



Edward D. Boit. *Looking into the Arno Valley*. Oil on canvas, 22" × 28".  
The Hainsworth Collection. Photo credit: R. H. Love Galleries, Chicago, Illinois.

*Several immune-mediated treatment approaches for malignant brain tumors, as well as the relevance and outcomes from recent clinical trials, are reviewed.*

# Recent Progress in Immunotherapy for Malignant Glioma: Treatment Strategies and Results From Clinical Trials

Moneeb Ehtesham, MD, Keith L. Black, MD, and John S. Yu, MD

**Background:** *Despite advances in surgical and adjuvant radiation therapy and chemotherapy strategies, malignant gliomas continue to be associated with poor prognoses.*

**Methods:** *We review immune-mediated treatment approaches for malignant glioma and the relevance of recent clinical trials and their outcomes. We specifically address the increasing evidence implicating the role of cytotoxic T cells in ensuring adequate immune-mediated clearance of neoplastic cells and the need for the optimization of therapies that can elicit and support such antitumor T-cell activity.*

**Results:** *The poor outcome of this disease has spurred the search for novel experimental therapies that can address and overcome the root biological phenomena associated with the lethality of this disease. The use of immunotherapy to bolster the otherwise impaired antitumor immune responses in glioma patients has received increasing attention.*

**Conclusions:** *An effective treatment paradigm for malignant gliomas may eventually require a multifaceted approach combining two or more different immunotherapeutic strategies. Such scenarios may involve the use of local cytokine gene therapy to enhance glioma-cell immunogenicity in conjunction with dendritic cell-based active vaccination to stimulate systemic tumoricidal T-cell immunity. Given the heterogeneity of this disease process and the potential risk of immunoediting out a selected, treatment-refractory tumor cell population, the concurrent use of multiple modalities that target disparate tumor characteristics may be of greatest therapeutic relevance.*

*From the Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center, Los Angeles, California. Dr Ehtesham is now at Vanderbilt University Medical Center, Nashville, Tenn.*

*Submitted June 2, 2003; accepted October 28, 2003.*

*Address reprint requests to John S. Yu, MD, Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center, 8631 West Third Street, Suite 800 East, Los Angeles, CA 90048. E-mail: John.Yu@csbs.org*

*No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.*

## Introduction

Despite dramatic advances in surgical technique, imaging, and adjuvant radiation therapy or chemotherapy, the prognosis for patients with malignant glial tumors remains poor.<sup>1,2</sup> The median survival following diagnosis of glioblastoma multiforme (GBM), the most common and aggressive subtype of malignant glioma, is under 1 year, with a 2-year survival rate approaching zero.<sup>3</sup> The dis-

seminated nature of these neoplasms makes current therapeutic interventions highly ineffective at eradicating all residual intracranial tumor reservoirs. This leads to near universal tumor recurrence that, in turn, contributes to the lethality of this disease.

The development of a successful treatment modality for malignant brain tumors will center on devising a means of eliminating all intracranial neoplastic foci left behind after surgical resection of the primary tumor mass. This is a daunting task, given the highly disseminated nature of the disease process and our current inability to adequately visualize and therapeutically target every remaining tumor cell. However, treatment approaches aimed at using the body's own immune system to combat intracranial neoplasms hold promise for achieving this objective. As demonstrated in numerous experimental settings,<sup>4,7</sup> therapeutic strategies that aim to stimulate immune recognition and clearance of glioma cells have the potential to generate powerful immune-mediated killing of all intracranial neoplastic foci. Many approaches toward bolstering antitumor immunity using a variety of immunostimulatory therapies have been tested in rodent brain tumor models. Although many of these strategies

have proven highly effective in animals, their translation into human patients has not been as successful. Nevertheless, promising data have been presented in the literature, and numerous clinical immunotherapy trials for patients with malignant brain tumors are currently underway. We discuss various immune-mediated treatment approaches for gliomas as well as the relevance of recent clinical trials and their outcomes.

## Relevance of Immunotherapy to the Treatment of Malignant Glioma

Oncogenesis and immune suppression are likely to be closely interlinked processes. It has been theorized that clearance of transformed neoplastic cells may be a routine physiological function of the normal, noncompromised immune system. This phenomenon, known as *immune surveillance*, is thought to be an evolutionary mechanism largely mediated by natural killer (NK) cells and to a lesser extent by cytotoxic T cells, that may serve to eliminate neoplastic cells that arise spontaneously due to genetic mutations or other oncogenic signals.<sup>8,9</sup> Cancerous cells

that successfully evade this initial immune killing are then able to subsequently propagate into established tumors. As they proliferate, these tumor cells accumulate additional mutations secondary to Darwinian selection, which may confer additional immuno-evasive survival advantages on the growing neoplasm. Consequently, by the time the tumor is clinically detectable, it has developed potent immunosuppressive qualities that enable it to depress host antitumor immunity. This may be particularly relevant in patients with malignant gliomas, who demonstrate significantly impaired immune function (Fig 1).<sup>10,11</sup> These defects include depressed peripheral T-cell responsiveness, as evidenced by cutaneous anergy to tumor antigens or nonspecific mitogens,<sup>10,12,13</sup> as well as depressed T-cell receptor-mediated signaling.<sup>14</sup> In addition, gliomas are associated with overall lymphopenia,<sup>15-17</sup> depressed antibody production,<sup>18,19</sup> and impaired antigen-presenting cell (APC) function.<sup>20,21</sup> It is postulated that many of these systemic immunosuppressive effects are mediated by tumor-expressed chemokines, possibly transforming growth factor beta (TGF- $\beta$ ),<sup>22,23</sup> prostaglandin E<sub>2</sub> (PGE<sub>2</sub>),<sup>24,25</sup> certain gangliosides,<sup>26,27</sup> and/or interleukin-10 (IL-10),<sup>28-30</sup> although evidence exists that a

