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REVIEW ARTICLE

CAR-T cell therapy: cell reprogramming to combat malignant neoplasms

Terapia com células CAR-T: reprogramação celular para o combate de neoplasias malignas

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ABSTRACT

The CAR-T cells are lymphocytes genetically modified to recognize a broader spectrum of cell surface antigens. In addition, they attack malignant tumor cells, which express these antigens, by activating cytoplasmic co-stimulation, cytokine secretion, tumor cell cytolysis and T cell proliferation. The aim of this study is to address immunotherapy with CAR-T cells, in order to explain its concept, manufacturing process and role in the treatment of hematological neoplasms and solid tumors. This is a literature review conducted through the PubMed portal, that uses the terms "car-t cell therapy" and "neoplasms" as descriptors, determined based on the DeCS (*Descritores em Ciências da Saúde*). To prepare this review, initially 10 articles were found and read in full. In addition, 3 updated clinical trials on the subject were added. For CAR-T cell therapy, T cells are collected from the patient, genetically modified to include specific antigen receptors, and later expanded in laboratories and transfused back to the patient. Thus, these receptors can recognize tumor cells that express a tumor-associated antigen. CAR-T cell therapy is best known for its role in the treatment of B cell hematological malignancies, with the CD19 protein being the most studied antigenic target to date. However, studies are being conducted to verify the effectiveness of this treatment, also, in solid tumors. Therefore, despite being formulated only for a selected group of patients, this therapy has great potential to act on a broader spectrum of patients.

Keywords: CAR-T Cell Therapy; Neoplasms; Immunotherapy.

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RESUMO

As células CAR-T são linfócitos geneticamente modificados para reconhecerem um espectro amplo de antígenos de superfície celulares. Além disso, atacam células tumorais malignas, que expressam esses antígenos, por meio da ativação da coestimulação citoplasmática, secreção de citocinas, citólise de células tumorais e proliferação de células T. O objetivo desse estudo é abordar a imunoterapia com células CAR-T, a fim de explicar seu conceito, processo de fabricação e papel no tratamento de neoplasias hematológicas e tumores sólidos. Foi realizada uma revisão através do portal PubMed, utilizando como descritores: "*car-t cell therapy*" e "*neoplasms*", determinados com base nos "Descritores em Ciências da Saúde". Foram obtidos, inicialmente, 10 artigos, os quais foram lidos integralmente para a confecção dessa revisão. Além disso, foram adicionados 3 ensaios clínicos atualizados sobre o tema. Na terapia com células CAR-T, as células T são coletadas do paciente, geneticamente modificadas para incluir receptores de antígeno específicos e, posteriormente, expandidas em laboratórios e transfundidas de volta para o paciente. Assim, esses receptores podem reconhecer células tumorais que expressam um antígeno associado a um tumor. A terapia com células CAR-T é mais conhecida por seu papel no tratamento de malignidades hematológicas de células B, sendo a proteína CD19 o alvo antigênico mais bem estudado até o momento. Entretanto, estudos estão sendo feitos para verificar a eficácia desse tratamento, também, em tumores sólidos. Portanto, apesar de inicialmente ser indicada apenas para um grupo seleto de pessoas, essa terapia tem demonstrado grande potencial para atuar em um espectro maior de pacientes.

Palavras-chave: Terapia CAR com Células T; Neoplasias; Imunoterapia.

Introduction

Despite the rapid development of science and the emergence of new technologies in the health area, tumor therapy is still a matter of difficult management. Conventional therapies such as surgery, chemotherapy and radiotherapy may provide short-term benefits but have side effects due to their invasiveness and biotoxicity. In addition, resistance to multiple chemotherapy drugs and the various toxicities of radiotherapy limit their healing effects. In this context, immunotherapies arouse the interest of several researchers.[1]

Typical immunotherapies include: tumor infiltrating lymphocytes (TILs), T cells with T-cell receptors (TCR) produced and T cells with chemistry antigenic receptors (CAR). Of these, CAR-T cell immunotherapy has received considerable attention in research areas, as it is highly effective in treating cancers, especially haematological cell malignancies B.[1,2] However, in solid tumors, there is a difficulty in the recognition of cancer cells by CAR due to their high phenotypic heterogeneity, among other factors.[1]

Until recently, CAR-T cell therapy was only available for a select group of patients with advanced haematological malignancies. Although the use of this emerging treatment has so far been largely restricted to small clinical trials, its advancement has been followed with great interest by researchers and clinicians.[3]

A CAR is a type of genetically constructed receiver[1,2,3] consisting of four parts. The first is an extracellular antigen recognition region with single-chain variable fragments (scFvs), which derive from antigen-specific monoclonal antibodies (mAb) and recognize and bind to specific antigens related to the tumor, independent of the molecular restriction of the main histocompatibility complex (MHC). The second is an area of extracellular stem (hinge) that usually

consists of crystallizable fragment regions (Fc) or the spacing region of the CD4 and CD8. The third is a transmembrane region normally derived from CD8, CD3-ζ, CD4, OX40, and H2-K^b. The fourth is an intracellular signalling tail, which includes the signal transducer component of the T-cell receptor (TCR) .[2] The design of the CAR and its structure have evolved over time, so that there are now four generations used in clinical practice, which vary in the composition of the co-stimulation domains. [2,4]

