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CAR-TCell Therapy: An Innovative Treatment Against Cancer

Sebastián Arguedas Chacón[1], Raquel Arguedas Herrera[1], Daniela González Rodríguez[1], Dayana Fuentes Herrera[1], Mariana Murillo Chinchilla[1], Juan José Mora Román[2]

[1]*Pharmacy student, Faculty of Pharmacy, Universidad de Costa Rica, San José, Costa Rica.*
[2]*Industrial Pharmacy Department, Faculty of Pharmacy, Universidad de Costa Rica, San José, Costa Rica.*
Corresponding author: Juan José Mora Román

Abstract
Cancer is characterized by uncontrolled growth, along with a spread of abnormal cells. The condition can be caused by various external and internal risk factors.Due to its high mortality rate, ways to treat it have been studied. Traditionally, the therapeutic triad has been used: surgery, radiotherapy, and chemotherapy. However, it generates a number of adverse effects on patients. Because of this, different methods and techniques have been investigated through which it can be attacked more effectively. Among these is the immunotherapy, a stimulation of the patient's immune system to fight cancer cells. T-cell therapy associated with a chimeric antigen receptor (CAR) is a powerful new option for cancer treatment. Patients who have been given this therapy have responded positively, indicating great effectiveness. There are evidences in both children and adults. Yet, scientists are working on the improvement of aspects such as adverse effects and an extension of the diseases for which the treatment can be utilized.

Key words:*CAR-T cells; immune system; cancer; antibody; immunotherapy.*

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I. Introduction

According to the World Health Organization (WHO), in 2015 cancer was the second leading cause of death worldwide (incidence of 8.8 million), with lung cancer being the main. 70% of these deaths were found in medium and low income countries[1]. Likewise, the National Cancer Institute (NCI) mentions that breast, lung, and prostate cancer as well as melanoma are among most common types[2]. Additionally, those related to infectious agents (stomach, liver, and cervical) have higher incidence and mortality rates in Latin American countries[3].

This disease is characterized by uncontrolled growth, along with a spread of abnormal cells. The condition can be caused by various external and internal risk factors. Among the external ones are nutrients deficiencyas a result of an unhealthy diet, contact with chemicals present in the environment, radiation, smoking, alcohol consumption, and infectious organisms such as viruses (capable of interfering with signals responsible of cell proliferation control). Some examples are: Epstein Barr, hepatitis B and C, human immunodeficiency (HIV), and human papilloma (HPV). Bacteria such as *Helicobacter pylori* can also be mentioned. Furthermore, aflatoxins in numerous grains of frequent consumption increase chances of suffering cancer. Similarly, within internal factors are inherited mutations, hormones, and immune system or metabolism problems[4].

Due to its high mortality rate, ways to treat it have been studied. Traditionally, the therapeutic triad has been used: surgery, radiotherapy, and chemotherapy[5]. However, it generates a number of adverse effects on patients. The decision of what approach will be employed depends on the disease stage[6].

Because of this, different methods and techniques have been investigated through which it can be attacked more effectively. Immunotherapy is a stimulation of the patient's immune system to fight cancer cells[7]. Among immunotherapyapproaches being developed are vaccines, immune checkpoint blockade therapy, andT-cell therapy associated with a chimeric antigen receptor (CAR)[6]. The latter is a combination of immune system cells and T lymphocytes. This process is performed by obtaining cells from the patient, which are genetically modified by an *ex vivo* transduction with a specific vector. Then, they are reintroduced into the patient, so that the attack against the tumor will be more efficient[8].

Therefore, the objective of this research is to describe one of the most recent therapies in cancer treatment, the CAR-T cells.

II. Immune system response to cancer

The immune system has been responsible for protecting organisms from pathogens. It has a range of mechanisms, molecules, cells, and pathways that allow this function to be performed with great efficiency. In addition, it is closely related to other systems such as endocrine and nervous. All its components perform elementary functions to maintain organism´s homeostasis and in its absence, humans and animals would contract a large number of infections that could be fatal[9].

