

# Current Status of Immunotherapeutic Strategies for Central Nervous System Tumors

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Malignant gliomas are the most common type of primary brain tumor and are in great need of novel therapeutic approaches. Advances in treatment have been very modest, significant improvement in survival has been lacking for many decades, and prognosis remains dismal. Despite "gross total" surgical resections and currently available radio-chemotherapy, malignant gliomas inevitably recur due to reservoirs of notoriously invasive tumor cells that infiltrate adjacent and non-adjacent areas of normal brain parenchyma. In principle, the immune system is uniquely qualified to recognize and target these infiltrative pockets of tumor cells, which have generally eluded conventional treatment approaches. In the span of the last 10 years, our understanding of the cancer-immune system relationship has increased exponentially; and yet we are only beginning to tease apart the intricacies of the central nervous system and immune cell interactions. This article reviews the complex associations of the immune system with brain tumors. We provide an overview of currently available treatment options for malignant gliomas, existing gaps in our knowledge of brain tumor immunology, and strategies that might be exploited for improved design of "custom immunotherapeutics." We will also examine major new immunotherapy approaches that are being actively investigated to treat patients with malignant glioma, and identify some current and future research priorities in this area.

**KEY WORDS :** Brain cancer · Biomarkers · Glioma · Immunotherapy · Antibody · Vaccines.

## Introduction

Gliomas are the most common tumors of the central nervous system (CNS) with an incidence of 5-10 per 100,000 population; and following stroke, they account for the second most common cause of death from neurological disease<sup>46</sup>. According to the 2005 annual statistics published by the American Cancer Society, an estimated 18,500 new cases of primary malignant brain and central nervous system (CNS) tumors were diagnosed in the United States in 2005, with an estimated 12,760 deaths attributed to this disease<sup>1</sup>. Gliomas are the most common solid (i.e., non-hematological) tumor in patients under 18 years of age.

Surgery, radiation, and chemotherapy have been the available treatment options for malignant gliomas and are the mainstays of current therapy. However, the recent advances in surgery, radiation oncology, neuro-oncology, neuro-pathology, and neuro-radiology have unfortunately not helped to significantly

increase survival rates of patients with high-grade gliomas. The vast majority of malignant brain tumors cannot be permanently eradicated by even the most meticulous surgery and aggressive radio-chemotherapy. As of 2002, patients with glioblastoma multiforme (GBM) still had an average survival of just under one-year<sup>67</sup>. Even with the addition of standard chemotherapy, survival rate for GBM at one year is only about 28%, with a two-year survival rate of 8% and a five-year survival of <3%<sup>67</sup>.

Over the past several years, a few chemotherapy drugs have been FDA-approved as adjuvant therapy to surgery and radiation for malignant glioma<sup>63</sup>. First, there was the Gliadel™, wafer, which is a biodegradable polymer that is inserted into the resection cavity at the time of surgery that continuously and locally delivers the chemotherapeutic agent carmustine (BCNU) over the next several weeks to the remaining tumor cells in the brain. Studies by Brem et al. have shown a survival advantage of only about 2 months, with Gliadel wafers improving survival from a median of 10.1 months to 12.6 months.

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ths<sup>8)</sup>. More recently, the oral systemic chemotherapeutic drug temozolomide (Temodar<sup>™</sup>) has been used following surgery, concomitant with radiation, and post radiation. Stupp et al. have demonstrated improved two-year survivals at 24% using adjuvant daily temozolomide during radiation followed by adjuvant five-day per month temozolomide in the post radiation setting. However, the overall survival advantage afforded by temozolomide for the majority of patients was still only about 2 months, from 12.1 to 14.6 months<sup>68)</sup>. It is unclear what accounts for the 10.1-month versus 12.1-month median survival in the control arms of the Gliadel<sup>™</sup>, and the temozolomide trials, respectively. However, it is well-known that historical/treatment controls often vary between institutions and study protocols. Regardless of whether the true median survival of glioblastoma patients is currently 10.1 versus 12.1 months, there clearly is still an unmet clinical need for further improving treatment outcomes for patients with these tumors.

#### Brain tumor immunology and the central nervous system

Despite currently available treatment modalities, malignant gliomas inevitably recur. Active tumor cells infiltrate adjacent areas of normal brain, and this reservoir of tumor likely leads to recurrence. A better method of killing these residual pockets of microscopic cells must be found to improve survival rates. In principle, the immune system is uniquely qualified to be an instrument for cancer therapy<sup>47)</sup>. An immune response directed against cells bearing tumor antigens could provide a specific and effective mechanism for killing unresectable, infiltrating residual tumor. While the theoretical background for immunotherapy as a treatment for brain tumors is elegant and persuasive, a substantial clinical breakthrough has yet to be made and most clinical investigations have not yet translated into FDA-approved therapies for brain tumor patients.

In the past five years, major work has been directed and evolved towards understanding the limitations and mechanisms of immune responses by cells of the human central nervous system<sup>73)</sup>. These areas include :

- active escape mechanisms by the tumor cell<sup>76,79)</sup>,
- interactions of antigen processing and effector T-cell function via the major histocompatibility complex(MHC)<sup>73,74)</sup>,
- biology of (ineffective) antigen processing events (APC, macrophage, microglia)<sup>65,70)</sup>,
- activation and migration of armed T cells<sup>21,39)</sup>,
- regulation of inflammation to prevent brain injury<sup>3,57)</sup>,
- role of soluble factors<sup>17,18)</sup>.

#### Types of immunotherapy for CNS neoplasms

Approaches to immunization and thus “tumor immunotherapy” are borrowed from the infectious disease model and can generally be classified into three categories<sup>22)</sup> :

1. Passive
2. Adoptive
3. Active

#### Passive immunotherapy

*Passive (serological) immunotherapy* consists of introducing target-specific exogenous antibodies into the patient. Clinically useful monoclonal antibodies(mAb) typically use a combination of mechanisms in directing their cytotoxic effects on tumor cells. Most mAbs interact with components of the immune system through antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity(CDC), while some may alter signal transduction within tumor cells or act to eliminate critical cell-surface antigens. Among these mechanisms, ADCC is the major method of cell killing by passive immunotherapy. ADCC occurs when antibodies bind to antigens on tumor cells and the antibody Fc domain engage Fc receptors(FcR) on the surface of immune effector cells (e.g., mononuclear phagocytes and/or human natural killer cells) to carry out tumor cell lysis<sup>2)</sup>. In addition to ADCC, some antibodies bind noncompetitively to antigens (or specific receptors) on tumor cells and are used to localize radio-isotopes and/or toxins to effect cytolysis of their target.

