

The Role of Cytokine IL-2 In Cancer Immunotherapy

Jinwu Li *

Henan University of Technology, Henan, China

* Corresponding Author Email: 202020040410@stu.haut.edu.cn

Abstract. Cancer remains a leading global cause of mortality, distinguished by its propensity for rapid proliferation, metastatic potential, and the ability to affect various organs with ease. If left untreated, it may lead to nerve compression, rupture of blood vessels, multi-organ failure, and may ultimately lead to the patient's death. The ability of immunotherapy to block signaling pathways that inhibit immune cell activation, enhance and amplify anti-tumor responses, or increase the amount of anti-tumor response cells makes it an important weapon in the fight against cancer. Among the key domains of cancer immunotherapy research, harnessing cytokines to invigorate anti-tumor reactions in cancer patients ranks as one of the foremost areas, given cytokines' capacity to regulate the host's immune reaction against cancer cells. Interleukin-2 (IL-2) is a recognized T-cell growth factor that has been used to treat metastatic melanoma. While extensive research has substantiated IL-2's potential in cancer therapy by impacting the immune system, its practical utility has encountered several constraints. This review centers on the diverse applications of IL-2-based cancer therapy, their underlying mechanisms, the associated limitations, and prospects for the future of IL-2 in the realm of cancer immunotherapy.

Keywords: IL-2; Cytokine; Immunotherapy; Cancer.

1. Introduction

According to the latest data from the World Health Organization (WHO), cancer ranks as the second leading cause of mortality worldwide. Each year, more than 19 million people develop, and more than 9.5 million people die. Conventional treatments for cancer include surgical resection, chemotherapy, radiotherapy, etc. Adoptive T-cell treatments (ACTs), cancer vaccines, and immune checkpoint inhibitors (ICIs) are all active areas of cancer immunotherapy research today. ICIs operate by removing the suppressive constraints on T-cells, fostering sustained immune system activation. Due to the release of inhibitory brakes on T cells, the immune system is activated longer, and an anti-tumor immune response is started. Three types of antibodies are commonly used to against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). These include antibodies targeting programmed cell death 1 (PD-1), as well as antibodies directed against PD-L1, which serves as the ligand for PD-1. ACT utilizes T-cells from the patient, modifies them to give them specific anti-tumor functions, and then "passes them on" back to the patient to treat the tumor. Cancer vaccines share similarities with traditional vaccines in that they trigger the body's immune response by utilizing tumor-associated antigens. These antigens can encompass various elements, such as surface markers on tumor cells, internal components of tumor cells, or substances released by tumor cells.

Exploring the utilization of cytokines as a therapeutic approach represents a novel direction in cancer treatment. Cytokines that belong to the common γ -chain family serve a pivotal role in regulating the immune system and exert diverse effects on both innate and adaptive immune cells. These cytokines not only modulate the host immune response against cancer cells but also have the capability to directly trigger apoptosis, or programmed cell death, in cancer cells. Among these cytokines, IL-2, IL-7, IL-9, IL-15, etc., all play significant roles. Of them, IL-2 and IL-7 have been used for a very long time to encourage the proliferation and division of T cells. In general, IL-2 acts as a "stimulator," causing T cell responses to be activated, while IL-7 acts as a "proliferator," increasing the T cell population.

In 1976, Doris Morgan, Robert Gallo, etc. demonstrated that activated T cell cultures contain specific mediators that stimulate antigen-activated T cell proliferation [1]. The protein in charge of this T-cell growth factor activity was subsequently effectively isolated, and its corresponding gene

was cloned. This milestone discovery affirmed that this biological activity originated from a solitary molecular entity [2]. Consequently, this cytokine was christened "IL-2". IL-2 belongs to the γ -chain cytokine family, with a molecular weight of 15 kDa, consisting of 133 amino acids divided into four α -helices. CD4⁺ and CD8⁺ T cells that have been activated are the main producers. By boosting the activity of NK cells and encouraging the proliferation and activation of antigen-activated T cells, it is essential for enhancing cellular and humoral immunity. [3].

