

Application of Cytokines in the Treatment of Lung Cancer

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Abstract. Lung cancer is one of the malignant tumors with a high incidence rate and mortality worldwide. Cytokines are a crucial component of the human immune system. In cancer treatment, cytokine-based cancer immunotherapy has shown promising clinical prospects. It is worth noting that over the past few decades, there has been extensive research on cytokines and their receptors as targets for cancer or cancer treatment methods. As proteins in the immune system, interleukin, interferons, and other cytokines play crucial roles in controlling the body's immunological response to cancer cells and triggering the death of cancer cells. For example, IL-12 has demonstrated powerful tumor suppression capabilities. In addition, because most cytokine therapy trials have started in late-stage cancer patients, this may to some extent limit the therapeutic effect of cytokine therapy. Therefore, using cytokine therapy to enhance the activity of traditional cancer therapies or mitigate the immunotoxic effects of therapy on the human body could be a direction for the development of cytokine therapy. Thus, the functions and principles of the different cytokines in the cancer-immune cycle are discussed here, with a focus on the clinical use of cancer therapy based on different cytokines in the management of lung cancer.

Keywords: Lung cancer, interleukin, interferon, immune response.

1. Introduction

Lung cancer is one of the cancers with the highest incidence and mortality rates in malignant tumors, with clinical manifestations such as coughing, hemoptysis, chest pain, fever, and dyspnea. The occurrence of lung cancer depends on numerous factors, possibly related to biological inheritance, carcinogens in the environment, autoimmunity, and lifestyle, among others [1]. Among these factors, immune factors have increasingly attracted the interest of scholars. In terms of lung cancer treatment, traditional therapies such as surgery, radiotherapy, and chemotherapy have many shortcomings and can bring serious side effects to patients in certain circumstances. Therefore, immunotherapy may be able to change the current situation of lung cancer treatment. Among the numerous immunotherapies, such as cancer vaccines, monoclonal antibodies targeting cancer antigens, chimeric antigen receptor T cell immunotherapy, immune checkpoint-related therapy, and cytokine therapy, have demonstrated significant therapeutic potential and to some extent provide alternative or improved approaches to traditional cancer therapies. Cytokine therapy, in particular, has shown superior and effective potential in numerous preclinical and clinical studies [2]. Cytokines are an important component of the immune system, produced by various cells throughout the body, acting as protein messenger molecules in cell-to-cell communication. Cytokines play a role in maintaining the body's normal immune balance but can become important mediators in the onset and development of diseases under abnormal conditions, including involvement in various inflammations and tumor formation [3]. At the same time, various cytokines play multiple roles in the immune cycle of cancer. For example, during cancer antigen release, the levels of interferon-alpha and interferon-beta in the body increase. When dendritic cells capture and present cancer antigens, various white blood cell interleukins in the body change, with significant increases in the levels of IL-1, IL-2, and IL-12. As effector T cells infiltrate cancer cells, the levels of interferon-gamma, IL-2, IL-15, IL-18, and IL-21 also increase in the body [4]. The organism's immune response affected by cytokines plays a dual role in tumors, as it can both inhibit tumor development and eliminate tumor cells, while also promoting cancer development by inducing immune tolerance or escape of tumor cells. For example, interleukin-6 can inhibit the body's anti-tumor immune response, promote lung cancer development, and is closely related to the occurrence, survival quality, and survival period of lung cancer patients. Targeted

therapy aimed at blocking the interleukin-6 signaling pathway provides a new approach to exploring more precise targets for lung cancer immunotherapy. Due to the varying effects of various cytokines in the treatment of cancer, and the complex biological characteristics of natural cytokines, multiple cell types often produce counteracting effects, limiting the therapeutic efficacy and dosage of cytokine therapy, and hindering the clinical development of cytokine therapy. In terms of application, cytokine therapy may apply to various cancers, but the majority of cytokine therapy scenarios are limited. Therefore, the precise application of cytokine therapy and its exploration in the treatment of lung cancer is urgent. This paper introduces and summarizes the recent progress of cytokine therapy in the research and application of lung cancer treatment, such as IL-2, IL-6, Interferons (IFNs), Granulocyte-macrophage colony-stimulating factor (GM-CSF), etc.

