Immunotherapy and biological modifiers for the treatment of malignant brain tumors

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The relative ineffectiveness of current therapies for malignant gliomas has led to the need for novel therapeutics. Therapies based on biologic modifiers are among a variety of cancer treatments currently in use or under experimental evaluation and have shown great promise, especially since several potent stimulators of the immune system have been cloned and are now available for clinical use. Early attempts at glioma therapy based on biologic modifiers, however, have failed to demonstrate significant effectiveness. In this review, we select and summarize the results of preclinical and clinical studies published during the past two years that focus on immunotherapy and biologic modifiers for treating gliomas. Despite limited clinical success, we conclude that an increased understanding of molecular biology and immunology from recent studies may pave the way for more effective approaches. Curr Opin Oncol 15:204–208

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Abbreviations

APCs	antigen presenting cells
BBB	blood–brain barrier
CIITA	class II transactivator
CNS	central nervous system
hTERT	human telomerase reverse transcriptase
iNOS	inducible nitric oxide synthase
LAKs	Lymphokine-Activated Killer cells
MHC II	major histocompatibility complex
PBMC	peripheral blood mononuclear cells
TGF	transforming growth factor

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Introduction

Malignant gliomas are the most commonly diagnosed primary brain tumors, with 16,800 new cases reported each year in the United States and 13,100 deaths [1]. Despite aggressive surgical resection and external beam irradiation, the median survival time for these patients is less than one year [2,3]. The addition of systemic chemotherapy may improve survival slightly, but such chemotherapy is associated with significant systemic toxicity. The relative ineffectiveness of current therapies for malignant gliomas has led to a widespread search for novel therapeutics.

Among the cancer treatments currently in use or under experimental evaluation, advances in molecular biology have spurred intense interest in strategies aiming at using the body's own defenses to fight cancer especially as many potent biologic modifiers have been cloned and are now available for use. By enhancing normal biologic processes, biologic modifiers can change the environment of cancerous cells and lead them to death or they can trigger the body's immune response toward cancer cells. The role of the immune system in the response to CNS tumors, however, still remains unclear.

It is well known that the blood–brain barrier (BBB) is capable of both limiting antigen escape from and lymphocyte entry into the CNS. For many years it was believed that this barrier and the limited availability of antigen presenting cells (APCs) actively prevented any interaction between the immune system and the brain parenchyma. In recent years, however, the results of several studies indicate that the immune system may play an important role in the body's natural response to brain tumors. The breakdown of the BBB that occurs with both malignant and metastatic brain tumors clearly causes infiltration of inflammatory cells within the proximity of the tumor. Moreover, there is evidence that activated T cells can cross the BBB and enter the CNS [4]. The identification of tight connections between the CNS and the immune system through cervical lymphatics has further demonstrated that both the afferent and efferent arms of the immune system are functional and that passage of lymphocytes into the CNS can occur via expression of specific adhesion molecules [5,6]. Taken as a whole, the results of these studies clearly indicate that the immune system plays an important although not fully understood role in the response to brain tumors.

In the present review, we select and summarize the results of preclinical and clinical studies published during 2001 and 2002 that focus on immunotherapy and biologic modifiers for treating gliomas.

Biologic findings

Cytokines

Cytokines are nonantibody proteins secreted by inflammatory leukocytes (and some nonleukocytic cells) that act as intercellular mediators, most often locally in a paracrine or autocrine fashion rather than an endocrine fashion. Cytokines play important roles in lymphocyte activation. Recent observations have strengthened the idea that the immune system's failure to recognize tumor cells may be attributed to tumor-associated cytokine dysregulation. Different T helper (Th) lymphocyte subsets secrete cytokines whose properties vary with the nature of the immune response generated. Th1 cytokine class, such as interferon γ (IFN- γ), interleukin-1 (IL-1), IL-2, IL-12, IL-15, lymphotoxin (LT), and tumor necrosis factor-α (TNF-α) promote cell-mediated immune responses and are capable of exerting antitumor effects. A recent study showed that high levels of the proinflammatory cytokine IL-1β within glioblastomas are associated with prolonged survival, suggesting that this cytokine is capable of suppressing tumor growth [7]. Moreover, a number of studies have shown that local delivery of the cytokines IL-2, IL-12, and GM-CSF can generate an antitumor immune response and, consequently, suppress tumor growth even within the CNS [8–18].

