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Modern immunotherapy using CAR-T cells in hemato-oncology and solid tumors

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Abstract

CAR-T immunotherapy, based on the genetic modification of T lymphocytes that contain a chimeric antigen receptor (CAR), has been a major breakthrough in the treatment of cancer patients since the 2017 approval of Kymriah™ (tisagenlecleucel) by the United States Food and Drug Administration (FDA). To date, six CAR-T cell therapies have been approved by the FDA, showing efficacy in patients with B-cell cancers and multiple myeloma. However, adverse events, such as cytokine release syndrome and neurotoxicity, present serious challenges to successful CAR-T cell therapy. The severity of adverse events correlates with the degree of tumor burden before treatment. This observation is supported by the use of CD19-specific CAR-T cells in autoimmune diseases such as systemic lupus erythematosus and anti-synthetase syndrome. These results indicate that early initiation of CAR-T cell therapy with a low tumor burden, or the use of a tumor-shrinking strategy prior to CAR-T cell infusion, may reduce the severity of adverse events. CAR-T cell therapy is expensive and has limited efficacy in solid tumors. Research is currently under way to increase the effectiveness of CAR-T therapy in patients with solid tumors not responding to conventional treatment.

Keywords: CAR-T cells, immunotherapy, chimeric antigen receptor, hematological malignancies, solid tumors

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Introduction

CAR-T cell therapy is the most advanced and modern form of immunotherapy using modified T lymphocytes, programmed in such a way as to redirect their natural action to anticancer activity [1]. This breakthrough technology involves the use of autologous, i.e. T lymphocytes taken from the patient, which are then modified in *ex vivo* conditions by transduction of a lentiviral vector encoding a chimeric antigen receptor (CAR) recognizing the target antigen on the surface of cancer cells. The modified CAR-T cells are grown *in vitro* and administered to the patient as an intravenous infusion.

CAR-T cell therapy is primarily used in the treatment of hematological cancers such as acute lymphoblastic leukemia, multiple myeloma, and B-cell malignant lymphomas, and offers a chance of prolonging life, and even of curing the disease, in patients who have exhausted available therapeutic methods [1–3]. As of April 2023, there were six United States (US) Food and Drug Administration (FDA)-approved CAR-T cell products: Kymriah™ (tisagenlecleucel), Yescarta™ (axicabtagene ciloleucel), Tecartus™ (brexucabtagene autoleucel), Breyanzi® (lisocabtagene maraleucel), Abecma™ (idecabtagene vicleucel), and Carvykti™ (ciltacabtagene autoleucel). Thanks to this clinical success, CAR-T cell therapy has become one of the most promising

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treatment options in the fight against hematological malignancies. Recent studies have focused on the effectiveness of this therapy in non-responding patients with solid tumors [4, 5].

CAR-T cell immunotherapy begins with a specialized process of separation of the patient's blood components (leukapheresis), during which leukocytes are isolated. Then, in a highly specialized laboratory, they are genetically modified. A fragment of genetic material is inserted into the DNA of the patient's lymphocyte, which encodes a chimeric receptor that recognizes the antigen specific to the cancer that the cell is supposed to fight. In this way, the modified lymphocyte is told to recognize and eliminate. The modified lymphocytes are multiplied to a quantity capable of fighting the cancer and then sent back to the clinic where the patient is waiting for them. The last stage of therapy consists of administering CAR-T cells to the patient by infusion [6, 7]. But like any other therapy, CAR-T cell therapy can cause side effects. Some patients may experience cytokine release syndrome or neurotoxicity during the first days of treatment. The growing clinical experience of treatment with CAR-T immunotherapy means that clinicians are developing a growing understanding of how to eliminate these complications [6].

