Interleukin-2 in the Therapy of Glioblastoma: Results and Prospects

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Abstract: Although recent advances in clinical oncology have shown fascinating achievements, the effectiveness of treatment regimens for glioblastoma (GBM) remains insufficient and the median overall survival is less than 1 year. Given limited effectiveness of the standard therapy, alternative treatment strategies are required and major expectations are connected with the immunotherapy. Over the last decade, several IL-2-based therapies have gained an increased interest. Newly developed clinical strategies for GBM treatment include IL-2-based approaches in combination with other immunotropic agents, such as a combination of immune checkpoint inhibitors (ICI) and oncolytic viruses. IL-2 is still regarded as a necessary component for the generation of activated killer cells used in the GBM therapy. A promising approach to the GBM treatment is based on CAR-T cells engineering that involves IL-2 activated cell expansion. As evidenced by the encouraging results of just a few clinical studies, this strategy can significantly improve the results of GBM treatment.
Key words: Interleukin -2, Glioblastoma, Adoptive immunotherapy

Introduction

Glioblastoma (GBM) is the most aggressive primary brain tumor originating from glial cells [1]. GBM accounts for about 75% of all malignant brain tumors. The tumor is characterized by the infiltrative growth and poor prognosis. Standard GBM therapy with surgical resection, radiotherapy and temozolomide treatment achieves an average survival of 15 months, while a 5-year survival reaches less than 5% [2]. Given limited effectiveness of the standard therapy, alternative treatment strategies are required and major expectations are connected with the immunotherapy [3, 4]. Clinical studies used a wide range of immunologic agents, such as interleukin-2 (IL-2) and other similar products, as well as novel checkpoint inhibitors and adoptive immunotherapies with activated and genetically modified lymphocytes [5].

IL-2 in the GBM treatment

In the 1990s, several clinical trials were initiated to evaluate the effectiveness of high-dose IL-2 antitumor immunotherapy. IL-2 is a pleiotropic cytokine that has a stimulating effect on the effectors of innate and adaptive immunity [6, 7]. IL-2 stimulates lymphocyte proliferation, induces expansion of cytotoxic T lymphocytes (CTL), and activates natural killer cells (NK) [8]. This cytokine plays an important role in the homeostasis of memory T cells by regulating their numbers and stimulates the generation of antigen-specific T cells, contributing to the survival of CD8+ memory T cells [9]. IL-2 enhances the therapeutic effects of other antitumor agents, including checkpoint inhibitors, and promotes the generation of tumor-specific immune clones [10, 11]. Taking into account these functions, IL-2 is considered a key cytokine in the regulation of antitumor immunity. High doses of IL-2 were recommended for the treatment of advanced renal cell carcinoma and melanoma. Following FDA approval, IL-2 systemic therapy was introduced in clinical settings; however, it failed to achieve the expected antitumor effects due to its short half-life and poor accumulation in the tumor [12]. Moreover, frequent systemic administration of high doses of IL-2 could lead to fatal adverse events [13].

Another approach of local IL-2 injections into the bed of the removed tumor or directly into the lesion was also practiced and showed good tolerance, but it was proved ineffective for the GBM treatment [14]. However, a case of tumor necrosis was described in a GBM patient after ineffective standard therapy who received 10 subcutaneous injections with genetically modified autologous tumor cells and fibroblasts secreting IL-2. The antitumor immune response was determined by the activation of IL-2 cytotoxic T-killers [15]. Combinations of cytokines interferon-α 2b (INF-α 2b) and IL-2 (in concentrations $10x10^6$ IU and $9x10^6$ IU, respectively) injected into a tumor in ten patients with GBM were also ineffective, neither was the local administration of IL-2 in a single mode [16].

