

Effectiveness of Cytokine-Related Combination Immunotherapy with Conventional Oncological Treatment

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Abstract. This review will provide an overview of cytokine and cytokine-based immunotherapy and focus on recent developments in new-era oncological treatment, including several combinations of both conventional cancer therapy and novel immunotherapy with cytokine, and their applications in clinical research. There are five main parts, (1) The technology of cytokine immunotherapy, (2) The function of cytokine immunotherapy and defects of cytokine as a monotherapy, (3) The specific cancers that are targeted by synergistic cytokine immunotherapy, and (4) Prospects of cytokine in immunotherapy.

Keywords: Immunotherapy, Efficacy, clinical, cytokine.

1. Introduction

Cytokines, as a group of soluble polypeptides, are capable of transporting and communicating from cell to cell, which dedicates to signaling diverse types of cells with proliferation, distinguishing pro-inflammation, or anti-inflammation actions in light of their expression with high-affinity receptors [1]. In the last century, the journey to cleanse cancer has initiated since the discovery of Radium and radiotherapy; in the recent twenty years, as the knowledge based on the human immune system exponentially rises, interest has been changed to immunotherapy, and efforts to exploit cytokines as a cancer therapy has intensified.

As a small, soluble signaling protein, cytokine has been applied in antiviral and anti-tumor studies with its rapid and efficient functions of promoting immune cell proliferation and activating immune cell effects since its discovery. Because of this, cytokines became the first tumor immunotherapy drug in history to be approved by the FDA: IFN α -2a and IFN α -2b were approved for multiple lymphomas in 1986, and the approval for high-dose IL-2 in 1992 and 1998 targeted malignant melanoma and renal cancer [2].

Several cytokines oppress tumor growth by directly signaling immune cells in an anti-proliferate manner. Nonetheless, data from clinical research failed to justify their high efficacy and compatibility with preclinical trials or animal models. The restrictions of cytokines per se, short half-life, potential overexpression, and increased toxicity are bound up for further progress in the monotherapy of cytokines. Based on existing mainstream preclinical models, synergetic recombination with other mainstream oncology treatments can be a promising approach and solution to the downsides of monotherapeutic cytokine.

This review describes how the current synergy treatments work on specific cancers according to existing research and clinical data and the role of cytokines in each treatment. Among both the immunostimulatory and immunosuppressive cytokines against tumor cells, we demonstrate their possible partners and cancer to deal with.

2. Cytokines as monotherapy

Immune cells used in the clinic mainly include natural killer cells and cytokine-induced killer cells $\gamma\delta$. Different cell therapy techniques include gamma delta T cells, DC dendritic cells, and chimeric antigen receptor T cells. Among these immune cells, distinctive functions and unique curative effects apply. At the same time, they also have their advantages, disadvantages, and risks.

NK cells exist in human blood. They are natural killer cells, which are immune cells. Cancer cells, aging cells, sick cells, germs, and viruses can all be killed by them directly in the body. NK cells can recognize and react with tumor cells. They are a significant antitumor immunotherapy effector, particularly in hematological malignancies. Human blood contains NK cells, which act as "first responders." In the body, they're like patrolmen on duty. Because aberrant cells can be detected wherever in the body, they will be dealt with stably, accurately, and mercilessly. They're called "natural killer" cells because, unlike T cells, they discover and kill infected and cancerous cells without needing to be activated or "trained" to do so. Although they can defend quickly and attack tumor cells directly, NK cells are only a small portion of the whole immune system. NK cell immunotherapy has thus become the key to activating their own immunity and defeating cancer by boosting the number of NK cells in vivo.

The disadvantage of NK natural killer cell therapy is that it is more effective for blood cancer (e.g., acute myeloid leukemia) and has no apparent effect on solid tumors. It can keep some patients stable. If only NK cells are administered alone, no matter the concentration, it can only improve immunity, but it is difficult to reduce the tumor significantly. If it enhances the patient's immunity and quality of life, it can be completed with NK cells to make it more physical to receive routine cancer treatment.

Disadvantages of CIK cytokine-induced killer cell therapy: the target of CIK is unknown, and its ability to identify cancer cells is poor. It needs a significant number of cells to kill cancer cells. If the number of cells is not enough, it will have no attack on cancer cells. The tumor interior is a highly immunosuppressive tumor microenvironment, which can easily lead to the poor efficacy of CIK alone. Therefore, CIK cells must be combined with chemotherapy, target drugs, or other immunotherapies (e.g., PD-1 inhibitors) to fight various cancers and improve efficacy. Combined treatment is vital.