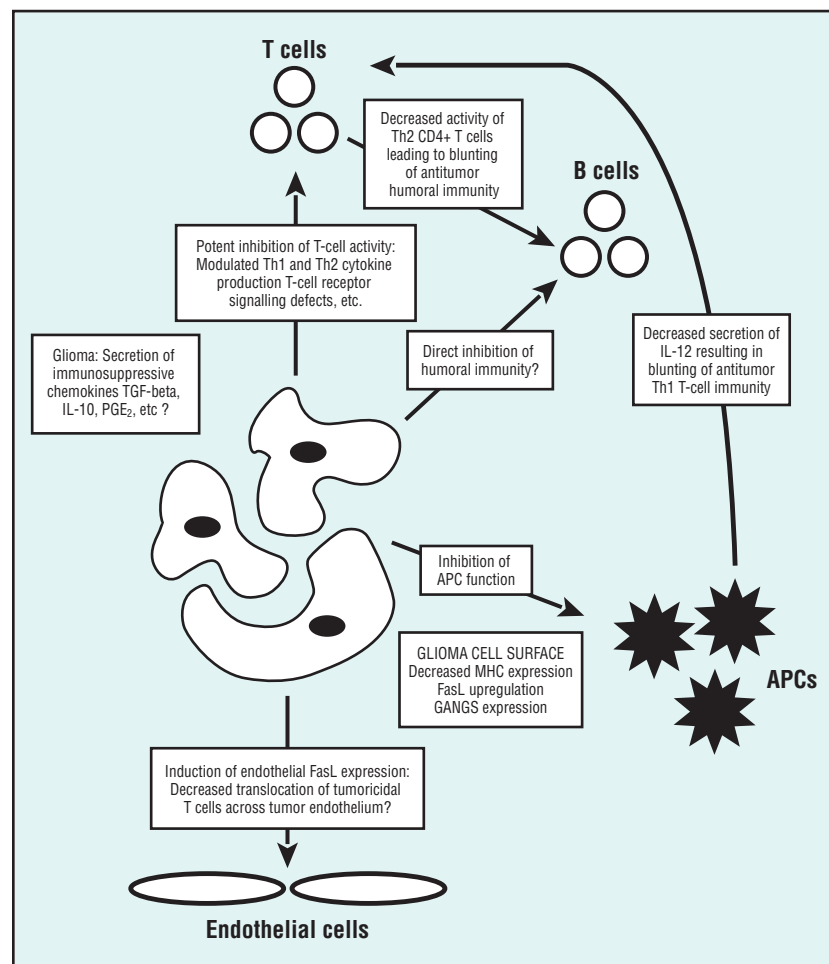


Fig 1. — Schematic representation of immunosuppressive mechanisms associated with malignant gliomas (GANGS = glioma-associated gangliosides, APCs = antigen-presenting cells).

significant portion of the immune-depressing effect may be associated with an additional, as of yet unidentified, glioma-secreted soluble factor.<sup>31</sup> In addition to inhibiting systemic antitumor immune activity, gliomas may also impair tumoricidal immune responses within the local tumor microenvironment. Glioma cells may downregulate their surface expression of major histocompatibility complex (MHC) molecules,<sup>32</sup> thereby depriving infiltrating immune cells of signals necessary for recognizing and clearing tumor cells. Localized deregulation of antitumor immunity is also evident in the polarization of the majority of glioma-infiltrating T cells toward a Th2 state of activation, at the expense of a Th1 response that is necessary for effective cell-mediated killing of neoplasms.<sup>33</sup> In addition, gliomas may induce the localized expression of certain proteins such as Fas ligand (FasL) that may inhibit the translocation of tumoricidal T cells into and through neoplastic tissue.<sup>34</sup> Recent evidence also indicates that gliomas may induce the upregulation of FasL on tumor-associated endothelium, thereby hindering translocation of antitumor T cells across vascular epithelium.<sup>4,35</sup>

It has long been recognized that boosting otherwise impaired tumor-specific immune responses can successfully eradicate neoplasms in experimental rodent models of intracranial glioma.<sup>4,5,7,36</sup> Immunotherapeutic approaches aim to bolster the arms of the immune system that have been affected by tumor-induced suppressive factors. Conceivably, if effective, a therapeutically relevant immune-mediated treatment strategy for glioma should successfully overcome tumor-associated immunosuppression to the extent that the immune system can adequately recognize and clear all residual neoplastic foci within the brain following surgical resection of the primary tumor mass. This would minimize the risk of tumor recurrence and therefore improve long-term survival. However, translation of these strategies into clinical scenarios has not duplicated the successes of the preclinical phase. In general, this underscores the complexity of treating an advanced malignant process in a poorly controlled clinical setting as opposed to the closely controlled therapy imparted in the laboratory. Despite the mixed outcomes, clinical trials have generated important data regarding immune system function and modulation in the setting of intracranial glioma, which can serve as a framework for further refining of future immunotherapeutic approaches. In the field of brain tumors, the term *immunotherapy* has been used widely and encompasses passive strategies utilizing immune molecules such as antibodies or cytokines as tumor-toxic vehicles or chemokines, as well as active strategies such as tumor cell-based or APC-based vaccination strategies that stimulate endogenous antitumor T-cell responses. In general, these various approaches can be divided into the following broad categories: passive serologic immunotherapy, adoptive cell transfer, cytokine therapy, and active cell-mediated immunotherapy.

