VIA MEDICA

REVIEW ARTICLE

Acta Haematologica Polonica 2022
Number 4, Volume 53, pages 241–248
DOI: 10.5603/AHP.a2022.0032
ISSN 0001–5814
e-ISSN 2300–7117

A living drug: application of CAR-T therapy for lymphoid malignancies and beyond

Anna Strzelec, Anna Klima, Natalia Gawlik-Rzemieniewska, Grzegorz Helbig

Faculty of Medicine in Katowice, Medical University of Silesia, Department of Hematology and Bone Marrow Transplantation, Katowice, Poland

Abstract

The ongoing development of novel personalized cancer therapies has resulted in the implementation of T-cells enriched with synthetic chimeric antigen receptors, known as chimeric antigen receptors T-cell (CAR-T) cells, into clinical practice. CAR-T cells are able to recognize and bind specific antigens present on the surface of target cells — so-called tumor-associated antigens. This innovative method has been approved for the treatment of hematological malignancies and may also serve as a bridge to hematopoietic stem cell transplantation. The production of the drug containing modified T-cells consists of several steps — leukapheresis, T-cell activation, transduction and expansion of the final CAR-T cells. Activation of CAR-T cells occurs through a pathway independent of the major histocompatibility complex, which is often associated with uncontrolled responses from the immune system and adverse reactions such as cytokine release syndrome. CAR-T therapy can only be performed in certified centers, and requires close cooperation between experienced specialists of different medical disciplines. This is what determines its effectiveness. Every step from collection and cryopreservation, through transport and modification, to thawing and infusion is strictly controlled because it has a critical impact on the quality and efficiency of the drug. Despite its proven benefits, CAR-T therapy remains available only to patients who meet well-defined criteria. These however are liable to change with the emergence of new indications.

Key words: CAR-T, efficacy, CRS, ICANS, side effects

Acta Haematologica Polonica 2022; 53, 4: 241–248

Introduction

The ongoing quest to develop more effective methods of cancer treatment has recently resulted in the implementation of a therapeutic modality based on the patient's cells, which combines the achievements of gene-, cell-, and immunotherapies.

Chimeric antigen receptors T cell (CAR-T) therapy, i.e. the use of T lymphocytes enriched with synthetic chimeric antigen receptors (CAR), has shown significant efficacy in the treatment of certain hematological malignancies, including mainly refractory and relapsed leukemias and lymphomas [1].

The motivation to engineer CAR-T cells was the antibody dependent cellular cytotoxicity (ADCC) process. This begins with the coating of the target cell by an antibody, which bridges a natural killer (NK) cell containing a Fc receptor (FcR). The activation of NK cells results in their degranulation, leading to the release of perforin, granzymes, and granulysins, followed by apoptosis of the target cell [2, 3]. In the late 1980s, the first studies were published describing the activation of T-cells without the involvement of major histocompatibility complex (MHC), i.e. through the combination of T-cell receptor (TCR) with variable antibody fragments [4, 5]. Three decades later, in

*Address for correspondence: Grzegorz Helbig, Department of Hematology and Bone Marrow Transplantation, Medical University of Silesia, 40–032 Katowice, Dąbrowskiego 25, fax +48 32 255 49 85, phone +48 32 259 13 10, e-mail: ghelbig@o2.pl

Received: 15.05.2022 Accepted: 12.06.2022

Copyright © 2022

The Polish Society of Haematologists and Transfusiologists, Insitute of Haematology and Transfusion Medicine.

All rights reserved.

This article is available in open access under Creative Common Attribution-Non-Commercial-No Derivatives 4.0 International (CC BY-NC-ND 4.0) license, allowing to download articles and share them with others as long as they credit the authors and the publisher, but without permission to change them in any way or use them commercially.

Table I. Food and Drug Administration (FDA)-approved chimeric antigen receptors T-cell (CAR-T) cell therapies (source [6])

Brand name (generic name)	Indications	Target	Date of approval
Kymriah™ (tisagenlecleucel)	r/r DLBCL r/r B-ALL up to 25 years	CD19	August 30, 2017
Yescarta™ (axicabactagene ciloleucel)	r/r DLBCL r/r PMBCL r/r FL	CD19	October 18, 2017
Tecartus™ (brexucabtagene autoleucel)	r/r MCL r/r B-ALL	CD19	July 24, 2020/October 1, 2021
Breyanzi® (lisocabtagene maraleucel)	r/r LBCL	CD19	February 5, 2021
Abecma® (idecabtagene vicleucel)	r/r MM	BCMA	March 26, 2021
Carvykti™ (ciltacabtagene autoleucel)	r/r MM	BCMA	February 28, 2022

r/r — relapsed/refractory; DLBCL — diffuse large B-cell lymphoma; B-ALL — B-cell acute lymphoblastic leukemia; PMBCL — primary mediastinal large B-cell lymphoma; FL —follicular lymphoma; MCL — mantle cell lymphoma; LBCL — large B-cell lymphoma; MM — multiple myeloma; BCMA — B-cell maturation antigen

2017, the US Food and Drug Administration (FDA) approved the two first drugs for relapsed or refractory (r/r) malignancies: for B-cell acute lymphoblastic leukemia (B-ALL) — Kymriah™ (Novartis) and for diffuse large B-cell lymphoma (DLBCL) — Yescarta™ (KitePharma). Since then, six CAR-T-based immunotherapies have been approved (see Table I) [6].

