Published in final edited form as:

Surg Oncol Clin N Am. 2007 October ; 16(4): 987–xii.

Current Immunotherapeutic Strategies for Central Nervous System Tumors

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Abstract

Immunotherapy has emerged as a promising tool in the management of malignant central nervous system (CNS) tumors. Despite the improvement in patient survival, traditional approaches - which consist mostly of surgery, radiotherapy, and chemotherapy - have been largely unsuccessful in permanently controlling these aggressive tumors. Immunotherapeutic strategies therefore offer not only a novel approach, but also an advantage in a way other modalities have been failing. Specifically, the capabilities of the immune system to recognize altered cells while leaving normal cells intact offer tremendous advantage over the conventional therapeutic approaches. This review summarizes our current understanding of immunotherapeutic treatment modalities employed in clinical trials for management of malignant CNS tumors.

Keywords

clinical trials; glioma; glioblastoma; immunotherapy; dendritic cells; cytokines; toxins

Immune system and the CNS

The immune system has the ability to recognize and destroy foreign cells via two separate modalities: innate and adaptive immunity. The innate component consists of macrophages, natural killer (NK) cells, monocytes, and granulocytes. These cells identify molecular patterns involved in cellular transformation and release various cytokines and inflammatory mediators. The innate response lacks the memory capability for foreign antigens, a feature present in adaptive immune response. This latter component of immune system also features specificity for foreign antigens, imparted by presence of receptors on lymphocytes. Antigen presenting cells (APCs) also play a role in the adaptive response – they engulf foreign antigens and present them to the lymphocytes in the context of major histocompatibility complex. CD4+ T cells

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bear receptors that recognize antigens in the context of MHC class II molecules, which then enables them to release cytokines and further activate CD8⁺ lymphocytes (CTLs) or B cells. CTLs are part of cell-mediated immunity and are capable of eliminating cells presented in the context of MHC class I molecules, via apoptosis or perforin-mediated cell lysis [1]. It is widely accepted that T-cell mediated immunity plays a vital role in the anti-tumor response, particularly when it comes to CNS tumors [2].

B cells are involved in release of immunoglobulins and as such are part of the humoral immune system. This latter system is not a significant part of immune response to CNS tumors, because antibodies largely interact with soluble antigens and therefore are not fully capable to respond to cytoplasmic tumor antigens. Additionally, immunoglobulins poorly penetrate the blood-brain barrier (BBB) [3].

The brain has historically been considered as an “immune privileged” site [4]. This theory was based on the fact that there is no organized lymphoid tissue or lymphatic drainage within the brain. Presence of the BBB and scarcity of MHC expression by neurons and astrocytes also lead to this assumption [1]. Such considerations alter the immune response in the CNS, however they do not render it completely immune privileged. It is now known that lymphocytes are present within the brain during infections and neoplastic processes, and glioma cells are capable of expressing a variety of tumor-associated antigens. Therefore, the immune-privileged state is overcome during such processes, and the immune responses within CNS are finely regulated. APCs are still necessary for the immune response to be mounted within the brain, although the exact mechanism of this process has not been fully elucidated.

The most likely APCs within the CNS are microglia [5], since they express MHC class I and II molecules, as well as other costimulatory factors [6]. Dendritic cells also play a part in antigen presentation within CNS, as demonstrated by primate studies of experimental allergic encephalomyelitis (EAE) [7]. Immune modulation within the brain with upregulation of MHC antigens takes place at sites of injury and after exposure to cytokines [8]. There is experimental evidence that human glioma cells express CD 95 and Fas ligand, which enables them to interact with T cells and initiate apoptosis [9,10]. These cells also present tumor-associated antigens, which are capable of producing immune responses

Additionally, it has been shown that brain tumor specific T cells are primed within cervical lymph nodes [11]. Despite the BBB, multiple types of lymphoid cells have been found within brain parenchyma during disease processes [12–14], which is possibly explained by the presence of pro-inflammatory cytokines affecting the BBB [15]. There is also evidence that astrocytes exert immunosuppressive function under normal conditions, which may produce anti-inflammatory state via repression of microglia [5,16].