Problems associated with monoclonal antibody therapy relate to creating truly tumor-specific antibodies, to delivery of sufficient doses of the antibodies to the invasive areas of tumor cells, and to the generation of potentially harmful immune reactivity to non-human (i.e., murine) components. Most of the antigens expressed by tumor cells are not tumor-specific, only tumor-associated antigens(TAA). Available target antigens consist mostly those differentially expressed at higher levels on tumor cells compared with normal cells, or antigens that are expressed in tumor and in undifferentiated, immature cell types. One target, which may be specific to tumor cells, is a mutant form of the epidermal growth factor receptor(EGFR) found in 17% of glioblastomas<sup>77)</sup>. EGFRvIII probably contribute to the proliferative abilities of glioma cells. Sampson et al. have described *in vivo* studies of an unarmed, tumor-specific monoclonal antibody against this mutant receptor, which could be a potential avenue for future therapy of brain tumors<sup>61)</sup>.

Antibodies also have the potential to reduce the tumor-induced immuno-suppression mediated by soluble receptors, major histocompatibility complex molecules, or other suppressive factors<sup>73,76,79)</sup>. Furthermore, recent advances in linking information generated from gene-expression profiling and proteomics of CNS tumors to the identification of precise immunogenic epitopes could allow such antigens to serve as useful targets for designing new tumor-specific monoclonal antibodies(mAb). The translation of passive immunotherapy

**Table 1.** Summary of clinical trials of passive immunotherapy for malignant gliomas

Therapy	Phase	Tumor histology	Responses	Survival	Reference
<sup>131</sup> I-labeled antitenascin mAb	Phase I (n=20); Phase II (n=91)	GBM (91); AA (10)	17.8% RR with bulky lesions; 66.6% RR with small lesions	Median OS = 19 mos.	Riva et al., 1999 [Ref. 54]
<sup>90</sup> Y-biotinylated antitenascin mAb	Phase I (n=24)	GBM (16); AA (8) [recurrent]	25% RR; 50% SD after 2 mos.	NR	Paganelli et al., 2001 [Ref. 42]
<sup>131</sup> I-labeled antitenascin mAb	Phase II (n=33)	GBM (27); AA/O (6) [newly diagnosed]	–	Median OS = 86.7 wks. (79.4 wks. for GBM)	Reardon et al., 2002 [Ref. 52]
<sup>125</sup> I-labeled 425 anti-EGFR mAb	Phase I/II (n=180)	GBM; AA [newly diagnosed]	NR	Median OS = 13.4 mos. GBM; Median OS = 50.9 mos. AA	Quang et al., 2004 [Ref. 48]

mAb = monoclonal antibody; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; AO = anaplastic oligodendroglioma; RR = response rate; OS = overall survival; NR = not reported

from the laboratory to the clinic has already occurred, with some encouraging results. Local-regional administration of specific antibodies has resulted in impressive clinical responses in certain brain tumor patients (Table 1).

### Radioactive antibody targeting

Direct targeting of radio-isotopes is a theoretically possible consequence of antigen-antibody specificity. An antibody specific for a tumor antigen conjugated to a radio-isotope delivers high-dose radiation to malignant cells, reducing the morbidity caused by less closely targeted radiotherapy (e.g., interstitial brachytherapy or external beam radiotherapy). Clinical experiences with radio-labeled anti-tenascin monoclonal antibody (mAb) 81C6 have resulted in statistically significant increases in survival and prolonged disease stabilization<sup>78</sup>. Tenascin, a large multi-domain extra-cellular matrix protein, is a glycoprotein found differentially in glioma cells versus normal brain tissue. <sup>131</sup>I-labeled murine mAbs against tenascin were assessed by Riva et al. in the treatment of recurrent and newly diagnosed gliomas<sup>54</sup>. 111 patients, 58 with newly diagnosed malignant gliomas and 53 with recurrent disease, were accrued between February 1990 and December 1997. Loco-regional radioimmunotherapy was administered via an indwelling catheter to the surgically created resection cavity at a dosage of up to 300 Gy per injection. Three courses of therapy were given at intervals of 30–60 days. This treatment produced no systemic or CNS toxicity, although there were a few cases of mild and transient headache and nausea. 69% of the patients developed human anti-mouse antibody (HAMA) responses. HAMA was dose-dependent, and all patients who completed three or more cycles of therapy were HAMA<sup>+</sup>. HAMA-positive patients did not exhibit allergic or anaphylactic symptoms on further therapy. Median survival for the patients was 19 months. Survival was increased by near-complete surgical resection prior to radioimmunotherapy. The response rate (complete response + partial response + no evidence of disease) was 17.8% for those patients with bulky lesions, with a median survival of 17 months; while response rate was 66.6% for patients with smaller lesions, with

a median survival of 25 months<sup>54</sup>. These results are encouraging, which have led to further clinical trials with this radio-labeled monoclonal antibody.

In a more recent trial, Reardon et al. concluded that median survival achieved with <sup>131</sup>I-labeled 81C6 exceeded historical controls treated with conventional radiotherapy and chemotherapy, even after accounting for established prognostic factors including age and Karnofsky performance status (KPS). In a Phase II trial, 120 mCi of <sup>131</sup>I-labeled murine anti-tenascin mAb 81C6 was injected directly into the surgically created resection cavities of 33 patients with newly diagnosed malignant gliomas (27 GBM, 4 anaplastic astrocytoma, and 2 anaplastic oligodendroglioma). Median survival for all patients and those with GBM were 86.7 weeks and 79.4 weeks, respectively. These median survival times compared favorably with either <sup>125</sup>I interstitial brachytherapy or stereotactic radiosurgery (SRS) and was associated with a significantly lower rate of re-operation for radionecrosis<sup>52</sup>.

In another study, patients with primary high-grade gliomas or recurrent astrocytomas were treated with an average of three intravenous or intra-arterial administrations of <sup>125</sup>I-labeled anti-EGFR mAb 425 over a two-week period. Rather than loco-regional administration, this study used systemic (i.v. or i.a.) administration of the anti-EGFR mAb. Although the integrity of the blood-brain barrier (BBB) is often compromised in gliomas, there are issues of accessibility to the tumor bed using systemic routes of antibody administration, which may account for the relatively unimpressive survival results of the glioblastoma patients in this study. Total <sup>125</sup>I dosing ranged from 40 to 296 mCi, with an average of 148 mCi. This regimen was of relatively low toxicity (one case of chronic hypothyroidism), with only one patient who received a single dose of more than 60 mCi experiencing acute toxicity. No evidence for human anti-mouse antibodies (HAMA) was found. Overall median survival for patients with glioblastoma (GBM, grade IV) and AA (anaplastic astrocytoma, grade III) was 13.4 and 50.9 months, respectively, with Karnofsky Performance Status (KPS) ranging from 40 to 100 and age ranging from 11 to 75 years.

