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REVIEW PAPER

Cytokines: General information, pharmacokinetics, indications, and limitations of its clinical use for cancer treatment

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ABSTRACT

Cancer is a genetic disease that ranks among the leading causes of death worldwide. It accounted for about 10 million deaths in 2020, representing one in six global fatalities. Although therapies such as surgery, chemotherapy, and radiotherapy are available, the survival rate of patients is low. Therefore, research to find new therapeutic strategies is a priority. As a result of these studies, immunotherapy was developed, through which it is sought that a person's immune system can fight cancer cells by administering substances such as cytokines. When secreted by the organism, these proteins function as mediators between effector cells, thereby regulating the inflammatory and immune response and acting as growth factors for cells such as hematopoietic ones. Despite its therapeutic potential, their high toxicity and complex pharmacokinetics have limited their consideration since only anti-cancer indications have been approved for IL-2 and IFN-α. Even with this, research by the pharmaceutical industry continues to assess safe and effective novel therapies against cancer and other pathologies involving these molecules.

Keywords: biotechnology, immune system, cytokine, cancer, immunotherapy.

INTRODUCTION

Cancer is among the leading causes of death worldwide. In 2020, there were around 10 million fatalities, representing one in six total deaths in the world. [1] This pathology is related to a genetic disorder since changes in gene function and expression can lead to mutations, with the consequent abnormal growth of the organism's cells. [2, 3] Major triggers include smoking (which provokes 25 to 30 % of all deaths), hereditary factors, diet, physical activity, radiation exposure, and infections by viruses and other microorganisms. [4]

The diverse treatments can be divided into conventional and advanced therapies. The

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conventional ones include chemotherapy, radiotherapy, and the resection of tumors by surgery. In contrast, the advanced ones cover biological treatments, including immunotherapy and stem cell therapies. [5]

Regarding immunotherapy, living beings are exposed to multiple microorganisms that can trigger an illness. [6] Therefore, there is an immune system, which consists of a dense network of connections between cells and molecules, responsible for guaranteeing homeostasis in the body. [7] Immunity can be divided into innate immunity and adaptive immunity. [8]

Innate immunity is fast-acting against non-specific microorganisms and represents the first line of defense. A set of components such as epithelium, enzymes, neutrophils, eosinophils, basophils, mast cells, and monocytes is necessary for its development. Macrophages, dendritic cells, Natural Killer (NK) lymphocytes, the complement system, and cytokines are also required. Along with destroying the infectious agent, it is processed and presented to the adaptive immune system. [6, 9] In addition, various defense mechanisms are mobilized thanks to the production of cytokines, which participate in the initial cellular recruitment and the local inflammation process, essential mechanisms for eliminating pathogens. [8]

Adaptive immunity acts later. Its reaction occurs when the first processes fail to eradicate the microorganism that originates the activation of the immune response. In this response, T and B cells are involved. [8]

Cytokines are a group of small proteins that, when secreted, function as mediators between distinct effector cells. [10] These proteins or glycoproteins regulate the inflammatory and immune response and are involved as growth factors for cells such as hematopoietic ones. [11] A cytokine can have several functions in different cells and be related to other group members to the point of being able to induce or inhibit other cytokines. [10]

Since these molecules broadly participate in the innate and adaptive immune system, they prevent neoplasia growth. These proteins allow cells to communicate over short distances while controlling leukocyte proliferation, cell differentiation, effector functions, and lifespan. [12] Thus, since the immune system can recognize and destroy cancer cells, cytokines are part of immunotherapeutic strategies to fight cancer.

Therefore, the review aims to describe the function of cytokines, their participation in the immune response against cancer, and their potential to treat this pathology.