Structure of CAR-T

The transmembrane CAR-T cells' receptor has a modular structure and is classically composed of five parts, which determine its durability and efficacy:

1) extracellular antigen-binding domain — a key component responsible for CAR specificity by recognizing a well-defined antigen, such as CD19, without the involvement of the MHC. It is derived from the single-chain variable fragment of the antibody (scFv), which is built from light and heavy regions linked by a peptide fragment;
2) hinge region — a linking element whose length and flexibility affect CAR functionality;
3) transmembrane domain — a hydrophobic fragment responsible for signal transduction into the cell and for receptor stability;
4) intracellular costimulatory domain — co-responsible for signal transduction. This structure reduces the risk of lymphocyte anergy, thus preserving the functionality, proliferation and survival of CAR-T cells. The drug Kymriah™ contains the 4-1BB domain, and Yescarta™ contains CD28. Studies indicate that the use of 4-1BB is associated with a later and smaller peak of expansion and higher longevity of CAR-T cells relative to those with the CD28 domain, which rapidly reach a maximum of activity, and subsequently become exhausted. It has been observed that the use of the 4-1BB domain promotes the differentiation of cells into central memory T-cells (T_{CM}), whereas the CD28 domain affects the differentiation into effector memory T-cells (T_{EM});
5) intracellular signalling domain — responsible for signal transduction into the cell, usually contains the TCR CD3ζ complex [7–10] (see Figure 1).

Subsequent modifications of CAR have led to the development of their five generations. The first generation lacks a costimulatory domain, making it insufficiently effective. The second generation contains one costimulatory domain, and the third generation contains two. The fourth generation has been further enriched with the ability to produce proteins such as cytokines — interleukins (IL) and chemokines, and the fifth generation with the expression of interleukin-2 receptor (IL-2R) β domain, which stimulates the STAT3/STAT5 signaling pathway. Currently, second generation CARs have been used in clinical practice [11].

Production of CAR-T cells and application in clinical practice

The production of a CAR-T cell drug is a multi-step process, and requires the collaboration of many specialists. Once the number of peripheral blood lymphocytes exceeds 0.3 × 10^3/µL, a patient eligible for CAR-T therapy is referred for leukapheresis targeting unmobilized CD3+ T-cells. This procedure is performed in transplant centers that routinely perform apheresis to collect hematopoietic stem cells from mobilized peripheral blood [12]. It is important to maintain an interval between the use of certain drugs indicated by the manufacturer and the apheresis, the so-called wash out period. For example, bendamustine impairs CAR-T cell production, but there are many others [13].

The main factors affecting the efficiency of leukapheresis are the patient's health status and age. In patients being treated for malignancies, lymphopenia, as a consequence of chemotherapy, may hinder the collection of sufficient numbers of cells [14]. An example is the concentration of memory T-cells in patients with ALL and non-Hodgkin lymphoma (NHL), which decreases with each course of standard treatment [15]. The possible contamination of

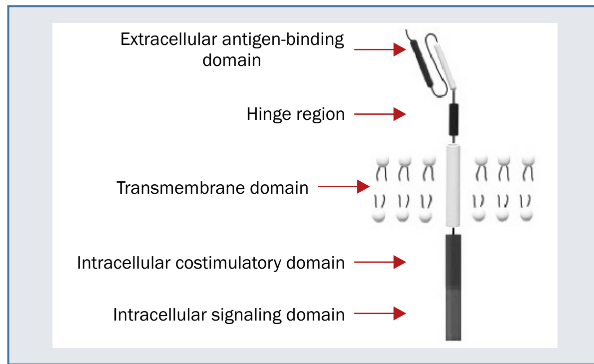

Figure 1. Schematic diagram of chimeric antigen receptors (CAR) structure [7–10]

the material by erythrocytes, monocytes and granulocytes can also be a problem [12]. The collected product should contain as many T-cells as possible. This has a beneficial effect on the efficiency of CAR-T cell production, and also reduces the possibility of accidental transduction of other populations, including tumor cells, inducing resistance to therapy. In infants and young children, the smaller volume of circulating blood remains an additional challenge [16].