**Table 2.** Summary of clinical trials of adoptive cellular immunotherapy for malignant gliomas

Therapy	Phase	Tumor Histology	Responses	Survival	Reference
LAK cells + IL-2	Phase I (n=20)	GBM (17); AO (2); sarcoma (1)	NR	PFS = 25 ± 6 weeks	Merchant et al., 1988 [Ref. 37]
Allo-CTL + IL-2	Phase I (n=5)	GBM (2); AA (1); AO (2) [recurrent]	NR	AA/AO patients with SD > 28 mos.	Kruse et al., 1997 [Ref. 28]
TILs + IL-2	Phase I (n=6)	GBM; AA [recurrent]	1 CR; 2 PR	NR	Quattrocchi et al., 1999 [Ref. 49]
Activated CTL from lymph nodes	Phase I (n=12)	GBM (6); AA(4); LGA (2) [newly diagnosed]	4 PR	3 patients > 2 yrs	Plautz et al., 2000 [Ref. 44]
Activated CTL from PBMC	Phase I (n=9)	GBM; AA [recurrent]	3 PR	2/9 patients > 4 yrs	Wood et al., 2000 [Ref. 80]
LAK cells + IL-2	Phase I (n=28)	GBM; AA [recurrent]	Lymphocytic infiltration; locally ↑ IL-2 & IFN-γ	6/28 patients > 2 yrs	Hayes et al., 2001 [Ref. 20]
NK cells	Phase I (n=9)	GBM (3); AA (6) [recurrent]	3 PR; 2 MR	NR	Ishikawa et al., 2004 [Ref. 25]
LAK cells	Phase I (n=40)	GBM [recurrent]	NR	34% 1-year survival; Median OS = 17.5 mos.	Dillman et al., 2004 [Ref. 14]

CTL = cytotoxic T lymphocyte; TIL = tumor-infiltrating lymphocyte; LAK = lymphokine-activated killer; NK = natural killer; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; AO = anaplastic oligodendroglioma; LGA = low-grade (grade II) astrocytoma; PFS = progression-free survival; CR = complete response; PR = partial response; MR = minimal response; OS = overall survival; NR = not reported

Prognostic factors (KPS and age) correlated positively with increased survival, with KPS the most important determinant of median survival. GBM and AA patients under age 40 years with a Karnofsky performance status >70 had an actuarial median survival of 22.5 and 65 months, respectively<sup>48</sup>.

More recent studies have used <sup>90</sup>Y-labeled biotin as part of a pre-targeting three-step method for the locoregional radioimmunotherapy of recurrent high grade gliomas, with some encouraging results<sup>42</sup>. When two cycles of temozolomide chemotherapy was added to this regimen, the overall outcomes were significantly improved, with a median progression-free survival of 10 months and a median overall survival of 25 months<sup>6</sup>.

**Targeted immunotoxins**

Clinical trials of immunotoxins for CNS neoplasia have been conducted using TFN-CRM107 (human diferric transferrin + *Diphtheria* toxin mutant CRM107)<sup>19</sup> and *Pseudomonas aeruginosa* exotoxin A mutants conjugated to interleukin-4 (IL-4-PE38KDEL)<sup>50</sup>, interleukin-13 (IL-13-PE38QQR)<sup>29,43</sup>, or transforming growth factor-alpha (TP-38)<sup>60</sup>. These initial trials of passive serotherapy have shown some significant anti-tumor effects, which have spurred further multi-center Phase II and Phase III trials.

Of particular note, IL13-PE38QQR cytotoxin was found to be highly potent and selective in killing IL-13R-expressing glioblastoma cells *in vitro*<sup>33</sup>. In contrast, normal cells (e.g., brain, immune cells, and endothelial cells) were generally not affected by this immunotoxin due to scant expression of IL-13R in non-tumor cells. *In vivo* pre-clinical studies for safety and toxicity

were performed in mice, rats, and monkeys, and IL-13 cytotoxin was found to be well-tolerated by both systemic and intracerebral administrations. IL13-PE38QQR (also known as cintredekin besudotox) was shown to mediate remarkable efficacy in pre-clinical animal models of human brain tumors<sup>24</sup>. Based on these pre-clinical studies, IL13-PE38QQR has been tested in four Phase I/II clinical trials in adult patients with recurrent malignant glioma, with some encouraging results<sup>29,30</sup>. These clinical trials involved convection-enhanced delivery (CED) of IL13-PE38QQR cytotoxin either intratumorally or intraparenchymally after resection of tumor<sup>43</sup>. At the time of this writing, a pivotal Phase III randomized, controlled clinical trial (PRECISE, sponsored by Neopharm, Inc.) comparing intraparenchymal CED of IL13-PE38QQR cytotoxin administration with Gliadel™, wafer placement has been completed and subjects are currently being monitored for safety and survival endpoints<sup>64</sup>.

**Adoptive cellular immunotherapy**

Adoptive transfer of immune cells is the cell-mediated analogue of passive immunotherapy : The introduction (adoption) into the patient of either autologous or allogeneic immune cells that may, or may not, have been stimulated *in vitro* with tumor antigens<sup>38,44,45</sup>. Sometimes, these cells are injected into the tumor cavity to maximize the exposure of the infused cells to tumor cells, while other strategies call for systemic infusion. Cytolytic effector cells are cultured *in vitro* with cytokines to activate them to attack tumor tissue *in vivo*. This approach was not clinically applicable until the discovery of interleukin-2

**Table 3.** Summary of clinical trials of active immunotherapy (Tumor vaccines) for adult malignant gliomas

Therapy	Phase	Tumor Histology	Responses	Survival	Reference
Tumor cells modified with Newcastle–Disease Virus (NDV)	Phase I (n=11)	GBM [newly diagnosed]	Local skin reaction	Median OS = 46 wks.	Schneider et al., 2001 [Ref. 62]
DC pulsed with acid-eluted tumor peptides	Phase I (n=9)	GBM (7); AA (2) [recurrent]	2/4 patients with ↑ lymphocytic infiltration	–	Yu et al., 2001 [Ref. 85]
DC–glioma cell fusions	Phase I (n=8)	GBM; AA	4/5 patients with ↑ CD16+ & CD56+ cells; ↑ IFN-γ in peripheral blood	NR	Kikuchi et al., 2001 [Ref. 27]
Tumor cell + IL4–transfected fibroblasts	Phase I (n=1)	GBM	Local CD4+, CD8+, and CD1a+ T cells ↑ with IL-4 produced at injection site	Patient survived 10 mos.	Okada et al., 2003 [Ref. 40]
DC pulsed with tumor lysate (intradermal + intratumoral via Ommaya reservoir)	Phase I/II (n=10)	GBM (7); AA (3)	2/5 patients with ↑ ELISPOT activity; 3/10 with ↑ DTH; 2 with ↑ lymphocytic infiltration	NR	Yamanaka et al., 2003 [Ref. 82]
DC pulsed with tumor lysate	Phase I (n=14)	GBM (8); AA (6) [recurrent]	6/10 with ↑ IFN-γ in peripheral blood; 3/6 with ↑ lymphocytic infiltration	Median OS = 133 wks.	Yu et al., 2004 [Ref. 84]
DC–glioma cell fusion + IL-12	Phase I (n=15)	GBM; AA	4/15 with > 50% ↓ tumor size	NR	Kikuchi et al., 2004 [Ref. 26]
Tumor cell modified with Newcastle–Disease Virus (NVD)	Phase II (n=23)	GBM	↑ DTH; ↑ tumor–infiltrating lymphocytes	Median OS = 100 wks. (vs. 49 wks. in controls, n=87); 39% 2–year survival rate	Steiner et al., 2004 [Ref. 66]
DC pulsed with acid-eluted tumor peptides	Phase I (n=12)	GBM (12) [7 newly diagnosed; 5 recurrent]	6/12 with ↑ CTL activity; 4/8 ↑ tumor–infiltrating lymphocytes	Median OS = 23.4 mos.; 50% 2–year survival rate	Liau et al., 2005 [Ref. 34]

DC = dendritic cell; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; DTH = delayed–type hypersensitivity; CTL = cytotoxic T lymphocyte; IFN = interferon; PR = partial response; OS = overall survival; NR = not reported

(IL-2) and other cytokines. Before that time, it was not possible to obtain sufficient numbers of immune cells and maintain them in culture during the *in vitro* sensitization period. IL-2, originally called T-cell growth factor, is a cytokine that mediates lymphocyte activation and stimulates division.