GENERALITIES OF CYTOKINES

Cytokines known to date comprise a wide range of substances. They are classified as interleukins (ILs), interferons (IFNs), tumor necrosis factor (TNF) and its related molecules, transforming growth

factors (TGFs), and hematopoietic growth factors. [10] ILs are expressed in many body cells, including leukocytes. Their primary function is to regulate immune system cells' growth, differentiation, and activation during inflammatory and immune responses. [13]

Moreover, IFNs are synthesized in response to viruses and other antigens. They have antiviral, immunomodulatory, and antiproliferative action by binding to membrane receptors and mediating intracellular signals. [14]

Regarding growth factors, they also bind to receptors and are involved in processes such as differentiation, embryo induction, cell motility, and cell death. [15] Finally, TNF is mainly produced by activated macrophages, NK cells, and T lymphocytes and, to a lesser extent, by fibroblasts, smooth muscle, and tumor cells. It participates in the organism's defense, including tumor surveillance. [16]

These molecules can be grouped into other categories without being mutually exclusive: [17]

- Growth factors: IL-1, IL-2, IL-3, IL-4, and colony-stimulating factors.
- Activation factors: IFN-α, IFN-β, and IFN-γ.
- Regulatory or cytotoxic factors: IL-10, IL-12, TGF-β, lymphotoxins, and TNF-α.
- Chemokines: IL-8, MIP-1α, and MIP-1β.

Chemokines are characterized by activating and attracting leukocytes to sites of inflammation through chemotaxis. Furthermore, they participate in other actions related to cell proliferation or apoptosis, angiogenesis, hematopoiesis, and the development of specific immune responses with the participation of Th1 (from T helper 1), Th2, and B lymphocytes. [18]

In the immune response, cytokines are necessary to amplify, modulate, and activate T cells. When macrophages secrete IL-1, T lymphocytes are stimulated and activated, expressing the IL-2 receptor and giving the cells clonal expansion. As a result of this process, IFN-γ is secreted, which leads to the expression of the major histocompatibility complex (MHC) class I and class II, increasing the response of T cells to the antigen. IL-2 secretion activates NK cells, and IL-3 secretion stimulates hematopoietic stem cells. IL-4 collaborates in the differentiation and expansion of B lymphocytes so that they secrete antibodies. [17]

In inflammatory processes, there is a balance between anti-inflammatory and pro-inflammatory cytokines. Within the second group, these molecules involving IL-8 participate in chemotaxis and cell activation. Additionally, IL-1 and TNF-α act synergistically, along with IFN-γ. To complete this balance, anti-inflammatory cytokines such as IL-10, IL-1 receptor antagonist, and soluble p68 (for IL-1), p55, and p75 receptors (both for TNF). [11] Other proteins and their biological effects are presented in **Table 1**.

Table 1. Known cytokines and their biological effects.

Cytokine	Biological effects
IL-5 [13]	-Growth and differentiation of B lymphocytes. -Activation and increase in the production of eosinophils.
IL-6 [11]	-Regulation of the immune response and participation in hematopoiesis and acute phase reactions.
IL-7 [13]	-Proliferation of B and T cells.
IL-9 [13]	-Improvement of T lymphocyte survival, mast cell activation, and synergy with erythropoietin.
IL-11 [13]	-Promotes osteoclast formation. -Increases platelets count *in vivo*. -Inhibits pro-inflammatory cytokines production.
IL-12 [13]	-Induction of Th1 cells and IFN-γ production by T and NK lymphocytes.
IL-13 [13]	-Increases mucus production by epithelial cells. -Augments collagen synthesis by fibroblasts. -Inhibits production of pro-inflammatory cytokines.
IL-15 [13]	-Proliferation of memory NK, B, and T lymphocytes.
IL-17 [13]	-Release of pro-inflammatory cytokines such as IL-6 and stimulation of chemokine synthesis by endothelial cells.
IL-21 [13]	-Proliferation and differentiation of B and T lymphocytes. -Enhancement of NK cell activity.
IL-22 [13]	-Inhibition of IL-4 synthesis. -Mucosal surface protection. -Tissue repair.
IL-23 [19]	-Development and maintenance of Th17 cells.
IL-25 [13]	-Stimulation of IL-4 and IL-13 synthesis (anti-inflammatory cytokines).
IL-27 [13]	-Stimulation of IL-10 production.
IFN-α [19]	-Antiviral activity. -Activation of NK lymphocytes.
IFN-β [19]	-Antiviral activity. -Activation of NK lymphocytes.
TNF-α [20]	-Involvement in the inflammation and autoimmunity processes.
TGF-β [19]	-Increases collagen production in fibroblasts.
GM-CSF [11]	-Stimulation of macrophage and granulocyte colonies formation, and to a lesser extent, that of eosinophils.
G-CSF [11]	-Induction of granulocyte colony formation.
M-CSF [11]	-Stimulation of macrophage colony formation.
MIP-1α [21]	-Induction of NK and B lymphocytes, cytotoxic T cells (CD8+), eosinophils, and basophils migration.
MIP-1β [22]	-Attraction of monocytes, helper T cells (CD4+), and NK lymphocytes.

