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CAR-T Immunotherapy in oncological treatment: literature review

Imunoterapia CAR-T no tratamento oncológico: revisão de literatura

Inmunoterapia CAR-T en tratamiento oncológico: revisión de la literatura

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Abstract
Introduction: Immunotherapies are developed to overcome limitations of conventional cancer therapy through stimulating innate immune response against tumor antigen. Objective: Elaborate a review on CAR-T Immunotherapy (Chimeric T Cell Antigen Receptor) in cancer treatment. Methods: Bibliographic survey of systematic reviews published in PubMed in the last 5 years. Results: 21 studies were selected. Antitumor effect occurs through cell lysis, due to CAR-T lymphocytes release of cytokines. Tumor escape is related to tumor's ability to not expose its Major Histocompatibility Complex (MHC) and/or inability of the adaptive immune system to recognize tumor antigens. These molecular targets may be proteins, carbohydrates, or glycolipids, with CD19 standing out as target of this therapy and in neoplasms as leukemia and lymphoma. Four generations of CARs have been described, which differ in terms of co-stimulatory domains and consequent functional efficiency. The therapy is indicated for cases of recurrence/refractoriness of hematological neoplasms, however applicability in solid tumors has been studied. Adverse events of CAR-T immunotherapy described were: cytokine release syndrome, neurotoxicity, anaphylactic shock, autoimmune reactions, B cell aplasia, tumor lysis syndrome and graft-versus-host disease. Conclusions: CAR-T immunotherapy is a promising therapy against relapsed/refractory cancer. It's mainly used in leukemias and lymphomas. Choice of dose and generation of CARs should be cautious, considering specific molecular targets of each neoplasm and its presence in healthy tissues, avoiding adverse events.
Keywords: Neoplasms; Adoptive immunotherapy; Chimeric antigen receptors; Lymphocytes; Immune system.

Resumo
Introdução: Imunoterapias são desenvolvidas para superar limitações da terapia convencional contra o câncer através do estímulo da resposta imune inata ao antígeno tumoral. Objetivo: Elaborar revisão sobre a Imunoterapia CAR-T (receptor quimérico de antígeno em células T) no tratamento oncológico. Métodos: Levantamento bibliográfico de revisões sistemáticas publicadas no PubMed nos últimos 5 anos. Resultados: Foram selecionados 21 estudos. O efeito antitumoral ocorre através da lise de células, a partir da liberação de citocinas pelos linfócitos CAR-T. O escape tumoral está relacionado à capacidade do tumor em não expor seu complexo principal de histocompatibilidade (MHC) e/ou incapacidade do sistema imune adaptativo em reconhecer antígenos tumorais. Esses alvos moleculares podem ser proteínas, carboidratos, ou glicolipídeos, sendo que o CD19 se destaca como alvo dessa terapia e em neoplasias como leucemias e linfoma. Foram descritas quatro gerações de CARs que apresentam diferenças quanto aos domínios co-estimulatórios e consequente eficiência funcional. A terapia é indicada para casos de recidivas ou refratariedade de neoplasias hematológicas, porém a aplicabilidade em tumores sólidos vem sendo estudada. Os eventos adversos da imunoterapia CAR-T descritos foram: síndrome de liberação de citocinas, neurotoxicidade, choque anafilático, reações autoimunes, aplasia de células B, síndrome de lise tumoral e doença do enxerto contra o hospedeiro. Conclusões: A imunoterapia CAR-T é uma terapia promissora contra o câncer recidivado ou refratário. Atualmente é utilizada principalmente em leucemias e linfomas. A escolha da dose e geração dos CARs deve ser criteriosa,

considerando os alvos moleculares específicos de cada neoplasia e sua presença em tecidos saudáveis, evitando eventos adversos.
Palavras-chave: Neoplasias; Imunoterapia adotiva; Receptores de antígenos quiméricos; Linfócitos; Sistema imunitário.