Current strategies against malignant gliomas

Malignant gliomas have an intrinsic capability to evade the immune response. This is due to several factors. First, the level of MHC class I expression by gliomas is very low [17]. However, this process can be up-regulated by reaction to interferon- ($\text{IFN-}\gamma$) [8]. Second, certain receptors on natural killer cells enable them to lyse tumor cells which intrinsically express low levels of MHCs [18]. Glioma cells also express human leukocyte antigen (HLA)-G, which is a non-typical class I MHC molecule [19]. Expression of HLA-G has been shown to lessen the susceptibility of tumor cells to immune responses [20]. Gliomas can also affect immune system constituents via secretion of certain tumor factors. This results in dysregulation of APC function and TCR signaling [21,22]. Among the better characterized immunosuppressive factors identified to date, transforming growth factor beta 2 (TGF- β 2) is notably over-expressed in patients with glioblastoma multiforme (GBM) and as such has arisen as the most likely candidate for immunosuppressive properties of gliomas [23,24]. TGF- β 2 hinders the cytotoxic

response of glioma cells to lymphocytes, IL-2 receptor induction, NK cell activity, and cytokine production [25–27]. Another contributing factor to TGF- β 2's ability to suppress gliomas' immune response is via down-regulation of HLA-DR expression on tumor cells, which is an important MHC antigen [28]. TGF- β 2 may additionally promote growth of glioma cells resulting in angiogenesis and stromal growth [29]. TGF- β 2 antagonists are currently being investigated and have so far been demonstrated to inhibit proliferation of glioma cells. In addition to TGF- β 2, another factor may be responsible for HLA-DR down-regulation, prostaglandin E2 [30]. This cytokine is also capable of decreasing lymphokine activated killer (LAK) cell activity, thereby contributing to the ability of malignant gliomas to evade immune response [31].

Recent advances in research on antigen presentation, specific cytokines, and T-cell cytotoxicity have given new direction to immunotherapeutic strategies against CNS tumors. Evidence of CNS manifestations after peripheral vaccination with brain-specific antigens in EAE and paraneoplastic cerebellar degeneration models have also lead to a search for possible immunotherapy regimens for management of malignant gliomas [32]. Current directions in immunotherapy investigations include the use of various cytokines, dendritic cells, viral and peptide vaccines, and toxins.

Cytokines

Cytokines augment T cell activation and MHC antigen expression. Antitumor effects mediated by expression of cytokines in cancer models have been demonstrated with IL-2, IL-4, IL-12, IL-23, IFN- α , and GM-CSF [33–40]. Cytokine gene therapy for malignant glioma has been investigated as a tool for enhancement of T cell activity and APC activation. Pre-clinical studies using peripheral cytokine-secreting vaccines have been shown to be efficacious against glioma in animal models, thereby leading to investigation of these cytokines in human studies [41].

Dillman et al., performed a study using IL-2 to generate autologous LAK cells [42]. After generation of LAK, these cells were injected intra-tumorally. That trial included 40 patients with GBM. The patients were compared to historical controls. Besides a positive conclusion regarding the safety of this approach, a clinical benefit was documented. The median survival after the therapy was 9.0 months and the 1-year survival rate 34%. Nine patients survived beyond 1 year and three patients survived beyond 2 years. Control patients had a median survival of 13.6 months from their initial diagnosis, with a 2-year survival rate of 20%; for the 31 patients who underwent LAK cell placement during the course of their disease, median survival was 17.5 months and the 2-year survival rate was 31% ($p = 0.012$). However, other studies using LAK cells could not reproduce these encouraging outcomes, and one study reported considerable toxicity [42].

IL-2 has also been used in clinical studies with cytotoxic T lymphocytes [43]. Initially, five patients with recurrent glioma were treated with intracavitary CTLs and IL-2. Alloreactive CTLs were sensitized to the MHC proteins of the patients thereby offering selective destruction of glioma cells that express MHC. The treatment was well tolerated. The trial included two patients with GBM (both had subsequently died), two patients with anaplastic oligodendroglioma (no evidence of tumor at 28 and 30 months follow-up), and one patient with anaplastic astrocytoma (no evidence of tumor at 28 month). A larger phase I trial utilizing CTLs is currently in planning stages.