Disadvantages of DC dendritic cell therapy: firstly, the tumor antigen is not easy to obtain. Due to cancer mutation, the effect of using only a single antigen is not good.

Disadvantages of chimeric antigen receptor T cell therapy: CAR-T cell immunotherapy has significantly improved treating hematological system tumors. However, CAR-T therapy still has many shortcomings in treating solid tumors. The main obstacles are the lack of stable tumor-specific antigens and high specificity; in particular, CAR-T cells sometimes mistakenly kill normal cells when they are supposed to attack tumor cells. The off-target effect is an urgent problem to be solved.

There are two ways to cure tumors: cold scalpel and hot radiation, but now these two tumor treatment methods have a ceiling effect, that is, tumor metastasis. Cancer can quickly metastasize to some parts, which may be related to the pre metastasis microenvironment. Targeted therapy or immunotherapy can effectively prevent tumor metastasis by accurately clearing the immunosuppressive cells or exocrine proteins in the exosomes and the pre metastasis microenvironment.

Therefore, researchers conclude that combination therapy can increase the curative effect. The "combination therapy" combined with multiple treatment methods is recently a new immunotherapy trend. In addition to CAR-T, as long as non-genetically modified immune cell therapy, including NK, CIK, and DC cell therapy, is combined with chemotherapy, radiotherapy, or PD-1 / PDL-1 inhibitors, the curative effect will increase because radiotherapy and chemotherapy can encourage cancer cells to release Neoantigens, which helps to improve the identification validity of immune cells.

In CAR-T cell therapy, there will be some adverse reactions, such as cytokine release syndrome, known as a common adverse drug reaction in CAR-T cell therapy. CAR-T therapy is new immunotherapy that modifies T cells of cancer patients through genetic engineering to express the chimeric antigen receptor (CAR), a receptor for specific tumor cell antigen. It then injects CAR-T cells back into patients to play an anti-cancer role. The increase of cytokines such as interleukin-6

(IL-6) is the main reason for the occurrence of CRS. CRS has a high probability and can be life-threatening [3].

Severe CRs (SCRs) were characterized by persistent fever for more than three days, selective cytokine elevation, and other clinical toxicity evidence; At the same time, hypotension, hypoxia, and or changes in the nervous system are the most common SCRs responses [4]. In case of SCRs-related toxic reactions, strict medical management shall be carried out, such as mechanically assisted ventilation, pressor drugs, antiepileptic drugs, antipyretics, etc.

The successful application of CAR -T cell therapy to malignant hematologic tumors has given a new direction to cancer treatment. Currently, CAR -T-cell therapy clinical trials are switching the first-line drug controlled by CRS from traditional hormone therapy to IL -6 targeted drugs - a targeted therapy based on Tozumab. Studies had shown that when TNF was used to treat nine adult patients with chronic lymphocytic leukemia (CLL) and one child ALL with CD19 CAR -T α and IL -6 receptor blockers (etanercept, tozumab), clinical symptoms improved rapidly and comprehensively. Meanwhile, other cytokine drugs and IL -6 targeted drugs in CRS are also under further investigation [5]. Meanwhile, other cytokine drugs and IL-6 targeted drugs in CRS are also under further study.

3. Advantages and applications

Despite being short-lived, with modest efficacy with risk in high dosage, cytokine-targeted treatments still succeed in many fields than conventional therapy, significantly when mitigating drug resistance and being biomarkers in the immunotherapy. Some implications in the recent studies demonstrated the causality between dysregulated cytokine expression and many drug resistance mechanisms. Cytokine is a general term defined as a broad category of secreted protein released by cells; if specified, other words describe some cytokine properties, like lymphokine, monokine, chemokine, and interleukin [6]. As an indispensable function in carcinogenesis and metastasis and mediating the cell immunity, the cytokine family enables cells of the immune system to communicate over short distances. In addition, some evidence from in vivo studies indicates that cytokines originated from cancer cells, and their stroma may be the clue to deciphering drug resistance mechanisms. Early diagnosis of resistance to conventional oncological therapy can be possible by regulating cytokine levels in chemotherapy, while minimally invasive methods can easily obtain cytokines in serum or plasma fluids. The results could lead researchers to observe tumor response to a specific medication and provide clues for possible corresponding strategies in chemotherapy.

In consideration of their devotion to the immune system, cytokines are feasible next-generation biomarkers as molecular parameters or imaging features to diagnose, monitor the progress of the diseases, and predict the treatment. Disorder of cytokine levels is associated with the development of multiple cancers, like gastric carcinomas and colorectal cancer. Considering the association, cytokines as a biomarker for cancer as assistance in detection and tracking the tumor are reassuring.