At the same time, the potential use of IL-2 is still an important approach in cancer treatment; in particular, it is considered for the immunotherapy with IL-2 in combination with other immunologic agents, especially with checkpoint inhibitors (ICI). The use of such combination is based on the results of the experimental studies. It was shown that IL-2 pretreatment of lymphocytes led to an enhanced expression of CTLA-4 and PD1, and the combination of IL-2 (Roncoleukin®) and ICI significantly increased the cytotoxic function of lymphocytes against tumor cells [11]. Another experimental study on mice with orthotopically implanted GBM demonstrated that low-intensity focused ultrasound-guided delivery of IL-2 in combination with the CXC 10 chemokine ligand (CXCL10) and PD-L1 into the brain increased the concentration of tumor infiltrating cytotoxic CD8+T cells in 3 times compared to the PD-L1 action alone. The therapeutic efficacy of ICI in combination with IL-2 significantly increased and led to the tumor regression, extended survival time and the formation of long-term immune memory in mice with GBM. According to the authors, this combination presented a promising strategy for immunotherapy of brain tumors [17]. Kartashev AV et al. [18] performed a course of IL-2 immunotherapy (Roncoleukin®) at the end of the chemo-radiotherapy cycle, administering a total of 10 million ME IL-2. A comparative analysis of the data of the patients in the control group who received standard treatment, as well as patients who did not undergo the full cycle of chemo-immunotherapy, and patients receiving accelerated chemo-radiotherapy showed that the accelerated cycle of chemo-immunoradiotherapy resulted in a longer survival as compared to the standard chemo-radiotherapy regimens.

An interesting potential approach to GBM therapy is the combination of IL-2 with oncolytic viruses. The experiments on mice with GBM xenographts investigated a combination of IL-2 and replicationdefective recombinant adenoviral vector encoding p53 (rAd-p53), which was considered a promising agent for p53-targeted gene therapy of GBM. The authors reported marked tumor regression and a significantly increased survival in GBM mice treated with IL-2 and rAd-p53 as compared with that of the control animal group and groups of animals treated with IL-2 or rAd-p53 in a single mode [2]. Another example of the potential use of IL-2 and oncolytic viruses is the recent research by Bommaredy et al. The authors evaluated the effectiveness of the administration of the oncolytic herpes simplex virus expressing IL-2 (G47Δ-mIL2) in the GBM mice. The results showed that the approach significantly increased median survival without any signs of systemic toxicity associated with IL-2. The therapeutic effect of G47Δ-mIL2 in the GBM model was associated with augmented intratumoral infiltration of CD8+T cells due to the local release of IL-2 in the tumor microenvironment [19].

IL-2 activated lymphocytes: LAK and CIK

To increase the effectiveness of antitumor immunotherapy, *ex vivo* activated IL-2 lymphocytes were used along with several cytokines, such as lymphokine-activated killer cells (LAK) or cytokine-activated killer cells (CIK) generated by culturing lymphocytes with IL-2 or a cytokine cocktail of IFN-γ, IL-2 and CD3 monoclonal antibodies (CD3mAb). These strategies demonstrated higher effectiveness compared to that of IL-2 alone used in various types of cancer [20, 21].

IL-2/LAK therapy showed a higher efficacy in GBM. After surgical operation, 40 patients with recurrent GBM multiforme received an injection of LAKs into the bed of the removed tumor; LAKs were generated *in vitro* by culturing peripheral blood mononuclear cells in the presence of IL-2 for 3-5 days. Patients received an average of 1.0-2.0 x 10^9 LAK cells. Some patients additionally received local injections of IL-2. The treatment proved satisfactory tolerance. The median survival of patients receiving IL-2/LAK therapy was 17.5 months compared with 13.6 months for the control group of patients with GBM [22].

Dillman et al. performed a clinical study of 33 patients with GBM with no signs of disease progression who received LAK injections into the tumor site after completion of the previous therapy cycles. Previous therapy included surgical tumor resection (97%), partial brain irradiation (97%), gamma knife radiosurgery (97%) and temozolomide chemotherapy (70%). LAK cell treatment was well tolerated; median survival reached 20.5 months with a 1-year survival rate of 75%. Higher survival was observed in patients who received a larger number of CD3+/CD16+/CD56+ LAK cells [23].

Another clinical study evaluated the effectiveness of a 5-day treatment with direct intratumoral injection of both LAK cells and IL-2 in patients with recurrent GBM. Ten patients were included in the study, nine of whom underwent 15 cycles of treatment with LAK cells (from 0.9 to 21.0x10^9 cells) and IL-2 (49-450x10^3 U/kg). Of the nine patients treated, one had a partial tumor response to immunotherapy. Neurological side effects were observed in all patients undergoing treatment and were associated with increased brain edema, which was apparently determined by the immunotherapy [24].