Colombo et al., described another trial using IL-2 as a transgene. In this case, the cytokine was tested in combination with HSV-tk transgene; the genes were carried by a retroviral virus on vector producing cells. Twelve patients with recurrent GBM were enrolled [44]. Treatment consisted of intra-tumoral injection of the construct followed by systemic administration of acyclovir. The treatment was well tolerated. Even though none of the patients achieved a

complete response, two had a partial response, as described below, four had a minor response, four had stable disease, and two patients had progressive disease. Of the two patients who showed a 50% reduction of mass size, one presented with a mass in the corpus callosum; this patient underwent stereotactic injection of the construct. After treatment, the patient experienced clinical improvement as well as regression of the tumor mass, demonstrated by magnetic resonance imaging (MRI) performed a month after completion of treatment. This patient died of pulmonary embolism 11 months after the treatment. The second patient that achieved partial response presented with GBM in the corpus callosum and a second small lesion in the left temporal lobe. The patient underwent intra-tumoral treatment into the corpus callosum lesion and subsequently experienced improvement of the neurological symptoms. Follow up by MRI showed regression not only of the injected mass, but also of the temporal lesion. Unfortunately, the patient showed tumor relapse and died 13 months after the therapy.

Okada et al., described a case where IL-4 was used in treatment of recurrent GBM [45]. The IL-4 transgene was expressed by transfected fibroblasts. The therapy consisted of the combination of transfected fibroblasts and irradiated autologous glioma cells injected intradermally. With regard to the immune response related to treatment, infiltration of dendritic cells (CD 1a+), CD4+, and CD8+ T cells was demonstrated at sites of injection. In fact, these cells increased proportionally to the amount of IL-4 produced at each of the injection sites. The patient demonstrated partial clinical and radiologic response as demonstrated by temporary symptomatic improvement and considerable resolution of enhancement and peritumoral edema relative to the pretreatment MRI. Treatment was well tolerated and the patient survived 10 months after initiation of this treatment.

IL-12 has also been tested in treatment of patients with gliomas. Kikuchi et al., conducted a study that included 15 patients with anaplastic astrocytoma (AA), anaplastic oligoastrocytoma (AOA), and GBM. Treatment consisted of subcutaneous administration of recombinant IL-12 in combination with a fusion of autologous dendritic and glioma cells [46]. In 2 patients, augmentation of CD8+ cytolytic activity on glioma cells was documented after treatment. With regards to clinical responses, four out of fifteen patients experienced deterioration in symptoms (three patients had worsening of the level of consciousness at the end of first course of vaccination and one patient's presenting symptom of hemiparesis worsened during the study). In these four patients, therapy was discontinued and steroids were administered. In the remaining 11 patients, clinical symptoms did not worsen during therapy. Radiologic findings showed that four patients had more than 50% reduction in size of tumors as documented by follow up MRI. This clinical trial therefore demonstrated that using a fusion product of dendritic and glioma cells with IL-12 resulted in no serious side effects and measurable radiographic response in four tumors.

IFN- α was tested in a phase III clinical trial for patients with high grade gliomas [47]. The study included 360 patients with AA, anaplastic AO, GBM and gliosarcoma. Of the 360 patients, only 214 met the eligibility criteria for further evaluation. The patients were initially treated with radiation and BCNU (β -chloro-nitrosourea, carmustine). Following radiotherapy, patients without disease progression were stratified and randomized into two groups: BCNU, and BCNU plus INF- α . Unfortunately, there was no difference in response rates between the two treatment arms in the final evaluation [47].

Table 1 summarizes the most recent clinical trials employing various cytokine based regimens against CNS tumors. More studies utilizing various cytokine-based protocols are currently underway, and given the encouraging results of animal studies, the hope for such approaches is appealing.

Dendritic cells

Dendritic cells (DCs) are hematopoietically derived leukocytes involved in immune surveillance [48,49]. They are effective antigen presenting cells, and as such have been the target of investigation for possible anti-glioma treatment strategies. Under normal conditions, DCs are absent from the brain parenchyma; they are however found within CNS under inflammatory conditions, most likely attracted by specific chemokines released by tissue astrocytes and microglia [50].