Bearing these limitations in mind, methods of purifying the material after apheresis can be used in the production of CAR-T therapy drug. One of these involves the selection of T lymphocytes, or their specific subpopulations, using magnetic beads conjugated with antibodies. This allows the separation of a pure population of required cells from a heterogeneous population of leukocytes [15]. A fully automated closed system CliniMACS Prodigy® (Miltenyi Biotec, Germany) using appropriate antibodies combined with microbeads is commercially available [17]. T-lymphocyte selection is mainly performed on material collected from patients with a high number of tumor cells in the peripheral blood, e.g. from untreated patients with chronic lymphocytic leukemia (CLL). Selection is also used in the later steps of drug production. Using anti-CD3, anti-CD4 or anti-CD8 antibodies, a final cell product with a specific CD4+/ /CD8+ lymphocyte ratio can be obtained, which positively influences the antitumor activity of CAR-T cells [18]. Furthermore, research to develop subcutaneous implants is in progress. They would be coated with antigens specific to receptors present on desired T-lymphocyte subpopulations, so that *in vivo* they could specifically capture cells necessary for CAR-T production. After a few days, such implants would be removed from the patient's body, thus replacing traditional apheresis [14].

The collected material is delivered to the Cell Bank and to the Hematological Laboratory, where CD3+ cell count and viability is determined by flow cytometry. After confirming the appropriate quality, the product is prepared for transport to the Cell Engineering Laboratory. Cells collected during leukapheresis can be transported unfrozen or frozen, although fresh lymphocytes have a short period of sufficiently high viability, and so in most cases cryopreservation of cell suspension in liquid nitrogen is recommended, using 5–10% dimethylsulfoxide (DMSO) as a cryoprotectant [15].

Proper cryopreservation of material with adequate cellularity is critical to maintaining product quality, and must be done under controlled conditions with a slow rate of temperature decrease. The transport of cells in an adapted dewar, a kind of vessel with a vacuum space between liquid nitrogen and the outer walls, is a critical moment for maintaining the viability of the required cytotoxic T-cells. Currently, pharmaceutical companies which are distributors of the drug in Poland cooperate with laboratories located in Switzerland, the United States of America (USA) and France, among others [19].

In pharmaceutical manufacture, the material is firstly thawed and washed to remove anticoagulants added during leukapheresis with the use of counterflow centrifugation. The T lymphocytes are then activated, which is a necessary step for subsequent transduction and expansion *ex vivo* [20]. The most common way to activate T lymphocytes is by stimulation using soluble anti-CD3 monoclonal antibodies or immobilized — on the surface of flasks or paramagnetic beads — anti-CD3 and anti-CD28 antibodies. The anti-CD3 antibodies are responsible for the proliferative signal, and the anti-CD28 antibodies are responsible for the costimulatory signal. Flasks form a relatively small surface area for T-cells to adhere to, so paramagnetic beads are more commonly used. The suspension containing such microbeads should then be exposed to a magnetic field in order to remove them from the finished product, which will be administered to the patient [15, 18]. Another way to activate T-cells uses retronectin, a recombinant fragment of human fibronectin that enhances gene transfer efficiency in retroviral transduction. Retronectin, combined with anti-CD3 and anti-CD28 monoclonal antibodies, is a promising method for proliferating less differentiated T-cell subpopulations, which may be beneficial for long-term persistence of CAR-T cells *in vivo*. However, retronectin activation should be performed with caution, especially in patients with a high percentage of tumor cells in the peripheral blood, as it may stimulate persistent malignant B cells within the cell product, especially if T-cell selection was not performed at the initial production step [18, 21].

In the next step, the CAR transgene is delivered into cells using lentiviral or retroviral vectors. The high transduction efficiency with these viruses requires the previously mentioned activation of T lymphocytes. Especially for retroviruses, which transduce only dividing cells, proliferation is essential for gene transfer [22]. Lentiviral vectors are usually produced by transient transfection using large amounts of plasmid DNA, making them more expensive than retroviruses which can be produced using stable packaging cell

lines [23]. However, it is important that viral vectors introduce genetic material into the genome in a manner that is random, otherwise we pose the risk of silencing a random gene or causing insertional oncogenesis [14].

Recent years have also seen the development of non-viral T-cell transfection techniques, which use transposon/ /transposase systems. A transposon is a DNA sequence that has the ability to change position within the genome via transposase-mediated excision and insertion [24]. To date, four transposons have been described: Sleeping Beauty (SB) and Frog Prince, which were reconstructed from inactive transposons derived from the fish and frog genomes, respectively; Tol2, which is the only vertebrate transposon of natural origin; and piggyBac (PB), which is derived from the insect *Trichoplusia ni*. SB and PB have high transposase activity in mammalian cells, with higher activity for PB and involving larger chromatin loops than in SB. In addition, PB does not leave gene excision marks, so that possible genome damage is less likely. Transposition involving PB is also simple to reproduce *in vitro* [25]. Targeted CAR transgene insertion can also be performed using the CRISPR-Cas9 genome editing system. Preclinical studies have shown promise in using this system to ablate the endogenous αβ TCR receptor on the surface of T-cells, and thus reduce the prevalence of graft-versus--host disease (GvHD) [26–28]. These modifications allow for the expression of CAR, which gives the T-cells the ability to recognize a specific antigen. In hematological malignancies, where CAR-T therapy is predominantly used, the receptor for the CD19 antigen is most frequently used [1].