IMMUNOLOGY OF CANCER

Cancer is the sum of genetic alterations that trigger abnormalities in the cell division process. Some cells in the body grow out of control, potentially moving into and invading other tissues. [23, 24]

The characteristics of this disease can be seen as a set of acquired and functional capacities established by human cells, which go from a normal state to one of neoplastic growth. These hallmarks are: sustaining proliferative signaling, evading growth suppressors, avoiding immune destruction, enabling replicative immortality, activating invasion and metastasis, inducing or accessing vasculature, resisting cell death, deregulating cellular metabolism, senescent cells, and unlocking phenotypic plasticity. [25, 26]

Given this scenario, the immune system seeks to destroy cancer cells. Such a process can be seen as the cycle of immunity to cancer, divided into seven steps. [24] In the first step occurs the release of cancer antigens and neoantigens. These peptides arise from a specific mutation process in tumors that the immune system has not previously recognized. These neoantigens are captured by dendritic cells for further processing. [24-26]

This process must be accompanied by signals that specify immunity, avoiding peripheral tolerance towards tumor antigens. These signals encompass pro-inflammatory cytokines and factors released by the tumor. This way, the dendritic cells present the antigen to the T lymphocytes in the second step. Thanks to the presentation, the third step involves the priming and activation of the effector T cells' response against said antigens. [24]

Subsequently, these activated effector T cells travel to and infiltrate the tumor. This activity promotes recognition and binding to cancer cells by interacting with the T cell receptor (TCR) and the antigen bound to MHC class I. Finally, the removal of these abnormal cells is engaged. [24, 26]

This elimination process is related to another process called immunosurveillance. The immune system can locate cells in the carcinogenesis process to eliminate them. The four stages involve the capture of tumor antigens, the stimulation of the lymphocyte-mediated immune response, the migration of these cells into the tumor microenvironment, and the elimination of neoplastic cells. [7]

Participation of cytokines in cancer treatment

The mix of cytokines in the tumor microenvironment is related to this disorder's pathogenesis. As indicated above, those released as a product of immune responses to infections and inflammatory and autoimmune processes with various cellular functions can inhibit tumor growth and progression. In contrast, they can be used by these malignant cells to promote their growth, decrease apoptotic processes, and promote invasion and metastasis. [29, 30]

Therefore, its employment seeks to generate a solid immune response. The purpose is to stimulate the production of CD4+ T cells against the tumor for its eradication and establish long-term anticancer immunity, primarily to deal with cases of recurrence and metastasis. [31]

Most tumor suppression studies evaluate cytokines capable of generating a Th1-type response (cellular response). [32, 33] Numerous proteins are being investigated to verify their functionality as monotherapy against cancer (discussed in depth later). Despite this, in 2021, only IL-2 and IFN-α were approved by the United States Food and Drug Administration (FDA). [34]

Cytokines can limit the growth of tumor cells directly, through an antiproliferative or pro-apoptotic activity, or indirectly by stimulating the cytotoxic activity of immune cells against these targets. [35] Such is the case of IFN-α. Some studies have expressed its immunomodulatory effects on dendritic cells and B lymphocytes, cooperating with antitumor immunity. [36] As a complement, IL-2 activates the immune system and can mediate the regression of tumors, such as metastatic ones. [37]

PRODUCTION OF CYTOKINES

Since the 1990s, the production of drugs to fight cancer has expanded. An example is the cloning of genes that encode cytokines. [38]

Recombinant deoxyribonucleic acid (DNA) technology consists of procedures to manipulate the genetic information of one organism and transfer it to another. For this technique, DNA cloning is performed. The gene of interest is isolated and attached to a vector that transports it to its destination, where it will replicate hundreds of times. This genetic material encodes the protein to be obtained (recombinant protein) [39] as a cytokine.