There are several issues which need to be resolved prior to large-scale clinical use of dendritic cells. First, appropriate source of DCs needs to be elucidated, whether it is direct isolation from patient-derived tumor antigens or from *in vitro*-derived dendritic cells. Second, mature and immature DCs have different antitumor properties, and optimal level of maturity is still not clear [51]. DC maturation is enhanced by addition of TNF- α [52] and clinical trials have shown that mature DCs are better at generating CTL responses in malignant melanoma [53]. Antigen-loading strategies present a third topic of investigation. Possible options include tumor-derived mRNA [54,55], tumor cell lysates [56–58], and whole tumor peptides [59]. Finally, optimal route of administration, and co-administration of cytokines, are further questions that arise with the use of DCs. Intravenous injection results in movement of DCs to reticular organs, while intradermal administration leads to homing of DCs to local lymph nodes [60]. Both routes can be beneficial, as the former results in Th2 activation of IL-10 and antibodies, while the latter results in Th1 activation of IL-2 [61,62]. Adjuvant administration of cytokines, such as IL-12 [63] and IFN- α [64], has shown promising results in animal experiments, and is currently a topic of investigation for human trials.

Genetically engineered DCs are the next venue of research [65]. This strategy involves introduction of genes encoding tumor antigens or certain cytokines into DCs [66]. Candidate genes include GM-CSF, IFN- α , IL-12, and IL-18, since transfection with these cytokine genes is expected to enhance the function of DCs.

The use of dendritic cells for treatment of brain tumors has also been tested in human trials. As described above, one of the treatments tested was the injection of a fusion product of dendritic cells and autologous glioma cells [52]. Fusion cells were injected intradermally close to a cervical lymph node in 8 patients with malignant gliomas. All patients received these cells every three weeks for a minimum of 3 and a maximum of 7 immunizations. With regards to the antitumoral immune response, five patients were assessed; in 4 out of 5 patients, immunization led to a modest rise of CD16 and CD56 positive cells in peripheral blood lymphocytes. In addition, peripheral blood mononuclear cells were incubated with irradiated autologous glioma and supernatants were harvested. In 6 cases analyzed, the concentration of interferon-gamma (IFN- γ) in the supernatant increased after immunization. Clinical results showed that there were no serious adverse effects and two partial responses [52].

Dendritic cells have also been injected after exposure to tumoral antigens ex-vivo. The rationale for this strategy was to promote tumoral antigenic presentation in order to trigger cellular immune response. DCs pulsed with peptides from patient's tumors have been tested in trials for malignant gliomas [67,68]. One of these trials evaluated 5 patients with GBM and AA [67]. In this study, dendritic cells were injected intradermally and intracranially. The mean number of vaccinations was 3.7 times intradermally (close to a cervical lymph node), and 3.2 times intratumorally via an Ommaya reservoir. With regards to the mounting of antitumoral response, the number of CD56+ cells in blood increased after treatment. Moreover, T-cell-mediated antitumor activity was also enhanced, as evaluated by the ELISPOT assay after vaccination, in two of five tested patients. Three patients showed delayed-type hypersensitivity reactivity to the autologous tumor lysate, two patients had a minor clinical response, and two had an increased ELISPOT result. Intratumoral CD4+ and CD8+ T-cell infiltration was

detected in two patients who underwent reoperation after vaccination. On the other hand, radiographic outcomes included two minor responses and four cases where no change was appreciated [67].

Another study included 9 patients with GBM and AA who were treated with intradermal vaccinations of peptide-pulsed dendritic cells [68]. The peptides were obtained by eluting autologous glioma cells. In the case of this study, the treatment was administered biweekly. Treatment related immune responses included systemic cytotoxicity in four of seven tested patients. Robust intratumoral cytotoxic and memory T-cell infiltration was detected in two of four patients who underwent reoperation after vaccination. With regards to clinical results, the study demonstrated this treatment regimen to be safe, as no major side effects were observed [68]. Increase in survival was also demonstrated; median survival times for the study and control groups were 455 and 257 days, respectively [68].

Table 2 summarizes current clinical trial using DC-based approaches for CNS tumors [52, 67–69]. Partial responses were observed in some trials, while two of the trials were also able to demonstrate increased survival. Dendritic cell based approach is clearly a very promising strategy in immunotherapeutic modalities against gliomas.

Viral vectors and peptide vaccines

Several viral vectors have shown promising results with regard to their potential to enhance immunotherapy of malignant gliomas. Replication competent and replication incompetent viruses have been used, with the latter group demonstrating more effective transgene expression. Herpes virus, adenovirus, vaccinia, reovirus, and New Castle Disease viruses have been used in various clinical studies. As an augmentation tool for immunotherapy, viral vectors have been utilized to deliver certain cytokines to brain tumors [70–72].