Passive serologic therapy involves the use of monoclonal antibodies, usually conjugated with a tumor-toxic payload, that are targeted against antigens expressed in abundance on cancerous cells. Adoptive cell transfer in the setting of gliomas has generally involved the transplantation of nonspecific lymphokine-activated killer (LAK) cells in patients with malignant brain tumors. Cytokine therapy utilizes either the intravenous or intratumoral administration of immunostimulatory chemokines with the aim of either causing direct toxicity to tumor cells, enhancing their immunogenicity, or directly supporting otherwise depressed antitumor cell-mediated immune responses. In contrast to these earlier strategies, active immunotherapy aims to promote effective tumor-specific cell-mediated immunity. This is achieved by sensitizing either endogenous APCs or naive T cells to tumor-expressed antigens. Successful active vaccination results in the expansion of cytotoxic T-cell clones specific for certain tumor antigens that are then able to exert their effector function by targeting and eliminating neoplastic cells. Apart from successful eradication of the entire tumor burden in experimental glioma models,<sup>5,37</sup> active immunotherapy can also confer long-term immunity against tumor, a cardinal sign of a successful acquired immune response.<sup>37</sup> In general, there has been accumulating evidence that of all these approaches, discussed in detail below, active immunization may be the most relevant and effective immunotherapeutic modality for malignant brain tumors, given its potency, ability to stimulate effective antitumor T-cell immunity and the capacity to produce long-term immune memory.

## Passive Serologic Immunotherapy

The use of antibodies to specifically target tumor cells represents a potential means of selectively treating neoplastic infiltrates within normal tissue. Theoretically, a highly specific monoclonal antibody could be designed to recognize a cell-surface epitope exclusively expressed on cancerous cells. This antibody could be conjugated with a toxic payload, such as a radioactive isotope, which could then be selectively delivered to tumor cells with minimal toxicity to normal tissue. Additionally, an antibody could be designed to neutralize a tumor-specific cell-surface receptor controlling downstream signaling pathways essential for neoplastic cell survival. However, in the setting of intracranial gliomas, this strategy has several key limitations. The first is the difficulty in definitively identifying a prominent cell surface protein that is ubiquitously expressed on malignant glioma cells but not on normal brain tissue. Many antigens have been identified whose expression is clearly upregulated in brain tumors, but none are exclusive to glioma, and therefore their use as an antibody target would endanger normal brain cells. Additionally, any tumor-specific antigen identified for this pur-

pose would have to exhibit stability of expression (eg, lack of shedding or internalization prior to antibody binding), and a minimum density ( $\geq 1 \times 10^5$  molecules per tumor cell) in order for antibody-mediated targeting to have a viable therapeutic effect.<sup>38</sup> Antigens on human gliomas that have been clinically targeted in this manner include a nondefined "glioma-associated antigen,"<sup>39,40</sup> neural cell adhesion molecule,<sup>41,42</sup> the chondroitin sulfate proteoglycan,<sup>43</sup> tenascin,<sup>43,44</sup> wild-type epidermal growth factor receptor (EGFR),<sup>45,46</sup> and a glioma-associated mutated form of EGFR designated as EGFRvIII.<sup>47</sup> Of these strategies, the most significant have involved the targeting of tenascin and EGFR.

### **Tenascin**

Tenascin is one of the most extensively targeted glioma-associated antigens. In malignant brain tumors, tenascin is principally elaborated as an extracellular matrix protein, and its deposition has been detected in up to 90% of all gliomas.<sup>48</sup> Riva and colleagues<sup>44,49-53</sup> have described the use of two monoclonal anti-tenascin antibodies, BC-2 and BC-4, in clinical trials. BC-2 was conjugated to <sup>131</sup>I and administered via an indwelling catheter into postresection tumor cavities in patients with newly diagnosed as well as recurrent grade 3 and 4 gliomas. BC-4 was linked to yttrium-90 (<sup>90</sup>Y), a pure beta radio emitter, and infused directly into the tumor bed or postresection tumor cavity. These scenarios yielded no major systemic or central nervous system (CNS) toxicity, although up to 69% of patients did develop human antimouse antibodies (HAMA) in a dose-dependent fashion.<sup>44</sup> Even though the HAMA responses were not associated with any overt allergic or anaphylactic symptoms, their presence could potentially impart a limiting effect on any therapeutic benefit derived from antibody-mediated glioma immunotherapy. Recently Cokgor et al<sup>54</sup> and Bigner et al<sup>55</sup> have conducted escalated-dose clinical trials with a <sup>131</sup>I-labeled 81C6 monoclonal anti-tenascin antibody that have yielded encouraging results. Thirty-four recurrent or metastatic brain tumor patients who had received initial surgery and external beam therapy were administered a single antibody dose directly into the postsurgical tumor cavity. Resulting median survival times were reported by the authors to be significantly longer than those associated with conventional surgery, high-dose rate brachytherapy, stereotactic radiosurgery, or external beam radiotherapy with carmustine wafer implantation.<sup>55</sup> An additional phase I trial by the same group involving newly diagnosed glioma patients with no prior radiation or chemotherapy also resulted in minimal toxicity and encouraging median survival rates (79 weeks for all patients with glioma and 69 weeks for those with GBM).<sup>54</sup> These studies were then extended into a phase II trial that again demonstrated encouraging treatment-related median survival (86.7 weeks for all patients with glioma and 79.4 weeks for those with GBM),