The ready, genetically modified lymphocytes are expanded in static or dynamic dishes or culture devices until the required therapeutic dose is reached. Ready-made culture media adapted to multiple cells of adaptive cell therapy, supplemented with e.g. IL-7 and IL-15 or human serum, are used. Cultures are monitored for bioanalytes – pH, glucose, lactate, electrolytes, pO_2, pCO_2, humidity – and for cell proliferation and volume change. This step can take place before or after gene transduction, depending on the drug manufacturer, and lasts approximately 10 days [20, 29]. Finally, cells are harvested and cryopreserved for further distribution. The frozen drug is received by the hospital pharmacy, which is responsible for its storage at a temperature ≤–130°C and subsequent delivery to the department. The completed product is intended only for a single autologous application in a particular patient [30].

The complete process of CAR-T cell production usually takes about four weeks (17 to 60 days). During this time, the patient may be considered for bridging therapy in the form of classical chemotherapy or immunotherapy and radiation therapy, based on disease burden. Promising results have been observed with the use of polatuzumab, but this requires further studies [31]. The lymphodepletion phase is between day –5 and day –3, and a regimen containing fludarabine/cyclophosphamide, which increases the expansion of CAR-T CD19 cells, is used. On the day of infusion, the drug is thawed in a 37°C water bath at the patient's bedside, as is done in cryopreserved hematopoietic stem cell transplantation, and immediately administered as an intravenous infusion. Additionally, premedication of oral paracetamol and intravenous diphenhydramine, or another H1 antihistamine, may be considered, but prophylactic use of systemic corticosteroids is not recommended – there is a possibility of drug interference.

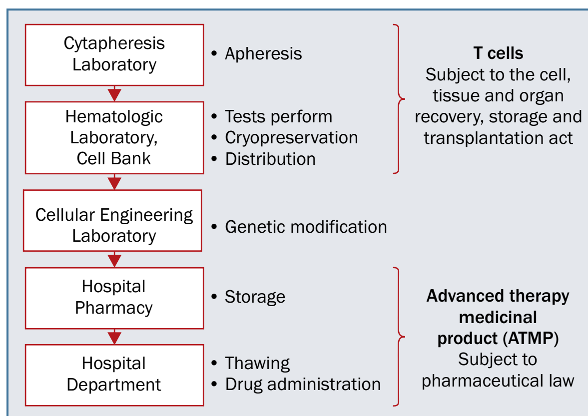

Figure 2. Schematic of collaboration in implementation of chimeric antigen receptors T-cell (CAR-T) therapy

A requirement for the use of the product is access to at least one dose of tocilizumab, which has an immunosuppressive effect. After the infusion, until at least day 10, the patient is hospitalized and thereafter for at least four weeks is obliged to remain near the center for close observation [19, 32, 33]. Figure 2 presents a schematic showing collaboration in the implementation of CAR-T therapy.

Mechanism of action

Modified CAR-T cells are able to recognize and bind specific antigens present on the surface of target cells, mainly tumor cells – tumor-associated antigens (TAAs) – however, it should be noted that normal antigen-presenting cells may also be targeted. Upon binding to the antigen, a signaling cascade activating CAR-T is induced through a conformational change. The release of perforins and granzymes, using immunological synapse, leads to the activation of cytotoxic mechanisms. Expression of Fas ligand (FasL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on the surface of CAR-T, by binding receptors containing the so-called death domain, induces apoptosis. Among others, caspase 8 and caspase 3 are involved in this process. Secretion of proinflammatory cytokines (IL-2, interferon-γ, tumor necrosis factor-α) activates other cells of the immune system. The reason for the superiority of the described processes in the therapy of hematological

malignancies compared to solid tumors is the lack of physical barriers — the localization of tumor cells and migrating T lymphocytes is usually the same — and the lack of an immunosuppressive microenvironment, which hinders the infiltration of CAR-T cells into the tumor site [34, 35].

CAR-T available for Polish patients

The cost of administering the drug exceeds $350,000 [36]. In Poland, CAR-T therapy has been reimbursed for patients with relapsed/refractory (r/r) B-ALL up to 25 years since September 2021 and for patients with r/r DLBCL since May 1 2022. A patient who meets the reimbursement criteria, in accordance with the relevant drug program (B.93), may apply through an accredited center for eligibility for treatment, which is subject to a final decision by the CAR-T Coordination Team.