For some years, most of the research has been responsible for studying the relationship between the immune system and cancer. This system protects throughrecognition between the own and the non-self, including malignant cells. The mechanisms mentioned above usually achieve their elimination, destroying these cells and not healthy tissues. Moreover, it creates memory, important in long-term recurrence situations[10,11].

Cancer is not a disease, but a set of them. Normally, body's cells divide to reproduce and replace those that are damaged or die of old age. Still, in cancer they lose their growth order and get out of control, having the ability to reach and spread to other body tissues. Unlike normal cells that replace old ones with new ones, aging carcinogens survive and form clusters. This is called a tumor[12].

The immune system ability to recognize abnormal cells is due to its immune surveillance. This process is carried out through four steps:

1. Specific antigens detection: they are detected in a danger signal context by antigen presenting cells (APC). During these early stages, cancer cells can be eliminated by macrophages, granulocytes, and NK (Natural Killer) lymphocytes. Though, part of the dendritic cells internalizes and processes antigens belonging to them, linked tomajor histocompatibility complex II (MHC-II), and presents them to effector cells[11,13].
2. Specific antitumor response activation mediated by helper (Th) or CD4+ and cytotoxic (Tc) or CD8+ T cells: these receive activation, proliferation, and functional differentiation signals when interacting with dendritic cells, through the MHC[11]. CD8+ T cells have the ability to recognize tumor antigens exposed on cancer cells surface and eliminate them[11,13]. These glycoproteins belong to a group called cluster of differentiation (CD), nomenclature utilized to name proteins found on the immune cells surface[14].
3. Activated lymphocytes migration towards the altered area and extravasation[11, 13].
4. Altered cells elimination: it is done through the CD8+, by producingperforins and granzymes release. Perforins allowgranzymes to enter the cytoplasm of the attacked cell, generating its apoptosis. As a complement, they express on the surface FAS ligand or Apo1L protein, which binds to FAS or Apo1 receptor, respectively, in the target cell, causing procaspases recruitment (cysteine proteases that lead to apoptosis)[11,13].

Despiteimmune surveillance specificity andimmune system high performance against carcinogenesis, there are circumstances in which tumors can develop resistance mechanisms and escape this monitoring. Some common ways to avoid immune recognition are: inadequate antigens presentation, immunosuppressive factors production, activation of negative stimulus signals such as galectin-1, expansion, activation, and migration of cells capable of suppressing antitumor response, including regulatory T lymphocytes (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and dendritic cells[15].

Evasion can be visualized against innate and adaptive immune system. In the case of innate one, processes are based on intrinsic alteration of certain signaling pathways such as WNT/β-catenin pathway activation, phosphatidylinositol 3-kinase (PI3K) activation, and liver kinase B1 (LKB1) signaling loss. Adaptive evasion is shown by MHC loss and lack of function of Janus kinases, causing interferon γ(IFN-γ) response inactivation[16].

Plus, inadequate antigens presentation is due to constant genetic variation of a tumor cell, changing its antigenic profile over and over again. This makes recognition more difficult, since being cells of the same individual allows them to present a certain immunotolerance degree[17].

In the case of Tregs (immune response modulators capable of suppressing antitumor response), tumor associated antigens induce and increase their number in it, becoming immunotolerant[18]. Besides, TAMs inhibit antitumor T response, attracting Tregs to the tumor site. In this way, they can block the effector response through expression of B7-H4, a transmembrane protein that negatively regulates T lymphocytes activation, stimulates new blood vessels formation, and allows tumor cells entry into the bloodstream, contributing to metastasis[15].

III. Traditional therapy against cancer

Traditional therapy to deal with cancer has focused on surgery, chemotherapy, and radiotherapy, which are nonspecific methods. These strategies, despite showing important benefits eliminating primary tumors, do not represent a definitive solution, since in many cases relapse occurs (malignant cells remain and there is a possibility of metastasis)[6,19]. Plus, in people with advanced stages, they did not provide a lasting benefit, with some exceptions such as testicular carcinoma, germ cell tumors, and some leukemias. Together with these transitory benefits, damage caused by total treatment doses, for example radiation, shows an impact on cells physiological functions[20].