although therapy was associated with hematologic and neurologic toxicity in 27% and 15% of patients, respectively.<sup>56</sup> These promising results illustrate the potential utility of antibody-mediated glioma immunotherapy. However, issues related to HAMA responses in treated patients and long-term toxicity issues secondary to direct infusion of high-dose radioisotopes still represent important problems associated with this approach. Additionally, the fact that tenascin in gliomas is largely an extracellular protein indicates that it may not be the best candidate for specific targeting of intracranial glioma cells.

### **Epidermal Growth Factor Receptor**

Another target utilized for antibody-mediated therapy in glioma is EGFR, a transmembrane glycoprotein with an extracellular domain ligand binding site for either EGF or TGF- $\alpha$ . EGFR is widely expressed in the body, particularly in epithelial cells in the liver.<sup>57</sup> With regard to gliomas, EGFR expression increases with grade and is immunohistochemically detectable in 27% to 57% of low-grade astrocytomas and in up to 90% of GBM.<sup>48</sup> An initial trial by Kalofonos et al<sup>45</sup> using intravenous or intracarotid administration of anti-EGFR or anti-placental alkaline phosphatase antibodies in 10 patients with GBM reported no association between outcome and therapy. Subsequently, a more comprehensive phase II trial used a <sup>125</sup>I conjugated monoclonal anti-EGFR antibody designated MAb 425 in patients with high-grade gliomas following surgery and external beam radiotherapy.<sup>46,58</sup> Therapy was associated with relatively low toxicity and no reported development of human antimouse antibody (HAMA) responses. However, median survival times were 56 weeks for 60 patients with newly diagnosed GBM and 52 months for 79 patients with anaplastic astrocytoma (AA), which did not represent a major improvement over conventional survival rates. Crombet et al<sup>59</sup> reported a phase I clinical trial utilizing a neutralizing anti-EGFR antibody compared with the radioisotope conjugates described earlier. Nine patients with active or recurrent gliomas or meningiomas were treated with intravenous doses of antibody. No objective responses to treatment were noted, and 1 patient developed a severe grade 4 allergic adverse response. Overall, experiences with EGFR targeting have not resulted in the same promising results that have been achieved with anti-tenascin therapy. Additionally, the widespread expression of EGFR makes its use as a therapeutically relevant treatment target highly questionable. However, a mutant variant of EGFR, designated EGFRvIII, has been identified in up to 50% of gliomas.<sup>57</sup> The mutation consists of an in-frame deletion of certain NH<sub>2</sub>-terminal residues from the extracellular domain of EGFR, resulting in a unique variant that is specifically expressed in gliomas. Sampson et al<sup>47</sup> described the successful use of an anti-EGFRvIII antibody in treating murine intracranial EGFRvIII overexpressing melanomas. Thus, the development of specific antibody-

mediated targeting of EGFRvIII as a specific means of delivering tumor toxic payloads to glioma is promising as a treatment option for intracranial brain tumors.

Despite some encouraging results from clinical trials utilizing anti-tenascin antibodies, the use of passive serologic immunotherapy is hampered by several key limitations. The failure to locate a unique tumor cell-surface protein with critical downstream survival-related function may eventually limit the utility of this therapeutic modality. The identification of EGFRvIII, as a glioma-specific variant of EGFR has been promising. However, it is unknown whether this mutant receptor plays any significant role in tumor cell survival, and the use of tumor surface antigens solely for targeted delivery of toxic radioisotopes in the brain remains a potentially contentious issue due to the possibility of long-term CNS toxicity. Additionally, given the significant cellular heterogeneity in gliomas, it is unclear whether a solitary antigen may ever suffice as a viable target for any form of immunotherapy.

## Adoptive Immunotherapy

Adoptive immune therapies transfer potentially tumoricidal T-cell populations into glioma-bearing hosts. Specific protocols have utilized either systemic infusion or local intracerebral inoculation as a means to deliver these cells to brain tumors. Initial approaches to adoptive cellular therapy involved the placement of autologous but nonactivated immune cells, using either intratumoral<sup>60,62</sup> or intrathecal<sup>63-65</sup> administration. Subsequent studies combined this approach with the administration of interferon,<sup>66,67</sup> but these strategies did not yield promising results. The identification of IL-2 as a potent T-cell growth factor led to the development of LAK cell-based adoptive therapies. LAK cells are peripherally isolated populations of lymphocytes capable of lysing NK cell-resistant tumor targets *in vitro* following stimulation with IL-2.<sup>68</sup> LAK cells are polyclonal in that their activation is nonspecific and not directed toward any particular tumor antigen(s). The use of LAK cells for the treatment of glioma has been investigated extensively, generally with transplantation of these cells into the post resection surgical cavity.<sup>69</sup> Hayes et al<sup>70</sup> reported encouraging results in 19 patients with GBM and AA treated with a combination of LAK cells and IL-2. Complete responses to treatment were observed in 1 patient with GBM and another with AA. Additionally, median survival for patients with GBM was 53 weeks following reoperation compared with 25.5 weeks for a matched control group that received chemotherapy post-surgery. Despite these encouraging results, other trials did not produce similar success,<sup>71</sup> and significant toxicity was also described in one study.<sup>72</sup>