Resumen

Introducción: Inmunoterapias están diseñadas para superar limitaciones de la terapia convencional contra el cáncer mediante estimulación de la respuesta inmunitaria innata al antígeno tumoral. Objetivo: Elaborar una revisión sobre inmunoterapia CAR-T (receptor de antígeno quimérico en células T) en el tratamiento oncológico. Métodos: Levantamiento bibliográfico de revisiones sistemáticas publicadas en PubMed en los últimos 5 años. Resultados: Se seleccionaron 21 estudios. El efecto antitumoral se produce a través de la lisis celular, con la liberación de citoquinas por los linfocitos CAR-T. El escape del tumor está relacionado con la capacidad a no exponer su complejo principal de histocompatibilidad y/o la incapacidad del sistema inmunitario adaptativo para reconocer los antígenos tumorales. Estas dianas moleculares pueden ser proteínas, carbohidratos, o glicolípidos, destacándose el CD19 como diana de esta terapia y en neoplasias como leucemias y linfomas. Se describieron cuatro generaciones de CARs que presentan diferencias en los dominios coestimuladores y eficiencia funcional. La terapia está indicada para casos de recurrencia o refractariedad de neoplasias hematológicas, pero se ha estudiado su aplicabilidad en tumores sólidos. Los eventos adversos de la inmunoterapia CAR-T descritos fueron: síndrome de liberación de citoquinas, neurotoxicidad, shock anafiláctico, reacciones autoinmunes, aplasia de células B, síndrome de lisis tumoral y enfermedad de injerto contra huésped. Conclusiones: Inmunoterapia CAR-T es una terapia prometedora contra el cáncer en recaída o refractario. Actualmente se utiliza principalmente en leucemias y linfomas. La elección de la dosis y generación de CARs debe ser juiciosa, considerando las dianas moleculares específicas de cada neoplasia y su presencia en tejidos sanos, evitando eventos adversos.
Palabras clave: Neoplasias; Inmunoterapia adoptiva; Receptores de antígenos quiméricos; Linfocitos; Sistema inmunitario.

1. Introduction

Cancer (CA) is defined as abnormal, autonomous and disordered growth of cells, which can invade other tissues. In underdeveloped countries, about 70% of morbidity and mortality occurs due to cancer. However, only 26% of low-income countries presented public services for diagnosis and treatment of this disease in 2017 (World Health Organization, 2018).

Cancer cells do not effectively perform their physiological metabolic activities and their proliferation causes functional and/or anatomical damage to the tissue, beyond alterations in the degree of specialization (Almeida *et al.*,2005).

CA may have external or internal causes. External causes can be related to poor diet (low intake of fruits and vegetables), sexual habits (infection by Human Papillomavirus), exposure to radiation (sun,bombs) and carcinogenic substances (alcohol, tobacco, nickel and benzene). On the other hand, internal causes are related to genetic factors (World Health Organization, 2018; Oppermann, 2014). Alteration of genes called oncogenes causes abnormal proliferation of cells. It's important to note that antioncogenes, which inhibit this multiplication, may mutate, resulting in cancer (Knudson,1993).

Conventional treatments like chemotherapy and/or radiotherapy and surgeries are not enough in about half of the cases, according to Weinberg cited by Werle. (Werle, 2016). Another challenge to the conventional treatment is relapse or refractoriness to hematological malignancies that resulted in FDA (Food and Drug Administration) approvals for CAR-T with evidence of remission and cure. (Shah NN, Fry TJ, 2019). Due to such conventional therapeutic limitation for CA, a new treatment modality, called Immunotherapy, is being developed from a chimeric T cell antigen receptor (CAR-T).

According to the literature, (Zhu *et al.*,2017; Dammeijer *et al.*, 2016; Sahlolbei *et al.*,2020; Zhao *et al.*,2017), immunotherapy is a treatment that develops or enhances the immune response and, in the case of CA, confers an anti-tumor effect. CAR-T therapy potentiates T cell receptors (TCR) of the innate immune system in an effective and specific way, promoting a response to a recognized antigen, and in CA an immune response to the tumor antigen (Zhu J *et al.*,2017; Dammeijer *et al.*, 2016; Sahlolbei *et al.*, 2020; Zhao *et al.*,2017; Zhu *et al.*,2016). In other words, a CAR- T cell is capable of recognizing and killing a cell with a specific antigen even in low levels. Its clinical use is historically related to cancer and

chronic virus infections like HIV. (Maus et al., 2014). However, CAR-T therapy also has some limitations to durable remissions like manufacture, expansion, persistence, loss or downregulation of the antigen provoking resistence (Shah NN, Fry TJ, 2019).

The main objective of the present study was to elaborate a bibliographic review of the literature on CAR-T Immunotherapy in cancer treatment, enlighten the following aspects: Definition, antitumor effect and tumor escape; molecular targets; structure and generations of CARs; therapy indications and adverse events.

2. Methodology

2.1 Data sources and research

Integrative literature review was conducted by the main author, by a bibliographic survey on PUBMED, searching systematic review articles published in the last 5 years (2015 to 2020). The medical subject heading (MESH) terms used were "CAR-T cell therapy", "cancer " (linked with "AND").

