CD19/22	Phase 1 52 participants	Zhang et al.54, 2020	1- 52 Pt with B-ALL r/r submitted to allo-HSCT post CR/CRi with CAR-T-19/22 2-CR: 9 cases with recurrence post allo-HSCT + CAR-T/ 6 Pt with recurrence used CAR-T19 (1) and 22 (5), 5 reached CR with MRD 3-EFS in 73% of PR with ≅ 12 months review; ≅90 (46) of alive Pt in ≅12 months post therapy	GVHD, CRS, abdominal infection, and hemorrhagic cystitis

Captions: allo-HSCT = allogeneic hematopoietic stem cell transplantation; DC = differentiation cluster; r/r = recurrent or refractory; B-NHL; B-cell non-Hodgkin lymphoma; CRi = complete remission with incomplete hematological restoration; ROR1 = Receptor tyrosine kinase-like orphan receptor 1; ORR = Overall response rate; DOR = duration of response; INS = insufficient number of events; PR = patients who responded to treatment; Pt = tested patients; Do = CAR-T tested dose; m = months; ≅ = approximately; MRD- = minimal residual disease; IS = immunological system; CNS = central nervous system; PNS = peripheral nervous system; GVHD = graft versus host disease; RFS = recurrence-free survival; EFS = event-free survival.

Table 3. Summary of studies used to evaluate clinical trials with CAR-T cells in patients with T-ALL

CAR-T cells/ target antigen(s) category	Targeted cancer	Polytherapy	Trial phase/ # of participants	Clinical trial reference / iD	Response rates/ current status of the clinical trial
CAR-T targeting CD7 (BE-CAR7)	T-ALL(r/r)	Lymphodepletion with fludarabine, cyclophosphamide Alemtuzumabe, BE-CAR7, allo-HSCT	Phase 1 3 participants	ISRCTN15323014 Chiesa et al., 2023[67]	1- Use of CAR-T-7 in 3 pediatric P with T-ALL r/r 2-CR: 1 P with CR on the 27th day, no MRD, and discharged 52 days after HPSCT An analysis 9 months later confirmed lasting remission 2 P on remission on days 19 and 25, with MRD 3 P with CR on the 28th day
CAR-T objective to CD7	T-ALL(r/r) with positive CD7	Allogeneic CAR-T-CD7 cells. Cyclophosphamide and fludarabine	Phase 1 20 participants	Tan et al., 2023[20]	1-Clinical course assessment (in ≅24 months) for 20 patients with T-ALL r/r who used CAR-T-CD7 cells 2- 17 P with CR in ≅27 months 3-OS: In ≅42% of patients after 2 years of therapeutic CAR-T infusion
CAR-T targeting CD70	T-ALL(r/r) with positive CD70	Fludarabine, Cyclophosphamide, CAR-T CD70 (autologous), Medication: Mesna	Phase 1 "24-participants estimated"	NCT06326463	Recruitment phase: inclusion of 24 patients with T-ALLr/r and other hematological neoplasms (r/r) positive for target-antigen-CD70 is projected
CAR-T anti-CD1a	T-ALL and acute lymphoblastic lymphoma of T-cells	CAR-T cells anti-CD1a.	Phase 2 "20 participants estimated"	NCT05745181	Recruiting: 20 patients aged 18-70 years old, with T-ALL r/r or acute T-cell r/r lymphoma that express target-antigen-CD1a are expected to subscribe
CAR-T anti-CD5/CD7	T-ALL	Fludarabine, cyclophosphamide, CAR-T cells CD5/CD7.	Phase 1/ 2 "60 participants estimated"	NCT06420076	Ongoing: 60 P diagnosed with T-ALL r/r or other specific hematological malignancies are expected to join the clinical trial
Allogeneic, compatible CAR-T-CD5	T-ALL(r/r)	Fludarabine, cyclophosphamide, CAR-T-CD5	Phase 1 16 participants	NCT05032599 Pan et al., 2024[50]	1- 19 Pm/16 Pt 2-CRi: 16 P obtained CRi 30 days after administration of CAR-T-CD5 3-EFS in 3 P with HPSCT in average observation of ≅14 months

Captions: allo-HSCT = allogeneic hematopoietic stem cell transplantation; DC = differentiation cluster; r/r = recurrent or refractory; P = patients; MRD = minimal residual disease; HPSCT = hematopoietic stem cell transplantation; Pe = patients enrolled; Pt = patients tested; CR = complete remission; CRi = complete remission with incomplete hematological restoration; OS = overall survival.

negative regulation of targets, and exhaustion of CAR-T cells, frequently interconnected with selection of targets with low specificity or pleiotropic features, that is, also considerably present in healthy cells and with important functions in the body[3,11,12,37,46,56,67]. Therefore, Table 4 highlights the studies that explore CAR expression aimed at diversified and extensively expressed targets in B-ALL /T-ALL[46-48,51,71,72-74].