2. The basic nature and classification of cytokines in immunotherapy

Cytokines are membrane-bound, secreted proteins that the innate and adaptive immune systems create in response to tumor antigens and pathogens. These proteins can facilitate intercellular communication. The immune system may be impacted by the concentration, incorporation into immune system pathways, and receptor expression patterns of various cytokines. Redundancy is an important aspect of cytokine signaling, where functionally similar cytokines compensate for each other in quantity to maintain balance, making it difficult to alter the quantity of a single cytokine in clinical therapy. Furthermore, the pleiotropy of cytokines may manifest as resistance in cytokine therapy [5], as a single cytokine may cause various cells to produce different effects. IL-2, for instance, functions as both an activator of T regulatory and T effector regions, which might lead to treatment resistance in clinical settings.

Based on the primary functions of cytokines, they can be classified as follows: 1. Interleukins (IL): the cDNA gene for interleukins was successfully cloned and expressed, leading to the identification of over 30 kinds of IL since 1979. 2. Colony-stimulating factors (CSF) enhance the maturation of mature cells and encourage the proliferation and differentiation of hematopoietic stem cells and progenitor cells at various phases. 3. Interferons (IFN) are generated by leukocytes, fibroblasts, and activated T cells and were identified in 1957. They share comparable biological activity. They fall under the IFN- α , IFN- β , and IFN- γ categories. 4. Lymphotoxin (LT), another name for tumor necrosis factor (TNF), is separated into two forms: TNF- β and TNF- α . Both have comparable biological activity; activated T cells create the latter, while monocyte-macrophages produce the former. 5. The Transforming Growth Factor- β Family (TGF- β family), which is mostly made up of bone morphogenetic protein, TGF- β 1, TGF- β 2, TGF- β 3, and TGF- β 1 β 2. 6. A variety of growth factors (GF), including platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). 7. The chemokine family, which is divided into four subfamilies: (1) C-X-C/ α subfamily, which primarily attracts neutrophils; members include IL-8, Platelet Factor-4 (PF-4), Platelet Basic Protein, proteolytic products like CTAP-III and β -thromboglobulin, Interferon-inducible Protein-10 (IP-10), and ENA-78; (2) C-C/ β subfamily, which primarily attracts monocytes; members include Macrophage Inflammatory Protein 1 α (MIP-1 α), MIP-1 β , RANTES, Monocyte Chemoattractant Protein-1 (MCP-1/MCAF), MCP-2, MCP-3, and I-309; (3) The C subfamily representatives are lymphocyte chemotactic factors; (4) The CX3C subfamily is represented by Fractalkine, a CX3C chemokine that has chemotactic effects on monocyte-macrophages, T cells, and NK cells. However, in the clinical application of cancer treatment, many of them have direct or indirect ways to stimulate immune cells to limit tumor cell growth, such as IFN- α , which has immune regulatory ability and can regulate angiogenesis, making it a good choice for cancer treatment [6]. Therefore, cytokine therapy is a promising method in cancer treatment. In the past decade, novel delivery systems have also promoted cytokine-based tumor therapy. Here, we focus on various cytokine therapies and clinical applications in lung cancer, discussing the current trends in cytokine therapy for cancer, particularly focusing on research and applications in lung cancer, and exploring the combination of cytokines with other therapeutic agents.

3. IL-2

Interleukin-2 plays a critical regulatory role in both adaptive and innate immunity. It can promote T-cell activation and proliferation, enhance or induce cytotoxic activity, stimulate immunoglobulin secretion, and participate in the generation of antibodies by B cells. Additionally, IL-2 serves as a growth factor for natural killer cells, regulating immune function through the modulation of immune cell proliferation [7], typically synthesized by Th1 cells.