The number of clinical trials for CAR-T therapy in 2022 was about 1,000, of which less than 10% are being conducted in Europe [37]. Despite excellent research facilities, European countries, compared to the USA or China, have complicated and time-consuming regulatory regulations, resulting in delays in clinical application of trials, as well as funding problems [38]. In Poland, the development of CAR-NET adoptive therapy is possible thanks to a grant from the Medical Research Agency worth more than $220,000, which will be implemented by a consortium between 2021–2026. This aims to improve therapeutic efficacy and significantly reduce the cost of CAR-T therapy, which would enable its application on a larger scale, rather than as before in the form of single cases often financed by public donations.

Difficulties associated with use of CAR-T therapy

Activation of CAR-T cells through an MHC-independent pathway may be associated with uncontrolled immune responses and some adverse reactions. The main ones include cytokine release syndrome (CRS), neurotoxicity with encephalopathy, headache, slurred speech and hallucinations, and also infections. Some patients develop transaminases increase, hypogammaglobulinemia, disseminated intravascular coagulation (DIC), and macrophage activation syndrome (MAS). These abnormalities are usually the manifestations of the expansion of CAR-T cells, which, after interaction with the patient's immune cells, activate each other, leading to increased toxicity [10].

CRS and ICANS

Binding of the CAR-T cell to the target TAA results in a cascade of reactions, including the production of proinflammatory cytokines. While desirable in limited amounts, in excess they can lead to serious clinical symptoms and threaten the patient's life. Symptoms of CRS include fever, decreased blood pressure, muscle and joint pain, accelerated heart rate, and tachypnea. The severity of CRS is assessed using a 5-grade classification. In extreme cases, CRS can lead to shock and multiorgan failure similar to hemophagocytic lymphohistiocytosis (HLH) or MAS. It is supposed that the severity of CRS correlates with previous allotransplantations, the percentage of blasts in the bone marrow before lymphodepletion, the dose of CAR-T cells, and the type of costimulatory domain used. An especially life-threatening condition is the rare blood-brain barrier injury leading to the development of immune effector cell-associated neurotoxicity syndrome (ICANS) with seizures, aphasia, brain edema, hypoxia, and elevated IL-15 levels. For treatment, in addition to IL-6 inhibitors, corticosteroids are used for patients with concomitant CRS. Preclinical studies on mouse models also indicate the involvement of IL-1 in the pathogenesis of CRS, and therefore the benefit of administering IL-1R antagonists or modification of 4th generation CAR-T to allow its release into the circulation. Another solution may be the inactivation of CAR-T cells by enriching them, e.g. with CD20 antigens, and then, in cases of CRS, administering rituximab; however, it should be remembered that this way of eliminating CAR-T cells will not be immediate [10, 39, 40].

On-target/off-tumor effect

Antigens (Ag) present on the surface of cancer cells are also found on some regular cells. The use of therapies targeting such Ag therefore runs the risk of attacking non-cancerous tissues. Due to the high heterogeneity of tumor cells, both inside cancerous and inter-individual, it is challenging to develop a unique set of TAAs to target CAR-T cells. This phenomenon is also an impediment when trying to develop bispecific CARs. One way to protect non-malignant cells may be to exploit the difference in expression of the same Ags on normal and cancer cells.

The administration of CAR-T cells whose scFv will have low affinity, and thus whose activation requires a high density of Ag on the surface of target cells, may prevent the destruction of normal tissues with low Ag expression [10, 41, 42].

Antigen loss

The complete or partial loss of Ag expression by tumor cells may be due to the proliferation of clones already present that lack specific Ag, or to the effect of treatment — the result of such a kind of selective pressure is the resistance of the patient to mono-targeted CAR-T therapy based on a single TAA. Some patients treated with anti-CD19 CAR-T cells may relapse with CD19- tumor cells due to mutations

or alternative gene splicing. Research is currently in progress to develop CAR-T cells that have CARs directed against several different Ags, including CD20 and CD22, to delay or avoid the difficulties associated with Ag loss [1, 10, 42].

T-cell exhaustion

An important factor limiting the efficacy of CAR-T therapy is T-cell exhaustion. CAR-T cell depletion is characterized by impaired effector function and increased expression of inhibitory receptors, such as the programmed death-receptor 1 (PD-1), due to chronic antigenic stimulation usually resulting from an ongoing chronic infection or from the neoplastic process itself. Aging or exhausted CAR-T cells are characterized by impaired proliferation and persistence *in vivo*. Strategies to detect, prevent, or reverse the effects of T-cell exhaustion — such as checkpoint blockade through ligand inhibition – are needed to enhance the efficacy of CAR-T therapy. One of them may include programmed death-ligand 1 (PD-L1) or PD-L2 by using nivolumab and pembrolizumab (anti-PD-1) [43–45].

Future of CAR-T therapy

Therapy of solid tumors

The effects of CAR-T therapy for solid tumors are currently not satisfying and are not associated with long-term responses. The problem of cancer cells heterogeneity precludes the success of available generations of CAR-T cells. In addition, the immunosuppressive microenvironment, including the extracellular matrix, constitutes a specific barrier and significantly limits the infiltration of CAR-T cells, leading to their depletion. Tumor cells are also characterized by increased expression of, among others, PD-L1, responsible for activation of signaling pathways impairing T-cell function.

