

IL-2 in Treatment of mRCC: Clinical Application and Improvement

Zicong Zhou *

Biomedical engineering, Sichuan University, Chengdu, 610065, China

* Corresponding Author Email: 20202211@stu.hebmu.edu.com

Abstract. Worldwide, the incidence of renal cell carcinoma ranks third among malignant tumors of the genitourinary system. Because renal cancer is not sensitive to radiotherapy or chemotherapy, immunotherapy has been the basic treatment for metastatic renal cancer for many years. For people with stage IV solid tumors, interleukin-2 (IL-2) was once one of the few medications that may result in complete responses (CRs), which were frequently long-lasting for decades without additional treatment. IL-2-related therapies have been used to treat kidney cancer for more than 20 years. Although its wide clinical application is still subject to various limitations, and new molecular targeted therapies have received more attention in recent years, IL-2, as the only drug that can cure metastatic kidney cancer, has great clinical value that cannot be ignored. This article summarizes the clinical application of IL-2 in renal cancer and the latest research progress, analyses data from several articles, and discusses the improvement methods, such as how to strive to find safer and more effective IL-2 treatment methods to reduce its side effects and enhance patient tolerance, and combining IL-2 with other immunotherapies, such as immune checkpoint inhibitors or CAR-T cell therapy, may result in a more potent anti-tumor effect. With a deeper understanding of individual patient's immune systems, there may be a trend towards personalized or customized IL-2 treatment to better meet specific patient needs.

Keywords: mRCC; IL-2; dose.

1. Introduction

Renal cell carcinoma belongs to solid tumors. Renal cell carcinoma is one of the 10 epidemic cancers and consists of diversified class of cancers stemmed from renal tubular epithelial cells, including ccRCC, pRCC, chrRCC and so on. Renal cell carcinoma can spread to bones, brain, pancreas, gallbladder, and adrenal gland which causes metastasis [1]. When distant metastasis of tumor cells occurs, it indicates that the patient's disease has entered an advanced stage and the prognosis is extremely poor. In modern medicine, many therapies have been developed to treat mRCC, such as radical nephrectomy, partial nephrectomy, radiation therapy, arterial embolization, targeted therapy, chemotherapy and so on [2].

Although many advances have been made in systemic therapies to cure mRCC, there are quite a lot of deficiencies in therapies that could threaten patients' health, and researchers have been trying to discover new targets and novel therapies to treat mRCC on some adverse conditions. Currently, Interleukin-2 (IL-2) has emerged as immunotherapeutic agents for treatment of metastasis renal cell carcinoma and this strategy is approved by FDA in 1992. High-dose IL-2 has been utilized in clinical application at scale and has shown efficacy to prolong overall survival of patients and a small portion of them have recovered from mRCC. IL-2 is not perfect and also has side effects, however, developing strategies to improve efficacy and diminish adverse influence of IL-2 has been research hotspots in future. This paper aims to illustrate the efficacy of IL-2 based therapy and explore the improvement of IL-2 based therapy, in order to provide reference for clinical diagnosis and treatment of this disease.

2. Biological characteristics of IL-2

Interleukins represent a class of cytokines that are generated by diverse cells and exhibit diverse cellular actions. To guarantee that hematopoiesis and immune modulation operate normally, they

collaborate and interact with hematopoietic growth factors. Important roles for interleukins include information transmission, immune cell stimulation and modulation, influencing T and B lymphocyte activation, proliferation, and differentiation, and playing a major role in inflammatory processes.

IL-2 is a member of the chemokine family and is produced by various cells, mainly by activated T cells. IL-2 manifests pleiotropic nature (mainly promoting the expansion, replication, and differentiation of lymphocytes). It plays crucial roles in orchestrating immune responses and combating viral infections. IL-2 induces the production of lymphokine-activated killer (LAK) cells, facilitates the proliferation and secretion of antibodies by B cells, and activates macrophages [3,4]. IL-2 is a kind of glycoprotein which encompasses 133 amino acid residues and possesses a relative molecular mass ranging from 15 to 35 kilodaltons. Natural IL-2 harbors a glycosyl group at the N-terminus, although glycosylation does not evidently influence on the biological activity of IL-2. Crucially, the IL-2 molecule harbors three cysteine residues situated at amino acid positions 58, 105, and 125. The intrachain disulfide bond formed between cysteine 58 and cysteine 105 significantly contributes to the preservation of IL-2's biological activity.

3. Mechanism of IL-2-based therapy

The mechanism that IL-2-based therapy treat mRCC is by eliciting responses in T cells, tumor-specific cytotoxic T lymphocytes (CTLs), NK cells, and potentially tumor-infiltrating lymphocytes. These immune effects are triggered by the binding of IL-2 to its receptor (IL-2R).