Therefore, CAR-T cells are genetically modified lymphocytes to recognize a wider spectrum of cell surface antigens, including glycolipids, carbohydrates and proteins. In addition, they attack malignant tumor cells that express these antigens by activating cytoplasmic co-stimulation, cytokine secretion, tumor cell cytolysis and T-cell proliferation.[1,5] It is essential for the development of a successful CAR-T cell that the chosen target allows the identification of an antigen associated with the tumor, in addition to being practically absent in normal cells and overexpressed in malignant cells, in order to minimize toxic effects in normal tissues (off target).[6]

The study aimed to address CAR-T cell immunotherapy, in order to explain its concept, its manufacturing process and its role in the treatment of haematological neoplasms and solid tumours, through a bibliographic review of the most recent publications on the subject, in order to contribute and encourage the expansion of research on this promising therapy.

Methodology

A literature review was conducted in which bibliographic and cross-sectional research was carried out through the PubMed (National Center For Biotechnology Information) portal, in which the terms "car t cell therapy" and "neoplasms" were used as descriptors, determined on the basis of the virtual library of health DeCS (*Descritores em Ciências da Saúde*). The search was restricted to free full articles, so that only the results that presented both terms in the title or abstract of the article were included. Thus, initially 10 articles were obtained, which were read in full for the preparation of this review. In addition, 3 updated clinical trials on the subject were added.

Results and Discussion

The manufacturing process begins with the collection of non-stimulated leukocytes via large-volume leukapheresis.[3,7] The separation of T cells can be achieved in several ways, some of them being the removal of red blood cells and platelet contaminants by density gradients, cell division by size and density, and elimination of monocytes and lymphocyte isolation.[3] The cells are then transferred to an enrichment cell processing facility, where they are incubated, generally, with viral vectors encoding CAR, which enter in the t cells and introduce the RNA of the same. This RNA is reverse-transcribed into DNA, which recombines into the T-cell genome, resulting in the permanent incorporation of the CAR gene.[7] Lentiviral vectors provide a safer genomic integration profile than gamma retrovirals and have therefore been commonly used in clinical trials of CAR-T cell therapies.[3]

Although lentiviral vectors have displayed these attractive features, they are complicated and expensive to access. For these reasons, researchers have been searching for more affordable gene transfer methods, and transposable elements (transposons) offer an alternative with vast potential for CAR-T cell therapy. The Sleeping Beauty (SB) transposon, one of the most explored non-viral gene transfer systems for CAR manufacturing, has shown comparable efficiency to viral vectors, offering a valid alternative for gene transfer.[4]

After gene transfer, the T cells modified by CAR are then expanded ex vivo and prepared as a pharmaceutical intravenous infusion product.[7] After blood collection and manufacturing of the patient-specific CAR-T cell product, the cells are frozen and delivered to the treatment center, where they are then thawed and infused into the patient.[3] Generally, the time between leukapheresis and administration of CAR-T cells is approximately 4 to 5 weeks, and the process of forwarding to infusion in the patient can take up to 2 months. Therefore, during this period, in order to minimize the rate of progression of the disease and the maintenance of the general condition of the patient, it is usually performed lymphodepletora chemotherapy.[7]

In summary, T cells are collected from the patient and genetically modified to include antigen receptors, which combine the unique chain variable fragment of an antibody with the intracellular signaling domains. Thus, they can recognize tumor cells that express an antigen associated with a tumor. The CAR-T cells designed are then expanded in the lab and transfused back to the patient.[3]

CAR-T cell therapy is best known for its role in the treatment of B-cell hematologic malignancies. CD19, a surface protein expressed on most B-lineage lymphocytes and not expressed on other normal tissues, is the best-studied antigenic target of malignancy-associated hematologic antigens. CD19-specific CAR-T cell therapy has shown great efficiency in inducing durable remissions of several hematological malignancies, including acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia (CLL) both malignant neoplasms of hematopoietic cells of lymphoid lineage,[1,7,8] and non-Hodkins lymphomas (NHL), which are malignant neoplasms that affect the lymphatic tissue in different ways (B cells, T cells, and natural killer cells).[5] In 90% of cases of ALL, in more than 50% of cases of CLL[1] and in 40 to 58% of cases of NHL, there has been complete remission of symptoms.[7]

In addition, there has been discussion regarding promising new candidate antigens for the treatment of refractory/recidivating multiple myeloma (MM) with CAR-T cells, such as: kappa light chain antigen, CD38, CD138, SLAMF7, GPRC5D and B-cell maturation

antigen. MM is a B-cell neoplasm with a variety of clinical manifestations and a very poor prognosis, and although CAR-T cell therapy has shown promise for its treatment, some challenges must be overcome, such as reducing the toxicity caused by the off-target effect. Although there is still no licensed CAR-T cell therapy for patients with MM, those heavily pretreated, ineligible for transplantation or relapsed early may benefit from this treatment.[6]