In the future, determination of the type of abnormal immune response may be used to enrich CAR-T cells with, for example, receptors for specific ILs and chemokines produced by the tumor microenvironment. With such modifications, cytokines released by cancer cells will serve as chemoattractants for CAR-T cells [41, 46].

Allogeneic CAR-T cells

All CAR-T cell products on the market or in clinical trials are autologous, i.e. are produced from T-cells from the same patient, so if they are manufactured from dysfunctional T-cells, they may not be effective. CAR-T therapy might be the only treatment for patients with resistant and relapsing forms of the disease, but it should be kept in mind that in patients with leukopenia it can be difficult or impossible to obtain sufficient numbers of cells. The CAR-T vein-to-vein process time is also an issue — patients in poor condition may not survive the waiting period required for genetic modification.

The solution to these obstacles is offered by potentially lymphocytes from a healthy allogeneic donor; however, as in the case of transplantation, this is associated with the risk of rejection of CAR-T by the host or the development of GvHD. Accordingly, one of the concepts is to modify CAR-T cells and turn off the mediators of GvHD — the TCRs. Researchers also aim to develop universal allogeneic CAR-Ts, ready to be administered to patients with a specific disease — in other words, 'off-the-shelf CAR-T' [41, 47, 48].

Summary

CAR-T therapy is performed only in specialized centers with extensive experience in allotransplantation, which have received appropriate accreditation at several levels. The whole procedure is complicated, not only in terms of the sophistication of the genetic modifications performed, but also given its logistical difficulties. Despite its remarkable innovativeness, due to some adverse reactions, high costs and limited number of eligible centers, currently it is not the gold standard of treatment, but this may change.

The development of CAR-T therapy is of enormous interest, and thanks to the cooperation of bioengineers and clinicians it will undergo more and more innovative improvements in response to real needs and problems arising in clinical practice. It is possible that this therapy, along with immune checkpoint inhibitors and bispecific antibodies, will become the standard of care for hematooncology patients.

Authors' contributions

AS, AK — analyzed data, conceived and wrote manuscript, drew figures. GH, NGR — analyzed data, conceived and wrote manuscript, critical review. All authors contributed to the article and approved the submitted version.

Conflict of interest

GH — Speaker's fee and Advisory Board for Novartis (GH). AS, AK, NRG — none.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/ EU for animal experiments; uniform requirements for manuscripts submitted to biomedical journals.