### Lymphokine-activated Killer(LAK) cells

The majority of previous clinical trials that have used adoptive immunotherapy for brain tumors have employed lymphokine-activated killer(LAK) or mitogen-activated killer(MAK) cells<sup>37</sup>. LAK cells are peripheral blood lymphocytes functionally defined by their ability to lyse natural killer(NK)-resistant tumor targets *in vitro* following stimulation with IL-2 and/or mitogens. LAK cells have been implanted into the resection cavity during surgery<sup>14,20</sup>. LAK cell therapy is non-specific in that the cells are not stimulated *in vitro* with any glioma-specific antigens. The limitations of LAK cell adoptive transfer to eradicate tumor

cells in clinical trials may be due to its non-specificity (i.e., not stimulated *in vitro* with any tumor-specific antigen) and the fact that cells do not migrate specifically to tumor sites.

### Cytotoxic T Lymphocytes(CTL)

One refinement to the previous adoptive transfer approaches is to stimulate lymphocytes *in vivo* with autologous tumor cells to generate MHC Class I-restricted cytotoxic T lymphocytes (CTL) that are then expanded *in vitro* and re-infused into patients<sup>28,36</sup>. The theory is that antigen-stimulated CTL would be more specific than LAK cells. Clinical trials using adoptive transfer of activated cytotoxic T-cell therapy have produced somewhat encouraging initial results<sup>28,44,80</sup>. However, because these pilot Phase I trials were designed primarily to demonstrate feasibility and safety, definitive evaluation of efficacy will require further study.

Often, pilot trials of adoptive immunotherapy have suggested

efficacy<sup>25,49</sup>), but they have not been followed up with Phase II or III larger trials designed to show true improvement in survival. Studies by Dudley and Rosenberg in melanoma have been particularly encouraging; however, the ability to clone and expand high-affinity TILs is currently restricted to a few centers around the world. Nevertheless, it appears that adoptive immunotherapy may offer certain advantages that active vaccination strategies cannot provide: 1) T-cells are expanded in the absence of tumor-derived soluble factors; and 2) CTLs can be grown to extremely large numbers sufficient for infusion<sup>75</sup>. A summary of recently published clinical trials of adoptive cellular immunotherapy for malignant gliomas is presented in Table 2.

#### Active immunotherapy (Tumor vaccines)

*Active immunotherapy* requires administration of the antigenic material to induce a (primary) immune response, in effect, a vaccination. Most tumor antigens are poor immunogens, and thus active immunotherapy usually includes the use of an "adjuvant" (e.g., *Bacille Calmette-Guerin* or cytokines) that enhances the immune response by prolonging the time of exposure to antigen and by increasing the activity of antigen presenting cells (APC). Dendritic cell (DC)-based therapies are one type of active immunotherapy<sup>32</sup>. Other approaches have included irradiated whole tumor cell vaccines and cytokine immuno-gene therapy strategies<sup>16,41</sup>, which have also been tested in Phase I trials for patients with malignant brain tumors (Table 3). One of the primary advantages of tumor vaccines (especially cell-based immunotherapies) is their favorable toxicity profiles compared with current chemotherapy and small molecule therapies.

#### Dendritic cell-based vaccines

Dendritic cells are the most potent professional APCs in the body, as evidenced by their abilities to stimulate allogeneic mixed leukocyte reactions and prime naive T lymphocytes. Approaches that combine adoptive transfer of antigen-pulsed DC for tumor vaccination in malignant glioma patients are currently under active investigation at several different centers around the world<sup>10,26,27,34,81-85</sup>. This strategy involves *ex vivo* exposure of a patient's dendritic cells to their tumor antigen followed by injection of these DCs primed with tumor antigen to stimulate an endogenous immune response<sup>32,47</sup>. The theory behind this therapeutic strategy is based upon evidence that tumor cells are poor APCs. The lack of activation especially affects the CD4<sup>+</sup> "helper" T-cells (Th) that require antigen presentation in association with MHC class II. Unless tumor antigens are secreted as soluble proteins and can be processed by professional APCs, the immune response will be handicapped by the lack of Th-produced cytokines required for

expansion of the cytotoxic T-cell population. Soluble antigen may be released by tumor cells in later stages with the development of necrotic areas; but by this time, the tumor is probably past the threshold when it can be checked by immune activity. To circumvent this, investigators are using *ex vivo* cytokine stimulation of DC and antigen exposure of autologous dendritic cells with autologous tumor lysate, tumor peptides, or tumor cell fusions (Table 3).

In a recent Phase I clinical trial with five-year follow-up, 12 patients with newly diagnosed (n=7) or recurrent (n=5) glioblastoma were enrolled. Patients received standard of care, which included surgery followed by external beam radiation therapy. The glioma cells were cultured *in vitro* and MHC-bound tumor peptides were eluted from the surface of the tumor cells<sup>32</sup>. The patient's own autologous DC were harvested from peripheral blood mononuclear cells (PBMC) and subsequently loaded with autologous acid-eluted tumor peptides. Three injections of peptide-pulsed DC were administered intradermally at two-week intervals. Although this Phase I study was not powered to detect clinical efficacy, it provides further evidence on the feasibility, safety, and *in vivo* bioactivity of autologous peptide-pulsed DC in patients with glioblastoma<sup>26,27,34,81,82,84,85</sup>. Although admittedly a select population of patients, prolonged survival times and significant immunological responses were observed in some of these patients, which support the possibility of an immune-related effect on tumor control. Proof of clinical benefit from DC-based vaccines remains to be established in future multi-center Phase II clinical trials for malignant glioma patients, which are currently underway [<http://www.nwbio.com>].

#### Cytokine immuno-gene therapy

Other studies of cancer vaccines have used irradiated whole tumor cells modified with cytokine genes<sup>16,40,41</sup> or viral vectors<sup>53,62,66</sup> to generate anti-tumor immunity. The pioneering studies were performed over a decade ago now and demonstrated that cytokine-secreting tumor cells could induce anti-tumor immunity.