Th2 class cytokines, such as IL-4, IL-6, IL-9, IL-10, and IL-13 stimulate humeral immune responses and thus down-regulate tumor-specific immunity. Other cytokines, belonging to the Th3 class and including members of the transforming growth factor (TGF) family, are strongly immunosuppressive.

The well-known, immune-suppressive intratumoral milieu of gliomas is partially owing to the action of cytokines. Malignant gliomas appear to secrete Th2 (IL-6, IL-10) and Th3 (transforming growth factors) cytokines whose immunosuppressive properties may abrogate cytotoxic anti-tumor immune responses [19–21]. A recent study confirmed the dominant expression of Th2/Th3 cytokines in human glioblastomas. This dominant expression accounts for the "immunosuppressive status" of these tumors [22]. Type beta transforming growth factor (TGF-β) is produced by human GBM cell lines and TGF-β mRNA has been demonstrated in experimental rat glioma cells *in situ* [23]. TGF-β appears to be involved in the suppression of antitumor immune surveillance and the regulation of angiogenesis in anaplastic gliomas [24]. Finally, survival is prolonged in glioblastoma patients with low levels of intratumoral TGF-β2 [7].

The role of the pleiotropic cytokine IL-6 in glioma progression is controversial. Under physiologic conditions, IL-6 expression within the CNS is very low but becomes significantly elevated in pathologic conditions, including brain tumors. IL-6 has been recognized as a potent autocrine growth factor in human glioblastoma multiform cell lines [21]. A recent study showed that glioblastomas display significantly higher IL-6 levels compared with lower-grade gliomas, suggesting that IL-6 overexpression is a marker of malignancy [25]. More recently, it has been reported that the expression of IL6 may correlate with resistance to ionizing radiation [26]. However, T9 glioblastoma cells genetically modified to express IL-6 are less tumorigenic when implanted into the brains of syngeneic animals, presumably due to a neutrophil-dependent antitumor response [27]. Recently, an *in vitro* study demonstrated that the Fas-FasL system induces IL-6 expression by CRT-MG glioma cells in a time- and dose-dependent manner [28]. In addition, glioblastoma samples were found to contain high levels of IL-6, which correlates with increased Fas levels [28].

Interferons

Interferons have held great promise in preclinical glioma studies. IFN-β exerts its antitumor effects by inhibiting glioma growth in S-phase, enhancing NK cell and cytotoxic T-cell activity, and synergizing with chemotherapeutic agents such as nitrosoureas. An additional mechanism relies on the enhancement of macrophage cytotoxicity toward glioma cells. Recent studies have demonstrated that macrophage activation is mediated by inducible nitric oxide synthase (iNOS) induction and the production of nitric oxide (NO)[29,30]. A recent study has also shown that there is a significant increase of iNOS expression and NO production at the immunization site and within brain tumors of rats immunized with rat glioma cells engineered to produce IFN-γ [31]. However, due to its pleiotropic effects, the full role of NO in the immune response is not completely understood.

Microglia and MHC II class molecules

In both human and experimental gliomas, a high proportion of macrophage/microglia cells reside around or within the tumor. Many such phagocytes are presumably derived from intrinsic microglia, but the exact role of these cells within gliomas has become very controversial. Microglia cells are considered the main immune effector cell population within the CNS. They are the primary cells capable of antigen presentation to T cells patrolling the CNS [32••]. This function, however, appears to be compromised by gliomas partially because of a down-regulation of the major histocompatibility complex (MHC II) class molecule presentation by microglia cells. Glioma culture supernatants are capable of down-regulating MHC II expression, but this can be reversed by anti-IL-10 antibodies and indomethacin. Thus, gliomas appear to be capable of suppressing the competence

of microglial-specific antigen presentation [32••]. Moreover, microglia have been recently identified as a major source of FasL expression suggesting, given the proposed role of FasL on immune escape, an additional contribution to the local immune suppressive status of gliomas [33]. Additional evidence suggests that microglia may support tumor cell migration and infiltration [34].