Different organisms, such as bacteria (*Escherichia coli*), yeasts (*Saccharomyces cerevisiae*), insect cells, plants, or mammals, can express the gene for obtaining these molecules. [40] A eukaryotic system is preferable if some post-translational modification is required to confer specific desired characteristics. Prokaryotic organisms do not require these modifications and do not normally carry them out. [41] An expression system such as embryonic kidney cells (HEK293) is ideal for human applications. [42]

When producing recombinant proteins on a large scale, the aim is to optimize processes from smaller scales through laboratory bioreactors, minimizing costs. The cell selection of the cell that will produce the protein and the vector is essential to increase the productivity and quality of the final product. For this reason, organisms that synthesize the molecule are identified and cultivated in these bioreactors, with the consequent evaluation of their growth and production levels. [43]

Another relevant aspect is the medium since it must provide the necessary nutrients for optimal cell growth and acceptable productivity. Furthermore, the bioreactor must be selected among those

available for producing biopharmaceuticals. During the process, it is necessary to control operating parameters such as temperature, pH, and gases in the environment (oxygen and carbon dioxide). [43]

In addition, steps must be taken to maintain similar performance when moving from small to large scale. Some critical parameters to know about bioreactors comprise heat and oxygen transfer, mixing, and shear force. [43, 44]

Next comes the purification step, which is the most expensive of the process (50 to 80 % of the total cost) and the one considered for the approval of a human-use product. In the case of proteins, precipitation, phase separation, and cell lysis chromatography are usually contemplated. [45]

Therapeutic cytokines are susceptible to physical and chemical instability, which originates concern as they are administered at low doses and have a narrow therapeutic index. Besides, their biological function and efficacy are compromised. Other challenges are hydrophobicity, the tendency to form aggregates, and the possibility of generating immunogenic degradation products. [46]

The cytokine must maintain its original structure so that the interaction with the cellular receptor can occur and the therapeutic effect is established. The alterations in the primary and tertiary structures enhance if the environmental conditions differ from the physiological ones. [46] Purification, filtration, filling, storage, and transport conditions can promote protein degradation by shear forces, temperature variations, and contact with several interfaces. [46, 47]

It should be noted that a critical aspect in the formulation to improve their stability is the choice of the ideal buffer system that maintains the optimal pH. The preparations are frequently liquid, but if they do not allow the required stability, the products are lyophilized, decreasing degradation reactions and augmenting resistance to high temperatures. Moreover, efforts are made to add the least excipients, including protein stabilizers, albumin, surfactants, antioxidants, chelating agents, tonicity modifiers, and preservatives. Modifications, such as conjugation with polyethylene glycol, are sometimes done to increase the protein half-life. [46]

PHARMACOKINETICS OF BIOLOGICAL DRUGS

Pharmacokinetics is the branch of pharmacology that studies the organism's action on a drug once they come into contact. It involves absorption, distribution, metabolism, and excretion. First, except for those administered intravenously, the drug must be absorbed from the administration site into the bloodstream. Then, the substance is distributed to the tissues. After that, the metabolic processes can activate or inactivate the administered molecule. Finally, the pharmaceutical product is excreted, with or without the intervention of metabolic reactions. [48]

Therapeutic cytokines are part of biological medicines because they are obtained from living organisms. Therefore, particular characteristics concerning their extraction, purification, and

modification processes must be evaluated. Biological drugs are more complex than chemical synthesis medicines due to their molecular weight, structure, and functions. They have diverse design, characterization, production, storage, and conservation approaches. [49] **Table 2** summarizes the main differences between chemical synthesis drugs and biological ones.

Table 2. Differences between chemical synthesis and biological synthesis drugs.