With CNS tumor models, cytokine-gene therapy studies have been able to demonstrate efficacious anti-tumor immunity to intracranial gliomas by secreting IL-2, IL-4, IL-6, IL-7, IL-12, GM-CSF, mM-CSF, and IFN- $\alpha/\beta/\gamma$ <sup>15,40,53</sup>. These studies reveal the complexity of the multiple mechanisms by which anti-tumor immunity can be generated.

#### Bacterial and viral tumor vaccines

*Live bacteria and/or viruses* may also serve as the basis for active immunotherapies against brain tumors<sup>11</sup>. Viral/bacterial infections and the resulting tissue damage can provide the appropriate "danger signals"<sup>35</sup> to attract professional APC

necessary for adequate antigen presentation. CTL-mediated immunity can be induced using live, attenuated bacterial or viral vectors that both stimulate the innate immune system and simultaneously deliver antigens<sup>11</sup>.

For instance, *Listeria monocytogenes* (*LM*) is a facultative, gram-positive intracellular bacterium that is able to enter host cells, escape from the endocytic vesicle, multiply within the cytoplasm, and spread directly from cell to cell without encountering the extracellular milieu. Antigens expressed by *LM* can access both MHC class I and class II processing pathways, and are presented to both CD8<sup>+</sup> and CD4<sup>+</sup> T cells. Additionally, *LM* has been shown to stimulate Toll-like receptors (TLR)<sup>7</sup> on the surface of APC and activate internal pattern recognition molecules, which may contribute to its immunostimulatory action<sup>72</sup>. In recent studies, it has been demonstrated that immunization with an attenuated, recombinant *Listeria monocytogenes* (*rLM*) expressing the lymphocytic choriomeningitis virus nucleoprotein (LCMV-NP) led to the rejection of a gliomas expressing the heterologous NP antigen<sup>33</sup>. Interestingly, animals clearing these tumors were subsequently immune to re-challenge with subcutaneous (s.c.) and intracranial (i.c.) glioma cells that did not express NP, suggesting that epitope spreading had occurred, which is a phenomenon whereby T cells can recognize shared endogenous glioma epitopes along with the targeted antigen(s)<sup>9,33</sup>. Given the heterogeneity of human brain tumors and the possibility of immune escape of tumor cells, exploiting the process of epitope spreading will obviously be valuable in the clinical context of designing tumor vaccines in the future.

In other studies, Rosenberg et al. have recently investigated an attenuated strain of *Salmonella typhimurium* (VNP20009) in pre-clinical studies and clinical trials<sup>55</sup>. *Salmonella typhimurium* genetically modified at the *purI* and *msbB* genes were found to specifically target and localize to transplantable murine tumors and partially inhibit tumor growth *in vivo*<sup>12,55</sup>. These pre-clinical results led to a Phase I clinical trial of the intravenous administration of attenuated *Salmonella typhimurium* VNP20009 to patients with metastatic melanoma. This clinical study showed that the VNP20009 strain of *Salmonella typhimurium* could be safely administered to patients and that some tumor colonization by *Salmonella* was observed at the highest tolerated dose. However, no anti-tumor effects were seen<sup>71</sup>.

Viral vaccines have also been investigated for the treatment of malignant gliomas. Csatory et al. recently reported on the use of a Newcastle Disease Virus vaccine (MTH-68/H) in four patients with advanced high-grade glioma, with purported survival times of 5~9 years<sup>13</sup>. More recently, Steiner et al. reported the results of a pilot clinical trial using a vaccine prepared from the patient's tumor cell cultures infected with Newcastle Disease Virus, followed by gamma-irradiation. This was a non-

randomized study of 23 vaccinated patients compared with 87 non-vaccinated controls, which showed a 39% two-year survival rate in the vaccinated patients compared with an 11% two-year survival in the controls. This viral-based vaccine appeared to be feasible and safe, and the improved prognosis of the vaccinated patients was substantiated by observed anti-tumor immune responses<sup>66</sup>.

Each of the immune-based therapies outlined above utilizes principles of basic immunology to find a strategy that hopefully brings us closer to the goal of killing the residual microscopic tumor cells that lead to inevitable recurrence of malignant gliomas. Major improvements in our understanding of glioma molecular biology and tumor immunology are now being translated into innovative clinical trials that provide new hope for patients with this devastating disease (see Table 1, 2, 3).

### Effects of corticosteroids on immunotherapy

With regard to incorporating experimental immunotherapeutic strategies with standard treatments, the use of corticosteroids needs to be considered. Although this is not directly an anti-cancer agent, steroids are almost always part of standard care for brain tumor patients at some point. Some immunotherapy trials appear to allow the use of dexamethasone, while others do not because corticosteroids are known to suppress the immune response.

In a recent study, an effect of dexamethasone on the efficacy of local IL-2 immunotherapy was examined by injecting IL-2-secreting cells intracranially into tumor-bearing mice treated with either 1mg/kg or 10mg/kg of dexamethasone. The results suggested that while high doses of dexamethasone can completely inhibit the immune response observed with IL-2, lower and more likely therapeutic doses of dexamethasone did not inhibit local IL-2 immunotherapy<sup>31</sup>. In another study, dexamethasone was added to cytotoxicity assays to test whether the immunosuppressive effects of corticosteroids affected the lytic function of alloreactive cytotoxic T lymphocytes (aCTL) against primary human glioma cell lines *in vitro*. This study also demonstrated that the addition of dexamethasone did not significantly impair the lytic function of adoptively transferred human aCTL<sup>51</sup>. These results suggest that low-dose steroids probably do not affect passive and adoptive immunotherapy approaches, which are relatively host-independent.

However, it is still unknown as to whether active vaccination strategies, which require the induction of a host anti-tumor response, may be negatively impacted by corticosteroid treatment. In one study, dexamethasone was found to abolish the inflammatory response in an experimental glioma animal model<sup>5</sup>, while another study showed that treatment with dexamethasone did not affect dendritic cell-induced tumor cell phagocytosis *in vitro* or inhibition of tumor growth *in vivo*<sup>69</sup>.

Because there is still controversy regarding whether dexamethasone significantly suppresses the induction of anti-glioma immunity or not, optimization of tumor vaccine trials should take advantage of the brief and precious window of opportunity during the post-operative period while tumor burden is minimal and when steroids are not clinically required.

## Conclusion

There is challenging work being done today to take basic immunology into the clinical realm. To date, clinical trials of immunotherapy for CNS gliomas have not yet demonstrated objective proof of clinical efficacy in rigorous multi-center Phase II and III studies. Nevertheless, such trials should be pursued because of encouraging results in many Phase I studies<sup>59</sup>. As future testing in this field continues, our ability to design effective, targeted immune therapies will mature and hopefully yield increased therapeutic success.

With this in mind, the priority areas of research and scientific investigation that appear to be most critical at this time involve : 1) developing techniques of antigen identification, resulting in readily available sources of information on the genes and gene products that are co-expressed in primary tumor cells<sup>56</sup> and that produce immunogenic antigens<sup>4</sup>; 2) characterizing both CNS and systemic immune responses in patients with brain tumors; and 3) considering the problems and challenges posed by patient and tumor heterogeneity.