In the case of radiotherapy, it is an ionizing radiation, which deposits its energy in cancer cells, generating its death, and producing mutations and damages in the genetic material that prevent its survival. It affects healthy and carcinogenic body's cells. Though, being the latter less efficient in repairing damage, normal ones are able to recover at a faster rate, maintaining their function. This therapy can be for disease cure and for palliative purposes[21].

With this type of conventional radiation therapy, normal tissues found nearby receive a dose, limiting their administration in a safe way. For this reason, there are diverse technical innovations, such as modulated intensity radiotherapy (IMRT) and image-guided radiation therapy (IGRT). They have achieved a more precise and focal radiation emission, with an improvement in the results obtained, as a result of toxicity decrease[22].

Moreover, chemotherapy treatment consists in triggering a toxic attack indiscriminately, that is directed to both malignant and normal cells, seeking to generate greater damage in cancer cells[20]. Once an initial treatment (radiation or surgery) has been administered, many patients receive adjuvant chemotherapy. This tries to eliminate microscopic cancer cells that may be present in other body´s areas. Besides, genetic evaluation of the patient is recommended, as it would be possible for a person that one treatment or another will work[23].

IV. Immunotherapy against cancer

As an alternative to the problems exhibited by traditional treatments, immunotherapy has been developed. It refers to a biological treatment that gives immune system different capacities to combat this disease. It is based on knowledge about cellular and molecular mechanisms that the body utilizes to fight cancer cells, seeking to improve immune response[24,25].

Recent studies suggest that by making a combination between immuno-oncological agents and chemotherapy therapies, the objective of the latter is perfected, because it is not focused solely ontumor cell destruction, but is directed towardsimmune clearance optimization, translated in a decrease in chemotherapy doses and tumor growth[20].

One way to classify immunotherapies is active, which includes everything related to vaccines (administration of tumor antigens in patients), and adoptive. This one stimulates effector T cells *in vitro* and then administers them intravenously[13].

Part of its current approaches consists in blocking immune evasion mechanisms employed by cancer cells. An example is the programmed cell death pathway. It inhibits antitumor response of T cells when they are positively regulated in the tumor microenvironment. Those therapies directed towards blocking this pathway have demonstrated efficacy[25].

Another therapeutic alternative isan antitumor vaccine. A tumor antigen is administered and through proper dendritic cells stimulation, the response is generated[26]. Immunization done in the patient can be active or passive. If an antibody is administered against a defined antigen or a reactive lymphocyte it is passive. In contrast, the active one is administered with the expectation that an immune response is generated against the tumor. This vaccine, specific or not, can be produced with one or more cell components. It should be noted thatvaccines potential are not employed as a form of treatment when cancer is present, but as prophylaxis that prevents its subsequent development[27].

In recent years, research has also been carried out to prove immunotherapies efficacy directed at specific immune checkpoints, allowing tumors to escape from immunovigilance. An example is the programmed cell death inhibitor protein (PD-1) or its ligand inhibitor (PD-L1)[28].

These checkpoints are T cells surface receptors, which trigger negative signals when they bind to ligands expressed in APC, silencing the response. The problem is that tumors uses this mechanism to evade the immune system, so that specific T lymphocytes against a tumor antigen, previously activated in lymph nodes, are deactivated when they come into contact with their microenvironment. Upon discovering that tumors express PD-L1, monoclonal antibodies were developed to interfere with the binding between this receptor and its ligand[29].

There is also an immune checkpoint protein receptor called CTLA-4. This suppresses T cells in the lymphoid compartment, unlike PD-1 that performs it in the cellular microenvironment. This action prevents expansion of antitumor type T cells responses. Therefore, anti CTLA-4 therapy prevents T cells from being inhibited[29].