In the treatment of cancer, the clinical application of IL-2 began in the mid-1990s, showing anticancer effects in high doses. It is worth emphasizing that IL-2 plays an important role in the proliferation of CD8+ T cells (primarily through stimulating CD8+ T cells and increasing receptor expression). IL-2 also demonstrates a certain ability to activate and expand NK cells, enhancing the cytotoxic function of NK cells. As IL-2 can induce the expansion of tumor antigen-presenting T cells, its combination with other cancer treatment drugs has improved treatment effectiveness. In recent years, IL-2 has been used in adjuvant therapy for various cancers, such as melanoma, ovarian cancer, and colorectal cancer. Notably, in the treatment of melanoma, the use of recombinant IL-2 combined with immune checkpoint inhibitors may improve immune system activation and enhance clinical efficacy. According to trials registered on ClinicalTrials.gov, IL-2 shows great potential in the treatment of melanoma and leukemia, surpassing other cancers in the trials.

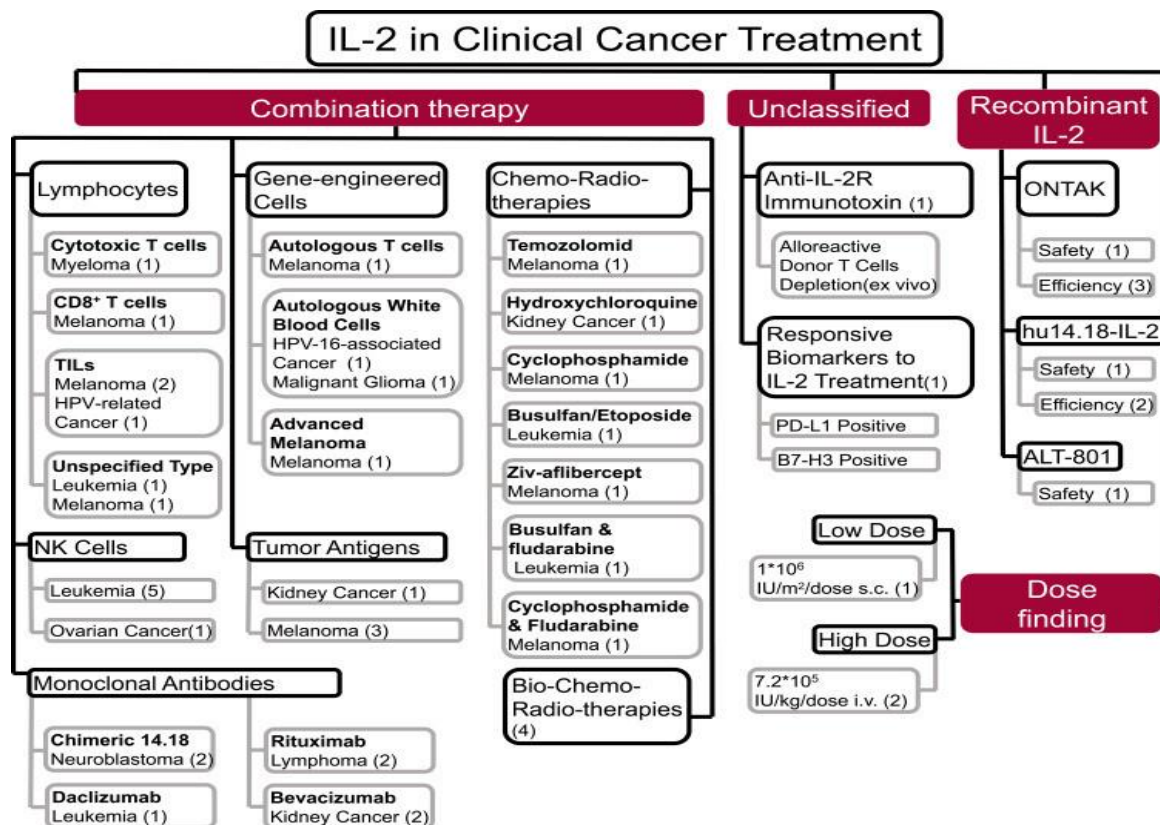


Fig 1. Application of IL-2 in 52 clinical trials for cancer therapy [4].