Drug	Chemical synthesis	Biological synthesis
Structure [50]	Chemical molecules.	Biomolecules such as proteins.
Complexity [49]	Minor.	Major.
Weight [51]	Less than 700 Da.	700 to 150,000 Da.
Specificity [51]	It tends to be low.	High to very high.
Route of administration [51]	Oral (usually).	Intravenous or subcutaneous.
Absorption [50]	Variable bioavailability depends on factors such as solubility, lipophilicity, specific transporters for certain substrates, and first-pass metabolism.	Slow absorption after parenteral administration.
Distribution [50]	Rapid diffusion, influenced by solute and efflux transporters diffusion.	Receptor mediated endocytosis (slow) or convection (slower).
Distribution volume [50]	Variable (from low to very high), depending on physicochemical properties, binding to proteins and transporters.	Very low due to its molecular size.
Access to the therapeutic target [50]	It reaches all tissues, including the central nervous system.	Limited access to tissues due to molecular size.
Elimination [50]	Hepatic (metabolism and excretion) and renal (filtration, secretion, and/or reabsorption) metabolism.	It is more complex because it includes the reticuloendothelial system (catabolism) and target mediated clearance. *Note: smaller therapeutic proteins (less than 60 kDa) undergo renal excretion and metabolism.*
Renal clearance [50]	High, influenced by the pathway and function of the organ.	Very low, depending on target-mediated processes.
Half-life [50]	Hours or days.	Weeks or months.

In the case of biological drugs, it is not correct to talk about generic drugs but biosimilars, as the substance obtained is distinct from the innovator. When a biosimilar is approved, it is ensured that it is similar regarding safety, quality, and efficacy concerning the reference product. There are no clinically

relevant differences when administering one or the other. Among the studies that must be done are pharmacokinetics investigations. [52]

These drugs have different pharmacokinetic mechanisms than synthetic ones, creating difficulties in administering them and reaching their site of action. For example, the oral route cannot be employed due to the large size of the molecules and the fact that the proteins are degraded at the gastrointestinal level, so its administration is preferably parenteral. However, the bioavailability for the subcutaneous and intramuscular routes can vary between 20 and 95 %, and absorption from these routes is usually slow through the lymphatic system. [50]

Additionally, these products can induce cellular or humoral immune responses because the protein exhibits differences for the organism. This characteristic leads to processes for the neutralization of the molecule action or its respective marking to facilitate phagocytosis or the complement action. [53] Consequently, treatment failure or severe cross-immunogenicity reactions with self-proteins occur. [49]

It should be noted that biological drugs hardly share metabolism or elimination pathways with synthetic ones, reducing the possibility of drug interactions between the two groups. [50] *In vitro* studies have shown that cytokines such as IL-1, IL-6, TNF-α, and INFs can affect cytochrome P450s function and modulate the expression of specific small molecule transporters and conjugated enzymes. [54] Nonetheless, the *in vivo* effects of potential interactions require further study.

Likewise, since its use is recent, there is less information on its long-term safety and efficacy, so pharmacovigilance is essential. [49] Thus, most health registries for these therapies require a pharmacovigilance program, where adverse reactions associated with their utilization are recorded. [55]

Pharmacokinetics studies of cytokines

Pharmacokinetic analysis of cytokines involves understanding methods of detecting these agents in biological fluids and recognizing factors that can impact concentration-time curves. The enzyme-linked immunosorbent assay (ELISA) is the most widely one, of which there are commercial kits for various cytokines. [56, 57] This procedure is commonly applied for detecting proteins, hormones, peptides, or antibodies. [58]

When performing pharmacokinetic studies, a series of factors must be studied, such as normal serum levels for each cytokine. For IL-2, the average value found in a study with 34 volunteers was 7.46 ± 2.98 U/ml prior to blood donation, determined by the ELISA test. [59]

It is essential to contemplate the presence of receptor antagonists, which may be receptor blockers such as the IL-1 receptor antagonist (IL-1Ra) that block the binding of IL-1α and IL-1β or cytokine

inhibitors. These structures are commonly soluble versions of the respective receptors, where the best characterized is the soluble form of IL-2 receptor (sIL-2R), released by activated mononuclear cells, which, by binding to IL-2, prevents its interaction with the membrane receptor responsible for the physiological response, controlling the excessive lymphocyte activation. [60]

The administration route must be considered when doing pharmacokinetic studies. Its selection can lead to alterations in cytokine levels and, in some cases, in the therapeutic effect, as a consequence of factors specific to these proteins. [57]

Pharmacokinetic studies have shown that injection into subcutaneous tissue makes the cytokine prone to protease degradation at these sites. Hence, its bioavailability is less than 70 %, preventing the drug from reaching its target cell or tissue. [57] Proteases are enzymes that break down proteins into smaller units, such as peptides or amino acids, and have essential roles in physiological processes, including immunity and cell death. [61]