V. Advances in therapy with antibodies

Monoclonal antibodies

Another type of passive immunotherapy includes antibody-directed therapy and its derivatives. These modulate tumor immune environment, so that the host is favored[30]. Antibodies were discovered in the late 19th century when Emil Von Behring and ShibasaburoKitasato discovered that serum produced by rabbits immunized against diphtheria or tetanus toxins prevented infection in other non-immunized rabbits[31].

When a substance presence outside the body occurs, the immune system through activated B lymphocytes produces antibodies for its elimination. These have the ability to recognize one or more antigen sections. The recognized area is called epitope. In the event that the antibody is only able to recognize a particular one, it is called monoclonal[19].

Antibody structure is similar to a Y. It is made up of light chains and heavy chains (two of each), which are linked by disulfide bridges. Chains have two regions, one variable and one constant. Its specificity will depend on the variable region. Heavy chains determine their isotype (IgG, IgD, IgE, IgM or IgA). Furthermore, antigen binding site is formed by the variable region of both heavy and light chains that are juxtaposed. So, there are two binding sites for each antibody[32].

With regard to monoclonal antibodies, they were discovered in the 1970s by Milstein and Kohler, who were investigating distinct molecular mechanisms by which antibody production occurred[33]. Their objective was to generate an immortal B cell with the ability to be specific for mutations in IgG genes. This was achieved throughhybridoma technique (fusion of murine myeloma cells with spleen cells of an immunized animal, selecting hybrid cells and clones as products that presented the specificity sought)[34, 35].

As part of the procedure, B cells from mice lymph nodes or spleen previously immunized with the desired antigen are cultured, together with those of myeloma and the fusion agent, all in a HAT culture medium (containing hypoxanthine, aminopterin, and thymidine). This combination caused fused cells survival, while non-hybrid myelomas and non-fused B lymphocytes die[36].

There are various types of monoclonal antibodies, among which are: murine (obtained from rodents), chimeric (combination between constant region of human being and variable region of rodent), humanized (possess only complementarity-determining regions of another species on the framework of human variable region), and human (100% human protein)[37]. For its design, like any pharmaceutical product, characteristics such as immunogenicity, affinity, stability, half-life, distribution, and elimination must be considered[38].

When the first human therapy was performed for the prevention of kidney rejection, it was recognized that murine type generated tolerance through human anti-murine antibodies, decreasing their efficacy[39]. As a way to counteract this effect, chimeric monoclonal ones were developed in the 1990s and since 2002, it was decided to develop and employ more humanized and human antibodies, to eliminate the barrier that a foreign agent implies in body and immune response against it[40].

Its utilization has led to treatments for various diseases, mainly in cancer area. Among existing antibodies are those directed against human epidermal growth factor receptor 2 (HER2) in breast cancer and non-Hodgkins lymphoma. In addition, they have been used in autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, and graft versus host disease[41].

Bispecific antibodies

Bispecific antibodies are another specific strategy for cancer treatment. With this, the aim is to direct CD8+ lymphocytes towards the tumor[42].

These structures are based on natural model of human Ig and have the potential to redirect effector cells to exert their function towards that expressed by the antigen. Effects generated are divided into three categories: redirection of cytotoxic effector cellsto tumor cells (T and NK lymphocytes), tumor immunomodulation (cells stimulation of immune system, so that the tumor is infiltrated), and dual immunomodulators (immune response modulation through two entities)[43]. Moreover, these structures are no longer based on T cell clones´generation or antigens presentation by dendritic cells. T lymphocytes activation is done by an antigen presented by thebispecific antibody, without requiring MHC-I expression[44].

Bispecific antibodies can be divided into two categories: those similar to IgG and those not similar to this immunoglobulin. The first ones possessantibodycrystallized fraction (Fc). This maintains functions such as antibody-dependent cellular cytotoxicity(ADCC), complement-dependent cytotoxicity (CDC), and phagocytosis. Besides, it makes its purification easier, its solubility and stability are improved, and due to their large size they have a half-life greater than those not similar to IgG. The othersdo not have an Fc region and are smaller (greater tissue penetration). Nevertheless, it loses all functions related to the Fc region[45].