IL-2 interacts with three types of receptor chains, known as IL-2R α / β / γ , which can form either a dimer or a trimer. Further, the high-affinity trimer IL-2R $\alpha\beta\gamma$ for IL-2 is produced by the amalgamation of IL-2R α (the CD25), IL-2R β (the CD122), and IL-2R γ (the CD132) subunits. CD122 and CD132, which are mostly expressed in NK cells, monocytes, macrophages, and CD4⁺ and CD8⁺ cells, facilitate signal transduction within this complex, with CD25 playing a pivotal role in increasing IL-2 affinity. Furthermore, CD25 is of crucial importance in immune suppression and the regulation of T cell proliferation. Nevertheless, in its absence, stimulation of NK and CD8⁺ cell proliferation and the destruction of IL-2-responsive cells can occur through IL-2R $\beta\gamma$ activity [5]. Moreover, CD25 plays a critical role in controlling T cell proliferation and immunological suppression. However, in its absence, IL-2R $\beta\gamma$ activity can stimulate the growth of NK and CD8⁺ cells and cause the death of IL-2-responsive cells. Apart from its initially identified function of inducing T cell proliferation, IL-2 has been demonstrated to notably augment the lytic activity of natural killer cells and trigger cytotoxicity mediated by lymphokine-activated killer cells. Other significant biological effects of IL-2 have now slowly come to light. The fact that IL-2 has been shown to promote the development of regulatory T cells and T helper 9 (TH9) cells is very significant. Additionally, it has the ability to suppress the differentiation of T follicular helper (TFH) cells and Th17 cells while promoting the differentiation of T cells into Th1 and Th2 cells. As a result, IL-2 is an important cytokine that shapes immune cell development and is necessary for the majority of cell types found in infections, autoimmune disorders, and cancerous settings [6]. Furthermore, IL-2 is essential for activation-induced cell death (AICD), a process that gets rid of potentially dangerous autoreactive cells. Each of these helps to make IL-2-based therapy effective in treating mRCC.

4. Efficacy of IL-2-based therapy

RCC patients receiving high-dose IL-2 (interleukin-2) treatment in the PROCLAIM registry database, using the International Metastatic RCC Database Consortium (IMDC) risk criteria assessment. In IMDC, the widely used prognostic assessment models in clinical practice mainly include six evaluation indicators: the interval between diagnosis and treatment, KPS score, hemoglobin levels, platelet count, neutrophil count, and serum calcium concentration. The follow-up period ranged from 0.2 to 124 months, with a median of 23.4 months. Subgroup analyses were carried out for patients who had either never been treated before, had received IL-2 alone, or had received

IL-2 treatment after receiving treatment. Two groups were formed out of some patients. The research examined 356 individuals who were given IL-2 alone, classifying the patients' outcomes according to risk factors. 59% of the 810 patients were categorized as intermediate risk, and 721 of them were left untreated. For patients at favorable risk, the 2-year survival rate was 77.6%, and the median overall survival (OS) was 63.3 months. Low-risk patients had a median OS of 14 months and a 2-year survival rate of 68.2%, whereas intermediate-risk patients had a median OS of 42.4 months. The 2-year survival rates and median survival for all risk groups in mRCC patients receiving high-dose IL-2 treatment were in line with recent studies on immunotherapy or targeted therapy for mRCC. In comparison to low-risk patients, favorable and intermediate-risk patients had longer OS, while high-risk patients had the longest OS. For high-risk patients receiving high-dose IL-2 therapy, the 2-year survival rate was 74% [7]. Given that IL-2 is both long-lasting and efficacious, these data maintain the advice that eligible mRCC patients get high-dose IL-2 therapy.

In IL-2-based immunotherapy, appropriate dosage controlling is important and high-dose IL-2 has been a major therapy in early treatment. However, a recent study conducted a comparative analysis to evaluate the efficacy and side effects of high-dose versus low-dose intravenous bolus interleukin-2 (IL-2) therapy in treating metastatic renal cell carcinoma. The research involved 125 patients who were randomly allocated to receive IL-2 intravenous bolus injections every 8 hours, with doses set at 720,000 IU/kg (high dose) or 72,000 IU/kg (low dose), until reaching the maximum tolerated dose or a limit of 15 doses. Results from the study revealed that those who received the high-dose IL-2 therapy were more likely to suffer from thrombocytopenia, malaise, and hypotension, whereas patients on the low-dose regimen showed a notably higher incidence of infections. Despite the lower incidence of complications with low-dose IL-2, its efficacy was found to be on par with the high-dose IL-2 treatment. This led to the recognition of low-dose intravenous bolus IL-2 as a viable and less harmful option for treating metastatic renal cell carcinoma [8].