Subsequent investigations focused on the adoptive transfer of more specific T-cell populations. Initial attempts to therapeutically utilize "presensitized" tumor-

infiltrating lymphocyte populations did not meet with much success,<sup>73</sup> although one clinical report by Quattrocchi and colleagues<sup>74</sup> described a somewhat encouraging outcome. However, in retrospect, the lack of a consistently encouraging outcome was predictable, given the multiple immune defects that have been observed in glioma-infiltrating immune cells, which may be secondary to inherent defects in the tumor-infiltrating lymphocytes themselves or a result of the action of glioma-elaborated immunosuppressive factors on these isolated populations. Further investigations involved the inoculation of *ex vivo* sensitized lymphocytes isolated from tumor-draining lymph nodes, which proved more encouraging in preclinical rodent studies.<sup>75,76</sup> Initial protocols utilized glioma cells to stimulate isolated lymphocyte populations *in vitro*.<sup>77</sup> More recently, Plautz and Shu<sup>78</sup> described the use of anti-CD3 antibodies and bacterial superantigens to directly stimulate T-cell activation. This approach was utilized in a clinical trial for glioma patients and used in conjunction with an autologous tumor cell vaccine. Results were encouraging, with 4 of 12 treated patients demonstrating partial tumor regression.<sup>79</sup> Kruse et al<sup>80</sup> forwarded an alternate approach utilizing allogeneic rather than autologous T-cell populations for adoptive transfer as a treatment for gliomas. This strategy is based on the rationale that MHC-mismatched cytotoxic T lymphocytes (CTLs) will produce a more potent tumoricidal response and has been validated by these investigators in preclinical studies. However, subsequent assessment of this strategy in a clinical setting has not proven as promising.<sup>81</sup>

The ability of these approaches to adequately generate tumor-specific T-cell clones is questionable since it is now known that antigen-specific T-cell activation results from a complex pathway involving not only the T-cell receptor (CD3 antigen), but also essential co-stimulatory signals that can be adequately provided only by professional APCs. Additionally, the harvest and *ex vivo* activation of lymphocytes from glioma patients is inherently problematic, given the plethora of T-cell defects that have been documented in these cases. Although popular during the early days of glioma immunotherapy, there has been increasing evidence justifying an evolution away from the nonspecific nature of adoptive cell transfer toward more targeted immunotherapeutic approaches using active vaccination protocols that can elicit tumor-directed T-cell responses *in vivo*. As demonstrated by Plautz et al,<sup>79</sup> the use of adoptive transfer strategies may still be relevant if used in combination with active immunotherapeutic approaches.

## Cytokine Therapy

The identification of cytokines — key effector molecules responsible for initiating, supporting, or blunting specific immune pathways — led to the assumption that the ther-

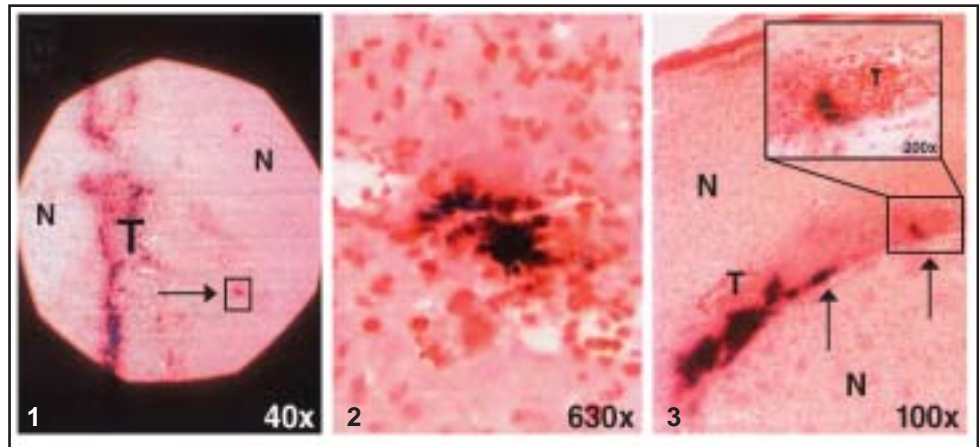
apeutic application of these chemokines could potentiate immune surveillance and induce cell-mediated antitumor immunity. The use of cytokine therapy for cancer has received considerable attention since then, with the use of a variety of agents in a wide range of neoplastic models. With particular regard to glioma immunotherapy, much attention has focused on cytokines involved in the promotion of antitumor T-cell activity. These have included IL-2,<sup>82,83</sup> IL-4,<sup>84,85</sup> the interferons (IFN- $\alpha$ , - $\beta$ , and - $\gamma$ ),<sup>86-88</sup> and IL-12,<sup>6,89</sup> among others. In addition, several studies have attempted to enhance the immunogenicity of *in vivo* glioma cells by treating with cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) or IFN- $\gamma$ , which induce upregulation of cell-surface MHC, thereby increasing "visibility" of the tumor cells to the immune system.<sup>4,90</sup> Cytokines such as TNF- $\alpha$  and its associated chemokine TRAIL (TNF-related apoptosis-inducing ligand) have also been utilized to induce direct tumor cell death, usually by means of triggering apoptotic cascades specifically within neoplastic cells.<sup>91-94</sup> Roles for certain cytokines including IFN- $\gamma$  have also been described in inhibiting intracranial tumor-induced neovascularization.<sup>95</sup> The use of cytokines was initially investigated utilizing systemic recombinant cytokine therapy as a means of delivering a high, potent dose to stimulate strong tumoricidal responses. This strategy, although highly effective at eradicating tumors in rodents,<sup>96,97</sup> has generally been of limited applicability in human cancers due to either issues of toxicity or subtherapeutic half-life.<sup>98,99</sup> For malignant brain tumors, the use of cytokine therapy must also take into account the privileged status of the central nervous system vis-à-vis the blood-brain barrier (BBB). Given these limitations, recent strategies employing cytokine therapy have evolved toward locoregional delivery paradigms that can circumvent the physiologic and pharmacokinetic barriers associated with earlier strategies.