Those similar to Ig were directed towards effector cells such as monocytes and macrophages, so that they recognized tumor cells that express antigens such as HER2, which favors tumor cells replication, or epidermal growth factor receptor (EGFR), responsible for joining epidermal growth factor (EGF) and making cell multiply. However, this therapy was not effective, as expected results were not obtained[46].

The first bispecific antibody developed was catumaxomab. It was approved in 2009 by the European Medicines Agency (EMA) and used for the treatment of malignant ascites, redirecting T cells of immune system by binding to EpCAM receptor of tumor cell and CD3 of T lymphocyte[47,48].

Other examplesarebispecific T-cell engagers (BiTEs). They are variable fragments of single strands ordered in tandem, allowing simultaneous binding to tumor cell and T lymphocyte[49]. An advantage is its high specificity at very small concentrations of up to 10 pm/ml. Despite this, it has a short lifespan, as a consequence

of the lack of Fc zone and its small size in blood serum, forcing injections at a higher frequency[47]. This therapy has been approved for diseases such as acute lymphoblastic leukemia (ALL) with the use of blinatumomab[32].

As part of the normal immune response in the body when immune cells encounter invading molecules, T lymphocytes come into action[50]. Knowing this, CAR-T cell therapy has been considered as a novel treatment. It combines T lymphocytes and the idea of monoclonal antibodies, having a single receptor where antigen binding occurs.

VI. CAR-T cells

CAR-T cell therapy is a powerful new option for cancer treatment[51]. It was approved in August 2017 by the Food and Drug Administration (FDA), using CAR-anti-CD19 cells for ALL treatment[52].

CARs are synthetic hybrid receptors that redirect T cells specificity, function, and metabolism[51]. Its structure has an extracellular region, a transmembrane domain, and an intracellular domain[53]. They can be employedagainst a specific antigen, constantly expressed by cells (CD19, CD20, CD22 of normal and malignant B lymphocytes), resulting in their elimination[54].

Extracellular domain consists of a signal peptide responsible for guiding proteins to endoplasmic reticulum[55], and an antigen recognition domain[54], which comes from a single chain fragment variable (scFV) of a monoclonal antibody[56]. It contains a variable heavy chain, a flexible linker, and a variable light chain region[54]. Variable fragment is the smallest functional unit of Ig that allows antigen binding to take place. This single chain fragment is formed by joining variable regions of heavy and light chains, which bind through a flexible linker peptide, increasing affinity properties and specificity alteration of scFV[57]. The domain binds to transmembrane domain through the spacer. This usually comes from the hinge region of IgG1[55], responsible for transmitting signals to the receptor when binding with its ligand occurs[58]. Short spacers have shown greater efficacy in inducing T lymphocytes function compared to those with a larger size[59].

On the other side, transmembrane domain is composed of hydrophobic alpha helices[60]. Its function is to provide stability to the receiver[61]. Depending on length and flexibility that gives, it may affect receiver function. Additionally, it can modify self-aggregation tendency, membrane surface density, and facility of other molecules to bind instead of its ligand[54].

Finally, there is the intracellular domain or endodomain. Once an antigen has been recognized, it undergoes conformational changes, allowing protein phosphorylation[60]. These are responsible for transmitting activation and co-stimulation signals[54].

It is precisely this domain that has undergone changes in its components in the generations of CAR-T cells[55]. The first only had a single CD3-ζ domain from a normal T cell receptor, responsible for sending cascade signals below[62]. Its presence facilitates artificial T lymphocyte receptors (TCRs) incorporationin native TCRs[55]. Plus, it has immunoreceptortyrosine-based activation motifs (ITAMs) sequences, whose function is to transduce signals[60]. This first generation had the problem of not showing good cytotoxicity, nor adequate proliferation of T cells *in vivo*[60,63]. Another drawback was a very short life time[61].

Thus, co-stimulation domains were added to second-generation receptors[61], with the intention of improving cytotoxicity and cell proliferation[60]. These domains are sections of glycoproteins such as CD28, 4-1BB (CD137), OX40 (CD134), and CD27[64,65,66]. In **Table 1**, functions of each one are mentioned.