Three different kinds of IL-2 receptors (IL-2R) are able to bind IL-2 with various affinities. IL-2 can bind to receptors that contain the IL-2R α subunit with low affinity, the IL2R β subunit with medium affinity, and IL-2R γ -containing receptors with high affinity (Figure 1) [4]. The high-affinity receptors for IL-2 are predominantly found on Treg cells (recently also found on activated CD4⁺/CD8⁺ effector T cells, select NK cells, and group 2 innate lymphoid-like cells). These high-affinity receptors are comprised of three subunits: CD25 (IL2R α), CD122 (IL2R β), and CD132 (IL2R γ). In contrast, intermediate affinity dimerization receptors devoid of the CD25 component are expressed by the majority of NK cells and CD4/C8 memory T cells. Only the CD25 subunit is present in low affinity receptors, which are typically expressed on activated cells [4]. By triggering the JAK/STAT, PI3K/Akt, and MAPK signaling pathways on T cells and Treg cells, IL-2 binding to dimerization receptors improves immune responses [5].

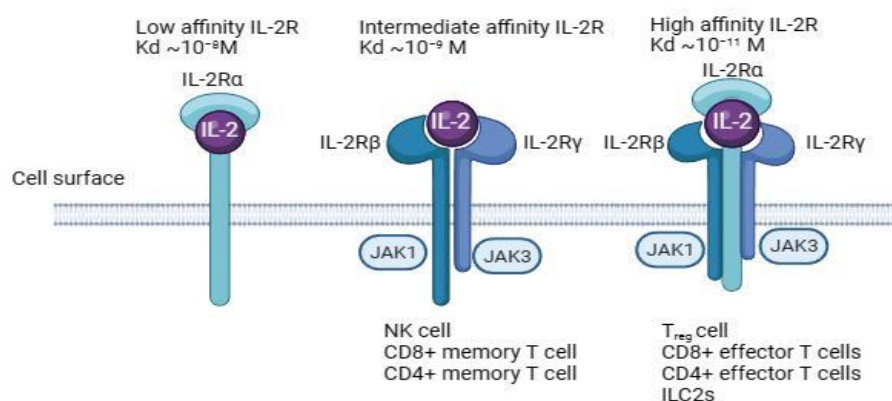


Figure 1: IL-2 receptors with different affinities: low, intermediate, and high. The low-affinity receptor, consisting solely of IL-2R α ; the intermediate-affinity receptor, formed by IL2R β and IL-2 γ ; and the high-affinity receptor, composed of IL-2R α , IL2R β , and IL-2 γ .

2. IL2 monotherapy in Cancer Treatment

In a clinical experiment in 1985, Rosenberg SA et al. selected 25 patients with metastatic cancer and administered high-dose (HD) IL-2 to them. They discovered that all 3 patients with metastatic kidney cancer and 4 out of 7 patients with metastatic melanoma had their metastatic tumors recede [6]. This study established the first-ever involvement of IL-2 in mediating tumor regression, which was a significant historical development. The possible therapeutic use of recombinant IL-2 in the treatment of acute leukemia has been proposed, in accordance with research by Lim et al. Acute myeloid leukemia patients in particular may benefit from IL-2's possible anti-leukemic actions, according to the study's results [7]. The Food and Drug Administration (FDA) authorized IL-2's usage in the treatment of metastatic renal cell carcinoma in 1992. After that, in 1998, the FDA widened the scope of its clearance to include the treatment of metastatic melanoma. The administration of high-dose interleukin-2 (HD IL-2) was observed in a cohort of 225 individuals diagnosed with metastatic renal cell carcinoma, as part of a phase II clinical trial. The treatment was delivered in multiple cycles.

The dosages were given between 600,000 and 720,000 IU/kg, and they may be given up to 15 times per 8 hours, or as often as the patients could handle. A noteworthy overall response rate of 15% was finally achieved by this treatment plan, with full responses occurring in 7% of the patients [8]. However, as mentioned earlier, despite the fact that IL-2 effectively increases the count of T cells, it also prompts the proliferation of Treg cells when it binds to the high-affinity trimeric receptor. This, in turn, dampens the activity of T cells. Therefore, IL-2 monotherapy will eventually cause unavoidable side effects in patients, leading to additional complications and reduced cancer treatment efficacy. Combining IL-2 with other therapies can improve this situation.