One of the first cytokines to be studied in the setting of gliomas was IL-2, an important T-cell growth factor involved in the proliferation of CD8<sup>+</sup> T cells and modulation of their cytotoxic activity. Various early *in vitro* studies supported a rationale for the use of IL-2 in patients with malignant brain tumors. This rationale was based largely on the ability of IL-2 to block the T-cell depressing capacities of TFG- $\beta$ , thought to be a key glioma-elaborated immunosuppressive chemokine.<sup>100-102</sup> Early clinical trials utilizing IL-2 for patients with glioma, frequently in combination with nonspecific LAK therapy, did not meet with much success.<sup>66,72,103,104</sup> These attempts involved systemic, intrathecal, and intratumoral administration of cytokine. CNS toxicity, including the development of cerebral edema secondary to IL-2 mediated increases in vascular permeability, were found to play a significant role in limiting the dose of cytokine that could be administered.<sup>105</sup> Several clinical trials have also focused on the use of IFN- $\alpha$ <sup>105-107</sup> and IFN- $\beta$ ,<sup>87,108,109</sup> often in addition to either radiation therapy or chemotherapy. One study reported up to

50% of treated patients demonstrating stable disease or tumor regression,<sup>109</sup> but subsequent trials did not yield encouraging results.<sup>86</sup> Additionally, many of the initial interferon trials were not well designed, and lack of uniformity regarding patient selection, imaging analysis, and tumor grading made it difficult to objectively assess whether the responses reported could truly be attributed to cytokine therapy.

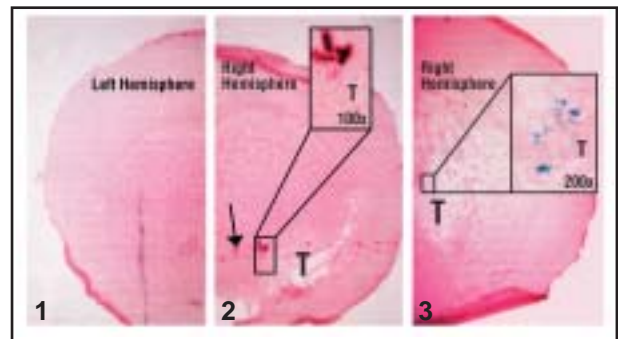
Early trials revealed the limitations of recombinant cytokine therapy related to issues of toxicity and short half-life. An attempt to address these concerns was made by engineering cells, using gene transfer techniques, to secrete relevant cytokines directly. These cells could then be transplanted into intracranial tumors, achieving a steady level and long-lasting localized production of cytokine, thereby circumventing the need to administer recombinant protein. Yu et al<sup>85</sup> described such a therapy involving engineered glioma cells secreting IL-4 inoculated into human glioma xenografts established in athymic T-cell incompetent rodents. Subsequently, Glick and colleagues<sup>82</sup> described the use of intratumorally administered IL-2-secreting fibroblasts in a GL261 rodent glioma model, with considerable therapeutic success. More recently, strategies based on gene therapy have evolved that utilize direct intratumoral cytokine gene transfer into intracranial gliomas, usually by means of engineered, cytokine gene-bearing attenuated viral vectors. This modality has the advantage of delivering high viral titers into the tumor bed with efficient subsequent infection and cytokine secretion by tumor tissue, and it obviates the concern of immune rejection of transplanted allogeneic cytokine-secreting cells. Benedetti and colleagues<sup>84</sup> described the use of retroviral-based delivery of IL-4, a supporter of Th2-mediated humoral immunity, in rodent gliomas. Subsequently, the uses of gene therapy approaches to deliver IL-12, a powerful stimulator of Th1-based antitumor cytotoxic immune responses, and the pluripotent immunomodulatory cytokines TNF- $\alpha$  and IFN- $\gamma$  to enhance tumor-cell immunogenicity, have been described.<sup>4,6</sup> Also, a role for retrovirally transduced glioma cells producing macrophage colony-stimulating factor (M-CSF) has been described.<sup>110</sup> These therapies have yielded encouraging preclinical results with clear evidence confirming the development of potent tumoricidal immunity. Recently, cell-based cytokine delivery paradigms have again come to light with the development of neural stem cell (NSC)-based delivery systems. NSCs are capable of exhibiting potent intracranial migratory activity and are tropic for migrating glioma microsatellites as they disseminate through the brain. Cytokine-secreting NSCs are therefore capable of tracking and delivering tumoricidal payloads directly to these neoplastic reservoirs. This targeted delivery represents a novel strategy that specifically addresses the highly invasive and disseminated nature of intracranial gliomas, a key factor that has limited the efficacy of most experimental and clinical therapies for this disease.