The ongoing studies that assessed CAR technology in the treatment of ALL and other hematological tumors focused on specific genetic editing methodologies to amplify response persistence and optimize CAR-T antineoplastic mechanisms[4,12,32,35,46-55,57,59,67-75]. The clinical trials listed in Table 4 are mostly in the recruitment phase or evaluation for publication. These studies aim at increasing specificity and minimizing AR, like CRS and GVHD in patients with acute leukemias. The focus is on improving the synthesis and mechanisms of action of CAR-T cells[46-48,71-74].

DISCUSSION

ALL has an aggressive nature, with significant resistance to traditional therapies[3,5,13,22,31,33,34]. Conventional treatments, like standardized chemotherapy and radiotherapy for patients with ALL, usually favor critical

Table 4. Summary of the main clinical trials referring to new methodological approaches using CAR-modified immune cells

Clinical trial start date and expected date of conclusion	CAR-variation cell models	Targeted molecule	Target cancer	Polytherapy	Trial phase/ # of participants	Clinical trial reference/ iD
1-Start date: 6/2017 2-Conclusion: 3/2023	NK-cells of the umbilical cord with CAR	CD19	B-ALL CD19+r/r and other hematological malignancies	Fludarabine, cyclophosphamide, CB-NK-iC9/CAR-19/ IL-15 cells, mesnex, and AP1903	Phase 1/2 49 participants	NCT03056339
1-Start date: 9/2021 2-Expected conclusion: 6/2026	Autologous-CAR-T anti-CD19/BCMA/CD123/CD7	CD19, CD123, BCMA, and CD7	ALL and CLL (r/r)	Autologous CAR-T, fludarabine, cyclophosphamide	Phase 1 "10 participants estimated"	NCT05528887
1-Start date: 3/2024 2-Expected conclusion: 12/2026	CAR-T + aHSC	TAA	ALL	CAR-T cells combined with aHSC	Phase 1 "20 participants estimated"	NCT05887167
1-Start date: 2/2014 2-Expected conclusion: 7/2036	CAR-T anti-CD19+ EGFR	CD19 and EGFR	ALL CD19+ (r/r) /EGFR+	CAR-T anti-CD19+/, fludarabine and cyclophosphamide	Phase 1/2 167 participants	NCT02028455
1-Start date: 4/2024 2-Expected conclusion: 12/2027	CB-NK cells synthesized with CAR-5/IL15	CD5	T-ALL	Fludarabine, Cyclophosphamide, CB-NK-CAR.5/IL15	Phase 1/2 "48 participants estimated"	NCT05110742
1-Start date: 12/2017 2-Conclusion: 12/2022	Allogeneic CAR-CIK anti-CD19	CD19	B-ALL(r/r)	Fludarabine, cyclophosphamide, CAR-CIK cells anti-CD19.	Phase 1/2 21participantes	NCT03389035 Magnani et al., 2020[47]

Captions: NK = natural killers; CLL = chronic lymphocytic leukemia; EGFR = epidermal growth factor receptor; EGFRt = truncated epidermal growth factor receptor; aHSC = autologous hematopoietic stem cells; CB-NK = Nk-cells derived from the umbilical cord; CAR-5/IL15; CAR anti-CD5 combined with interleukin-15; CB-NK-CAR.5/IL15 = Nk-cells derived from umbilical cord synthesized with IL-15 and modified with CAR-CD5; CAR-CIK anti-CD19 = anti-CD19 CAR-modified cytokine-induced killer cells; CB-NK-iC9/CAR-19/IL15 = Nk-cells derived from the umbilical cord synthesized with interleukin-15, susceptible to caspase-9 induction and CAR-CD19-CD28-zeta-2A domains; BCMA = B-cell maturation antigen; TAA = tumor associated antigen.

reactions in individuals and present varied remission responses[1,3,8,22,31,33,34,36]. The application of chemotherapy drugs in onco-hematology has shown to be insufficient for prolonged remissions[1,31,33,34,36,42,44], which drives the investigation of new therapeutic strategies, like CAR-T cells therapy[1,3,22,33]. These stand out for precisely targeting malignant cells, reducing collateral damage observed in conventional treatments[1,3,7,35,36,38,42,76].