An almost inevitable side effect of CAR-T cell immunotherapy is cytokine release syndrome (CRS), also known as "cytokine storm", which is related to the systemic inflammatory process induced by the reaction between the infused CAR-T cells and the target antigens. The presentation of CRS can range from a flu-like, self-limited syndrome to multiple organ dysfunction that, without prompt intervention and intensive supportive treatments, can lead the patient to death. CRS toxicity usually develops within the first week after infusion of CAR-T cells[9] and peaks within 1 to 2 weeks, coinciding with the maximum in vivo expansion of T cells.[3] When severe, vasopressors, mechanical ventilation, antiepileptics, and hemodialysis may be required. A reliable indicator of CRS severity is C-reactive protein (CRP).[1] Researchers can now control most cases of CRS with anti-interleukin 6 antibody, such as tocilizumab, approved in 2017 by the Food and Drug Administration (FDA) for the treatment of CRS induced by CAR-T therapy.[1,3,9]

Also in 2017, relying on safety and efficacy results from the ZUMA-1 clinical trial (table 1), the FDA approved the use of axicabtagene ciloleucel for the treatment of adults with relapsed or refractory B-cell NHL.[10] In 2021, the results of the ZUMA-3 clinical trial (Table 1) lead to the approval of brexucabtagene autoleucel (KTE-X19) for the treatment of adult patients with relapsed or refractory B-lineage ALL, the first CAR-T cell therapy for this disease.[11] Currently, the phase 3 TRANSFORM clinical trial, expected to end in late 2023, is evaluating whether the use of lisocabtagene maraleucel in adults with relapsed or refractory high-grade B-cell NHL is as safe and effective as After the success of CAR-T cell therapy in hematological malignancies, more research has been done to extend this treatment to other neoplasms, such as solid tumors.[3] However, among the difficulties encountered in its use are: the absence of antigenic targets specific to solid tumors, the heterogeneity of these tumors, and the hostility of the tumor microenvironment (immunosuppressive) to T cells, making it difficult for these cells to infiltrate and persist in these tissues long enough to generate an efficient response.[1]

Despite the difficulties, CAR-T cell therapy has achieved promising results for specific types of solid tumors, such as non-small cell lung cancer, malignant pleural mesothelioma, cholangiocarcinoma, epithelial ovarian cancer, glioblastoma, and sarcomas. However, some key points must be considered to translate the success of CAR-T cell therapy for solid tumors, such as the discovery of a specific antigen, the manufacturing of CAR-T cells with receptors for multiple antigens, the adaptation of T cells to face the hostility of the tumor microenvironment.[1] Soluble immunosuppressive factors of this environment, such as transforming growth factor β (TGF-β), are believed to be responsible for the inhibition of the cellular immune response. Combining TGF-β receptor blockade with CAR-T cell therapy is expected to result in improved outcomes for cancer patients.[13]

Finally, another major challenge of CAR-T cell therapy to overcome is its affordability. In the United States of America (USA), the total cost of treating B-cell ALL, taking into account the product and the expense of the necessary supportive measures, is about 1 million dollars.[9]

Table 1. Main clinical data concerning ZUMA-1 and ZUMA-3.

ZUMA-1[10]		**ZUMA-3**[11]	
Tested drug	Axicabtagene ciloleucel	**Tested drug**	Brexucabtagene autoleucel
Number of treated patients	101 (phase 2)	**Number of treated patients**	55
Follow-up period	25,7-28,8 months	**Follow-up period**	13,8-19,6 months
Response type	Complete: 58% Partial: 25% Stable disease: 10% Progressive disease: 5% Could not be assessed: 2%	**Response type**	Complete remission (CR): 56% CR with incomplete haematological recovery: 15% Blast-free hypoplastic or aplastic bone marrow: 7% No response: 16 % Unknown or not evaluable: 5%

Source: authors' own table based on data from the ZUMA-1 and ZUMA-3 clinical trials.

Conclusion

CAR-T cell therapy was initially indicated only for a select group of patients with advanced hematologic malignancies. However, this therapy has advanced and shown great potential to work in an increasing spectrum of patients. With regard to hematological malignancies, therapy with CD19-specific CAR-T cells has shown particular efficacy in inducing durable remissions in ALL, CLL and NHL. In addition, new antigens have been studied for the treatment of refractory/recidivating MM with CAR-T cells. However, the intense immune activation characteristic of this therapy must be properly managed to allow its successful clinical use, as it may present severe repercussions. On the other hand, the results in solid cancers have not been homogeneous regarding its efficacy so far, since the therapy for these tumors is more complicated than for hematological neoplasms and, although there are some cases of temporary remission of solid tumors, its clinical application faces some technical difficulties, such as the hostility of the tumor microenvironment, as well as it could benefit from the development of more economical solutions for this type of therapy.

Author's Contribution

All authors participated significantly in the design of the study, in the analysis and interpretation of data, in the preparation, review and translation of the manuscript.

Additionally, the author José Eduardo Palacio Soares was also responsible for answering the reviewers and updating the manuscript, when requested.

Finally, the author Fernanda Cardoso Parreiras was responsible for the orientation and final approval of the manuscript.

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