Fig 2A-D. — (A) Neural stem cells demonstrate tropism for disseminating glioma in vivo. C57Bl/6 mice bearing intracranial GL26 gliomas were inoculated intratumorally with  $\beta$ -galactosidase expressing neural stem cells (NSCs). Subsequently, histologic sections were stained with X-gal and counterstained with neutral red. NSCs appear blue as they express  $\beta$ -galactosidase whereas tumor appears as hypercellular areas staining intensely with neutral red. T designates tumor mass, outgrowths, and microsattellites. Arrows indicate disseminating NSCs closely following migrating pockets of tumor. Several distinct patterns of tumor spread were detected, and NSCs were found tracking migrating glioma in each case. Panel 1 demonstrates thin outgrowth of tumor cells deep into adjacent normal brain. Panel 2 represents direct extension of tumor mass into adjacent tissue. Panel 3 illustrates migration of glioma cells away from the primary tumor bed along a white matter tract. (B) NSCs can migrate to the contralateral hemisphere toward existing intracranial tumor. NSCs were



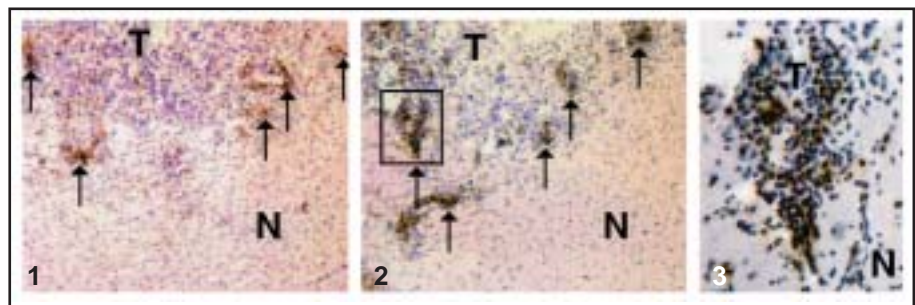
2A

inoculated contralateral to established murine intracranial gliomas. Panel 1 represents portion of left cerebral hemisphere where NSCs were inoculated and illustrates that NSCs do not randomly dissipate into adjacent non-tumorous tissue. Panel 2 shows tumor-bearing portion of right cerebral hemisphere demonstrating specific nonrandom migration of NSCs across the brain into the vicinity of the tumor in the opposite hemisphere (inset box). Panel 3 demonstrates a second tumor-bearing portion of a similarly treated brain.  $\beta$ -galactosidase expressing NSCs are visible, interspersed within the tumor mass (inset box), contralateral to their site of inoculation. (C) NSC-mediated delivery of IL-12 in established gliomas induces robust intratumoral T-cell infiltration. Established intracranial murine gliomas were inoculated with IL-12 expressing NSCs. Subsequent immunohistochemical analysis of tumor sections revealed robust infiltration of tumors with CD4<sup>+</sup> (panel 1) and CD8<sup>+</sup> (panel 2 and panel 3) T cells, with numerous aggregates along the tumor/normal tissue boundary (arrows). T designates tumors and N represents normal brain tissue. Panel 3 is a high-power image of the boxed area in panel 2. Magnification = 100 $\times$  for panels 1 and 2 and 400 $\times$  for panel 3. (D) NSCs utilized to deliver TRAIL to intracranial gliomas elicit overwhelming apoptosis in main tumor mass and tumor satellites. Established intracranial human glioma xenografts in mice were treated with intratumoral inoculation of TRAIL-expressing NSCs. Brains were then analyzed histologically for apoptotic activity using a TUNEL assay (red staining). Slides were counterstained with hematoxylin. Panel 1 demonstrates a treated tumor exhibiting almost complete cellular apoptosis in the main tumor mass (T). Panel 2 represents a higher magnification of the boxed area in panel 1, demonstrating specificity of staining in the apoptotic tumor and lack of apoptosis in adjacent normal tissue. Panel 3 illustrates a section from another TUNEL-stained tumor-bearing brain treated with TRAIL-expressing NSCs. Note the highly apoptotic primary tumor mass (T, demarcated by arrowheads). Significant apoptosis is also visible within a tumor satellite (t) at considerable distance from the main tumor mass. Panel 4 is a higher magnification of the boxed area in panel 3, demonstrating specificity of TUNEL staining in the tumor satellite.