Malignant glioma cells also express MHCII class molecules suggesting that they may be capable direct antigen presentation to CD4+ T cells. Soos *et al.* [35] recently demonstrated that glioma cell stimulation by IFN-γ up-regulates MHC II expression through the activation of the MHC class II transactivator (CIITA), the key intermediate that controls class II expression. They also found that glioma cells can process native antigens for presentation to CD4 (+) MHC class II-restricted Th1 cells [35]. Thus, CIITA may be a key target for future malignant glioma therapeutics.

Telomerase

Telomerase, a ribonucleoprotein enzyme, is another potential target for cancer treatment. Telomerase activity within malignant gliomas is greatly increased, while expression in normal brain is nearly absent. Telomerase expression is tightly regulated at the transcriptional level by human telomerase reverse transcriptase (hTERT). Two different approaches to telomerase-based therapies have been proposed [36••]. One approach involves the direct inhibition of the telomerase activity, which indirectly leads to apoptosis. The other aims at targeting telomerase-expressing cells to selectively kill tumor cells. This strategy includes, among others, immunotherapy as well as gene therapy transfer of suicide genes under the control of the hTERT promoter [37–40].

Immunotherapy

Many immunologic approaches have been proposed for treating gliomas. The advent of recombinant DNA techniques first opened the doors to nonspecific immune therapy strategy via the administration of cytokines. These immune adjuvants have been administered systemically to boost the immune response in patients harboring brain tumors. A recent study has determined both the safety and efficacy of high-dose intravenous recombinant IL-2 therapy for treating patients with brain metastases from renal cell carcinoma and melanoma. Treatment, however, was associated with significant dose-related adverse effects, which must be monitored closely. Nevertheless, this study has determined that selected patients can safely receive high-dose IL-2 therapy and have a clinically significant response to the treatment [41]. Systemic administration of cytokines for the treatment of primary brain tumors, however, has not produced similar results. Conversely, direct local injection often results in considerable toxicity. For instance, intrathecal administration of IFN-α in a group of patients affected by neoplastic meningitis showed modest therapeutic results associated with considerable toxicity [42].

In an attempt to more closely mimic their natural bioactivity, cytokines are generally delivered locally either by implanting genetically transduced cells or by using *in vivo* gene transfer techniques. Genetically engineered cells acting as a sort of biologic pump could deliver biologic modifiers in a paracrine fashion. Intratumorally injected allogeneic fibroblasts genetically engineered to secrete IL-2 were effective in the treatment of established primary and metastatic brain tumors in mice [43,44]. INF-β has been successfully delivered to the CNS by using bone marrow cells [45]. Most exciting is the current work with neural stem cells. Recently, neural stem cells were successfully engineered to deliver IL-12 [46]. Given the highly tropism for glioma cells displayed by neural stem cells, this vehicle represents an appealing modality of tracking infiltrating tumor cells [47,48].

Tumor cells can be genetically engineered using either *in vivo* or *ex vivo* gene transfer techniques to express not only cytokines but also co-stimulatory molecules (B7-1, B7-2) or MHC molecules. In addition, tumor cells can be genetically modified to produce antisense molecules against glioma-derived immunosuppressive factors such as TGF-β. Tumor cells *ex vivo* transfected to express antitumor biologic modifiers must be re-injected into the brain. This strategy combines the potential of the vaccination (active immunotherapy) with the actions exerted by the biologic modifier. The use of tumor cells however, raises obvious concerns about the possibility of introducing active tumor. To address this issue, neoplastic cells after gene transfection undergo irradiation thereby inhibiting any further cell division and the onset of secondary tumors. Furthermore, irradiation (200 Gy) of tumor cells genetically transduced to express either GM-CSF, or IL-12, or B7-2 molecule does not appear to alter therapeutic gene expression [49]. On the other hand, most cells are not viable by the tenth day after irradiation, suggesting the possible need for multiple vaccinations [49].