Table 1. CD markers used as co-stimulation domains.

Marker	Function
CD28	Increases glycolytic speed of immune cells[67] Activates T cells[66] Allows T cells proliferation[67]
CD137 (4-1BB) **CD134 (OX40)**	Recruits factors associated with tumor necrosis factor[68] Increases T lymphocytes proliferation, survival, and cytotoxicity[68,69] Allows cytokines secretion[68,69]
CD27	Promotes regulatory T lymphocytes proliferation[70] Allows B cells formation[71]

The addition of these domains allowed dual signaling. It consists of a first signal that recognizes MHC in APC, and a second one that stimulates IL-2 synthesis, whose function is to promote T cells activation[55]. In a study in which first and second generation receptors were compared, second generation receptors were significantly better, as a result of this dual signaling[72].

As for third generation receivers, they followed second generation steps, but two co-stimulation domains were utilized instead of only one[73]. The idea was to increase power, due to a greater cytokines production. However, studies carried out so far do not show a significant improvement with respect to those of second generation[55].

Finally, fourth generation receptors are called T cells redirected for universal cytokine-mediated killing (TRUCKs)[74]. They are constituted, like those of second generation, by a co-stimulatory domain. Nonetheless,

they have the addition of pro-inflammatory proteins, either constitutively or with the ability to send signals that promote their production and release[60,74]. Its development usually involves a nuclear factor of activated T-cells (NFAT), whose function is to induce pro-inflammatory proteins[66,74]. This signaling usually involves interleukin 12 (IL-12)[74], IL-18 or IL-15 release[60].

IL-12 is a proinflammatory cytokine responsible for inducing IFN-γ secretion, aiding in helper T lymphocytes 1 (Th1) differentiation, and increasing NKcytotoxicity[75]. It has been observed that TRUCKs using IL-12 are very effective in fighting cancer, because of their ability to influence IFN-γ secretion[76]. Yet, there is the question abouttoxicity that can be induced[60]. In a study in humans, where lymphocytes were administered with a gene encoding IL-12 expression, severe dose-dependent toxicity was found, including liver dysfunction, fevers, and hemodynamic instability[77].Though, in other investigations, CAR-T cells with IL-12 have not presented such toxicity[60]. To clarify this uncertainty, phase 1 clinical studies are being conducted to determine its safety[78].

As a consequence of the toxicity that could be generated by IL-12 use, IL-18 is available as an alternative. Its functions include increasing IFN-γ production[79], and activating NK and cytotoxic T lymphocytes[80]. Recent studies show that using CAR-T with IL-18 increases antitumor activity and immune cell proliferation[80,81].

As a complement, IL-15 increasesNK and CD8+ lymphocytes survival, proliferation, and activation[82]. In high concentrations, it favors the T cells´ lytic activity, in addition to expressing granzyme B[83].

As in third generation, the fourth generation´s efficacy has not been fully demonstrated and there is a significant improvement in treatment with respect to the second generation[66].

Production

Preparing CAR-T cells is a multi-stage process and must be under strict quality control[55]. It begins with leukapheresis procedure, performed with a continuous-flow blood cell separator[84]or an autologous blood recovery system[85]. Through them, immune cells, mainly leukocytes, present in patient's blood[86] or from healthy donors (previously anticoagulated) are removed. Then, the other blood cells are returned to the body. At the end of the production process, modified T cells are introduced to the patient[62,87,88].

The cells of interest are T lymphocytes. Therefore, a wash is performed to remove added substances, such as anticoagulants. Obtaining or selecting T lymphocytes from samples is done through a process called enrichment. This is made by counterflow centrifuges, separating cells by size and density. Other techniques such as separating columns followed by washes and dilutions allow purification of these cells in their variousforms, either by expressing CD4+ molecules (bind to MHC-II receptors) or CD8+ (bind to MHC-I receptors), and generating attack responses in infected cells that express it[89]. CD25+ is another component, specifically of IL-2 receptor, which participates inregulation and proliferation of T cells[90]. There is also CD62L+, a marker found in T lymphocytes (responsible of the differentiation between central memory and effector function[87,91]).