In recent years, the development of cytokine detection progressed at an astounding rate as various modern biosensors were introduced, like capacitive biosensors, photonic resonators, and nanodielectric detectors [7]. Respectively detecting cytokine levels depends on capacitive values, refractive index, and cellular bioelectricity. Both are prospective candidates to target and trace cytokines more economically, sensitive, and straightforwardly than the traditional ELISA method.

3.1. Application in Melanoma

Cytokines first appeared as tumor immunotherapy drugs in 1984 when a female patient with metastatic melanoma received an infusion of interleukin-2. She had been treated with various therapies, but her disease worsened. The patient was cured within a few months after receiving this infusion of this recombinant IL-2 version. Years after being in CR, the woman was credited with being the first person to cure cancer by boosting a patient's immune system [8].

Since then, IL-2 has been considered the most promising drug in tumor immunotherapy precisely because it has been found clinically that large doses of IL-2 can be used to induce a sustained immune

response in patients with metastatic melanoma and renal cell carcinoma. The FDA has approved two types of cytokines as a single drug for cancer treatment: The first is IFN α -2A and IFN α -2b, which were approved in 1986 to treat multiple lymphomas. The second is high-dose interleukin-2, approved for metastatic melanoma in 1992 and kidney cancer in 1998. Interleukin-2 can play a vital role in immune regulation, which can not only stimulate the proliferation and differentiation of activated T cells, enhance the cytotoxic activity of T cells, but also stimulate the cytotoxic activity of mononuclear macrophages, enhance the growth of NK cells, and enhance the killing activity of NK cells.

As time goes on, the disadvantages of cytokine monotherapy continue to emerge. The main reason for these problems lies in the pluripotency and redundancy of the functions of cytokines and their different sources and terminals of action. In simple terms, these cytokines weave a rich interaction and extremely complex cytokine network, plus many cytokines have the double function of immune activation and immunosuppression, which leads to uncomplicated cytokine treatment patients could produce all kinds of uncontrolled adverse reactions, a low degree of drug resistance and high toxicity, Patients with hypotension, neurotoxicity, arrhythmia and other symptoms of discomfort, suggesting that our natural cytokines may not be suitable as drug targets.

Despite the disadvantages of cytokine monotherapy for cancer, it still cannot be ignored that the crucial role of cytokines in the immune response. Therefore, it has become an essential direction of tumor immunotherapy in recent years to precisely design modified cytokines and combine them with other immunotherapy methods. For example, it has been common to design cytokines as effector molecules in CAR-T.

There are two main types of cytokine therapy: one is to combine the two approved drugs with other radiotherapy chemotherapy drugs; The other is a new modification and fusion protein drugs, such as NKTR-214, Pegilodecakin, and AIT-803. With the in-depth study of various therapeutic mechanisms and dealing with the toxicity of cytokine therapy, the combination strategy between different immunotherapies has become a trend. Compared with monotherapy, the combination of immunotherapy overcomes multiple immunosuppressive mechanisms, reduces toxicity, and makes cancer treatment more comprehensive [9].

The most notable example is the next generation of Aldesilkin derivatives in clinical studies, including the interleukin-2 recombinant precursor BEMPEG (BEMPEG, NKTR-214). In several clinical studies, it has been selected as a representative cytokine for melanoma therapy in combination with other immunotherapies. Next, to illustrate the feasibility of cytokine combination therapy in more detail, we will review existing cases in which cytokine therapy is used in conjunction with other immunotherapies.

First, cytokine therapy with monoclonal antibody drugs has been tested in mouse models, using NKTR-214 and anti-CTLA-4 monoclonal antibodies, showing synergistic solid effects.

Secondly, clinical trials of cytokines in combination with PD-1 antibody drugs have been conducted. In the Pivot 02 trial, BEMPEG and nivolumab were the combination drugs of choice in 38 patients with untreated melanoma [10]. The ORR and CR of the combination were 59.5% and 18.9%, respectively. This trial demonstrates the powerful synergistic effect of combining cytokines with monoclonal antibodies. As a result, A Phase 3 trial comparing nKTR-214 to nivolumab monotherapy has begun at Bristol-Myers Squibb. The preliminary completion date is 2024, but the results have not yet been released. Preliminary completion is expected in 2024, with results not yet available.