2.2 Study selection

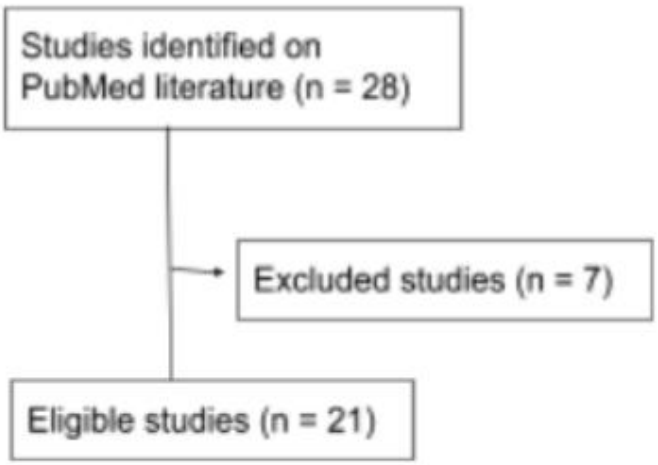
Studies were considered eligible for inclusion once they contained data about CAR-T immunotherapy. Articles containing only other treatments such as dendritic cells, tumor infiltrating lymphocytes and killer cells instead of CAR-T immunotherapy were excluded.

3. Results

3.1 Search strategy

The search strategy retrieved a total of 28 studies. Of these, seven articles were excluded, as they addressed other types of immunotherapy. Thus, 21 articles met the eligible criteria on the application of CAR-T immunotherapy in the CA (Figure 1).

Figure 1- Flowchart of studies selection.



Sources: Authors.

Table 1. Characteristics of the selected studies for the literature review according to, author, year, title, journal, keywords, study objective and study design.

Author/year	**Title**	**Journal**	**Keywords**	**Study Objective**
	Systematic Review			
Anwer F *et al.*, 2017	Donor origin CAR T cells: graft versus malignancy effect without GVHD, a systematic review	Immunotherapy	Allogenic stem cell transplantation; chimeric antigen T cells; graft versus leukemia; hematological malignancy; relapse; salvage.	To identify the current evidence for efficacy and safety of donor-derived CAR T cells after failure of an allogenic hematopoietic stem cell transplant and whether or not there is associated high risk of of acute and chronic graft-versus-host disease flare with this treatment.
Cao G, Lei L, Zhu X, 2019	Efficiency and safety of autologous chimeric antigen receptor T-cells therapy used for patients with lymphoma: A systematic review and meta-analysis	Medicine	CAR T cells; lymphoma; meta-analysis.	To assess the safety and efficacy of CAR T-cells in lymphoma treatment and determine the relationships between potential factors and efficacy.
Cao JX *et al.*, 2019	The efficacy of anti-CD19 chimeric antigen receptor T cells for B-cell malignancies	Cytotherapy,	CAR-T; acute lymphocytic leukemia; chronic lymphocytic leukemia; lymphomas; meta-analysis.	To performe a systematic review in terms of the clinical response treated with CAR-T cells in acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL) and lymphomas patients.
Dammeijer *et al.*, 2016	Efficacy of Tumor Vaccines and Cellular Immunotherapies in Non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis	Journal Of Clinical Oncology	-	To assess the efficacy of tumor vaccination and cellular immunotherapy in NSCLC.
Drokow EK *et al.*,2019	Survival outcomes and efficacy of autologous CD19 chimeric antigen receptor-T cell therapy	Therapeutics and Clinical Risk	CAR-T; CD19; autologous; hematological malignancies;	To estimate the survival outcome, response rate and toxicity of autologous CD19 CAR-

	in the patient with diagnosed hematological malignancies: a systematic review and meta-analysis	Management	response rate.	T cell therapy and predict other factors associated with a better prognosis.
Grigor EJM *et al.*, 2019	Risks and Benefits of Chimeric Antigen Receptor T-Cell (CAR-T) Therapy in Cancer: A Systematic Review and Meta-Analysis	Transfusion Medicine Reviews	CAR-T cell therapy; Systematic review; Cancer; Hematologic.	To summarize the efficacy and safety of CAR-T cell therapy in patients with relapsed or refractory hematologic or solid malignancies.
Hao L *et al.*, 2019	Adoptive Immunotherapy for B-cell Malignancies Using CD19- Targeted Chimeric Antigen Receptor T-Cells: A Systematic Review of Efficacy and Safety	Current Medicinal Chemistry	B-cell malignancies; Chimeric antigen receptor; adoptive T cell therapy; leukemia; lymphoma; safety.	To evaluate the efficacy and side effects of CAR-T on refractory and/or relapsed B-cell malignancies, including leukemia and lymphoma
Holzinger A, Barden M, Abken H, 2016	The growing world of CAR T cell trials: a systematic review	Cancer Immunology, Immunotherapy	Adoptive cell therapy; CAR; Chimeric antigen receptor; Clinical trial; T cell.	To list ongoing trials that envolve CAR T cells targeting hematopoietic malignancies and solid cancer.
Jin Z *et al.*,2018	The severe cytokine release syndrome in phase I trials of CD19-CAR-T cell therapy: a systematic review	Annals of Hematology	Acute lymphoblastic leukemia; B-cell non-Hodgkin lymphoma; CD19 chimeric antigen receptor T cell; Chronic lymphoblastic leukemia; Cytokine release syndrome.	To estimate the factors that might affect the cytokine release syndrome and to evaluate the incidence depending on the type of B-cell malignant disease.
Li J, Wu Z, Zhao N, 2019	Individual Patient Data Meta-Analysis from 16 Trials for Safety Factors in Cytokine Release Syndrome After CAR-T Therapy in Patients with Non-Hodgkin Lymphoma (NHL) and Acute Lymphoblastic Leukemia	Advances in Therapy	Acute lymphoblastic leukemia; Chimeric antigen receptor T cell; Generalized additive model; Individual patient data meta-analysis.	To investigate the association of severe CRS with CAR-T dose and baseline factors.