T lymphocytes require activators of complementary DNA transduction of CAR, which will be introduced through viral genetic material[85]. Within the body, dendritic cells behave as activators. Nevertheless, at *ex vivo* level its employment is difficult, since there is variability in the cellspower from one patient to another[86], making the process more laborious, complicated, and suboptimal[87]. Activation should be prepared in a proliferative environment with antibodies of interest, IL-2, anti-CD3[92], or anti-CD28. T cell cultures are more successful when done in presence of IL-2 and APCs, according to the affinity require with a cell line (polarizing cells to a specific phenotype). In a standardized and efficient manner, coated beads with monoclonal antibodies, called artificial APCs, are used[86,87]. Its novelty lies in the ability to select and activate T cells in the same step. These beads are easily removed by magnetic separation[87].

Simultaneous to the activation process, expansion process occurs for several days. In this, T cells are incubated together with viral vectors encoding the CAR during a minimum of two weeks[87]. CARs are synthesized as fusion proteins, which facilitate vectors insertion[93]. Genetic material in the form of RNA is introduced in a very stable manner into cells through lentiviral or retroviral genes[87], due to its ease in transfecting cells and expressing genes that produce the protein of interest. Yet, it requires many studies to prove safety[87,93]. Lentiviruses have shown to have a safer insertion site than retroviruses. Moreover, it is possible to introduce genetic material as transposes or mobile genetic units present in the chromosomes[94]. These are simpler to produce, require fewer safety studies, and are introduced through plasmids[93]. Still, there is uncertainty regarding relative efficiency compared to viral vectors[87].

Subsequently, it is transcribed by reverse transcriptase to DNA[86,92], and genetic material is integrated into cell genome, so that receptors are generated. In general terms, these therapies are highly dependent on CARstability at administration time to activated T cells. Once expansion process is finished, they are concentrated and applied to the patient[87]. It is necessary to ensure permanence of genetic material belonging to CAR, for which polymerase chain reaction (PCR) technique is used[87,95,96]. In it, copies of the CAR gene are amplified in a thermal cycler in order to identify the gene more easily[97]. Fluorescent *in situ* hybridization

(FISH)[98] can be employed, too. This technique detects nucleic acid sequences using a fluorescently labeled probe. This probe hybridizes specifically with its complementary target sequence, allowing chromosome labeling[87,99].

Applications

Most applications are related to cancer treatment. These include myeloid neoplasms, large vascularizedmetastatic melanomas (application of tumor-infiltrating lymphocytes), tumors associated with Epstein-Barr virus (virus-specific T cells, useful in Hodgkin's disease), lymphoma of Burkitt, and nasopharyngeal cancer[100,101].

As mentioned earlier, therapy against ALL using CAR-T anti-CD19 has already been approved by the FDA. There are studies that show a remission rate of up to 80% in patients with this treatment[102,103]. Due to their success, they are currently being tested as possible treatment against diffuse large B-cell lymphoma[60], non-Hodgkin lymphoma, and chronic lymphocytic leukemia (CLL)[104].

Additionally, there are researches conducted against pediatric lymphoblastic leukemia and CLL has been studied[101,105]. Plus, for solid tumors (taking into account non-physical tumor microenvironment), its utilization in gioblastoma is under study, through epidermal growth factor receptor variant III (EGFRvIII)[106]. Besides, they are being evaluated in hematologic malignancies such as multiple myeloma and show great promise in melanoma, breast cancer, and sarcoma[88,101].

As a complement, there are currently 500 studies in different clinical stages. Of these, only 7.4% have been completed. The rest is in process or in recruitment[101], demonstrating a broad field of knowledge to discover about these cells.

Adverse effects

Despite being such an innovative and hopeful treatment, it does not escape adverse effects associated with its employment, as with all drugs in general. The most frequent are described below.