Recently, gene therapy has emerged as a feasible route for the local administration of cytokines because their expression is generated directly in tumor cells. As a consequence of this strategy, the concentration of the desired elements occurs locally, decreasing their toxicity as a consequence of the release at a systemic level. [62]

On the other hand, studies indicated that certain cytokines concentrations increase during neutropenia. In the same way, neutropenia leads to elevated production of these substances. [57] This condition consists of a decreased absolute number of neutrophils in blood circulation. The characteristic lower limit of the neutrophil count is about 1500 cells/µl in those over 12 years of age. Nevertheless, this number may be inferior in individuals of African descent and some Middle-Eastern ethnic groups. [63] During neutropenia, the number of receptors on neutrophils may also be diminished. [57]

Knowledge of the assay techniques and the many factors that affect the pharmacokinetics of these drugs allows a better understanding of the concentration-effect relationship so that optimal dosing regimens for treating diseases can be established. [57]

CLINICAL APPLICATIONS OF CYTOKINES

Clinical applications cover two areas: cancer and inflammation. The latter is a consequence of allergic or autoimmune reactions or the action of pathogens that invade the organism. [64] Studies show that G-CSF, GM-CSF, M-CSF, and IFN-γ have the potential to treat infections. Its administration increases the activity of polymorphonuclear leukocytes, such as monocytes or macrophages, fulfilling a relevant role in defense against pathogens. [65] Some therapeutic cytokines and their indications are presented in **Table 3**.

Table 3. Cytokines utilized clinically and their indications.

Cytokine	Indications
G-CSF [66]	Chronic neutropenia, myelosuppression, and patients receiving chemotherapy.
GM-CSF [66]	Patients receiving chemotherapy.
IFN-α2a [66]	Chronic hepatitis B and C.
IFN-α2b [66]	Chronic hepatitis B and C, malignant melanoma, hairy cell leukemia, acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma, follicular lymphoma.
IFN-β [67]	Multiple sclerosis.
IFN-γ [67]	Chronic granulomatous disease, osteopetrosis.
IL-1 [66]	Rheumatoid arthritis, cryopyrin-associated periodic syndrome.
IL-2 [66]	Metastatic renal cell carcinoma, metastatic melanoma.
IL-11 [66]	Prevention of thrombocytopenia, reduction of platelet transfusion after myelosuppressive chemotherapy.
TNF-α [68]	Soft tissue sarcoma.

Sometimes, it is sought to counteract their activity, becoming therapeutic targets. In allergic disorders, the inflammatory process is decreased by inhibiting its function and preventing binding to its receptors, delaying illness course and tissue damage. [69] In the case of asthma, clinical trials have been developed. The therapeutic targets have been IFN-α, IFN-γ, IL-4/IL-13, IL-5, IL-9, IL-10, IL-12, and TNF-α. They are expressed in large quantities during the pathology progression as part of the response to allergens. These therapies aim to reduce exacerbations and improve respiratory function. [70]

Regarding some diseases with an inflammatory component, it is beneficial to inhibit the action of specific cytokines, for which monoclonal antibodies are considered. [71] These are proteins made by a single clone of B lymphocytes against a particular antigen. [72]

In rheumatoid arthritis, IL-1, IL-6, and TNF-α are therapeutic targets. Other pathologies with a high degree of inflammation where this therapy has been explored include psoriasis, osteoarthritis, and irritable bowel syndrome. Therapeutic advances provide an alternative to patients with a standard treatment of anti-inflammatory drugs or other medications that are not effective enough in all cases and generate appreciable adverse effects. [71]

In cancer, these substances can limit cell growth directly (through pro-apoptotic or antiproliferative activity) or indirectly (stimulation of immune cell cytotoxicity). Pro-inflammatory cytokines act at all

stages of the cancer immune cycle, enhancing antigen presentation and increasing the number of effector immune cells in the tumor microenvironment. [35]

Cancer immunotherapy stimulates a person's immune system to fight abnormal cells by administering different compounds. These treatments try to reduce the tumor burden and generate memory in the immune system. [73, 74]