Nagle K *et al.*,2018	Effect of transplant status in CD19-targeted CAR T-cell therapy: a systematic review and meta-analysis	Medical Oncology	CAR T-cell therapy; Hematopoietic stem cell transplant; MRD-negative complete remission; Cytokine release syndrome; Neurotoxicity	To systematically evaluate the likelihood of achieving optimum response, severe cytokine release syndrome (sCRS) and neurotoxicity in the context of CAR T-cell therapy for hematopoietic stem cell transplant (HSCT) - naïve patients versus those with prior HSCT.
Petrou P,2019	Is it a Chimera? A systematic review of the economic evaluations of CAR-T cell therapy	Expert review of pharmacoeconomics & outcomes research	CAR-T; axicabtagene; economic evaluation; tisagenlecleucel.	To provide a systematic review on economic evaluations of the chimeric antigen receptor T – cell treatment.
Pettitt D *et al.*,2018	CAR-T Cells: A Systematic Review and Mixed Methods Analysis of the Clinical Trial Landscape	Molecular Therapy	CAR-T cells; cell therapy; translational medicine.	To identify and criticize published CAR-T cell clinical trials and to examine the critical factors required to enable CAR-T cells to become a standard therapy.
Riaz IB *et al.*,2017	Anti-CD 19 and anti-CD 20 CAR-modified T cells for B-cell malignancies: a systematic review and meta-analysis.	Immunotherapy	Chimeric antigen T cells; hematological malignancy; leukemia; lymphoma; refractory; relapse.	To conduct a systematic review and meta-analysis of all published clinical trials studying the role of efficacy and safety of CD-19 and CD-20 chimeric antigen receptor-T therapy for B-cell hematologic malignancies.
Sohail A et al., 2018	Emerging immune targets for the treatment of multiple myeloma	Immunotherapy	adoptive cell therapy; antibodies; antibody therapeutics; chimeric antigen T cells; immunotherapy; multiple myeloma.	To summarize the current state of knowledge on the efficacy of non-US FDA approved MoAbs, CAR T cells and their targets.
Sahlolbei *et al.*,2020	Evaluation of targetable biomarkers for	International	Animal model; CAR T-cell;	Identify experimental animal studies that

	chimeric antigen receptor T-cell (CAR-T) in the treatment of pancreatic cancer: a systematic review and meta-analysis of preclinical studies	Reviews of Immunology	antigen; meta-analysis; pancreatic cancer.	investigated the antigens targeted by CAR T-cell for pancreatic cancer treatment.
Zeng Y *et al.*, 2016	Adoptive Immunotherapy in Postoperative Non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis	PLoS One	-	To provide more reliable and up-to-date evidence on the effect of postoperative adoptive immunotherapy in non-small-cell lung cancer (NSCLC) patients.
Zhang J, Wang L, 2019	The Emerging World of TCR-T Cell Trials Against Cancer: A Systematic Review	Technology in Cancer Research & Treatment	Adoptive T-cell therapy; TCR-T; tumor immunotherapy; tumor antigen; clinical trial.	To create an unbiased, comprehensive, and scientific report.
Zhao *et al.*,2017	Adoptive immunotherapy shows encouraging benefit on non-small cell lung cancer: a systematic review and meta-analysis	Oncotarget	adoptive immunotherapy; meta-analysis; non-small cell lung cancer.	to evaluate the efficacy of adoptive immunotherapy for non-small cell lung câncer.
Zhu *et al.*,2017	Immunotherapy (excluding checkpoint inhibitors) for stage I to III non-small cell lung cancer treated with surgery or radiotherapy with curative intent	Cochrane Database of Systematic Reviews	-	To evaluate the effectiveness and safety of immunotherapy in patients with localised NSCLC (stages I to III) who receive surgery or radiotherapy with curative intent.
Zhu Y *et al.*,2016	Anti-CD19 chimeric antigen receptor-modified T cells for B-cell malignancies: a systematic review of efficacy and safety in clinical trials	European Journal of Haematology	B-cell malignancy; CD19; chimeric antigen receptor; survival analysis	To investigate the critical parameters affecting efficacy and evaluated the safety of using CAR T cells targeting CD19 in B-lineage malignancies.