It should be noted that, as with any other targeted treatment modality for brain tumors, immunotherapy trials might only realize significant potential clinical efficacy if given to the appropriate subgroup of patients and/or if administered in combination with other therapies. With the current experience in cancer treatments, it appears that simultaneously targeting several components essential to the neoplastic process should provide maximal chances of tumor control. Therefore, therapies based on immuno-enhancement and cancer vaccines could be combined with the traditional surgery, radiation, and chemotherapies, along with molecularly targeted biological agents. Such integrated treatment strategies may prove to be of low toxicity and should be synergistic. In addition, the combined use of conventional treatments within the context of clinical trials of immunotherapy will allow evaluation of efficacy, yet retain the ethical requirements for human investigation.

Over the next decade, the concept of stimulating a patient's natural immunity to produce anti-tumor responses may lead to the approach of "customized immunotherapy"<sup>58</sup> for patients with malignant glioma. Such, "personalized therapeutics" may be a potential solution to deal with the important observations of patient and tumor heterogeneity. However, there are still significant obstacles for developing highly patient-selective

treatments : the patient accrual on clinical trials is slower, the potential market for an approved product is lower, and so development times to clinical applications are prolonged.

Furthermore, there are significant manufacturing challenges that face the clinical development of many immunotherapeutics, especially with regard to patient-specific vaccines. This is arguably the greatest technical hurdle to getting these types of treatments into large-scale, pivotal trials. Because so-called GMP-level facilities on many academic campuses do not meet the stricter regulations of the FDA for non-pilot studies, further clinical development of cellular therapies and biologic agents for brain tumors will require partnerships between international academic medical centers, government agencies, and biotechnology companies in order to overcome the manufacturing challenges that currently impede large-scale, multi-center Phase III clinical trials.

As we have pointed out above, there is already a plethora of small-scale Phase I/II brain tumor immunotherapy trials that still await confirmation of clinical efficacy. In order to obtain meaningful biological and clinical data from more complicated trials of customized immunotherapy in selected patient groups, larger multi-institutional studies need to be performed with more structured collaborations from academic and community medical centers, the biotechnology industry, and patient advocacy groups.

Currently, immunotherapy is cautiously and deliberately making its way to the patient bedside, as adjuncts to standard modalities of surgery, radiotherapy, and chemotherapy. While the number of clinical trials evaluating such immunotherapeutic strategies is still limited, current advances in the high-throughput production of clinical-grade cellular/biologic therapeutics and molecular/genetic target identification will hopefully spur future clinical development.

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## References

1. American Cancer Society : **Cancer Facts and Figures 2005**, Atlanta, GA : American Cancer Society, 2005
2. Adams GP, Weiner LM : Monoclonal antibody therapy of cancer. **Nat Biotechnol** 23 : 1147-1157, 2005
3. Aloisi F, Ambrosini E, Columba-Cabezas S, Magliozzi R, Serafini B : Intracerebral regulation of immune responses. **Ann Med** 33 : 510-515, 2001
4. Andersen MH, Pedersen LO, Capeller B, Brocker EB, Becker JC, Straten PT : Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes in situ as well as ex vivo in cancer patients. **Cancer Res** 61 : 5964-5968, 2001
5. Badie B, Schartner JM, Paul J, Bartley BA, Vorpahl J, Preston JK : Dexamethasone-induced abolition of the inflammatory response in an experimental glioma model : a flow cytometry study. **J Neurosurg** 93 : 634-639, 2000
6. Bartolomei M, Mazzetta C, Handkiewicz-Junak D, Bodei L, Rocca P,