HSV/IL-4 combination was shown to augment the oncolytic effect of the virus, when compared to HSV used alone in animal models [73]. Such beneficial effects were also observed in studies of HSV with IL-12, IL-2, and TNF- α [74,75]. Human studies are needed to confirm these encouraging combination regimens for targeting malignant gliomas.

The amplification of epidermal growth factor receptor (EGFR) gene is present in approximately 40% of gliomas, and is most frequently associated with EGFRvIII (variant III) gene rearrangement. Attenuated measles vaccines displaying EGFRvIII have potent antitumor activity in GBM, while having no significant toxicity on normal cells [76]. Investigations are ongoing exploring efficacy of EGFRvIII-retargeted oncolytic measles virus vaccines in human trials.

Of note, the EGFRvIII peptide has also been used as an antigen to elicit antitumoral immune response. A phase I study was conducted with this peptide as a vaccine (study presented at the American Society of Clinical Oncology Meeting 2006). This trial consisted in vaccinating 12 newly diagnosed glioblastoma multiforme patients with an EGFRvIII peptide vaccine. The study demonstrated minimal toxicity and showed that this vaccine generates cellular as well as humoral immune response against this peptide. With respect to clinical outcome, median time to progression from surgery for vaccinated patients was 12 months, suggesting a therapeutic benefit when compared to that of historical matched control cohort that had a median time to progression of 7.1 months (n=39) (p=0.0058). Interestingly, among the recurrent tumors that were evaluated by immunohistochemistry, 100% lacked expression of EGFRvIII, suggesting that treatment might have eliminated EGFRvIII + cells.

Peptide vaccines directed at IL-13 receptor alpha-2 (IL-13Ralpha2) have been investigated as potential tool against glioma cells [77]. These receptors are highly expressed in majority of

malignant gliomas, which makes them an attractive target for vaccination. Pre-clinical studies have found that vaccines containing IL-13 α 2 are highly immunogenic and are effective at expanding tumor reactive CTLs [78].

Toxins

Convection-enhanced delivery of toxins has been the topic of extensive and encouraging research in glioma therapies. The technique implies administration of fusion proteins under image guidance with the aid of positive pressure microinfusion [79,80]. Currently investigated toxins include bacteria-derived products, such as diphtheria or pseudomonas exotoxin. These are fused to a carrier ligand that can recognize a tumor cell-surface marker [81]. The rationale for selecting a specific cytokine derives from the fact that certain cytokine receptors are over expressed in brain tumors, thus, the toxic complex preferentially binds to tumor cells.

A dose-escalation trial of intratumoral administration of IL-4 Pseudomonas exotoxin (NBI-3001) in patients with recurrent malignant glioma was conducted. Thirty one patients with grades 3 and 4 astrocytoma were studied (25 with GBM, 6 with AA). Patients were assigned to one of four dose groups. NBI-3001 was administered via convection-enhanced delivery intratumorally using stereotactically placed catheters. In this study no drug-related systemic toxicity was noted. Nevertheless, some central nervous system related adverse effects were present. Grade 3 or 4 toxicity was seen in 39% of patients in all dose groups and 22% of patients at the maximum tolerated dose; no deaths were attributable to treatment. With regards to clinical efficacy, median survival was 8.2 months overall, with a median survival of 5.8 months for the GBM patients. Six-month survival was 52% and 48%, respectively. Post-treatment MRI showed decreased signal intensity within the tumor consistent with tumor necrosis in many patients [82]. Nevertheless, neurotoxicity of this agent is a concern that needs to be addressed. A case report of a patient with recurrent GBM that followed the same protocol from this study was published in 2004 [83]. The maximal dose was used. The agent was injected intracranially by high flow bulk infusion. Side effects included seizures, clinical signs of meningeal reaction, hydrocephalus, increase in motor deficits, a severely impaired vision on both eyes and bilateral hearing loss [82]. On the other hand, the patient had follow-up MRI 4 weeks after the toxin infusion that demonstrated necrosis with shrinkage of tumor; months later, the tumor had shrunk and almost disappeared, leaving less than 5% residual contrast enhancement surrounded by scar. Follow-up MRI confirmed the durable tumor response. Three years after the toxin infusion, the patient presented with a local recurrence and died 4 months later. In spite of toxicity, this patient survived 3 years after treatment with this toxin [82].