Although highly effective, the above strategies often fail to eradicate established tumors. Inadequate antigen presentation is one of the proposed mechanisms underlying incomplete tumor eradication obtained by cytokine-based immunotherapy. To facilitate antigen presentation, Iwadate *et al.* [50] proposed a strategy based on subcutaneous vaccination with replication-incompetent tumor cells and local transplantation of IL-2 secreting cells at the tumor site. This combinatory strategy has proven effective in eliminating established brain tumors. In their experiments, locally delivered IL-2 from either genetically engineered 9L cells or xenogenic neuroblastoma cells seems to act as a chemo-attractant for activated T cells with tumor-specific cytotoxicity. On the

other hand, glioma cell exposure to professional antigen-presenting cells such as the dendritic cells, which are abundant in subcutaneous tissues, is essential in priming effector T cells. The up-regulation of MHC class I antigen may also be involved in this mechanism [50].

Many practical considerations, however, may ultimately prevent the translation of gene therapy strategies for the local delivery of cytokines or other biologic modifiers into clinical use. Indeed, the *in vitro* gene-transduction process is not uniformly successful and can require up to several months to perform successfully. Transduced cells must then be selected and re-implanted, necessitating a second surgical operation. Furthermore, *in vivo* gene therapy models utilizing viral vectors encoding cytokine genes are limited by the low efficiency of *in vivo* gene transfer. Therefore an alternative approach have been developed based on the controlled local release of cytokines using synthetic, biodegradable polymers. Hanes *et al.* [51] have developed a method to encapsulate IL-2 into microspheres composed of gelatin and chondroitin-6-sulfate. This polymeric system has proven to be capable of delivering active recombinant IL-2 in a controlled fashion following intratumoral injection for up to 21 days *in vivo*. IL-2 releasing microspheres have also shown to be at least equally effective in treating animals bearing either primary or metastatic brain tumors as IL-2 transduced tumor cells. The process of manufacturing IL-2 microspheres is relatively easy and may provide a more controlled product as compared to genetically engineered cells. The use of a synthetic system should greatly facilitate the eventual translation of cytokine-based immunotherapeutic approaches into the clinical field.

Passive immunotherapy is based on the administration of antibodies directed against tumor-specific antigens. Preclinical studies have identified several glioma antigens and consequently several specific monoclonal antibodies have been produced. These antibodies can be conjugated with drug, toxin, or radioisotope. Grana *et al.* [52] have described an interesting three-step radioimmunotherapy method as an alternative to the intralesional injection of radiolabeled antitenascin monoclonal antibodies previously proposed by Riva *et al.* [53–55]. This system exploits the high affinity of the avidin-biotin complex. In the first step, the patient is injected with biotinylated monoclonal antibodies against tenascin. In the second step, streptavidin is injected and binds to the biotinylated antibodies over the tumor surfaces. In the third step, radiolabeled Yttrium-90 biotin is injected to target streptavidin molecules. Preliminary clinical observations using this method have shown encouraging results without major side effects [52]. According to the authors, this system has the advantage of decreasing the load of targeting monoclonal antibodies, while at the same time increasing the specific delivery of the radioactive agents to the target.

Adoptive immunotherapy is based on effector cell targeting. Autologous lymphocytes, derived from peripheral blood or from the tumor, are pulsed either with IL-2 (Lymphokine-Activated Killer cells or LAKs) or with autologous tumor cells and then re-infused intravenously or intratumorally. Recently, there has been great interest in using adoptive dendritic cells derived from peripheral blood and pulsed *in vitro* with relevant tumor antigens to generate CTL response that is tumor specific [56]. In an interesting study, Yoshida *et al.* [57] generated mature dendritic cells from peripheral blood mononuclear cells (PBMC) of patients affected by a variety of malignant brain tumors using GM-CSF, IL-4, and TNF-α. Dendritic cells were then pulsed with autologous tumor lysate prepared from freshly resected brain tumors. After pulsation with tumor lysate, dendritic cells were shown to induce cytotoxic T lymphocytes, and produce a strong and specific antitumor effect [57]. In a recent clinical phase I trial, dendritic cells from patients bearing high-grade gliomas were pulsed with peptides eluted from the surface of autologous tumor cells. Patients subsequently underwent biweekly intradermal injection of peptide pulsed dendritic cells. This technique elicited systemic cytotoxicity in four of seven patients. In two patients who underwent re-operation, a strong cytotoxic and memory-T cell infiltration was detected in the tumor bed. This study demonstrated the safety and potential bioactivity of this novel approach [58].