The role of IL-2's anti-tumor mechanism in the onset and progression of lung cancer is increasingly being recognized, and it is widely used to treat a variety of malignancies (Figure 1). Patients with advanced cancer have more hope when IL-2 is used alone or in conjunction with other medicines for cancer therapy. Research has indicated a strong correlation between the body's IL-2 levels and the aggressiveness of lung cancer. When lung cancer is severe, IL-2 levels decrease. The reason for this may be that the body's immune activity and anti-tumor efficacy decrease with the decrease in IL-2 levels, making the infiltration and metastasis of cancer cells in the body smoother, thereby worsening the condition of lung cancer. According to a meta-analysis, IL-2 improves patients' overall survival rates for non-small cell lung cancer without having a major negative impact on their health. This benefit is shown when IL-2 is administered in combination treatment.

4. IL-6

IL-6 was discovered in 1986 to be a B cell immunoglobulin induction factor. It is mainly generated by fibroblasts, monocytes, T lymphocytes, B lymphocytes, keratinocytes, epithelial cells, and different tumor cells (including lung cancer cells). With its many biological functions, IL-6 may both increase and reduce inflammation depending on the situation. It influences both immune and non-immune cells in a broad range of ways, including wound healing, aging, inflammation, immunity, and regulation of embryonic development. IL-6 is immediately produced in the body's stable state when it is infected to activate acute and immune responses for emergency prevention. However, its excessive or sustained synthesis imbalance affects the pathogenesis of various diseases. Furthermore, IL-6 is particularly highly expressed in almost all types of tumors, including in the advanced stages of lung cancer (Figure 2). Increased levels of IL-6 can promote immune tolerance, tumor proliferation, infiltration, and metastasis, making it a key factor in proliferation and anti-apoptosis [8]. IL-6 can increase the amount of cancer cells in primary lung cancer tissue and circulating blood because it is a crucial T-cell co-stimulatory factor that directly targets lung cancer stem cells. It is an important issue because of its strong correlation with lung cancer incidence, cachexia, and patient survival quality. Lung cancer may advance more quickly as a result of its immune system suppression, either directly or indirectly. This article discusses the latest findings on IL-6 immunotherapy for lung cancer and how it may be used in targeted therapy.

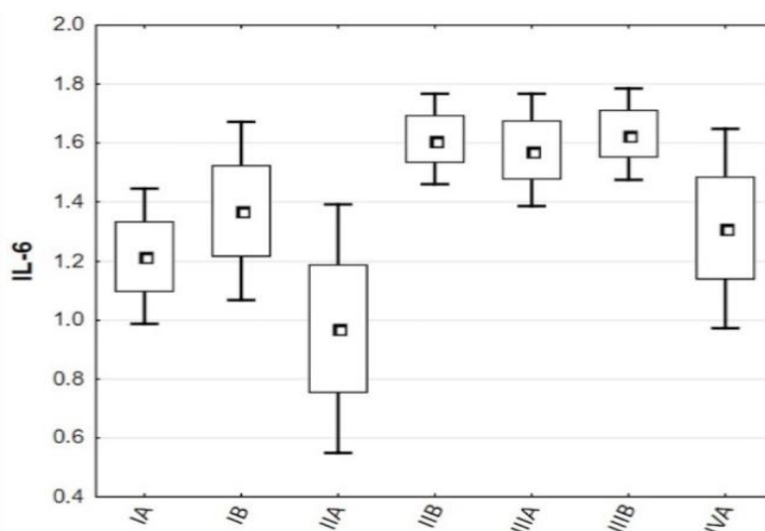


Fig 2. Distribution of IL-6 concentrations among lung cancer stages [9].