Generations of chimeric antigen receptor

CAR is a genetically modified receptor protein consisting of an extracellular fragment that binds a target cellular antigen, a spacer domain, a transmembrane domain, and an intracellular signaling domain. Recognition of the target antigen by CAR-containing T-cells (CAR-T) leads to their activation independently of major histocompatibility complex (MHC) proteins. Thanks to this, CAR-T lymphocytes can recognize antigens and destroy cancer cells without prior recognition of antigens presented by MHC molecules [2, 8]. The gene construct introduced into T lymphocytes by a suitable viral vector (e.g. lentivirus) comprises an extracellular domain binding a cellular target antigen (scFv) derived from a monoclonal antibody, a spacer domain, a transmembrane domain, and intracellular signaling domains e.g. CD3ζ, 4-1BB and CD28. The presence of the co-stimulatory domain 4-1BB ensures increased survival of T cells and stability *in vivo*, the co-stimulatory domain CD28 ensures proliferation of T lymphocytes and cytokine secretion, the CD3ζ domain determines the cytotoxicity of CAR-T cells, and the presence of the extracellular domain binding the target cellular antigen (scFv) ensures the possibility of recognizing and binding to specific antigens e.g. CD19, CD22.

The preparations available on the market today have only one 4-1BB or CD28 costimulatory domain. For example, Kymriah™ has a 4-1BB domain, while Yescarta™ has a CD28 domain. These are preparations containing the second-generation chimeric CAR receptor [2, 5, 6]. There are at least four generations of CAR-T cells. The number of co-stimulating domains of the CAR receptor determines its belonging to the next generation. In the CAR molecules of the first generation, signaling is carried out only through a fragment of the CD3ζ chain. In the second generation, in addition to the presence of the CD3ζ chain, there is an additional co-stimulating domain e.g. CD28, OX40, 4-1BB, CD27 or DAP10. The third generation of CAR receptors, apart from the CD3ζ chain, is composed of two costimulatory domains in various combinations [2, 9–11]. The fourth generation is a diverse group, including, for example, T cells redirected for universal cytokine killing (TRUCK) [12, 13] or CAR-T with suicide genes [14, 15].

CAR-T cell therapies approved in EU

CAR-T technology is currently one of the most modern and advanced medical procedures. The first CAR-T cell therapy was approved by the US FDA on 30 August, 2017 and was tisagenlecleucel (Kymriah™) produced by the Novartis Pharmaceuticals Corporation for the treatment of children and young adults with acute lymphoblastic leukemia [16]. Subsequently, three more CAR-T formulations were approved by the FDA for the treatment of various B-cell malignancies: axicabtagene ciloleucel (Yescarta™), brexucabtagene autoleucel (Tecartus™), and lisocabtagene maraleucel (Breyanzi®) [17–19]. In April 2021 and February 2022, two BCMA-specific CAR-T cell therapies were approved for the treatment of multiple myeloma, namely idecabtagene vicleucel (Abecma™) and ciltacabtagene autoleucel (Carvykti™) [20, 21]. CAR-T cell therapy has been reimbursed in Poland since September 2021 for the treatment of B-cell acute lymphoblastic leukemia in patients under the age of 25. In addition, since May 2022, the therapy has been reimbursed in the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma. Table I sets out details of each EU-approved CAR-T cell product.

CAR-T cell therapy and serious adverse events

CAR-T cell therapy has shown great promise in the treatment of hematological malignancies. One major concern regarding this therapy is the potential for life-threatening adverse events. The most frequently occurring of these adverse events are cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), CAR-T-related encephalopathy syndrome (CRES), infections, tumor lysis syndrome, allergic reactions, prolonged neutropenia, hypogammaglobulinemia, and aplasia-related hematological toxicity [22]. Of these, CRS and ICANS pose the greatest threat to patients undergoing CAR-T cell therapy. CRS arises from the activation of CAR-T cells, the

Table I. List of chimeric antigen receptor (CAR) T-cells preparations registered in European Union (EU) in treatment of hematological malignancies (sources [5, 6])