References

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • Of special interest
- •• Of outstanding interest

1 Legler JM, Ries LA, Smith MA, et al.: Cancer surveillance series [corrected]: brain and other central nervous system cancers: recent trends in incidence and mortality. J Natl Cancer Inst 1999, 91:1382–1390.

2 Barker FG, 2nd, Chang SM, Gutin PH, et al.: Survival and functional status after resection of recurrent glioblastoma multiforme. Neurosurgery 1998, 42:709–720; discussion 720–723.

3 Mohan DS, Suh JH, Phan JL, et al.: Outcome in elderly patients undergoing definitive surgery and radiation therapy for supratentorial glioblastoma multiforme at a tertiary care institution. Int J Radiat Oncol Biol Phys 1998, 42:981–987.

4 Trojan J, Johnson TR, Rudin SD, et al.: Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I RNA. Science 1993, 259:94–97.

5 Cserr HF, Knopf PM: Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. Immunol Today 1992, 13:507–512.

6 Weller RO, Engelhardt B, Phillips MJ: Lymphocyte targeting of the central nervous system: a review of afferent and efferent CNS-immune pathways. Brain Pathol 1996, 6:275–288.

7 Cuny E, Loiseau H, Penchet G, et al.: Association of elevated glial expression of interleukin-1beta with improved survival in patients with glioblastomas multiforme. J Neurosurg 2002, 96:294–301.

8 Andreansky S, He B, van Cott J, et al.: Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. Gene Ther 1998, 5:121–130.

9 Benedetti S, Bruzzone MG, Pollo B, et al.: Eradication of rat malignant gliomas by retroviral-mediated, in vivo delivery of the interleukin 4 gene. Cancer Res 1999, 59:645–652.

10 DiMeco F, Rhines L, Hanes J, et al.: Paracrine delivery of interleukin-12 against intracranial 9L gliosarcoma in rats. J Neurosurg 2000, 92:419–427.

11 Ewend MG, Thompson RC, Anderson R, et al.: Intracranial paracrine interleukin-2 therapy stimulates prolonged antitumor immunity that extends outside the central nervous system. J Immunother 2000, 23:438–448.

12 Giezeman-Smits KM, Okada H, Brissette-Storkus CS, et al.: Cytokine gene therapy of gliomas: induction of reactive CD4+ T cells by interleukin-4-transfected 9L gliosarcoma is essential for protective immunity. Cancer Res 2000, 60:2449–2457.

13 Glick RP, Lichtor T, Kim TS, et al.: Fibroblasts genetically engineered to secrete cytokines suppress tumor growth and induce antitumor immunity to a murine glioma in vivo. Neurosurgery 1995, 36:548–555.

14 Glick RP, Lichtor T, Mogharbel A, et al.: Intracerebral versus subcutaneous immunization with allogeneic fibroblasts genetically engineered to secrete interleukin-2 in the treatment of central nervous system glioma and melanoma. Neurosurgery 1997, 41:898–906; discussion 906–7.

15 Lichtor T, Glick RP, Kim TS, et al.: Prolonged survival of mice with glioma injected intracerebrally with double cytokine-secreting cells. J Neurosurg 1995, 83:1038–1044.

16 Parney IF, Petruk KC, Zhang C, et al.: Granulocyte-macrophage colony-stimulating factor and B7-2 combination immunogene therapy in an allogeneic Hu-PBL-SCID/beige mouse-human glioblastoma multiforme model. Hum Gene Ther 1997, 8:1073–1085.

17 Saleh M, Wiegmans A, Malone Q, et al.: Effect of in situ retroviral interleukin-4 transfer on established intracranial tumors. J Natl Cancer Inst 1999, 91:438–445.

18 Yu JS, Wei MX, Chiocca EA, et al.: Treatment of glioma by engineered interleukin 4-secreting cells. Cancer Res 1993, 53:3125–3128.