One strategy for treating cancer is to inhibit IL-6R, to inhibit abnormal cell growth or excessive angiogenesis. The IL-6 signaling pathway plays a crucial role in the growth and metastasis of cancer, and signal transducer and transcriptional activator 3 (STAT3) are important factors in this pathway. In the IL-6 signaling pathway, GP130 also plays an important role as a signal transduction receptor. When mesenchymal stem cells activate the IL6/JAK2/STAT3 signaling pathway mediated by GP130, it promotes the occurrence of lung cancer. The sustained activation of this pathway has been regarded as an important factor in inducing cancer. For this pathway, researchers have attempted to use small interfering RNA (siRNA) or monoclonal antibodies to block IL-6R, thereby achieving the effect of inhibiting lung cancer stem cell proliferation. In addition, IL-6 can promote the development of non-small cell lung cancer by activating STAT3 [10]. Therefore, inhibiting the IL6/JAK2/STAT3 signaling pathway is considered an effective method to reduce cancer cell growth. In clinical studies and therapies, metformin has demonstrated encouraging potential in blocking the IL-6 signaling pathway, and when combined with gefitinib, it has produced positive outcomes in the treatment of lung cancer [11]. The combined treatment of cottonseed acetate and cisplatin, specifically the R-(-) enantiomer, has been shown to decrease IL-6/STAT3 signal transduction, which in turn reduces IL-6-related chemotherapy resistance in lung cancer [12]. Additionally, ribavirin has been shown in

recent studies to reduce the carcinogenic impact by downregulating IL-6 expression in lung adenocarcinomas in vitro [13].

5. Interferons (IFNs)

Since their discovery in 1957, interferons (IFNs) are useful cytokines in the immunotherapy of cancer, particularly the management of lung cancer. They may be primarily divided into IFN- α , IFN- β , and IFN- γ groups based on their target receptors and function. IFN- α and IFN- β are examples of type I interferons; IFN- γ is referred to be a type II interferon. IFNs have demonstrated positive effects on tumor cell growth by directly inhibiting it, improving the antigenicity of tumor cells, and increasing the activity of Th1 cells, NK cells, and macrophages. They have also been shown to increase the affinity of tumor necrosis factor for its receptors, which improves chemotherapy efficacy. By attaching to certain receptors, IFNs start signal transduction. This activates gene transcription and several downstream signaling pathways, which results in a variety of cellular responses, such as cell cycle arrest and death in tumor cells. It is interesting to note that type I interferons function by promoting the expression of major histocompatibility complex (MHC) class I molecules on tumor cells as well as the activation and development of dendritic cells in tumor antigens. Immune interferon, or type II interferon IFN- γ , is a critical component in halting and preventing the growth of cancer. It can improve antigen presentation, stimulate the production of distinct cell MHC class I and II molecules, activate monocytes and macrophages during the immune response, and encourage the development of Th0 cells into Th1 cells [14]. On the other hand, IFN- γ stimulates the production of chemotactic factors in cells to activate immune responses by inducing the infiltration of M1 macrophages into tumor tissues, reducing the levels of M2 macrophages, inhibiting tumor proliferation, promoting apoptosis, regulating immunity, and decreasing telomerase activity in tumors.

IFNs by themselves are not as successful in treating lung cancer systemically; however, when applied in conjunction with immunotherapy and targeted therapy, or with conventional treatments (radiation, chemotherapy, surgery), the efficacy of treatment of lung cancer is much increased (Figure 3). In recent studies, it has been found that the JAK2-STAT1 pathway in lung adenocarcinoma interferes with the response to IFN- γ , which promotes the design of combined targeted therapy and immunotherapy for lung adenocarcinoma [15]. Furthermore, research indicates that IFN- γ induces apoptosis of lung cancer cells by inducing endoplasmic reticulum stress damage to autophagy, representing a new mechanism of IFN- γ -mediated anti-cancer effects [16]. According to a recent study, IFNs may be crucial in the treatment of lung cancer by causing cancer cells to undergo cell cycle arrest and develop repair mechanisms [17].