- Grana C, et al : Combined treatment of glioblastoma patients with locoregional pre-targeted 90Y-biotin radioimmunotherapy and temozolomide. *Q J Nucl Med Mol Imaging* 48 : 220-228, 2004
7. Barton GM, Medzhitov R : Toll-like receptors and their ligands. *Curr Top Microbiol Immunol* 270 : 81-92, 2002
  8. Brem H, Gabikian P : Biodegradable polymer implants to treat brain tumors. *J Control Release* 74 : 63-67, 2001
  9. Calzascia T, Di Berardino-Besson W, Wilmotte R, Masson F, de Tribolet N, Dietrich PY, et al : Cutting edge : cross-presentation as a mechanism for efficient recruitment of tumor-specific CTL to the brain. *J Immunol* 171 : 2187-2191, 2003
  10. Caruso DA, Orme LM, Neale AM, Radcliff FJ, Amor GM, Maixner W, et al : Results of a phase 1 study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. *Neuro-oncol* 6 : 236-246, 2004
  11. Chabalgoity JA, Dougan G, Mastroeni P, Aspinnall RJ : Live bacteria as the basis for immunotherapies against cancer. *Expert Rev Vaccines* 1 : 495-505, 2002
  12. Clairmont C, Lee KC, Pike J, Ittensohn M, Low KB, Pawelek J, et al : Biodistribution and genetic stability of the novel antitumor agent VNP 20009, a genetically modified strain of *Salmonella typhimurium*. *J Infect Dis* 181 : 1996-2002, 2000
  13. Csatory LK, Gosztanyi G, Szeberenyi J, Fabian Z, Liszka V, Bodey B, et al : MTH-68/H oncolytic viral treatment in human high-grade gliomas. *J Neurooncol* 67 : 83-93, 2004
  14. Dillman RO, Duma CM, Schiltz PM, DePriest C, Ellis RA, Okamoto K, et al : Intracavitary placement of autologous lymphokine-activated killer (LAK) cells after resection of recurrent glioblastoma. *J Immunother* 27 : 398-404, 2004
  15. Giezeman-Smits KM, Okada H, Brissette-Storkus CS, Villa LA, Attanucci J, Lotze MT, 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* 60 : 2449-2457, 2000
  16. Glick RP, Lichtor T, Cohen EP : Cytokine-based immuno-gene therapy for brain tumors, in Liau LM, Becker DP, Cloughesy TF, Bigner DD (eds) : *Brain Tumor Immunotherapy*, Totowa, New Jersey : Humana Press, 2001, pp 273-288
  17. Gorelik L, Constant S, Flavell RA : Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 195 : 1499-1505, 2002
  18. Gorelik L, Flavell RA : Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2 : 46-53, 2002
  19. Hall WA : Targeted toxin therapy for malignant astrocytoma. *Neurosurgery* 46 : 544-551, 2000
  20. Hayes RL, Arbit E, Odaimi M, Pannullo S, Scheff R, Kravchinskiy D, et al : Adoptive cellular immunotherapy for the treatment of malignant gliomas. *Crit Rev Oncol Hematol* 39 : 31-42, 2001
  21. Hickey WF : Basic principles of immunological surveillance of the normal central nervous system. *Glia* 36 : 118-124, 2001
  22. Hong Y, Rha H, Jung C, Lee S, Jo T, Baik M, et al : Brain tumor immunology -- Part II : Immunotherapy of malignant brain tumors. *J Korean Neurosurg Soc* 19 : 1389-1396, 1990
  23. Husain SR, Joshi BH, Puri RK : Interleukin-13 receptor as a unique target for anti-glioblastoma therapy. *Int J Cancer* 92 : 168-175, 2001
  24. Husain SR, Puri RK : Interleukin-13 receptor-directed cytotoxin for malignant glioma therapy : from bench to bedside. *J Neurooncol* 65 : 37-48, 2003
  25. Ishikawa E, Tsuboi K, Saijo K, Harada H, Takano S, Nose T, et al : Autologous natural killer cell therapy for human recurrent malignant glioma. *Anticancer Res* 24 : 1861-1871, 2004
  26. Kikuchi T, Akasaki Y, Abe T, Fukuda T, Saotome H, Ryan JL, et al : Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. *J Immunother* 27 : 452-459, 2004
  27. Kikuchi T, Akasaki Y, Irie M, Homma S, Abe T, Ohno T : Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. *Cancer Immunol Immunother* 50 : 337-344, 2001
  28. Kruse CA, Cepeda L, Owens B, Johnson SD, Stears J, Lillehei KO : Treatment of recurrent glioma with intracavitary alloreactive cytotoxic T lymphocytes and interleukin-2. *Cancer Immunol Immunother*. 45 : 77-87, 1997
  29. Kunwar S : Convection-enhanced delivery of IL13-PE38QQR for treatment of recurrent malignant glioma : presentation of interim findings from ongoing Phase I studies. *Acta Neurochir Suppl* 88 : 105-111, 2003
  30. Kunwar S, Chang SM, Prados MD, Berger MS, Sampson JH, Croteau D, et al : Safety of intraparenchymal convection-enhanced delivery of cintredekin besudotox in early-phase studies. *Neurosurg Focus* 20 : E15, 2006
  31. Lesniak MS, Gabikian P, Tyler BM, Pardoll DM, Brem H : Dexamethasone mediated inhibition of local IL-2 immunotherapy is dose dependent in experimental brain tumors. *J Neurooncol* 70 : 23-28, 2004
  32. Liau LM, Black KL, Prins RM, Sykes SN, DiPatre PL, Cloughesy TF, et al : Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 90 : 1115-1124, 1999
  33. Liau LM, Jensen ER, Kremen TJ, Odesa SK, Sykes SN, Soung MC, et al : Tumor immunity within the central nervous system stimulated by recombinant *Listeria monocytogenes* vaccination. *Cancer Res* 62 : 2287-2293, 2002
  34. Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, et al : Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local CNS tumor microenvironment. *Clin Cancer Res* 11 : 5515-5525, 2005
  35. Matzinger P : The danger model : a renewed sense of self. *Science* 296 : 301-305, 2002
  36. Merchant RE, Baldwin NG, Rice CD, Bear HD : Adoptive immunotherapy of malignant glioma using tumor-sensitized T lymphocytes. *Neurol Res* 19 : 145-152, 1997
  37. Merchant RE, Grant AJ, Merchant LH, Young HF : Adoptive immunotherapy for recurrent glioblastoma multiforme using lymphokine activated killer cells and recombinant interleukin-2. *Cancer* 62 : 665-671, 1988
  38. Mitchell DA, Fecci PE, Sampson JH : Adoptive immunotherapy for malignant glioma. *Cancer J* 9 : 157-166, 2003
  39. Ochsenbein AF, Sierro S, Odermatt B, Pericin M, Karrer U, Hermans J, et al : Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. *Nature* 411 : 1058-1064, 2001
  40. Okada H, Lieberman FS, Edington HD, Witham TF, Wargo MJ, Cai Q, et al : Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of recurrent glioblastoma : preliminary observations in a patient with a favorable response to therapy. *J Neurooncol* 64 : 13-20, 2003
  41. Okada H, Pollack IF : Cytokine gene therapy for malignant glioma. *Expert Opin Biol Ther* 4 : 1609-1620, 2004
  42. Paganelli G, Bartolomei M, Ferrari M, Cremonesi M, Broggi G, Maira G, et al : Pre-targeted locoregional radioimmunotherapy with 90Y-biotin in glioma patients : phase I study and preliminary therapeutic results. *Cancer Biother Radiopharm* 16 : 227-235, 2001
  43. Parney IF, Kunwar S, McDermott M, Berger MS, Prados M, Cha S, et al : Neurodiagnostic changes following convection-enhanced delivery of the recombinant cytotoxin interleukin 13-PE38QQR for recurrent malignant glioma. *J Neurosurg* 102 : 267-275, 2005
  44. Plautz GE, Miller DW, Barnett GH, Stevens GH, Maffett S, Kim J, et al : T cell adoptive immunotherapy of newly diagnosed gliomas. *Clin. Cancer Res* 6 : 2209-2218, 2000.
  45. Plautz GE, Mukai S, Cohen PA, Shu S : Cross-presentation of tumor antigens to effector T cells is sufficient to mediate effective immunotherapy of established intracranial tumors. *J Immunol* 165 : 3656-3662, 2000
  46. Preston-Martin S, Davis F, McKean-Cowdin R : Epidemiology of primary brain tumors, in Liau LM, Becker DP, Cloughesy TF, Bigner DD (eds) : *Brain Tumor Immunotherapy*, Totowa, New Jersey : Humana Press, 2001, pp 47-71
  47. Prins RM, Liau LM : Immunology and immunotherapy in neurosurgical disease. *Neurosurgery* 53 : 144-152, 2003
  48. Quang TS, Brady LW : Radioimmunotherapy as a novel treatment regimen : 125I-labeled monoclonal antibody 425 in the treatment of high-grade brain gliomas. *Int J Radiat Oncol Biol Phys* 58 : 972-975, 2004
  49. Quattrocchi KB, Miller CH, Cush S, Bernard SA, Dull ST, Smith M, et al : Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. *J Neurooncol* 45 : 141-157, 1999
  50. Rand RW, Kreitman RJ, Patronas N, Varricchio F, Pastan I, Puri RK : Intratumoral administration of recombinant circularly permuted inte-