Sources: Authors.

3.2 Definition, antitumor effect and tumor escape

CAR-T immunotherapy is a treatment that enhances the immune system, through genetically modified T lymphocytes activity (Holzinger A, Barden M, Abken H, 2016), which have chimeric antigen receptors (CARs) on their surface.

Antitumor effect occurs through using and mechanism of action of these receptors in cancer treatments, which allows the connection with a antigen and elimination of a specific tumor cell (Grigor EJM *et al.*, 2019; Sahlolbei *et al.*,2020; Zhang J, Wang L, 2019; Drokow EK *et al.*,2019) recognized as "non self" (Zhu J *et al.*,2017). In addition, it promotes regression of metastatic cases (Sahlolbei *et al.*,2020; Zeng Y *et al.*, 2016) and prevents recurrence (Sahlolbei *et al.*,2020; Zhu Y *et al.*,2016).

Specific cellular recognition of tumor antigens (Dammeijer *et al.*, 2016) activates the release of pro-inflammatory cytokines, such as perforins and granzymes (Holzinger A, Barden M, Abken H, 2016; Riaz IB *et al.*,2017), by T lymphocytes, resulting in lysis of the cancer cell. Unlike conventional treatments, such as chemo and radiotherapy, specificity is a characteristic of CAR-T therapy (Pettitt D *et al.*,2018). The modified lymphocytes also have the capacity to remain as memory cells (Drokow EK *et al.*,2019; Zeng Y *et al.*, 2016; Riaz IB *et al.*,2017).

Tumor escape is a phenomenon in which tumor antigens are not submitted to immune response act. This is related both to the tumor's ability to not expose it's Main Histocompatibility Complex (MHC) and the inability of the adaptive immune system to recognize tumor antigens (Zhu Y *et al.*,2016; Pettitt D *et al.*,2018). In the last case, antigen-presenting cells, such as dendritic and macrophage cells, do not process and present antigens to T lymphocytes due to both MHC failures and immunosuppression (Zhu J *et al.*,2017).

3.3 Molecular targets

Allogeneic or autologous CAR-T lymphocytes can recognize antigens whose composition is proteins, carbohydrates or glycolipids (Zhu Y *et al.*,2016). Therefore, specific antigens that may be present in the CA are molecular targets of the CAR-T lymphocytes, as indicated below:

- **BCMA:** Protein expressed in malignant plasma cells and B cells (Sohail A et al., 2018);
- **K light chains:** Related to B cell neoplasms (Cao G, Lei L, Zhu X, 2019);
- **CD19** (Riaz IB *et al.*,2017; Cao G, Lei L, Zhu X, 2019; Nagle K *et al.*,2018): Surface glycoprotein present in young B cells (Drokow EK *et al.*,2019; Cao JX *et al.*, 2019). Hematopoietic stem cells and other tissues do not express this molecule, presenting specificity to malignancies (Zhu Y *et al.*,2016; Cao JX *et al.*, 2019; Hao L *et al.*, 2019), such as multiple myeloma, acute or chronic leukemia (Sohail A *et al.*, 2018; Hao L *et al.*, 2019) and lymphoma (Hao L *et al.*, 2019);
- **CD20:** Related to B cell neoplasms (Riaz IB *et al.*,2017; Cao G, Lei L, Zhu X, 2019);
- **CD30:** Related to Hodgkin's lymphoma (Cao G, Lei L, Zhu X, 2019);
- **CD138:** Expressed in plasma cells (Sohail A *et al.*, 2018);
- **CEA:** Carcinoembryogenic antigen (Holzinger A, Barden M, Abken H, 2016);
- **HER2:** Related to breast (Cao G, Lei L, Zhu X, 2019) and pancreas (Sahlolbei *et al.*,2020) cancer;
- **MUC1:** Protein that influence the activity of the tyrosine kinase receptor and usually epithelial cells metabolism (Holzinger A, Barden M, Abken H, 2016; Zhu J *et al.*,2017)
- **PSCA:** Expressed in prostate, bladder and pancreas neoplasms (Sahlolbei et al.,2020);
- **PSMA:** Prostate membrane specific antigen (Holzinger A, Barden M, Abken H, 2016);
- **KLC18:** Expressed in B cells or plasma (Sohail A *et al.*, 2018);

Polysaccharide or lipid antigens generally develop weak and non complex immune responses, without the involvement of T-lymphocyte recognition to activate B-lymphocytes, which classifies them as thymo-independent agents, unlike protein molecules that are generally thymo-dependent and activate the response of the CD4 T lymphocyte. Thus, CAR-T cells have a greater margin of recognition of antigens in terms of their chemical nature compared to their own lymphocytes (Zhu Y *et al.*,2016).