Another study investigated the safety and activity of directly infusing IL-4(38–37)-PE38KDEL, a chimeric protein composed of circularly permuted IL-4 and a truncated form of Pseudomonas exotoxin (PE), into recurrent malignant high-grade gliomas [84]. In this case, IL-4-toxin complex was infused over a 4 to 8 day period into gliomas of nine patients via one to three stereotactically placed catheters. No apparent systemic toxicity occurred in any patient. The infusion of IL-4-toxin in six of nine patients showed glioma necrosis as evidenced by diminished enhancement on MRI. On the other hand, seven of nine patients underwent craniotomy because of increased intracranial pressure after the beginning of infusion. In six of these seven patients, partial-to-extensive tumor necrosis with edema was confirmed pathologically. Local toxicity seemed attributable mainly to tumor necrosis or occasionally to the volume of infusion. With regards to the two patients who did not undergo surgery, MRI showed gadolinium enhancement in one, while the other patient showed extensive necrosis of tumor leading to complete remission; this patient remained disease-free 18 months after the procedure [84].

Some studies have tested the ability of IL-13 to target toxins into gliomas [85]. This strategy is based on taking advantage of the over expression of IL-13 α 2 receptor on these tumors

[86]. IL13PE38QQR is a recombinant toxin composed of the enzymatically-active portion of Pseudomonas Exotoxin A conjugated with human IL-13. A report on preliminary results from three phase I studies that tested this strategy was published [85]. These studies were done on 46 patients with recurrent malignant gliomas. The trials suggest that intratumoral infusion with or without resection is fairly well-tolerated. Post-resection infusion into the peritumoral brain parenchyma also appears to be very well tolerated. Histopathological tumor effect was seen at drug concentrations of 0.5–2.0 microg/mL. Nonetheless, a phase III study of IL-13 and Pseudomonas toxin was recently performed and it showed no therapeutic benefit of this system.

The fusion protein TGF- α /pseudomonas (transforming growth factor- α) exotoxin was demonstrated to bind EGFR on human glioma cells. This construct, TP-38, was tested in one patient with recurrent bifrontal GBM that was refractory to other treatments [87]. The treatment was administered via two multiport pediatric ventricular catheters placed stereotactically into the left and right frontal cortices, respectively. Twenty milliliters of TP-38 were infused through each catheter. The TP-38 concentration was 25 ng/ml, resulting in a total delivered TP-38 dose of 1 mg. No significant increases in intracranial pressure were observed throughout the duration of the infusion. The patient tolerated the TP-38 infusion without complication and was discharged. After therapy, she improved clinically, was weaned off steroids and anticonvulsants, and experienced a progressive decrease in enhancing tumor volume. To the time of publication the patient remained without progression for 43 months after TP-38 therapy. The results of this case remain to be corroborated in further studies, but the clinical response as well as the tolerability of this treatment are encouraging [87].

Table 3 presents a summary of recent clinical trials using cytokine/toxin constructs.

Future Directions

As a result of enormous progress in tumor immunobiology, immunotherapeutic approaches to treatment of CNS tumors have entered an assertive and exciting phase of research. Many novel strategies are currently being investigated in human trials, and have already been found safe and efficacious in preliminary studies. Dendritic cell-based approaches appear promising for increasing survival in patients with glioma. Peptide vaccine and various cytokine based protocols are currently in different stages of investigation. Development of effective immunotherapy protocols is the goal of current research. The progress is being hampered by our lack of full understanding of glioma-specific antigens, the heterogeneity of tumor cells, and the poor understanding of the exact mechanisms of glioma-induced immunosuppression. The potential for immunotherapeutic strategies in treatment of CNS tumors, as an adjunct to the current regimens, or as a stand-alone approach, is tangible; however, the challenges of applying our knowledge of laboratory research to patient care remain real and formidable.

Acknowledgements

Support: This work was supported by a grant from the National Institutes of Neurological Disorders and Stroke (K08 NS046430, ML), and the Elsa U. Pardee Foundation (ML).