There are many different drugs available for cytokine therapy and monoclonal antibody therapy. Ipilimumab and high-dose interleukin-2 are also commonly used in combination immunotherapy for melanoma. In a Phase Ib/II trial, the two drugs were combined with standard HD IL-2 and incremental dose ipilimumab for metastatic melanoma. The initial ORR was 22%, the follow-up response rate 28%, and the complete response rate 17%. This indicates that the combination of IL-2 and ipilimumab has significant significance [11].

In addition to the combination of monoclonal antibodies, there are tremendous possibilities for the therapeutic selection of cytokines in melanoma, such as immune checkpoint therapy. In NCT00204581 and NCT03435640, IL-2 (intratumoral or systemic NKTR-214) and immune checkpoint therapy will be used to treat metastatic melanoma [12].

Cytokine therapy can undoubtedly combine with conventional treatment, intratumoral IL-2, and cryotherapy also proved to be significantly effective with a one-year remission period.

These combination therapies that are currently underway or have been proven to be effective reveal many possibilities in tumor biology and demonstrate the feasibility and advantages of combining cytokine therapy with other immunotherapies. In addition, these trials and cases suggest the future of local and systemic therapies for melanoma.

3.2. Application in Renal Cancer

Renal cell carcinoma (RCC) is a malignant tumor originating from renal tubular epithelium, accounting for 80% ~ 90% of malignant renal tumors. Pathologic types include clear cell carcinoma, papillary renal cell carcinoma, chromophobe cell carcinoma, and collecting duct carcinoma, among which clear cell carcinoma is the most common. The incidence of kidney cancer is second only to prostate and bladder cancer, accounting for the third urinary system tumor. Worldwide, kidney cancer accounts for about 3 percent of adult malignancies [13]. In recent decades, the incidence of kidney cancer has been increasing in most countries and regions.

Treatment for kidney cancer varies depending on the patient's tumor location, stage of development, and health status, including surgery and systemic combination therapy. Surgery, laparoscopy, and ablation are often used to treat early-stage renal cancer. At the same time, systematic treatments such as targeted therapy and immunotherapy are preferred for patients with advanced or metastatic renal cancer. In radical nephrectomy and renal preservation surgery, postoperative disease recurrence is a clinical problem that needs attention.

Some past clinical studies observed occasional spontaneous regression in patients with metastatic renal cancer after postoperative tumor recurrence. Because of this sign, kidney cancer is often considered a good target for immunotherapy, which uses the body's immune system to help kill cancer cells. Therefore, in the past few years, cytokines have been widely used to deal with the recurrence of renal cancer after surgery [14]. The application of cytokines can shrink kidney cancer in approximately 10-20% of patients and provide lasting remission in these patients. Today, however, such non-specific immunological methods using cytokines alone are rarely used due to severe side effects and few cases of complete long-term response.

As researchers continue to explore and understand the various immune mechanisms, the systematic treatment of renal cell carcinoma has rapidly developed and changed in the past decade. Initially, researchers focused on the use of cytokine therapy. Later, with the popularization of targeted therapy, researchers abandoned the commonly used cytokines and used vascular endothelial growth factor (VEGF) in copious quantities. In recent years, immunotherapy has developed rapidly and has become the preferred treatment for kidney cancer. One of the most representative is immunocombinational therapy. In particular, the combination of PD-1/PD-L1 and CTLA-4 checkpoint inhibitor therapy with cytokines is an essential treatment for metastatic or advanced renal cancer.

In the kidney cancer diagnosis and Treatment Guidelines issued in China in 2022, it suggests that cytokine therapy can be used as an alternative therapy for patients with metastatic clear cell renal carcinoma who cannot receive targeted drug therapy. Among them, large doses of interleukin-2 can be used to treat patients with metastatic clear renal cells in good condition and have a normal cardiopulmonary function. In the first-line treatment strategy for metastatic or unresectable clear cell renal cell carcinoma, immunotherapy is effective in high-risk groups. The combination of bevacizumab with IFN- α -2b and high dose IL-2 is recommended.

4. Conclusion and prospects

Cancer immunotherapy has rapidly progressed in the last decades with extraordinary achievements, which led to the development of distinctive immunotherapies that are synergistically applicable with potentially improved efficacy, reduced risks, and manipulative side effects. A growing number of combinational trials like radiation therapy and immunotherapy or a combination of immunotherapies open up a new era of cancer therapies with highly personalized approaches and identifications of genetic and immunological features. Boasting the recognition of the innate immune system, implementing these approaches can be a critical part of controlling the immune response and against drug resistance. As the understanding of immunotherapies advances, it is more likely to alleviate the dangers of cancer treatment and have better experiences with clinical care.

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