Commercial name, generic name, company	Product features: generation, origin, target antigen	Costimulatory domains	Primery indications (EU)	Today's indications (May 2023) (EU) (*USA)	Trial	Approval date
Kymriah™ Tisagenlecleucel **Novartis**	2nd Autologous CD19	4-1BB + CD3ζ	B-ALL, DLBCL	B-ALL, DLBCL, FL, HGBL*	ELIANA (NCT02228096)	US: 30 August 2017 EU: 23 August 2018
Yescarta™ Axicabtagene ciloleucel **Kite a Gilead company**	2nd Autologous CD19	CD28 + CD3ζ	PMBCL, DLBCL	PMBCL, DLBCL, HGBL, FL	ZUMA-1 (NCT02348216)	US: 18 October 2017 EU: 23 August 2018
Tecartus™ Brexucabtagene autoleucel **Kite a Gilead company**	2nd Autologous CD19	CD28 + CD3ζ	MCL	MCL, B-ALL	ZUMA-2 (NCT02601313)	US: 24 July 2020 EU: 14 December 2020
Abecma™ Idecabtagene vicleucel **Bristol-Meyers Squibb**	2nd Autologous BCMA	4-1BB+ CD3ζ	MM	MM	KarMMa (NCT03361748)	US: 26 March 2021 EU: 18 August 2021
Breyanzi® Lisocabtagene maraleucel **Bristol-Meyers Squibb**	2nd Autologous CD19	4-1BB+ CD3ζ	FL3B, PMBCL, DLBCL	FL3B, PMBCL, DLBCL, HGBL*	Transcend NHL001 (NCT02631044)	US: 05 February 2021 EU: 4 April 2022
Carvykti™ Ciltacabtagene autoleucel **Janssen-Cilag International**	2nd Autologous BCMA	4-1BB+ CD3ζ	MM	MM	CARTITUDE-1 (NCT03548207)	US: 28 February 2022 EU: 25 May 2022

B-ALL — acute lymphoblastic leukemia; DLBCL — diffuse large B-cell lymphoma; FL — follicular lymphoma; FL3B — stage 3B follicular lymphoma; HGBL — high-grade B-cell lymphoma; MCL — mantle cell lymphoma; MM — multiple myeloma; PMBCL — primary mediastinal large B-cell lymphoma

destruction of target cells post-antigen recognition, and the release of tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and interferon gamma (IFNγ). This triggers uncontrolled cascades without natural inhibition mechanisms. CRS symptoms include fever, hypotension, hypoxia, and tachycardia, potentially leading to multi-organ failure or coagulation disorders. The severity of CRS correlates with cancer cell mass. Based on the concentration of cytokines in the serum, prediction schemes for the severe course of CRS and neurological toxicity have been developed, the use of which may contribute to the inhibition of undesirable phenomena at an early stage [23]. Patients with severe neurotoxicity exhibit increased endothelial activation, disseminated intravascular coagulation (DIC) symptoms, and heightened blood-brain barrier (BBB) permeability [22, 23]. Elevated protein levels in cerebrospinal fluid (CSF) indicate BBB permeability, allowing CAR-T cell penetration and excessive cytokine production in the CSF [23]. Another expected side effect is the depletion of B lymphocytes expressing the CD19 marker, in the case of a response directed against this molecule. Aplasia of healthy cells expressing this marker leads to hypogammaglobulinemia requiring the use of immunoglobulin supplementation, which provides them with the necessary antibodies to fight

pathogens [24]. To mitigate the risk of CRS and neurotoxicity, the FDA in 2017 approved the use of a recombinant humanized monoclonal antibody, tocilizumab, for CAR-T cell therapy. Tocilizumab is a genetically engineered monoclonal antibody that targets the IL-6 receptor and is approved for treating patients with systemic connective tissue diseases. By blocking IL-6 receptor signaling, the drug inhibits inflammatory reactions. Tocilizumab rapidly reverses cytokine release syndrome (CRS) and has become the standard of care for this complication. Corticosteroids are used to suppress inflammation in patients who do not respond to IL-6 receptor blockade. Preliminary findings indicate that using IL-6 receptor blockade or steroids does not increase the risk of lymphoma relapse compared to patients without CRS following CAR-T therapy. Ongoing research is testing new drugs that block signal pathways in CRS, including siltuximab (an anti-IL-6 monoclonal antibody), anakinra (a human interleukin-1 receptor antagonist), dasatinib (a tyrosine kinase inhibitor), adenosine A3 receptor agonists, JAK–STAT inhibitors (namodenosone, piclidenosome), and lenzilumab (an anti-GM-CSF monoclonal antibody) [22].