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Table 1
Current clinical trials in cytokine therapies for CNS tumors

Cytokine / Cell Used	Study Description	Route of administration	n	Tumor	Outcome	Ref.
IL-2 / HSV-tk transgenes on retroviral vector-producing cells (RVPCs) and ganciclovir	Phase II	Intratumoral	12	Recurrent GBM	Safety was demonstrated. Tumor responses in 50% of cases. Intratumoral and plasma Th1 cytokine levels were noted.	[44]
IL-2 generated LAK cells	Phase I	Intratumoral	40	GBM	Safety was demonstrated; median survival increased	[42]
IL-2 with CTLs	Phase I	Intratumoral	5	GBM, AOA, AA	Mild toxicity	[Kushen et al., 2008]
IL-4 expressed by transduced fibroblasts / irradiated autologous glioma cells	Phase I	Intradermal	1	Recurrent GBM	Infiltration of CD4, CD8 and CD1a cells at injection sites.	
IL-12 / Fusion of dendritic and glioma cells	Phase I	Subcutaneous	15	Malignant gliomas (GBM, AA, AOA)	10 months survival after treatment	[46]
IFN- α / BCNU and radiation	Phase III	Subcutaneous	275	AA, anaplastic AO, GBM and gliosarcoma	No serious adverse effects were observed. In four patients, MRI showed a greater than 50% reduction in tumor size	[47]
					Patients receiving IFN- α experienced more fever, chills, myalgias, and neurocortical symptoms including somnolence, confusion, and exacerbation of neurologic deficits. There was no significant difference with regard to time to disease progression or overall survival between the two groups.	

Abbreviations: AA: anaplastic astrocytoma; AO: Anaplastic oligodendroglioma; AOA: anaplastic oligoastrocytoma; BCNU: β -chloro-nitrosourea (carmustine); CTL: cytotoxic T lymphocytes; GBM: glioblastoma multiforme; HSV-tk: herpes simplex virus-thymidine kinase; IL: interleukin; LAK: lymphokine-activated killer cells; MRI: magnetic resonance imaging; n: number of patients in the study.

Table 2
Current clinical trials using dendritic cell vaccines for CNS tumors

Cell / vaccination strategy	Study Description	Route of administration	n	Tumor	Outcome	Ref.
Fusions of dendritic and glioma cells	Phase I	Intradermal	8	Malignant gliomas	No serious adverse effects. Two partial responses.	[52]
DCs pulsed with tumor lysate	Phase I/II	Intradermal and intracranial	5	GBM, AA	Treatment was well tolerated. There were two minor responses and four no-change cases evaluated by radiological findings. T-cell-mediated anti-tumor activity in two of five tested patients. Three patients showed delayed-type hypersensitivity reactivity to the autologous tumor lysate, two of these had a minor clinical response.	[67]
DCs with peptide eluted from tumor cells	Phase I	Intradermal	9	GBM, AA	Evidence of intratumoral infiltration; increased survival	[68]

Abbreviations: AA: anaplastic astrocytoma; GBM: glioblastoma multiforme; DC: dendritic cells.

Table 3
Current clinical trials in toxin therapies for CNS tumors

Ligand / Toxin	Study Description by authors	Route of administration	n	Tumor	Outcome	Ref.
IL-4 / Pseudomonas toxin	Case report	Intracranial	1	Recurrent GBM	There were some permanent neurological side effects resulting from toxin infusion. Survived 3 years after toxin infusion	[82]
IL-4 / Pseudomonas toxin	Open-label, dose-escalation trial	Intracranial	31	Recurrent malignant glioma, (grade 3 and 4 astrocytoma)	Acceptable safety and toxicity: No drug-related systemic toxicity/ Treatment-related neurotoxicity.	[83,88]
IL-4 / Pseudomonas toxin	Phase I	Intracranial	9	Recurrent GBM	Occasional increase in intracranial pressure attributed to volume of infusion, otherwise well tolerated	[84]
IL 13/ Pseudomonas toxin	Phase I	Intracranial	46	Recurrent malignant glioma	Well-tolerated with corticosteroids prophylaxis. Occasional prolongation of survival times have been observed	[85]
TGF- α / Pseudomonas toxin	Case report	Intracranial	1	Recurrent GBM	Clinical improvement, tumor has not progressed for >43 months	[87]

Abbreviations: GBM: glioblastoma multiforme; IL: interleukin; TGF: transforming growth factor.