Chernykh E.R. et al. carried out a series of clinical studies in patients with GBM, in particular, a research on adoptive immunotherapy with LAKs in combination with IL-2 (Roncoleukin®) that were injected in the bed of the removed tumor in order to destroy the remaining tumor cells. According to the presented results, this type of the immunotherapy demonstrated a fairly high effectiveness. The report showed that 24 months after the treatment, 5.1% of patients survived in the control group, and 55.5% of patients - in the main group, with 25% of patients who survived a 3-year follow-up period [25-27].

The research of Boiardi et al. studied nine patients with recurrent glioblastoma who received injections with autologous LAKs and IL-2 directly into the tumor through an Ommaya tube installed during surgery/biopsy. The immunotherapy was well tolerated, and the response rate reached 33% (one complete response, two partial responses, four patients had disease stabilization and two patients developed disease progression). Successive determinations of IL-2 (Proleukin) in the tumor cavity during the course of treatment showed that IL-2 concentrations were sufficient to maintain lymphocyte activation [28].

A randomized phase III clinical trial involving 180 patients with GBM evaluated the effectiveness of CIK immunotherapy. CIKs were generated from mononuclear blood cells of patients during cell culturing in the presence of IL-2 and monoclonal antibody against CD3 for 12-21 days [29]. The CIK cell product contained 1x10^9-2x10^{10} cells. CIKs were infused intravenously for 60 minutes. The immunotherapy cycle included 14 CIK injections, namely, 4 injections once a week, 4 - every 2 weeks and, finally, 6 injections every 4 weeks). The immunotherapy with autologous CIK cells in combination with standard chemo-radiotherapy with temozolomide (TMZ) was associated with an increase in the median progression-free survival (PFS) by 2.7 months. Patients receiving autologous CIK immunotherapy showed a higher disease control rate (DDR) than the control group (82.4% vs. 63.4%, P = 0.0058). In addition, CIK immunotherapy in combination with TMZ did not worsen the quality of life.

Later, these authors analyzed the results of treatment of 180 patients with GBM. The analysis established that the immunotherapy and TMZ treatment could increase overall survival (OS) and FPS in patients with GBM. Side effects of CIK immunotherapy included mainly toxicity of grade 1 or 2, such as fever, chills, fatigue, headache, and skin rash. Thus, the study demonstrated the efficacy and safety of adjuvant CIK immunotherapy in combination with traditional chemo-radiotherapy in patients with newly diagnosed pathologically pure GBM [30].

CAR-T cell therapy

CAR-T cell therapy has demonstrated revolutionary results in the treatment of B-cell hemoblastosis and has shown basic effectiveness in the treatment of gliomas, although significant results have not been achieved, yet. The technology of CAR-T cell manufacturing involves activation of lymphocytes and expansion of genetically modified cells. The cell culturing stages should use IL-2 for production of both LAKs and CIKs. Phase II/III clinical trials of GBM treatment with CAR-T cells targeting glioma-associated antigens, such as interleukin-13 receptor subunit alpha-2 (IL13-Rα2) [31], human epidermal growth factor receptor 2 (HER2) [32] and the epidermal growth factor receptor variant III (EGFRvIII) [33], showed the potential effectiveness and relative safety of the strategy.

Thus, recent studies have shown that locoregional administration of tandem CAR-T cells targeting concurrently two tumor antigens increases the effectiveness of the immunotherapy. However, additional randomized clinical trials of CAR-T cells are required to develop a promising strategy for GBM treatment [34].

Conclusion

Over the last years, IL-2-based therapies gained an especially high interest. This cytokine with antitumor activity is undergoing reincarnation after years of neglect. Novel strategies for the clinical use of IL-2 imply abandoning the high-dose immunotherapy, associated with pronounced adverse events, but using the immunostimulating doses of IL-2 in combination with other immunotropic agents. In particular, clinical studies found effective combinations of IL-2 for GBM treatment, such as the combination of ICI and oncolytic viruses. IL-2 has its respective place in the rapidly developing adoptive cell-based immunotherapy. This cytokine is an essential component for the production of activated killer cells, LAKs and CIKs, and is used in combination with these cellular products.

Locoregional IL2/LAK (CIK) therapy has demonstrated reliable clinical efficacy. CAR-T cells generated and expanded in the presence of IL-2 provide a promising new strategy in the GBM treatment. The encouraging results of yet a few clinical studies gave evidence that the presented approach can significantly improve GMB therapy.

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