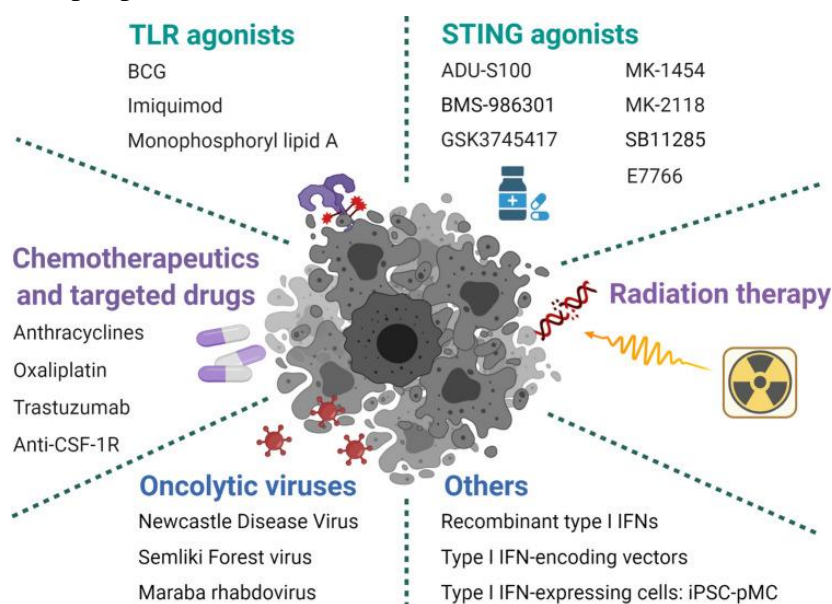


Fig 3. Immunotherapies based on Type I IFNs [18].

6. Granulocyte-macrophage colony-stimulating factor (GM-CSF)

GM-CSF (granulocyte-macrophage colony-stimulating factor) is a cytokine produced by lymphocytes, macrophages, fibroblasts, tumor cells, chondrocytes, and endothelial cells. It is expressed on myeloid, non-hematopoietic, and B cells and has been used extensively in clinical trials for cancer immunotherapy and anti-tumor vaccines. GM-CSF has been identified as a hematopoietic growth factor that plays a critical role in driving the production of myeloid cells such as neutrophils, macrophages, and dendritic cells. In addition to its effects on the hematopoietic system, GM-CSF regulates inflammatory and autoimmune responses by stimulating endothelial cells and fibroblasts. When GM-CSF is activated, it triggers a series of biological processes, including cell proliferation, differentiation, and functional activation, that help the body respond to infection, trauma, or other stimuli.

Through its actions on a variety of immune and non-immune cells, including suppression of angiogenesis, direct reduction of tumor growth, and immunological activation, GM-CSF demonstrates specific anti-tumor properties in reducing tumor formation. Firstly, GM-CSF suppresses tumor development, angiogenesis, and metastasis by blocking angiogenesis and endothelial cell migration through its action on soluble vascular endothelial growth factor receptor-1 [19]. Second, non-small cell lung cancer (NSCLC) may be diagnosed with the help of aberrant GM-CSF production. In NSCLC, GM-CSF suppresses the growth of malignant cells and promotes their differentiation, which lowers the likelihood that these cells will metastasize [20]. Furthermore, GM-CSF's immune-stimulatory capabilities are crucial in lung cancer treatment, including activation of anti-tumor macrophages, NK cells, and dendritic cells; restoration of neutrophil-driven immune response capability; and promotion of T cell anti-cancer responses. The therapeutic and pathogenic effects of GM-CSF on anti-cancer immune surveillance are shown in Figure 4.