- releukin-4-Pseudomonas exotoxin in patients with high-grade glioma. *Clin Cancer Res* 6 : 2157-2165, 2000
51. Read SB, Kulprathipanja NV, Gomez GG, Paul DB, Winston KR, Robbins JM, et al : Human alloreactive CTL interactions with gliomas and with those having upregulated HLA expression from exogenous IFN-gamma or IFN-gamma gene modification. *J Interferon Cytokine Res* 23 : 379-393, 2003
  52. Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE, et al : Phase II trial of murine (131)I-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J Clin Oncol* 20 : 1389-1397, 2002
  53. Ren H, Boulilikas T, Lundstrom K, Soling A, Warnke PC, Rainov NG : Immunogene therapy of recurrent glioblastoma multiforme with a liposomally encapsulated replication-incompetent Semliki forest virus vector carrying the human interleukin-12 gene--a phase I/II clinical protocol. *J Neurooncol* 64 : 147-154, 2003
  54. Riva P, Franceschi G, Frattarelli M, Lazzari S, Riva N, Giuliani G, et al : Loco-regional radioimmunotherapy of high-grade malignant gliomas using specific monoclonal antibodies labeled with <sup>90</sup>Y : a phase I study. *Clin Cancer Res* 5 : 3275s-3280s, 1999
  55. Rosenberg SA, Spiess PJ, Kleiner DE : Antitumor effects in mice of the intravenous injection of attenuated *Salmonella typhimurium*. *J Immunother* 25 : 218-225, 2002
  56. Ryu J, Kim C, Cheong J, Bak K, Kim J, Oh S : Co-expression of survivin and Bcl-2 in primary brain tumors : their potential effect on anti-apoptosis. *J Korean Neurosurg Soc* 40 : 1-5, 2006
  57. Saas P, Walker PR, Quiquerez AL, Chalmers DE, Arrighi JF, Lienard A, et al : A self-defence mechanism of astrocytes against Fas-mediated death involving interleukin-8 and CXCR2. *Neuroreport* 13 : 1921-1924, 2002
  58. Salgaller ML : American Association for Cancer Research : dendritic cells : strategies and vaccines. April 1-5, 2000, San Francisco, CA, USA. *Expert Opin Investig Drugs* 9 : 1407-1412, 2000
  59. Salgaller ML, Liau LM : Current status of clinical trials for glioblastoma. *Reviews on Recent Clinical Trials* 1 : in press, 2006
  60. Sampson JH, Akabani G, Archer GE, Bigner DD, Berger MS, Friedman AH, et al : Progress report of a Phase I study of the intracerebral microinfusion of a recombinant chimeric protein composed of transforming growth factor(TGF)-alpha and a mutated form of the *Pseudomonas* exotoxin termed PE-38 (TP-38) for the treatment of malignant brain tumors. *J Neurooncol* 65 : 27-35, 2003
  61. Sampson JH, Crotty LE, Lee S, Archer GE, Ashley DM, Wikstrand CJ, et al : Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors. *Proc Natl Acad Sci USA* 97 : 7503-7508, 2000
  62. Schneider T, Gerhards R, Kirches E, Firsching R : Preliminary results of active specific immunization with modified tumor cell vaccine in glioblastoma multiforme. *J Neurooncol* 53 : 39-46, 2001
  63. Sehati N, Liau LM : Adjuvant treatment for gliomas. *Contemporary Neurosurgery* 25 : 1-9, 2003
  64. Shimamura T, Husain SR, Puri RK : The IL-4 and IL-13 *Pseudomonas* exotoxins : new hope for brain tumor therapy. *Neurosurg Focus* 20 : E11, 2006
  65. Smits HA, van Beelen AJ, de Vos NM, Rijsmus A, van der Bruggen T, Verhoef J, et al : Activation of human macrophages by amyloid-beta is attenuated by astrocytes. *J Immunol* 166 : 6869-6876, 2001
  66. Steiner HH, Bonsanto MM, Beckhove P, Brysch M, Geletneký K, Ahmadi R, et al : Antitumor vaccination of patients with glioblastoma multiforme : a pilot study to assess feasibility, safety, and clinical benefit. *J Clin Oncol* 22 : 4272-4281, 2004
  67. Stewart LA : Chemotherapy in adult high-grade glioma : a systematic review and meta-analysis of individual patient data from 12 randomised trials. *Lancet* 359 : 1011-1018, 2002
  68. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al : Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352 : 987-996, 2005
  69. Takagi Y, Kikuchi T, Niimura M, Ohno T : Effects of glucocorticoids on antitumor effects of immunizations with fusions of dendritic and tumor cells. *Anticancer Res* 23 : 2553-2558, 2003
  70. Taniguchi Y, Ono K, Yoshida S, Tanaka R : Antigen-presenting capability of glial cells under glioma-harboring conditions and the effect of glioma-derived factors on antigen presentation. *J Neuroimmunol* 111 : 177-185, 2000
  71. Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ, et al : Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *J Clin Oncol* 20 : 142-152, 2002
  72. Ulevitch RJ : Therapeutics targeting the innate immune system. *Nat Rev Immunol* 4 : 512-520, 2004
  73. Walker PR, Calzascia T, Dietrich PY : All in the head: obstacles for immune rejection of brain tumours. *Immunology* 107 : 28-38, 2002
  74. Walker PR, Calzascia T, Schnuriger V, Scamuffa N, Saas P, de Tribolet N, et al : The brain parenchyma is permissive for full antitumor CTL effector function, even in the absence of CD4 T cells. *J Immunol* 165 : 3128-3135, 2000
  75. Wang LX, Huang WX, Graor H, Cohen PA, Kim JA, Shu S, et al : Adoptive immunotherapy of cancer with polyclonal, 108-fold hypere-expanded, CD4+ and CD8+ T cells. *J Transl Med* 2 : 41, 2004
  76. Wiendl H, Mitsdoerffer M, Weller M : Hide-and-seek in the brain : a role for HLA-G mediating immune privilege for glioma cells. *Semin Cancer Biol* 13 : 343-351, 2003
  77. Wikstrand CJ, Cole VR, Crotty LE, Sampson JH, Bigner DD : Generation of anti-idiotypic reagents in the EGFRvIII tumor-associated antigen system. *Cancer Immunol Immunother* 50 : 639-652, 2002
  78. Wikstrand CJ, Zalutsky MR, Bigner DD : Radiolabeled antibodies for therapy of brain tumors, in Liau LM, Becker DP, Cloughesy TF, Bigner DD (eds) : *Brain Tumor Immunotherapy*, Totowa : Humana Press, 2001, pp 205-229
  79. Wischhusen J, Schneider D, Mittelbronn M, Meyermann R, Engelmann H, Jung G, et al : Death receptor-mediated apoptosis in human malignant glioma cells: modulation by the CD40/CD40L system. *J Neuroimmunol* 162 : 28-42, 2005
  80. Wood GW, Holladay FP, Turner T, Wang YY, Chiga M : A pilot study of autologous cancer cell vaccination and cellular immunotherapy using anti-CD3 stimulated lymphocytes in patients with recurrent grade III/IV astrocytoma. *J Neurooncol* 48 : 113-120, 2000
  81. Yamanaka R, Abe T, Yajima N, Tsuchiya N, Homma J, Kobayashi T, et al : Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses : results of a clinical phase I/II trial. *Br J Cancer* 89 : 1172-1179, 2003
  82. Yamanaka R, Homma J, Yajima N, Tsuchiya N, Sano M, Kobayashi T, et al : Clinical Evaluation of Dendritic Cell Vaccination for Patients with Recurrent Glioma : Results of a Clinical Phase I/II Trial. *Clin Cancer Res* 11 : 4160-4167, 2005
  83. Yamanaka R, Yajima N, Abe T, Tsuchiya N, Homma J, Narita M, et al : Dendritic cell-based glioma immunotherapy (review). *Int J Oncol* 23 : 5-15, 2003
  84. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ : Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 64 : 4973-4979, 2004
  85. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, et al : Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61 : 842-847, 2001