In this sense, several studies attribute the efficiency of CAR-T to its molecular structure, consisting of autologous or allogeneic CD8-T-cells[3-7,12,14,35,37,42,76]. In traditional CAR-T development, T-lymphocytes are extracted from the patient, cultivated in the laboratory, and exposed to antigens found specifically in the tumoral tissue[8,10,12,16,18,31,77]. To avoid compromising healthy cells, pre-clinical trials verified that CD19, CD20, and CD22 antigens are broadly expressed in mutated B-cells, becoming extremely useful targets for CAR-T cells in the treatment of B-ALL[1-5,10,22-29,35,36,52,64,75,78,79]. However, challenges continue, especially regarding negative regulation of antigens, like CD19, which results in recurrences, facts observed in the studies described in Table 2[1,52,64,65,78].

The use of CAR-T aiming multiple targets, like CD19/CD22, showed greater durability of response, as reported by Liu et al.[10] and Zhang et al.[54]. This methodology suggests being more effective in overcoming target loss and reducing recurrences, highlighting the importance of combined strategies to maximize therapy efficacy[1,6,10,43,54,75,79].

For T-ALL, CAR-T therapy faces additional obstacles, like the simultaneous expression of CD7-antigen in CAR-T cells, which may lead to fratricide of modified cells[12,20,21,49,50,55,67]. Methods like the use of CAR-T-CD5 with deletion of the CD5 gene through CRISPR-Cas9 proposed by Pan et al.[50] showed favorable initial results, with high remission rates; however, with infectious complications and resistance to therapy[50].

The use of TCTH combined with CAR-T has improved clinical results, as reported by Zhang et al.[54]

and Pan et al.[50]. However, the high incidence of serious AR, like CRS and neurotoxicity, highlights the need for a more rigorous risk assessment before treatment, especially in pediatric, elderly, and/or patients with other comorbidities and heterogeneous tumors[10,12,19,20,44,50,54,67,76]. In light of the available evidence, it was verified that ARs occur greatly from the fabrication protocol adopted by CAR-T, like the type of reagent used for growing T-cells, and dosage errors of the reagents used in the construction of CAR[1,3,4,12,16,17,18,31,37,74]. Perceptible effects on the NCT02028455 study, divided into two phases, with 43 patients treated in phase 1 and 21 in phase 2, in which different reagents were used in the production of the material[74]. It was evident that changing the reagent caused early CRS, however, the alternation of reagents increased activity and duration of the drug on the body, as demonstrated by superior EFS and LFS rates in the second phase, projecting, in this phase, an OS around 80% with average follow-up of 1 year, above the 67% reported in phase 1[74].

For protocol purposes, it is proposed that the construction of CAR-T cells with selectivity to target anomalous blasts involves the development of four domains: the specific extracellular interaction domain via single-chain variable fragment (scFv), composed of one or more synthetic proteins, responsible for the individualized recognition of mutated antigens present in leukemic cells; and the hinge region domain, which together with the scFV forms the ectodomain.[1,16,18,31,34-37].

The other two domains include transmembrane and the co-stimulatory domain(s) (composed by CD28 and/or 4-1BB), acting as essential mediators in the translation of the extrinsic signal to the intrinsic cellular medium[1-4,10,17,34,-40,45]. This new synthesized molecule aims to overcome tumoral immunomodulation, like negative regulation of the main histocompatibility complex (MHC), responsible for the presentation of antigens to the T-cell receptor (TCR)[1,31,35,37,52,53,77].

Moreover, conventional T-cells are commonly inhibited by TIM immunoregulatory molecules (ex.: PD-L1, CTLA-4, TGF-β, and IL-6). New studies are investigating the combination of CAR-T with immunological checkpoint blockers like PD-1-knockout, with the aim of overcoming this tumoral resistance[7,18,42,50,64,77]. Another documented approach consists of CAR-T cells added with co-stimulatory domains of interleukin induction, like IL-12, IL-15, and/or IL-18; this increment may promote the death of aberrant cells in TIM, cooperating with a greater T-cell activation, increasing its expansion and persistence *in vivo*[16-18,31,35,38,44,46,53,71,77].