3. IL-2-Related Combination Therapy in Cancer Treatment

3.1. IL-2 combined with chemotherapeutic agents

Chemotherapy is a common method for cancer treatment today, but it inevitably harms the patient's organism in the process of removing cancer cells. In an effort to enhance treatment effectiveness and extend patient survival, U. Keilholz's team incorporated cisplatin into a cytokine therapy regimen that included IFN- α and HD IL-2. This combination was created to assess how it might affect patients with metastatic melanoma's survival rates. The addition of cisplatin to immunotherapy with IFN- α and IL-2 did not result in any appreciable improvement in the overall survival of patients with advanced metastatic melanoma, while noticing a significant increase in remission rates and progression-free survival [9]. Patients with metastatic melanoma underwent tamoxifen, cisplatin, and dacarbazine-based chemotherapy in Steven A. Rosenberg's clinical study, which was followed by injections of interferon alfa-2b and IL-2. The findings demonstrated that this combination of therapies enhanced toxicity without increasing survival rates [10]. In another clinical trial, Michael B. Atkins et al. administered cisplatin, vincristine, and temozolomide to patients with metastatic melanoma coupled with continuous intravenous (IV) injections of IL-2 on days 1-4 and continuous IV IFN- on days 1-5, 8, 10, and 12. The treatment protocol demonstrated effectiveness and was generally well-tolerated among individuals. However, this intervention did not result in an improvement in the overall prognosis for patients [11]. Moreover, the combined outcomes of various studies for the assessment of the IL-2 and cisplatin-based bio-chemotherapy indicated that 50% of patients responded to the treatment, with 10% of them attaining a persistent, full response that could not be sustained [12].

3.2. IL-2 combined with cancer vaccines

Due to its immune-boosting qualities, IL-2 has been shown to have a synergistic impact when paired with tumor vaccinations. The therapeutic effectiveness of the vaccination against cancer may be significantly increased by adding IL-2 to cancer vaccines such as recombinant viruses, bare DNA, or peptide antigen vaccines. In a prior phase II clinical research, it was shown that people diagnosed with metastatic melanoma who were administered a therapeutic regimen comprising of HD IL-2 in combination with a gp100 peptide immunization had comparatively greater rates of positive response in contrast to those who got IL-2 alone [13]. In a murine model of colorectal cancer, the combined administration of a MUC1 DNA vaccine together with CpG and IL-2 adjuvants exhibited enhanced activation of cytotoxic T lymphocytes (CTL). Furthermore, this intervention shown a notable ability to successfully restrict tumor development and metastasis [14]. In another study, the use of recombinant adenovirus (rAd) that co-expressed a fusion antigen combining MUC1 and survivin along with IL-2 as an immune adjuvant resulted in a remarkable tumor inhibition rate of up to 50.1%. Additionally, this approach elicited a robust response of CTL among tumor-bearing mice [15].

The vaccination TG4010 is designed to express both MUC1 and IL-2 using a recombinant vaccinia virus. During the first phase of clinical testing, this vaccine had a positive safety profile. Furthermore, it induced substantial immune responses against tumor cells in a group of 13 patients who were diagnosed with advanced solid tumors [16]. In phase II trials, TG4010 has been employed in patients with advanced non-small cell lung cancer (NSCLC) who were concurrently receiving chemotherapy.

The results show that TG4010 not only maintained a high level of safety but also exhibited superior efficacy compared to chemotherapy administered alone [17]. This implies that the utilization of IL-2 as an adjuvant in vaccinations or as a constituent of vaccines exhibits potential in enhancing the therapeutic effectiveness of vaccines. Additionally, the exploration of various vaccine combinations could offer alternative approaches for malignancy therapies.

3.3. IL-2 combined with immune checkpoint inhibitors.

As has been said, it has been shown that the effectiveness of cancer therapy may be improved by suppressing the immunological checkpoints that are present on the immune cells. The study conducted by Ajay V. Maker et al. evaluated the effectiveness and autoimmune adverse effects of the combination of anti-CTLA-4 antibodies and IL-2 in individuals diagnosed with metastatic melanoma, as part of a phase I/II clinical trial. The objective remission rate of 22% was seen among the 36 patients who participated in the experiment, when they were treated with a combination of anti-CTLA-4 antibodies and IL-2 [18]. Based on the historical remission rate of 15% seen in patients with metastatic melanoma undergoing IL-2 treatment, there is now insufficient meaningful data to support the existence of a synergistic effect when combining IL-2 treatment with anti-CTLA-4 therapy [8,18]. Research conducted in a mouse model revealed that IL-2 therapy exhibited a synergistic effect in conjunction with PD-L1 blockers. The concurrent administration of this combination has been shown to augment CD8⁺ T cell responses and significantly diminish viral load. [19]. A experiment was undertaken by Buchbinder, whereby individuals who had previously received therapy with PD-1 or PD-L1 inhibitors were supplied HD IL-2. The findings of the study suggest that the administration of HD IL-2 shown long-lasting effectiveness in suppressing tumor growth in individuals who had not received prior immune checkpoint blockade (ICB) therapy. Furthermore, it was observed that there was no identifiable pattern indicating a rise in toxicity [20]. The results of this study indicate that HD IL-2 may serve as a feasible therapeutic alternative for those who are undergoing disease advancement after ICB therapy.