Additionally, for immunotherapy, immune checkpoint inhibitors such as Ipilimumab plus nivolumab and Nivolumab are proved useful to prolong overall survival. In fact, combined Immune checkpoint Inhibitors and antiangiogenic targeted therapies show higher OS and median survival compared with combined high-dose IL-2 and interferon-alpha with fewer adverse events rate [3]. Therefore, it's urgent to discover novel strategies to enhance efficacy of IL-2-based immunotherapy and deal with challenges IL-based therapy faces.

5. Strategies to enhance efficacy of IL-2

5.1. Immunocytokines

Over the past 15 years, multi-antibody-cytokine fusion constructs with IL-2, called immunocytokines, have been designed and are being tested in clinical trials for the treatment of various types of cancer. Fusing the IL-2 gene to tumor-targeting antibodies can increase the therapeutic window of cytokines and limit toxicity [9, 10]. For instance, combined IL-2 and GD2-specific antibody have been used to treat melanoma and neuroblastoma, and GD2 and epidermal growth factor receptor (EGFR) targeted IL-2 immunocytokines can inhibit cancer cells from spreading to other organs such as liver and lung, prolonging overall survival. In addition, Immunocytokines targeting a variety of antigens, such as EDA-IL-2, prostate-specific membrane antigen (PSMA), programmed death ligand-1 (PD-L1), EGFR, carbonic anhydrase IX (CAIX), CD20, and DNA, have been developed using Interleukin-2. The author notices that the specific antigens expressed on RCC (Renal Cell Carcinoma) cancer cells include, but are not limited to Carbonic anhydrase IX (CAIX, Highly specific and highly expressed antigen in RCC cancer cells, and serves as a target for FDA-approved targeted therapies); Renal Cell Carcinoma (RCC) associated antigen: An antigen known to be associated with RCC; Tumor associated antigen (TAA): Antigens that are overexpressed on the surface or inside cancer cells and can potentially serve as targets for immunotherapy; Programmed Death-Ligand 1 (PD-L1): PD-L1 is an immune checkpoint molecule,

and its high expression is commonly observed in RCC. Targeted therapies against PD-L1 have been widely used in the treatment of RCC.

Therefore, by fusing IL-2 to antibodies targeting antigens above people can hopefully trigger T cells and NK cells in RCC and kill cancer cells more effectively.

5.2. Nanomedicine

IL-2 is an important immune regulatory factor that promotes the proliferation and activation of immune cells. In recent years, scientists have conducted a series of research and exploration on IL-2 using nanotechnology, aiming to improve its drug efficacy and reduce adverse reactions.

One study suggests that encapsulating IL-2 in nanoparticles can enhance the drug's stability and bioavailability in the body. In the study, a novel IL-2 nanoassembly formulation (nano-IL-2) has been developed using 1, 2-distearoyl-Sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (PEG2000-DSPE). This formulation demonstrates prolonged stability and minimal vascular leakage or inflammatory damage at the injection site due to the high binding affinity of the two components (at the level of 10^{-8} M). Furthermore, nano-IL-2 exhibits superior solubility, lymphatic targeting properties, an extended and stable range of serum IL-2 concentrations, and lower toxicity compared to commercial formulations. Mono-therapies employing nano-IL-2 have demonstrated optimal efficacy in controlling the growth of melanoma and colon carcinoma in mice. In summary, this study provides a more suitable design strategy for subcutaneous administration and improved safety for lymphatic-targeting IL-2 formulations. Additionally, nanoparticles can prolong the drug's action time by controlling the release rate, reducing the need for frequent injections. The nanoparticle carrier can also enhance IL-2 specificity by targeting immune cells, reducing damage to non-target cells [11].

Furthermore, some research has found that using nanotechnology, IL-2 can be combined with other immune regulatory factors or anti-tumor drugs to form nano-complexes, further enhancing therapeutic effects. These nano-complexes can achieve multiple therapeutic effects in the body by promoting immune cell activation and tumor cell death through different pathways, and the author will concentrate on mRNA-based cytokine therapy which is designed basing on nanotechnology.

There have been significant advancements in mRNA-based cytokine (including IL-2) therapy in recent years. By using nanotechnology as a delivery vehicle for mRNA, scientists have successfully achieved effective delivery of cytokines. This approach holds promise as a new strategy for cancer immunotherapy.