The severity of adverse events associated with CAR-T cell therapy depends on many factors, including tumor mass before treatment, the intensity of the conditioning regimen, and the received dose of CAR-T lymphocytes [25, 26]. The tumor mass itself does not seem to affect the expansion of CD19 CAR-T cells, but it may negatively affect remission rates and overall survival [27]. A relationship between the severity of adverse events and tumor mass has also been observed with the use of CD19 CAR-T cell therapy in autoimmune diseases. In patients with systemic lupus erythematosus (SLE) who have received CAR-T cell therapy, despite a significant expansion of CAR-T cells *in vivo*, rapid relief of lupus symptoms has been observed, with few or no side effects [28, 29]. Similarly, a 41-year-old man with antisynthetase syndrome (ASyS) reported low severity of side effects after receiving CD19 CAR-T cell therapy, despite a significant expansion of CAR-T cells [30].

It is worth emphasizing that patients with CLL and ALL have a significantly increased number of B cells in their peripheral blood [31, 32], while patients with autoimmune diseases such as SLE or AsyS have a reduced or unchanged number of B cells [33–35]. The lack of serious adverse events in patients with SLE or AsyS who have received CAR-T therapy suggests that the increased adverse events seen in cancer patients are not directly caused by CAR-T cells. Rather, these adverse events seem to be related to the massive destruction of tumor cells by CAR-T cells. This may result in the release of intracellular content into the bloodstream and thus trigger tumor lysis syndrome (TLS) [36, 37]. This discovery offers an opportunity to prevent serious adverse events by treating patients with CAR-T cell therapy at an early stage of the disease, when the tumor mass is low.

Cost of autologous CAR-T cell production and allo-CAR-T as a new cost-effective strategy

One constraint on CAR-T cell therapy is its high cost of production, which can reach up to $500,000 [38]. CAR-T cell therapy is prominent as being one of the most expensive cancer treatment options available. The price for a single dose of approved CAR-T cell therapy ranges from $350,000 to $500,000 (Table II). Unfortunately, this high cost severely limits the accessibility and applicability of the therapy. Several factors contribute to the high cost of CAR-T cell therapy, with the primary factor being the intricate and labor-intensive manufacturing process. The development of CAR-T cells involves multiple steps performed within a good manufacturing practice (GMP) facility, necessitating the use of GMP-grade materials and highly skilled personnel. Systems like the CliniMACS Prodigy® enable comprehensive manufacturing and expansion in a single device. However, each automated system can handle only one patient's product at a time, leading to higher capital costs for manufacturing. In contrast to traditional small molecule-based drugs, every patient's CAR-T product must undergo rigorous quality control testing before being reintroduced into the patient's body, further escalating manufacturing cost. Another significant challenge to establishing a robust CAR-T platform is manufacturing failure. This arises because many cancer patients receiving CAR-T cells have undergone extensive prior treatments, depleting their T-cells. Consequent manufacturing failures lead to suboptimal efficacy. Thus, finding cost-effective strategies for CAR-T cell production and delivery, along with ensuring equitable access to this therapy, remains a critical concern [39].

Table II. Cost of autologous chimeric antigen receptor (CAR) T-cell therapies (source [39])

Commercial name	Today's indications (May 2023) (EU) (*USA)	Cost ($)
Kymriah™	B-ALL, DLBCL, FL, HGBL*	437,927–475,000#
Yescarta™	PMBCL, DLBCL, HGBL, FL	375,000–399,000#
Tecartus™	MCL, B-ALL	373,000
Abecma™	MM	441,743
Breyanzi®	FL3B, PMBCL, DLBCL, HGBL*	470,940
Carvykti™	MM	465,000

#Depends on indication; B-ALL — acute lymphoblastic leukemia; DLBCL — diffuse large B-cell lymphoma; FL — follicular lymphoma; FL3B — stage 3B follicular lymphoma; HGBL — high-grade B-cell lymphoma; MCL — mantle cell lymphoma; MM — multiple myeloma; PMBCL — primary mediastinal large B-cell lymphoma

Therefore, to make treatment more accessible, several new strategies are being tested, including the use of ready-made allogeneic CAR-T cells (allo-CAR-T) and the generation of CAR-T cells *in vivo*.