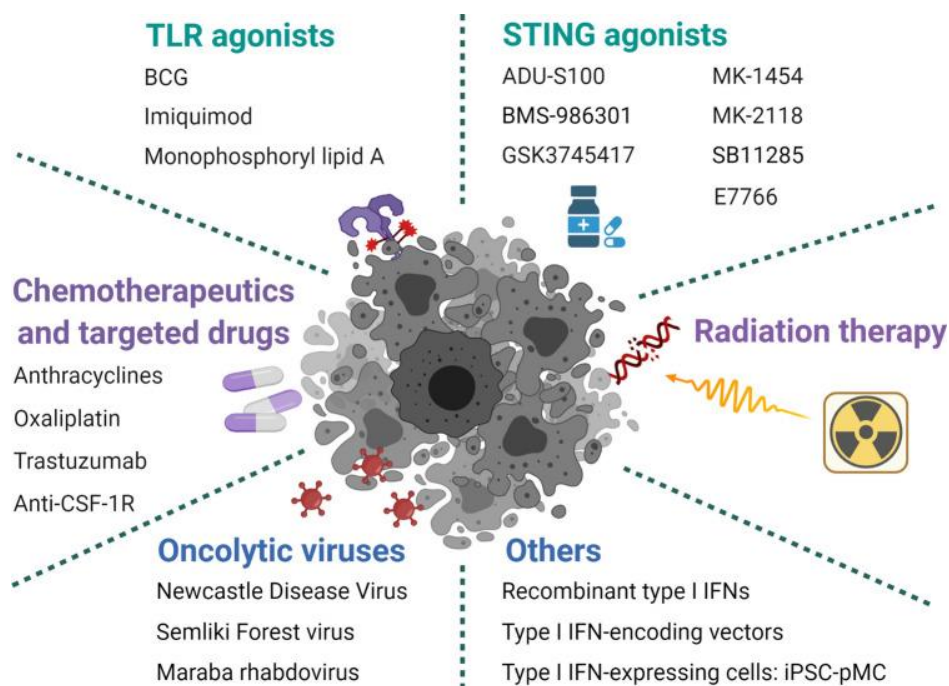


Fig 4. Therapeutic and pathogenic effects of GM-CSF on anti-cancer immune surveillance [21].

7. Conclusion

One of the malignant tumors with the highest morbidity and fatality rates in the world is lung cancer. Immunotherapy based on cytokines is valuable in the treatment of lung cancer. Upon activation, immune cells and certain non-immune cells primarily manufacture and release these tiny molecules that resemble proteins. By stimulating immune cells, encouraging their growth and differentiation, and controlling the magnitude and duration of the immunological response, they

strengthen the body's defense against lung cancer cells. Furthermore, several cytokines can directly suppress the formation of tumors. They can also suppress lung cancer cells' ability to proliferate and survive by several different mechanisms, such as by triggering apoptosis or obstructing the cell cycle.

The cytokine network and its mechanism of action are extremely complex. The interactions between different cytokines can lead to changes in the level of a single cytokine which can impact the entire cytokine network. Additionally, the application of cytokines does not follow a simple dose-response relationship. Different doses may produce opposite effects. However, it is undeniable that cytokines have been proven to be effective in cancer treatment and regulate the human immune system by modulating different immune cell populations to support anti-tumor responses. Furthermore, in the future development and improvement of cytokine therapy, cytokines can be used in combination with other therapies (surgery, chemotherapy, radiotherapy) to enhance clinical efficacy. The use of biotechnology to improve the targeting and pharmacokinetics of cytokines is also a promising direction. For example, upgrading cytokine gene therapy and cytokine-guided therapy to molecularly improve cytokines to enhance their anti-tumor activity and reduce toxic side effects. According to the development trend of cancer immunotherapy, cytokines may also play an important role when used in combination with other drugs, such as immune checkpoint inhibitors or cancer vaccines.

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