Therefore, it is postulated that the accurate synthesis of CAR-T with affinity for the mutated target biomolecules in ALL, together with the biochemical mechanisms exerted by the CAR signaling domains, corroborate a sustained remission[1,3,17,34-37,56,75-77].

However, it is understood, from investigative studies, that ALL presents a high degree of molecular and histological heterogeneity, reflected in the different results among the distinct patient profiles, provoking variations in the recurrence, OS, and DOR rates, compromising therapeutic success[1,5-12,14,15,20,35,41,45,47,54,64-67]. This hypothesis may be explained by the formation of resistant subclones during treatment, repression of target antigens, and genetic and epigenetic mutations on the TIM, effects that, in addition to AR, culminated in lethal intercurrences in the tested individuals[10,20,37,46,47,50,54,64-67,74].

Considering these limitations, the NCT03056339 study aimed to administer a CAR-anti-CD19 connected to an atypical model of NK-cells, extracted from the umbilical cord of allogeneic sources, with vigilance, cytotoxicity, and communication between adaptive and innate immune system properties[1,3,12,18,42,46]. This proposal aimed to solve GVHD and syndromic complications, tied to TIM and incompatibility between allogeneic donor and patient cases[42,46,55-58,77].

Results were promising, demonstrating considerable safety in 11 individuals, with the absence of GVHD and CRS cases[46]. However, extrapyramidal symptoms like cardiac and blood disturbances, in addition to persistent infections, were observed, which is similar to AR identified in CAR-T studies[46]. CR was detected in 7 of the 11 patients tested with CAR-NK, within ≅13 months of treatment, with clinical response being recorded one month after beginning therapy[46].

The study by Magnani et al.[47] used a non-viral vector-modified allogeneic CAR-CIK (CAR-modified cytokine-induced killer cells) model to express a CAR-anti-CD19, designed to reduce production time and alleviate or avoid AR caused by transfusion, achieving most of the proposed objectives. The study verified the effects of the drug in 13 patients. It documented the absence of direct GVHD reactions, attributing CR to 61% of participants, with *in vivo* persistence for up to 10 months[47]. These data corroborate, partly, the findings from research NCT03056339, which, although derived from different allogeneic models, proposes new methodologies for medication production and implements never-before-seen genetic modulation strategies to confer more *in vivo* persistence, tumor resistance, and compatibility with the patient[18,31,42,46,47,57].

CAR-T cell therapies are showing significant potential for remission in the treatment of ALL, but therapeutic success depends on the precise identification of more molecular targets and rigorous control of AEs.[3,4,10,13,19,

[28,35,39,44,50,54,59,76]. Disease heterogeneity and mechanisms of tumoral evasion require a customized approach to minimize complications and maximize therapeutic efficacy[4,5,8,14-16,34,35,40,45,46,50,57]. The combination of CAR-T with complementary therapies, like TCTH, proves pertinent, but further studies are still needed to validate these approaches on a large scale[10,19,20,21,54,67]. The results show that, despite significant progress, CAR-T therapy still needs improvement to increase its safety[3,5,8,10,15,16,22,35,40,46,40,19].

CONCLUSION

Further studies aimed at molecular immunology and oncology are crucial for developing more precise treatments. Based on the current literature, employing CAR-T to treat ALL represents a promising strategy, with the ability to eliminate the tumor, preserve healthy cells, and restore the health of many individuals.

The validation of this therapy requires more evidence, since, despite remission reports, the number of recurrences, mortality, and AR were significant. Many clinical trials demonstrated tumoral remission rates above 85% and durable response in over 50% of cases. However, complications, such as CRS and GVHD, are present in most of the studies, reinforcing the need for further investigation and developing prophylactic strategies for serious reactions.

AE, loss of target antigen, and tumoral immunosuppression are the main causes of misfortunes in CAR-T immunotherapy. To face these challenges, new generations of these cells incorporating interleukins and enzymes are being developed. However, the production of this medication is still complex, due to the process of extracting cells from the patient and possible errors in the construction process. As perspectives, innovative techniques, such as the use of iPSC and genetic engineering with CRISPR-Cas9, are being tested to improve the efficiency of therapy, enabling a longer OS or even a cure.

Therefore, a holistic assessment of the numerous variables present in the treatment of ALL is essential, assessing the suitability of complementary therapies to overcome the challenges imposed by the tumor and the limitations of CAR-T therapy, to improve results and reduce complications associated with the disease and treatment.

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Guilherme dos Santos Elias participated in every step of the article construction, from design to the approval of the final version to be published.

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There is no conflict of interest to declare.

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