When allogeneic CAR-T cells are given to patients, there is a risk of graft-versus-host disease (GvHD) and rejection of the CAR-T cells by the host immune system. To minimize this risk, TCR genes are removed from CAR-T cells using the CRISPR/Cas9 system. In addition, to avoid host rejection of CAR-T cells, the formation of HLA class I molecules is blocked by deleting the beta$_2$-microglobulin (B2M) gene [40]. Nevertheless, a challenge in therapy containing allogeneic CAR-T cells is that allo-CAR-T cells do not survive for a long time in the body of patients, exposing them to limited anticancer efficacy [41]. There are several explanations for the reduced persistence of allo-CAR-T cells *in vivo*. One possibility is that allo-CAR-T cells, which do not express HLA class I molecules, may be targeted and eliminated by natural killer (NK) cells of the recipient. In addition, HLA class I molecules may also play a role in the survival of mature T cells *in vivo* [42]. Another strategy for lowering the cost of treating patients is the generation of CAR-T cells *in vivo*. CAR-T cells can be produced *in vivo* by delivering mRNA encoding CAR targeted to fibroblast activation protein (FAP) in lipid nanoparticles [43, 44]. With this approach, CAR-encoding lentiviral vectors are directly infused into the patient, and viral transduction of T cells occurs spontaneously *in vivo*.

Thus, *in vivo* generation of CAR-T cells would eliminate the need for *ex vivo* production of engineered cells and would therefore significantly reduce the cost of CAR-T therapy.

In addition, shortening the production of autologous CAR-T cells may also reduce costs by introducing new bioengineering solutions. The standard CAR-T cell production process involves several steps including T cell activation, viral transduction, and *ex vivo* expansion lasting at least one week. Expansion of huCART19-IL18 (humanized anti-CD19 CAR co-expressing IL-18) in *ex vivo* conditions is shortened to three days. As demonstrated in the first phase of the clinical trial, CAR-T cells, despite the shortened production time, are produced with good efficiency [45]. Another study has shown that CAR-T cells in mice can be generated within 24 hours by transduction of non-activated T cells without additional expansion, and still show antitumor efficacy [46].

Solid tumors and CAR-T cell therapy

Solid tumors account for c.90% of cancers in adults and 30% in children. Despite unprecedented success in hematological malignancies, CAR-T cell therapy remains a challenge in solid tumors. There are two main problems related to the effectiveness of therapy, which include the immunosuppressive tumor microenvironment (TME) and the limited set of unique antigens that CAR molecules can target [5, 47].

TME is enriched with regulatory T cells, tumor-associated macrophages (TAMs) and CD4+ 2 (Th2) T helper cells that produce anti-inflammatory cytokines such as IL-4, IL-10 and TGF-β. To offset the effects of TME, CAR-T cells are designed to express various helper molecules that can enhance the antitumor activity of CAR-T cells by reducing immunosuppression in the tumor microenvironment. To date, numerous CAR constructs capable of promoting the expression of helper molecules have been described in the literature, including cytokines such as IL-7, IL-12, IL-15, IL-18 and IL-21, as well as the constitutively activated IL-7 receptor C7R, non-coding RNA RN7SL1, BATF, CD40L, PRODH2, and c-Jun [48–52]. The CAR-T cells described above are often referred to as 'armored'.