The cytokine release syndrome deals with toxicity, not related to antigens, but as a consequence to great immune response activation[107]. First generation CAR-T cells, lacking co-stimulation domains, have been shown to have deficiencies in proliferation and cytokine secretion. On the other hand, in the second generation, addition of CD28 or 41BB domains promoted their activation and cytokines release. Nonetheless, high increase in IFN-γ causes fever, fatigue, malaise, anorexia, tachycardia/hypotension, capillary effusion, cardiac dysfunction, kidney damage, liver failure, and disseminated intravascular coagulation[108,109].

Neurological toxicity has been generated, too. Although its mechanism is unknown, it is associated with the great cytokines release. Confusion, delirium, expressive aphasia, absence of normal stimuli for a period of time, involuntary muscle movement, and seizures have been reported[108,110].

Other adverse effects are generated by recognition on-target/off-tumor, an autoimmune toxicity that occurs when healthy tissues express an antigen associated with the tumor that wants to be attacked[107,110], causing its destruction. As a result, manageable B-cell aplasia to severe toxicity occurs, leading to death. Pulmonary, gastrointestinal, and hematological systems are affected[108].

Finally, rejection of CAR-T cells by the immune system, known as anaphylaxis, develops. This is produced because of the great immunogenicity of foreign murine proteins[108].

Advantages and disadvantages

Chimeric receptors have the advantage that they can be directed towards antigens of all types (proteins, carbohydrates, and lipids). This increases system versatility[111].

Still, since they are not dependent or restricted by human leukocyte antigen (HLA) system (a molecule involved in antigens presentation), it can be applied to different patients with distinct haplotypes. The benefit of being independent of HLA is that tumors normally prevent immunological surveillance of T cells, hiding HLA or other molecules involved in antigens processing and presentation. Hence, it would not affecttreatment operation[88,111].Another advantage is that system methodology is much more efficient and allows the generation of specific antigen T cells in a shorter period[112].

In accordance with the above, these modified cells have flexible intracellular signaling domains. Such a situation counteracts inhibitory molecules released by cancer cells[88].

Though, as part of its limitations it can only recognize antigens expressed in the membrane[26,101].

Plus, when activation of antitumor immunity is given by therapy application, an over-activation of the immune system occurs, causing cytokine release syndrome. Furthermore, neurotoxicity has been presented in patients. Hallucinations, delirium, dysphasia, and epilepsy are shown. These two side effects are the most common among patients. IFN-γ and tumor necrosis factor (TNF) are causes of acute neurotoxicity induction. So, treatment can be accompanied by tocilizumab, suppressing inflammatory pathways mediated by IL-6[88]. IL-6 is responsible forIgs production in activated B cells[113].

Besides, some patients suffer from B-cell aplasia(CD19 antigen is present in both healthy and malignant ones)[114], causing a decrease in antibody production. That is why those who are more prone to infections may be given Ig replacement therapy. But, it is not strictly necessary, since this depletion is temporary. What should be made is a long-term follow-up that allows evaluation of possible effects, due to this decrease[115,116].

A final disadvantage is its high cost. Estimations calculate a total cost between US 150,000 and 300,000 USD, taking into account their generation using current manufacturing methods[117].

VII. Conclusions

Immunotherapy based on CAR-T cells shows great potential, thanks to the ability of T lymphocytes to recognize antigens present in tumor cells, without being restricted by HLA. This manages to induce their apoptosis and allows the patient's immune system to generate memory.

Patients who have been given this therapy have responded positively, indicating great effectiveness, evidenced in both children and adults. However, scientists are working on improvement of aspects such as adverse effects and an extension of the diseases for which this treatment can be used.

Although good therapeutic results have been obtained, it is not available to the entire population, due to its high cost. Therefore, the way to reduce costs should be sought.

Finally, in despite that cancer treatment with CAR-T cells has been tested, only 7.4% of clinical studies have been successfully completed, which shows how much remains to be discovered from this pharmacological therapy. That is important for the individualized approach of a non-traditional therapy against cancer.

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