Cancer cell antigens are often not recognized by lymphocytes (Pettitt D *et al.*,2018), in addition, these cells undergo constant mutations and therefore expose new epitopes, which should be considered in CAR engineering for neoplasms (Holzinger A, Barden M, Abken H, 2016).

3.4 Structure and generations of CARs

The chimeric receptor is formed by the following general structure:

- ***Extracellular domain or ectodomain:*** Fragment of a single chain monoclonal antibody capable of recognizing a specific antigen expressed by a cell (Zhang J, Wang L, 2019; Pettitt D *et al.*,2018; Cao G, Lei L, Zhu X, 2019; Jin Z *et al.*,2018). In other words, antigens recognition does not depend on an MHC (Sahlolbei *et al.*,2020; Zhu Y *et al.*,2016; Riaz IB *et al.*,2017; Anwer F *et al.*, 2017), but on a specific antibody to hit the target (Riaz IB *et al.*,2017; Hao L *et al.*, 2019), which suggests a possible expansion of therapy for other neoplasms and diseases in the future (Zhu Y *et al.*,2016; Riaz IB *et al.*,2017; Anwer F *et al.*, 2017);
- ***Transmembrane domain:*** Fix the ectodomain and endodomain on the lymphocyte cell membrane (Zhu Y *et al.*,2016);
- ***Intracellular domain, endodomain or cytoplasmic domain:*** composed of the CD3Z chain that promotes the activation of antitumor functions (lymphocyte activation, secretion and degranulation of cytokines and cytolysis) specific to the target from a primary signal (Zhu Y *et al.*,2016; Cao G, Lei L, Zhu X, 2019; Jin Z *et al.*,2018; Zhang J, Wang L, 2019). In addition, it presents a set of immunoreceptors with a tyrosine-based activation motif (Zhang J, Wang L, 2019).

Four generations or classes of CARs have been described, which differ due to presence or absence of extra signaling domains responsible for sustaining the activation of T lymphocytes (Holzinger A, Barden M, Abken H, 2016).

The first class have only the CD3Z domain and the ectodomain while other generations have extra intracellular domains that can be co-stimulatory, T cell activators or promoters to increase cytokine release (Pettitt D *et al.*,2018). The second generation has only one extra domain (CD28 or 4-1BB) and the third has two (it can be: CD27; CD28; 4-1BB; ICOS or OX-40) (Holzinger A, Barden M, Abken H, 2016; Riaz IB *et al.*,2017; Pettitt D *et al.*,2018; Cao G, Lei L, Zhu X, 2019; Nagle K *et al.*,2018; Cao JX *et al.*, 2019; Hao L *et al.*, 2019). These extra domains are also known as a secondary signal and enhance anti-tumor effects as well as proliferation and persistence (Zhu Y *et al.*,2016).

Expansion and persistence is greater for the 4-1BB domain compared to CD2823, (Jin Z *et al.*,2018), although CD28 also promises this effect (Zhu Y *et al.*,2016) promisingly when associated with CD3Z and CD137 (Hao L *et al.*, 2019).

In clinical studies, second and third generations showed high evidence of effectiveness with no significant difference (Cao G, Lei L, Zhu X, 2019). However, other authors suggest that there is still a lack of data to determine if there is really no difference between these generations (Drokow EK *et al.*,2019) and reinforce that both have better response and effectiveness when comparing them with the first generation. First generation is less effective in cell division stages, optimal production of cytokines, expansion and support of antitumor effects (Zhu Y *et al.*,2016; Riaz IB *et al.*,2017).

Fourth-generation of CARs are capable of producing self-stimulating protein factors, such as IL-12, or safety, for example, caspase 9 ligands. (Holzinger A, Barden M, Abken H, 2016). To induce the elimination of CAR-T in cases of toxicity, as well as to regulate its persistence, antibodies whose target is CAR-T activates the expression of genes for caspase 9 incorporated in the construction of the receptor (Drokow EK *et al.*,2019; Holzinger A, Barden M, Abken H, 2016).

3.5 Therapy indications

CAR-T treatment is more effective in hematological malignancies, however its application in solid malignancies has been studied (Cao JX *et al.*, 2019) as well as the possibility of remission of the disease, new molecular targets (Grigor EJM *et al.*, 2019; Sahlolbei *et al.*,2020; Zhu J *et al.*,2017), adjuvants (Zhu J *et al.*,2017) and cure in addition to an increased survival rate already presented (Pettitt D et al.,2018).

Some neoplasms may have relapses and therefore impact on mortality because of the resistance to conventional treatments (Hao L *et al.*, 2019). Among the neoplasms indicated for treatment with CAR-T immunotherapy, it is worth mentioning relapse or refractoriness in hematological malignancies such as Leukemias (Acute Lymphocytic Leukemia and Agute/Cronic lymphoblastic leukemia) and lymphomas (Diffuse B-cell, Non-Hodgkin and Large Mediastinal B Large Cells) (Riaz IB *et al.*,2017; Cao G, Lei L, Zhu X, 2019; Petrou P, 2019).