Preclinical studies have shown that cell therapies using 'armored' CAR-T cells have greater anticancer activity against solid tumors. However, the critical issue is to maintain immune homeostasis and prevent autoimmunity and inflammation. Persistent disruption of cell signaling could unbalance and cause unintended consequences [5]. The ideal target for CAR-T therapy for solid tumors would be a membrane protein or glycolipid that is only expressed on the surface of tumor cells, and not in normal tissues. However, in theory, such a target does not exist. Currently, there are several target sites for solid tumor therapies that are in various stages of clinical trials. These include GD2, mesothelin, HER2, IL13Ra2, GPC3, EGFRvIII, CEA, claudin-18.2, MUC1, PSCA, and PSMA [53]. These target sites are highly expressed in solid tumors but are also present in some normal tissues. Even CD19, a target for CAR-T cell therapy in B-cell malignancies, is expressed in normal B-cells. Thus, patients receiving therapy with CD19-specific CAR-T cells develop B-cell lymphopenia and are more susceptible to infection, even with immunoglobulin replacement therapy [54]. One way to increase the specificity of CAR-T cell therapy is genetic modification of T cells capable of recognizing two different antigens present on tumor cells [55]. This approach can effectively eliminate tumor cells expressing only one antigen.

Currently, several clinical trials are under way to evaluate the effectiveness of CAR-T cell therapy in solid tumors. Promising research results have been obtained for neuroblastoma and diffuse intrinsic pontine glioma (DIPG) using GD2 CAR-T cell therapy [56, 57]. In a phase I clinical trial involving four patients with DIPG, GD2 CAR-T cell infusion was used. Three out of four patients showed clinical improvement and, interestingly, no signs of toxicity to non-cancerous cells were observed [58]. The lack of significant toxicity in GD2 CAR-T cell therapy has been attributed to the observation that CAR-T cells require a high level of antigen density for full effector function [59].

Polish experience with CAR-T

The Polish experience in the use of CAR-T cell therapy is worth mentioning. CAR-T cell therapy may only be used in certified healthcare facilities. Due to the restrictive procedures for preparing and administering products, appropriate infrastructure of the center and trained specialist staff are required for its use. The completed certification process confirms the center's full readiness to use CAR-T cell therapy.

The first clinic to obtain such a certificate in Poland was the Department of Hematology and Bone Marrow Transplantation of the Poznan University of Medical Sciences [60]. Other Polish hematology clinics followed suit. CAR-T cells administered to patients are commercially available drugs, manufactured abroad.

The Medical Research Agency has launched a program aimed at financing research and development activities in Poland in the field of non-commercial clinical trials on CAR-T. Currently, three projects are being implemented, the aim of which is to produce the first CAR-T cells in Poland. The first center which obtained authorization to manufacture the tested medicinal products of advanced technology and completed the validation trials was the Regional Blood Center in Poznań which is a member of the consortium under the CARMEN project 'Development of an optimal strategy for the production and administration of CAR-T lymphocytes in adults and children with B-cell non-Hodgkin lymphomas and acute lymphoblastic leukemia' No. 2020/ /ABM/01/00107-00 together with the Department of Hematology, Blood Cancer and Bone Marrow Transplantation, Wroclaw Medical University.

Conclusions

The use of CAR-T cells is considered a highly innovative and effective method of therapy, and undoubtedly represents one of the most groundbreaking achievements in cancer treatment of recent years. Methodology development, including gene transfer to human T lymphocytes, the development of a cell culture strategy, the identification of new targets, rational supportive therapy, the selection of biomarkers, the prevention of high toxicity, and an innovative approach to constructing CAR cells, represents an opportunity for further improvement of the prognosis of cancer patients' hematology and solid tumors.

CAR-T cell therapy has made significant progress in the treatment of cancer, but several challenges still need to be overcome in order to make this treatment more widely available and effective.

Ongoing research into the development of CAR-T cell therapy in solid tumors, the general availability of CAR-T cell therapy, and the safety, cost and application of CAR-T therapy in non-cancer diseases, will all be critical to the future success of this treatment.

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AB — conceptualization, literature analysis, original draft preparation, review and editing. AB, MB — literature analysis, original draft preparation, review and editing. KO, AŁ, RK — review and editing. KJ — conceptualization, review and editing. All authors have read and agreed published version of manuscript.

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