19 Constam DB, Philipp J, Malipiero UV, et al.: Differential expression of transforming growth factor-beta 1, -beta 2, and -beta 3 by glioblastoma cells, astrocytes, and microglia. J Immunol 1992, 148:1404–1410.

20 Huettner C, Paulus W, Roggendorf W: Messenger RNA expression of the immunosuppressive cytokine IL-10 in human gliomas. Am J Pathol 1995, 146:317–322.

21 Goswami S, Gupta A, Sharma SK: Interleukin-6-mediated autocrine growth promotion in human glioblastoma multiforme cell line U87MG. J Neurochem 1998, 71:1837–1845.

22 Hao C, Parney IF, Roa WH, et al.: Cytokine and cytokine receptor mRNA expression in human glioblastomas: evidence of Th1, Th2 and Th3 cytokine dysregulation. Acta Neuropathol (Berl) 2002, 103:171–178.

23 Kiefer R, Supler ML, Toyka KV, et al.: In situ detection of transforming growth factor-beta mRNA in experimental rat glioma and reactive glial cells. Neurosci Lett 1994, 166:161–164.

24 Platten M, Wick W, Weller M: Malignant glioma biology: role for TGF-beta in growth, motility, angiogenesis, and immune escape. Microsc Res Tech 2001, 52:401–410.

25 Rolhion C, Penault-Llorca F, Kemeny JL, et al.: Interleukin-6 overexpression as a marker of malignancy in human gliomas. J Neurosurg 2001, 94:97–101.

26 Dubost JJ, Rolhion C, Tchirkov A, et al.: Interleukin-6-producing cells in a human glioblastoma cell line are not affected by ionizing radiation. J Neurooncol 2002, 56:29–34.

27 Graf MR, Prins RM, Merchant RE: IL-6 secretion by a rat T9 glioma clone induces a neutrophil-dependent antitumor response with resultant cellular, antiglioma immunity. J Immunol 2001, 166:121–129.

28 Choi C, Gillespie GY, Van Wagoner NJ, et al.: Fas engagement increases expression of interleukin-6 in human glioma cells. J Neurooncol 2002, 56:13–19.

29 Wang B, Xiong Q, Shi Q, et al.: Intact nitric oxide synthase II gene is required for interferon-beta-mediated suppression of growth and metastasis of pancreatic adenocarcinoma. Cancer Res 2001, 61:71–75.

30 Kito T, Kuroda E, Yokota A, et al.: Enhancement of macrophage cytotoxicity against murine gliomas by interferon beta: increase in nitric oxide production in response to glioma-derived soluble factors. J Neurosurg 2002, 97:619–626.

31 Johansson AC, Hegardt P, Janelidze S, et al.: Enhanced expression of iNOS intratumorally and at the immunization site after immunization with IFNgamma-secreting rat glioma cells. J Neuroimmunol 2002, 123:135–143.

32 •• Graeber MB, Scheithauer BW, Kreutzberg GW: Microglia in brain tumors. Glia 2002, 40:252–259.
Extensive review of microglia.

33 Badie B, Schartner J, Prabakaran S, et al.: Expression of Fas ligand by microglia: possible role in glioma immune evasion. J Neuroimmunol 2001, 120:19–24.

34 Bettinger I, Thanos S, Paulus W: Microglia promote glioma migration. Acta Neuropathol (Berl) 2002, 103:351–355.

35 Soos JM, Krieger JI, Stuve O, et al.: Malignant glioma cells use MHC class II transactivator (CIITA) promoters III and IV to direct IFN-gamma-inducible CIITA expression and can function as nonprofessional antigen presenting cells in endocytic processing and CD4(+) T-cell activation. Glia 2001, 36:391–405.

36 •• Komata T, Kanzawa T, Kondo Y, et al.: Telomerase as a therapeutic target for malignant gliomas. Oncogene 2002, 21:656–663.
Interesting review of telomerase-based therapy for malignant gliomas.

37 Komata T, Koga S, Hirohata S, et al.: A novel treatment of human malignant gliomas in vitro and in vivo: FADD gene transfer under the control of the human telomerase reverse transcriptase gene promoter. Int J Oncol 2001, 19:1015–1020.