References

1. Han D, Xu Z, Zhuang Y, et al. Current progress in CAR-T cell therapy for hematological malignancies. J Cancer. 2021; 12(2): 326–334, doi: 10.7150/jca.48976, indexed in Pubmed: 33391429.
2. Kohrt HE, Houot R, Marabelle A, et al. Combination strategies to enhance antitumor ADCC. Immunotherapy. 2012; 4(5): 511–527, doi: 10.2217/imt.12.38, indexed in Pubmed: 22642334.
3. Chen Y, You F, Jiang L, et al. Gene-modified NK-92MI cells expressing a chimeric CD16-BB-ζ or CD64-BB-ζ receptor exhibit enhanced cancer-killing ability in combination with therapeutic antibody. Oncotarget. 2017; 8(23): 37128–37139, doi: 10.18632/oncotarget.16201, indexed in Pubmed: 28415754.
4. Kuwana Y, Asakura Y, Utsunomiya N, et al. Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. Biochem Biophys Res Commun. 1987; 149(3): 960–968, doi: 10.1016/0006-291x(87)90502-x, indexed in Pubmed: 3122749.
5. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci USA. 1989; 86(24): 10024–10028, doi: 10.1073/pnas.86.24.10024, indexed in Pubmed: 2513569.
6. FDA. Cellular and Gene Therapy Products. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products (April 10, 2022).
7. Zhang C, Liu J, Zhong JF, et al. Engineering CAR-T cells. Biomark Res. 2017; 5: 22, doi: 10.1186/s40364-017-0102-y, indexed in Pubmed: 28652918.
8. Abate-Daga D, Davila ML. CAR models: next-generation CAR modifications for enhanced T-cell function. Mol Ther Oncolytics. 2016; 3: 16014, doi: 10.1038/mto.2016.14, indexed in Pubmed: 27231717.
9. Zhao Z, Condomines M, van der Stegen SJC, et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell. 2015; 28(4): 415–428, doi: 10.1016/j.ccell.2015.09.004, indexed in Pubmed: 26461090.
10. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol. 2020; 17(3): 147–167, doi: 10.1038/s41571-019-0297-y, indexed in Pubmed: 31848460.
11. Golubovskaya V. CAR-T cells targeting immune checkpoint pathway players. Front Biosci (Landmark Ed). 2022; 27(4): 121, doi: 10.31083/j.fbl2704121, indexed in Pubmed: 35468680.
12. Gee AP. GMP CAR-T cell production. Best Pract Res Clin Haematol. 2018; 31(2): 126–134, doi: 10.1016/j.beha.2018.01.002, indexed in Pubmed: 29909913.
13. Nastoupil LJ. The evolving use of CAR T-cell therapy in follicular lymphoma. Clin Adv Hematol Oncol. 2021; 19(11): 684–686, indexed in Pubmed: 34807012.
14. Piscopo NJ, Mueller KP, Das A, et al. Bioengineering solutions for manufacturing challenges in CAR T cells. Biotechnol J. 2018; 13(2), doi: 10.1002/biot.201700095, indexed in Pubmed: 28840981.
15. Abou-El-Enein M, Elsallab M, Feldman SA, et al. Scalable manufacturing of CAR T cells for cancer immunotherapy. Blood Cancer Discov. 2021; 2(5): 408–422, doi: 10.1158/2643-3230.BCD-21-0084, indexed in Pubmed: 34568831.
16. Lee G, Arepally GM. Anticoagulation techniques in apheresis: from heparin to citrate and beyond. J Clin Apher. 2012; 27(3): 117–125, doi: 10.1002/jca.21222, indexed in Pubmed: 22532037.
17. Poorebrahim M, Sadeghi S, Fakhr E, et al. Production of CAR T-cells by GMP-grade lentiviral vectors: latest advances and future prospects. Crit Rev Clin Lab Sci. 2019; 56(6): 393–419, doi: 10.1080/10408363.2019.1633512, indexed in Pubmed: 31314617.
18. Stock S, Schmitt M, Sellner L. Optimizing manufacturing protocols of chimeric antigen receptor T cells for improved anticancer immunotherapy. Int J Mol Sci. 2019; 20(24), doi: 10.3390/ijms20246223, indexed in Pubmed: 31835562.
19. Kymriah. Therapy for leukaemia/lymphoma. https://www.us.kymriah.com (April 21, 2022).
20. Master A, O'Connor RS. T cell media: a comprehensive guide to key components. https://cellculturedish.com/t-cell-media-comprehensive-guide-key-components/ (April 21, 2022).
21. Stock S, Hoffmann JM, Schubert ML, et al. Influence of retronectin-mediated T-cell activation on expansion and phenotype of CD19-specific chimeric antigen receptor T cells. Hum Gene Ther. 2018; 29(10): 1167–1182, doi: 10.1089/hum.2017.237, indexed in Pubmed: 30024314.
22. Ghorashian S, Pule M, Amrolia P. CD19 chimeric antigen receptor T cell therapy for haematological malignancies. Br J Haematol. 2015; 169(4): 463–478, doi: 10.1111/bjh.13340, indexed in Pubmed: 25753571.
23. Ramanayake S, Bilmon I, Bishop D, et al. Low-cost generation of Good Manufacturing Practice-grade CD19-specific chimeric antigen receptor-expressing T cells using piggyBac gene transfer and patient-derived materials. Cytotherapy. 2015; 17(9): 1251–1267, doi: 10.1016/j.jcyt.2015.05.013, indexed in Pubmed: 26212611.
24. Ivics Z, Hackett PB, Plasterk RH, et al. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. Cell. 1997; 91(4): 501–510, doi: 10.1016/s0092-8674(00)80436-5, indexed in Pubmed: 9390559.
25. Lin Z, Liu X, Liu T, et al. Evaluation of nonviral *piggyBac* and *lentiviral* vector in functions of CD19+ chimeric antigen receptor T cells and their antitumor activity for CD19 tumor cells. Front Immunol. 2021; 12: 802705, doi: 10.3389/fimmu.2021.802705, indexed in Pubmed: 35082789.
26. Mirones I, Moreno L, Patiño-García A, et al. Grupo de Inmunoterapia y Terapias Avanzadas de la Sociedad Española de Hematología y Oncología Pediátricas, Grupo de Inmunoterapia y Terapias Avanzadas de la Sociedad Española de Hematología y Oncología Pediátricas. [Immunotherapy with CAR-T cells in paediatric haematology-oncology]. An Pediatr (Engl Ed). 2020; 93(1): 59.e1–59.e10, doi: 10.1016/j.anpedi.2019.12.014, indexed in Pubmed: 32107177.
27. Freitag F, Maucher M, Riester Z, et al. New targets and technologies for CAR-T cells. Curr Opin Oncol. 2020; 32(5): 510–517, doi: 10.1097/CCO.0000000000000653, indexed in Pubmed: 32657796.
28. Razeghian E, Nasution MKM, Rahman HS, et al. A deep insight into CRISPR/Cas9 application in CAR-T cell-based tumor immunotherapies. Stem Cell Res Ther. 2021; 12(1): 428, doi: 10.1186/s13287-021-02510-7, indexed in Pubmed: 34321099.
29. Miltenyi Biotec. Engineering of CAR T cells for research use. https://www.miltenyibiotec.com/_Resources/Persistent/761c3be7be470fc995206924676e45625273581a/IM0022471.pdf (May 05, 2022).
30. Vormittag P, Gunn R, Ghorashian S, et al. A guide to manufacturing CAR T cell therapies. Curr Opin Biotechnol. 2018; 53: 164–181, doi: 10.1016/j.copbio.2018.01.025, indexed in Pubmed: 29462761.
31. Liebers N, Duell J, Fitzgerald D, et al. Polatuzumab vedotin as a salvage and bridging treatment in relapsed or refractory large B-cell lymphomas. Blood Adv. 2021; 5(13): 2707–2716, doi: 10.1182/bloodadvances.2020004155, indexed in Pubmed: 34196677.
32. FDA. Package Insert: Kymriah. https://www.fda.gov/media/107296/download (April 21, 2022).
33. FDA. Package Insert: Yescarta. https://www.fda.gov/media/108377/download (April 21, 2022).