4. Novel IL-2 variants via biochemical modifications and cytokine engineering

Given the dual characteristics of interleukin-2 (IL-2) in relation to effector T cells and regulatory T cells (Treg cells), it exerts both enhancing and suppressing influences on immunological responses. The utilization of diverse permutations or modifications of IL-2 presents a viable approach to augment the effectiveness of IL-2 in the context of cancer treatment. According to reports, the F42A mutation in IL-2 significantly diminishes the affinity of IL-2 for IL-2R α . Conversely, several mutations in the IL-2 gene have been seen to increase the propensity for receptor binding, such as the L80F and R81D variants. All of these alterations increase the probability of IL-2 β binding [21]. A hypermutant IL-2-Fc variation was generated by fusing the IL-2 molecule with the Fc component and introducing particular mutations (F42A, L80F, R81D, L85V, I86V, and I92F). The engineered variation was specifically tailored to preferentially induce activation of effector T cells rather than regulatory T cells (Treg cells). In Sun's study, the researchers examined many IL-2 variants, including wild-type (WT) IL-2, F42A mutant IL-2, super-IL-2, and hypermutant IL-2-Fc, to assess their capacity for binding to both Treg cells and CD8⁺ T cells [5]. When compared to activated CD8⁺ T cells, it was shown that WT IL-2 had higher affinity for Treg cells across these variations. The IL-2 variation, F42A mutant, showed a reduction in IL-2's capacity to attach to regulatory T (Treg) cells but did not show a noticeably higher capacity to bind to CD8⁺ T cells. Only the super-mutant IL-2-Fc demonstrated a preferential binding preference for CD8⁺ T cells, with little binding to Treg cells, while super-IL-2 shown a high binding capacity for both cell types. These findings indicate that hypermutated IL-2-Fc has enhanced capacity for CD8⁺ T cell activation, while demonstrating less capacity for Treg cell activation compared to alternative IL-2 variants. While the enhanced form of IL-2 has more efficacy in stimulating an immune response against cancer, it has also been linked to adverse consequences when administered in large quantities. These symptoms include fluid retention,

compromised organ function, and even mortality directly attributable to the therapy. The dual functionality of T cells persists. Hence, the pursuit of integrating various therapy methods may provide a more advantageous approach.

5. Conclusion

The biological function of IL-2 is to stimulate and enhance the proliferation of T lymphocytes and NK cells. The intervention of IL-2 alters the immune response in individuals with tumors, both in laboratory settings and within living organisms. This intervention specifically enhances the reactivity of T cells that target tumors or prolongs the survival and increases the population of these T cells. As a result, IL-2 was employed as a cancer monotherapy. Additionally, it can enhance the prognosis of cancer patients because of the immunosynergistic impact it has with other therapy approaches. Other treatment modalities, including cancer vaccines, and immunosuppressive sites, can be utilized in conjunction with IL-2. More efforts and trials are required to fully understand how IL-2 interacts with immunosuppressive sites, since the findings of recent research have shown that the combination enhanced the therapeutic efficiency.

However, there are still many issues that need to be addressed. IL-2 has been studied more deeply in combination with chemotherapeutic agents, but it currently does not improve the prognosis of tumor patients. The IL-2 not only enhances the immune effects of T cells but also triggers immunosuppressive cells, such as Treg cells, that trigger a decline in the immune response, a weakening of the therapeutic effect, and even strong side effects for the patient. Current research focuses on the modification of IL-2 to improve binding affinity, but serious side effects have been observed. Exploration of various combinations of IL-2 with different therapeutic modalities could be a future direction for the IL-2 and related cytokine therapeutics.

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