Complete remission of Acute Lymphoid leukemia was achieved in up to 2 years for 90% of patients who underwent anti-CD19 intervention, but this data may vary according to the sample size, source of lymphocytes, dose, previous chemotherapy and lymphodepletion of lymphocytes received, according to Maude cited by Drokow (Drokow EK *et al.*,2019).

Another example of hematological malignancy is an advanced multiple myeloma, which has achieved a total global response to CAR-T therapy for anti-BCMA (Sohail A et al., 2018).

3.6 Adverse events

Adverse events for this therapy are:

Cytokine release syndrome (Anwer F *et al.*, 2017; Li J, Wu Z, Zhao N, 2019; Grigor EJM *et al.*, 2019) inflammatory reaction that usually occurs days (first 24 hours) (Drokow EK *et al.*,2019) or weeks (2-3 weeks) (Jin Z *et al.*,2018) after infusion of CAR-T lymphocytes (Zhu Y *et al.*,2016). The duration of this event varies according to the expansion capacity and persistence of CAR-T cells (Jin Z *et al.*,2018). It is characterized by nausea, fever, hypotension, hypoxia, myalgia, cytopenias, increased vascular permeability, coagulopathies and multiple organ failure (Holzinger A, Barden M, Abken H, 2016; Nagle K *et al.*,2018; Hao L *et al.*, 2019; Li J, Wu Z, Zhao N, 2019).

In addition, there is a high release of pro-inflammatory cytokines, such as INFγ and TNF-α, by T lymphocytes in accentuated activation and proliferation (Holzinger A, Barden M, Abken H, 2016; Cao G, Lei L, Zhu X, 2019; Li J, Wu Z, Zhao N, 2019). Activated macrophages and monocytes secrete IL-6, favoring this toxicity, whose syndrome can be mitigated by neutralizing this interleukin by immunosuppression or monoclonal antibodies like Tocilizumab type, except in cerebral parenchyma (Holzinger A, Barden M, Abken H, 2016; Zhu Y *et al.*,2016; Jin Z *et al.*,2018). Immunosuppression can restrict the effectiveness of the treatment, since the clinical presentation of this syndrome is related to a better response (Zhu Y *et al.*,2016; Hao L *et al.*, 2019).

Cytokine Release Syndrome is possibly related to high doses of CAR-T cells (Cao G, Lei L, Zhu X, 2019) and proliferation of T lymphocytes (Hao L *et al.*, 2019).

Neurotoxicity (Grigor EJM *et al.*, 2019): related to the existence of CAR-T cells in the central nervous system due to the possibility of migration through the blood-brain barrier and cerebrospinal fluid (Hao L *et al.*, 2019). Hao suggests, (Hao L *et al.*, 2019), that this adverse event reached 26 to 50% of the cases with manifestations of: tremors, myoclonus, gait disorders, paralysis, aphasia, confusion, hallucinations, delirium and encephalopathy.

Anaphylactic shock (Petrou P ,2019).

Autoimmune reactions: if tumor antigens are also present in healthy cells (Pettitt D *et al.*,2018) an "on-target off-tumor" reaction occurs. Therefore molecular targets must be carefully studied to avoid adverse reactions in vital organs and impaired selectivity to neoplasms (Holzinger A, Barden M, Abken H, 2016; Sahlolbei *et al.*,2020). This exclusivity to attack only tumors is a challenge and new studies aim to improve it with other approaches such as: co-expression of inhibitory CARs and activation of CAR by antigen pairs (Holzinger A, Barden M, Abken H, 2016).

B cell aplasia (Cao G, Lei L, Zhu X, 2019; Anwer F *et al.*, 2017) is a condition in which insufficient B cell production favors opportunistic infections (Zhu Y *et al.*,2016).

Tumor lysis syndrome (Anwer F *et al.*, 2017) is characterized by hydroelectrolytic disorders resulting from extensive destruction of hematological tumor cells, being an emergency.

Graft-versus-host disease that may occur in the case of allogeneic lymphocytes after hematopoietic stem cell transplantation (Anwer F *et al.*, 2017).

4. Discussion

4.1 Discussion of the results obtained

The limitation of patient samples in the articles and small dose range evaluated in clinical studies limit the evidence on the efficacy, efficiency and safety of the therapy, whether in hematological tumors or in solids (Li J, Wu Z, Zhao N, 2019). These differences result in different proportions regarding the assessment of adverse events and the degree of response.

The main advantages of this therapy compared to the conventional treatment are: specificity, metastasis regression and a treatment for relapses and refractoriness. However, the literature describes a variety of adverse events that may be: cytokine release syndrome, neurotoxicity, anaphylactic shock, autoimmune reactions, B cell aplasia, tumor lysis syndrome and graft versus host disease.