34. Benmebarek MR, Karches CH, Cadilha BL, et al. Killing mechanisms of chimeric antigen receptor (CAR) T cells. Int J Mol Sci. 2019; 20(6), doi: 10.3390/ijms20061283, indexed in Pubmed: 30875739.
35. Cartellieri M, Bachmann M, Feldmann A, et al. Chimeric antigen receptor-engineered T cells for immunotherapy of cancer. J Biomed Biotechnol. 2010; 2010: 956304, doi: 10.1155/2010/956304, indexed in Pubmed: 20467460.
36. Lin JK, Muffly LS, Spinner MA, et al. Cost effectiveness of chimeric antigen receptor T-cell therapy in multiply relapsed or refractory adult large B-cell lymphoma. J Clin Oncol. 2019; 37(24): 2105–2119, doi: 10.1200/JCO.18.02079, indexed in Pubmed: 31157579.
37. Clinical trials. CAR-T. https://www.clinicaltrials.gov/ct2/results?cond=car-t (April 04, 2022).
38. Van Norman GA. Drugs and devices: comparison of European and U.S. approval processes. JACC Basic Transl Sci. 2016; 1(5): 399–412, doi: 10.1016/j.jacbts.2016.06.003, indexed in Pubmed: 30167527.
39. Sheth VS, Gauthier J. Taming the beast: CRS and ICANS after CAR T-cell therapy for ALL. Bone Marrow Transplant. 2021; 56(3): 552–566, doi: 10.1038/s41409-020-01134-4, indexed in Pubmed: 33230186.
40. Giavridis T, van der Stegen SJC, Eyquem J, et al. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nat Med. 2018; 24(6): 731–738, doi: 10.1038/s41591-018-0041-7, indexed in Pubmed: 29808005.
41. Zhao Z, Condomines M, van der Stegen SJC, et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell. 2015; 28(4): 415–428, doi: 10.1016/j.ccell.2015.09.004, indexed in Pubmed: 26461090.
42. Abbott RC, Hughes-Parry HE, Jenkins MR. To go or not to go? Biological logic gating engineered T cells. J Immunother Cancer. 2022; 10(4), doi: 10.1136/jitc-2021-004185, indexed in Pubmed: 35379738.
43. Beider K, Itzhaki O, Schachter J, et al. Molecular and functional signatures associated with CAR T cell exhaustion and impaired clinical response in patients with B cell malignancies. Cells. 2022; 11(7), doi: 10.3390/cells11071140, indexed in Pubmed: 35406703.
44. Titov A, Kaminskiy Y, Ganeeva I, et al. Knowns and unknowns about CAR-T cell dysfunction. Cancers (Basel). 2022; 14(4), doi: 10.3390/cancers14041078, indexed in Pubmed: 35205827.
45. Chong EA, Alanio C, Svoboda J, et al. Pembrolizumab for B-cell lymphomas relapsing after or refractory to CD19-directed CAR T-cell therapy. Blood. 2022; 139(7): 1026–1038, doi: 10.1182/blood.2021012634, indexed in Pubmed: 34496014.
46. Miliotou AN, Papadopoulou LC. CAR T-cell therapy: a new era in cancer immunotherapy. Curr Pharm Biotechnol. 2018; 19(1): 5–18, doi: 10.2174/1389201019666180418095526, indexed in Pubmed: 29667553.
47. Khurana A, Lin Yi. Allogeneic chimeric antigen receptor therapy in lymphoma. Curr Treat Options Oncol. 2022; 23(2): 171–187, doi: 10.1007/s11864-021-00920-6, indexed in Pubmed: 35212892.
48. Lin H, Cheng J, Mu W, et al. Advances in universal CAR-T cell therapy. Front Immunol. 2021; 12: 744823, doi: 10.3389/fimmu.2021.744823, indexed in Pubmed: 34691052.