Studies have shown that delivery of cytokine mRNA can achieve specific, effective, and safe cytokine expression in vivo. In a study, scientists have developed a porous silicon dioxide nanoparticle (PPSN) delivery platform based on polyethyleneimine-modified, carrying cytokine mRNA for in vivo local immune therapy. The delivery platform is much more efficient in local mRNA translation compared to FDA-approved lipid nanoparticles. The study found no instances of mRNA translating in unintended organs or signs of systemic toxicity. Injecting PPSN with cytokine mRNA directly into tumors led to a significant increase in protein expression in mRCC and induced a type of cancer cell death that activates the immune system. Furthermore, when cytokine mRNA was used alongside immune checkpoint inhibitors, there was a notable improvement in the anti-tumor effects in mRCC, including the suppression of tumors that had metastasized. These findings highlight the promise of using PPSN for targeted, efficient, and safe mRNA delivery in the context of cancer immunotherapy. Delivery of cytokine mRNA can activate the immune system, trigger anti-tumor immune responses, and reverse immune tolerance in tumors [12]. Furthermore, researchers have combined immune checkpoint inhibitors with cytokine mRNA delivery to enhance immune responses and observed inhibitory effects on distant metastatic tumors. Thus, the progress in mRNA-based cytokine therapy confirms the potential of this strategy in cancer immunotherapy and opens up new possibilities for cancer treatment. Despite the need for further clinical research and experimental validation, this treatment strategy holds great promise in the field of cancer immunotherapy.

In summary, nanotechnology provides new approaches and strategies for improving the drug efficacy of IL-2, which is expected to become an important means of developing immune therapy in the future. However, current research is still in the experimental stages and requires more clinical validation and safety assessment before it can be applied in clinical practice.

6. Conclusion

mRCC is a kind of cancer affected by immune regulation, prone to recurrence and metastasis, and insensitive to radiotherapy and chemotherapy. In recent years, people's understanding of the biological mechanism of kidney tumor has been deepening, and with the emergence of targeted therapy and immunotherapy, a new means for the treatment of advanced kidney cancer has been provided. Among them, IL-2 related immunotherapy, although the toxic side effects cannot be neglected, and the sensitive population is relatively small, it has always been able to produce lasting remission in a small number of patients. For a long time, scholars have tried various methods to reduce the toxicity and improve the efficacy of IL-2, including changing the drug route, reducing the dose of IL-2, combining with other anti-tumor drugs, even change the crystal structure of IL-2, but many treatment methods still need a large number of clinical trials to support the evidence, and the individual differences of patients for immunotherapy are large, and there is a lack of effective biological indicators to guide clinical treatment. It is believed that IL-2 will play a more important role in the treatment of mRCC with further research on tumor immune mechanism and more clinical trials.

References

- [1] Rafiah Kanwal, Metastasis in renal cell carcinoma: Biology and treatment, *Advances in Cancer Biology – Metastasis*, 2023.
- [2] Fares, J., Fares, M.Y., Khachfe, H.H. et al. Molecular principles of metastasis: a hallmark of cancer revisited, 2020.
- [3] Benczik M, Gaffen SL. The interleukin (IL)-2 family cytokines: survival and proliferation signaling pathways in T lymphocytes. *Immunol Invest*, 2004, 33(2): 109-42.
- [4] Boersma B, Jiskoot W, Lowe P, Bourquin C. The interleukin-1 cytokine family members: Role in cancer pathogenesis and potential therapeutic applications in cancer immunotherapy. *Cytokine Growth Factor Rev*, 2021, 62: 1-14.
- [5] Spolski, R., Li, P. & Leonard, W.J. Biology and regulation of IL-2: from molecular mechanisms to human therapy, 2018, 18: 648–659.
- [6] Hwang, JR., Byeon, Y., Kim, D. et al. Recent insights of T cell receptor-mediated signaling pathways for T cell activation and development, 2020, 52: 750–761.
- [7] Fishman M, Dutcher JP, Clark JI, et al. Overall survival by clinical risk category for high dose interleukin-2 (HD IL-2) treated patients with metastatic renal cell cancer (mRCC): data from the PROCLAIMSM registry. *J Immunother Cancer*, 2019, 7(1):84.
- [8] Harris, K.E., Lorentsen, K.J., Malik-Chaudhry, H.K. et al. A bispecific antibody agonist of the IL-2 heterodimeric receptor preferentially promotes in vivo expansion of CD8 and NK cells, 2021.
- [9] Raeber ME, Sahin D, Boyman O. Interleukin-2-based therapies in cancer. *Sci Transl Med*, 2022.
- [10] Díaz-Montero, C.M., Rini, B.I. & Finke, J.H. The immunology of renal cell carcinoma, 2020, 16: 721–735.
- [11] Shin, Hojeong, Kang, Seounghun, Won, CheolheeMin, Dal-Hee, Enhanced Local Delivery of Engineered IL-2 mRNA by Porous Silica Nanoparticles to Promote Effective Antitumor Immunity. *American Chemical Society*, 2023.
- [12] Hotz C, Wagenaar TR, Gieseke F, et al Local delivery of mRNA-encoded cytokines promotes antitumor immunity and tumor eradication across multiple preclinical tumor models. *Sci Transl Med*, 2021.