The use of this modality generally occurs in hematological malignancies, however more studies are needed to evaluate its applicability in solid tumors. The efficacy in the response of solid tumors to CAR-T therapy leaves something to be desired when compared with hematological neoplasms, according to Zhang (Zhang J, Wang L, 2019). According to the literature, the limitation of this comparison is mainly due to researcher's bias, heterogeneity (Grigor EJM et al., 2019) and small sample sizes (Cao G, Lei L, Zhu X, 2019). However, other therapies such as T cell receptors (TCR) show a more satisfactory performance in the treatment of solid neoplasms (Zhang J, Wang L, 2019).

Other factors determining the effectiveness of the therapy in solid tumors, reported by Holzinger (Holzinger A, Barden M, Abken H, 2016) are the status of the disease and the immune system, tumor burden, infiltrative capacity of CAR-T and recruitment of other cells. In addition, adversities should be assessed regarding systemic or local application via endoscopy

(Holzinger A, Barden M, Abken H, 2016). Cao (Cao JX et al., 2019) relates efficacy and safety to gene transfer, persistence and toxicity.

The second generation, compared to the first, has better activation, expansion and persistence of cells (Drokow EK et al.,2019), which may allow its application for infiltration of tumors from the peripheral circulation in the future (Zhu Y et al.,2016).

An example of a solid neoplasm that has been studied regarding the applicability and effectiveness of CAR-T therapy is pancreatic cancer in which the recognized antigen is PSCA (Sahlolbei et al.,2020).

Some variables show a positive impact with an increased response rate for CAR-T anti-CD19 therapy in B-cell malignancies. Among them, the administration of IL-2 (expansion stimulatory cytokine) and lymphodepletion that prevents rejection of CAR-T cells and exhibits improvement in free tumor progression survival time (Drokow EK et al.,2019; Cao G, Lei L, Zhu X, 2019).

On Grigor's review (Grigor EJM et al., 2019), for hematological malignancies, more than half of the patients presented cytokine release syndrome (55.3%) and obtained a complete response (54.4%) for the CAR-T therapy anti-CD19 ; meanwhile approximately a quarter (24.4%) had a complete response unrelated to CD19. As for solid tumors, a minority (4.4%) showed a complete response to the treatment.

On the other hand, Riaz's review, (Riaz IB et al.,2017) addressed both CD-19 and CD-20 as a target for B cell hematological malignancies therapy. In this study, the analysis suggested a complete response in 42% of patients and partial response in 19% (Riaz IB et al.,2017).

In Drokow's review, (Drokow EK et al.,2019), for hematological malignancies treated with anti-CD19 CAR-T, more than half showed toxicity related to cytokine release syndrome (60.15%) and complete response (71.88%).

Meanwhile, Zhu's review, (Zhu J et al.,2017), indicated that less than half (24%) showed a complete response to hematological malignancies treated with CAR-T anti-CD19.

For Hao's meta-analysis (Hao L et al., 2019), the percentage of complete response in patients with leukemia and lymphoma relapses was also over half (55%) and the partial was 25%.

There are several interpretations of the dose / effect relationship: while some researchers suggest a better response to higher doses (Cao G, Lei L, Zhu X, 2019), others reveal that the relationship between high dose and cytokine release syndrome only occurs in individuals aged 25 years or less and that this adverse event can be reduced from previous chemotherapy by controlling and reducing tumor burden (Li J, Wu Z, Zhao N, 2019).

Although there is a potential in the treatment of other malignancies and the therapy has shown favorable cost-effectiveness, it implies a high cost for the systems and other health sectors (Petrou P ,2019).

4.2 Future prospects and open questions

The new therapy requires further studies on molecular targets and mechanisms that guarantee specificity against adverse events "on-target off-tumor", activation of the expression of genes for caspase 9, improvement of the infiltrative capacity in solid tumors, persistence and release of cytokines. In addition, a possible expansion of therapy for other neoplasms and diseases in the future may occur, in view of the antigen-antibody specificity without the involvement of MHC (Zhu Y et al.,2016; Riaz IB et al.,2017; Anwer F et al., 2017).

As for CD19, further studies are needed to investigate its physiological expression and neurological cancer cells (Hao L et al., 2019).

5. Conclusion

Immunotherapy is a promising treatment that enhances the immune system of cancer patients, with advantages over the conventional method in indications of hematological relapse or refractoriness in view of the specificity to a molecular target. It is currently indicated for hematological neoplasms, mainly leukemias and lymphomas, which have the best therapeutic success rates.

The choice of the use of therapy, dose and generation of CARs with their corresponding co-stimulatory domains must be careful, considering the molecular targets characteristic of the neoplasia and adverse events if the targets are expressed in healthy tissues.

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