Two types of immunotherapies can be defined. The passive consists of the transfer to persons with tumors of cells or antibodies generated *in vitro* against the tumor. In contrast, in the active, the goal is to generate an immune response by the patient. [75]

Regarding passive immunotherapy, IL-2 is essential for the maintenance of regulatory T lymphocytes, for the differentiation of CD4+ T cells and for optimizing the generation of CD8+ T lymphocytes, and for differentiation into memory cells. It targets adaptive immune cells, such as T and B cells. As an adjunct, administration of IFN-α induces IL-4 secretion and consequent activation of B lymphocytes. It also promotes caspase-dependent apoptosis of tumor cells. [73] As a complement, clinical trials continue to increase cytokines indications at the clinical level.

Cytokine-induced killer (CIK) cells are an antitumor treatment in cancer immunotherapy. [76] They were first described in 1991, emerging as a safe and feasible possibility for advanced tumors. [77] CIK therapy consists of inoculating these cells, which mediate tumor cell apoptosis or directly generate cell death. In addition, CIK cells have characteristics such as rapid proliferation, high tumoricidal activity because of their cytolytic features, a broad spectrum of tumor killing, and strong recognition ability. [77, 78]

In 2010, the FDA approved the first autologous cellular immunotherapy, Sipuleucel-T. [77, 79] This approval indicates the enormous potential for cancer treatment, and the hope is that more of these therapies will continue to be approved. [77]

Among the investigations found, there is a multicenter, open-labeled phase IV trial with the participation of patients who underwent curative surgical resection or radiofrequency ablation and were later divided to receive adjuvant immunotherapy with CIK cells or assigned to the control group (without adjuvant therapy). The primary endpoint was recurrence-free survival, while the secondary ones involved overall survival and safety. [80]

The immunotherapy administration protocol consisted of injecting a 200 ml aliquot of the CIK cells agent through the intravenous route for 60 minutes, followed by a 30-minute monitorization to identify any side effects. The therapy was scheduled to be injected 16 times over 59 weeks (four treatments every one week, four treatments every two weeks, four treatments every four weeks, and four treatments every eight weeks). The therapy was discontinued when hepatocellular carcinoma recurrence was detected. The main result obtained was that adjuvant immunotherapy after curative

treatment prolonged recurrence-free survival in patients who suffered from hepatocellular carcinoma, after curative treatment. [80]

CHALLENGES OF IMMUNOTHERAPY WITH CYTOKINES

Cytokine immunotherapy often results in severe and dose-restricting side effects. These proteins are pleiotropic, influencing more than one type of cell. Besides, they have a short serum half-life, resulting in high doses administered to achieve their therapeutic effects. These doses increase pleiotropic activities, manifesting as adverse effects in patients. An example of this associated toxicity is excessive vascular permeability, which progresses to an adverse state known as vascular leak syndrome. [68] It corresponds to a disorder characterized by sudden outbreaks of massive plasma and protein leakage from the circulatory system into the interstitial space, giving rise to shock and massive edema. The result is a drop in blood pressure that can be fatal if left untreated. [81]

Another problem is that the ELISA assay, commonly utilized for determining cytokines, cannot act on the biologically active protein. Plus, bioassays can measure a biological event (proliferation or cytotoxicity). However, they are not considered due to their high cost, the long time to perform them, the lack of specialization, the low sensitivity, and the influence of environmental conditions on the result. [57]

Although cytokines' immunomodulatory properties are helpful against several conditions, their pharmacokinetics and toxicity have limited their therapeutic potential. This scenario has triggered the number of approved medicines to be low. [81]

CONCLUSIONS

Traditional treatments such as surgery, chemotherapy, and radiotherapy often do not improve the patient's prognosis and statistically show low overall survival. Therefore, the search for new, more effective therapies is constant. An example is cytokine immunotherapy for the treatment of distinct types of cancer. Unfortunately, these drugs' high toxicity and complex pharmacokinetics have limited their applications to the point that only IL-2 and IFN-α have been approved against this illness. Nonetheless, research by the pharmaceutical industry continues, as is the case with CIKs administered together with cytokines. The purpose is to find safe and effective novel therapies against cancer and other pathologies.

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