38 Koga S, Hirohata S, Kondo Y, et al.: FADD gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter to restrict induction of apoptosis to tumors in vitro and in vivo. Anticancer Res 2001, 21:1937–1943.

39 Komata T, Kondo Y, Kanzawa T, et al.: Treatment of malignant glioma cells with the transfer of constitutively active caspase-6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. Cancer Res 2001, 61:5796–5802.

40 Komata T, Kondo Y, Kanzawa T, et al.: Caspase-8 gene therapy using the human telomerase reverse transcriptase promoter for malignant glioma cells. Hum Gene Ther 2002, 13:1015–1025.

41 Guirguis LM, Yang JC, White DE, et al.: Safety and efficacy of high-dose interleukin-2 therapy in patients with brain metastases. J Immunother 2002, 25:82–87.

42 Chamberlain MC: A phase II trial of intra-cerebrospinal fluid alpha interferon in the treatment of neoplastic meningitis. Cancer 2002, 94:2675–2680.

43 Lichtor T, Glick RP, Tarlock K, et al.: Application of interleukin-2-secreting syngeneic/allogeneic fibroblasts in the treatment of primary and metastatic brain tumors. Cancer Gene Ther 2002, 9:464–469.

44 Deshmukh P, Glick RP, Lichtor T, et al.: Immunogene therapy with interleukin-2-secreting fibroblasts for intracerebrally metastasizing breast cancer in mice. J Neurosurg 2001, 94:287–292.

45 Makar TK, Wilt S, Dong Z, et al.: IFN-beta gene transfer into the central nervous system using bone marrow cells as a delivery system. J Interferon Cytokine Res 2002, 22:783–791.

46 Ehtesham M, Kabos P, Kabosova A, et al.: The use of interleukin 12-secreting neural stem cells for the treatment of intracranial glioma. Cancer Res 2002, 62:5657–5663.

47 Aboody KS, Brown A, Rainov NG, et al.: Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci U S A 2000, 97:12846–12851.

48 Park KI, Ourednik J, Ourednik V, et al.: Global gene and cell replacement strategies via stem cells. Gene Ther 2002, 9:613–624.

49 Parney IF, Farr-Jones MA, Koshal A, et al.: Human brain tumor cell culture characterization after immunostimulatory gene transfer. Neurosurgery 2002, 50:1094–1102.

50 Iwadate Y, Yamaura A, Sato Y, et al.: Induction of immunity in peripheral tissues combined with intracerebral transplantation of interleukin-2-producing cells eliminates established brain tumors. Cancer Res 2001, 61:8769–8774.

51 Hanes J, Sills A, Zhao Z, et al.: Controlled local delivery of interleukin-2 by biodegradable polymers protects animals from experimental brain tumors and liver tumors. Pharm Res 2001, 18:899–906.

52 Grana C, Chinol M, Robertson C, et al.: Pretargeted adjuvant radioimmunotherapy with yttrium-90-biotin in malignant glioma patients: a pilot study. Br J Cancer 2002, 86:207–212.

53 Riva P, Arista A, Sturiale C, et al.: Treatment of intracranial human glioblastoma by direct intratumoral administration of 131I-labelled anti-tenascin monoclonal antibody BC-2. Int J Cancer 1992, 51:7–13.

54 Riva P, Arista A, Tison V, et al.: Intralesional radioimmunotherapy of malignant gliomas. An effective treatment in recurrent tumors. Cancer 1994, 73:1076–1082.

55 Riva P, Franceschi G, Arista A, et al.: Local application of radiolabeled monoclonal antibodies in the treatment of high grade malignant gliomas: a six-year clinical experience. Cancer 1997, 80:2733–2742.

56 Plautz GE, Shu S: Adoptive immunotherapy of CNS malignancies. Cancer Chemother Biol Response Modif 2001, 19:327–338.

57 Yoshida S, Morii K, Watanabe M, et al.: The generation of anti-tumoral cells using dentritic cells from the peripheral bloood of patients with malignant brain tumors. Cancer Immunol Immunother 2001, 50:321–327.

58 Yu JS, Wheeler CJ, Zeltzer PM, et al.: Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res 2001, 61:842–847.