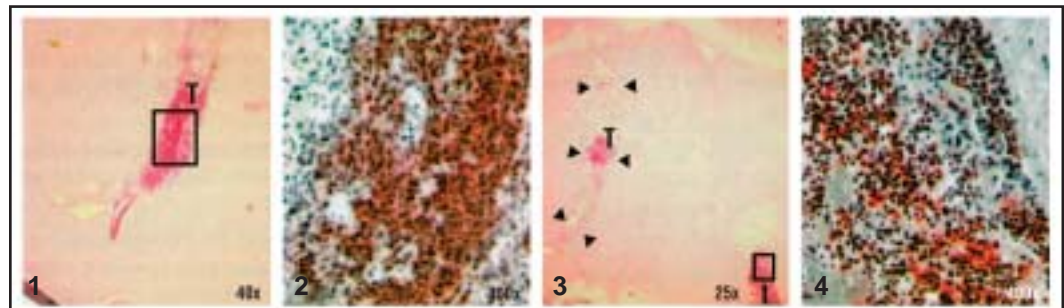
2B

inoculated contralateral to established murine intracranial gliomas. Panel 1 represents portion of left cerebral hemisphere where NSCs were inoculated and illustrates that NSCs do not randomly dissipate into adjacent non-tumorous tissue. Panel 2 shows tumor-bearing portion of right cerebral hemisphere demonstrating specific nonrandom migration of NSCs across the brain into the vicinity of the tumor in the opposite hemisphere (inset box). Panel 3 demonstrates a second tumor-bearing portion of a similarly treated brain.  $\beta$ -galactosidase expressing NSCs are visible, interspersed within the tumor mass (inset box), contralateral to their site of inoculation. (C) NSC-mediated delivery of IL-12 in established gliomas induces robust intratumoral T-cell infiltration. Established intracranial murine gliomas were inoculated with IL-12 expressing NSCs. Subsequent immunohistochemical analysis of tumor sections revealed robust infiltration of tumors with CD4<sup>+</sup> (panel 1) and CD8<sup>+</sup> (panel 2 and panel 3) T cells, with numerous aggregates along the tumor/normal tissue boundary (arrows). T designates tumors and N represents normal brain tissue. Panel 3 is a high-power image of the boxed area in panel 2. Magnification = 100 $\times$  for panels 1 and 2 and 400 $\times$  for panel 3. (D) NSCs utilized to deliver TRAIL to intracranial gliomas elicit overwhelming apoptosis in main tumor mass and tumor satellites. Established intracranial human glioma xenografts in mice were treated with intratumoral inoculation of TRAIL-expressing NSCs. Brains were then analyzed histologically for apoptotic activity using a TUNEL assay (red staining). Slides were counterstained with hematoxylin. Panel 1 demonstrates a treated tumor exhibiting almost complete cellular apoptosis in the main tumor mass (T). Panel 2 represents a higher magnification of the boxed area in panel 1, demonstrating specificity of staining in the apoptotic tumor and lack of apoptosis in adjacent normal tissue. Panel 3 illustrates a section from another TUNEL-stained tumor-bearing brain treated with TRAIL-expressing NSCs. Note the highly apoptotic primary tumor mass (T, demarcated by arrowheads). Significant apoptosis is also visible within a tumor satellite (t) at considerable distance from the main tumor mass. Panel 4 is a higher magnification of the boxed area in panel 3, demonstrating specificity of TUNEL staining in the tumor satellite.



2C

inoculated contralateral to established murine intracranial gliomas. Panel 1 represents portion of left cerebral hemisphere where NSCs were inoculated and illustrates that NSCs do not randomly dissipate into adjacent non-tumorous tissue. Panel 2 shows tumor-bearing portion of right cerebral hemisphere demonstrating specific nonrandom migration of NSCs across the brain into the vicinity of the tumor in the opposite hemisphere (inset box). Panel 3 demonstrates a second tumor-bearing portion of a similarly treated brain.  $\beta$ -galactosidase expressing NSCs are visible, interspersed within the tumor mass (inset box), contralateral to their site of inoculation. (C) NSC-mediated delivery of IL-12 in established gliomas induces robust intratumoral T-cell infiltration. Established intracranial murine gliomas were inoculated with IL-12 expressing NSCs. Subsequent immunohistochemical analysis of tumor sections revealed robust infiltration of tumors with CD4<sup>+</sup> (panel 1) and CD8<sup>+</sup> (panel 2 and panel 3) T cells, with numerous aggregates along the tumor/normal tissue boundary (arrows). T designates tumors and N represents normal brain tissue. Panel 3 is a high-power image of the boxed area in panel 2. Magnification = 100 $\times$  for panels 1 and 2 and 400 $\times$  for panel 3. (D) NSCs utilized to deliver TRAIL to intracranial gliomas elicit overwhelming apoptosis in main tumor mass and tumor satellites. Established intracranial human glioma xenografts in mice were treated with intratumoral inoculation of TRAIL-expressing NSCs. Brains were then analyzed histologically for apoptotic activity using a TUNEL assay (red staining). Slides were counterstained with hematoxylin. Panel 1 demonstrates a treated tumor exhibiting almost complete cellular apoptosis in the main tumor mass (T). Panel 2 represents a higher magnification of the boxed area in panel 1, demonstrating specificity of staining in the apoptotic tumor and lack of apoptosis in adjacent normal tissue. Panel 3 illustrates a section from another TUNEL-stained tumor-bearing brain treated with TRAIL-expressing NSCs. Note the highly apoptotic primary tumor mass (T, demarcated by arrowheads). Significant apoptosis is also visible within a tumor satellite (t) at considerable distance from the main tumor mass. Panel 4 is a higher magnification of the boxed area in panel 3, demonstrating specificity of TUNEL staining in the tumor satellite.



2D

inoculated contralateral to established murine intracranial gliomas. Panel 1 represents portion of left cerebral hemisphere where NSCs were inoculated and illustrates that NSCs do not randomly dissipate into adjacent non-tumorous tissue. Panel 2 shows tumor-bearing portion of right cerebral hemisphere demonstrating specific nonrandom migration of NSCs across the brain into the vicinity of the tumor in the opposite hemisphere (inset box). Panel 3 demonstrates a second tumor-bearing portion of a similarly treated brain.  $\beta$ -galactosidase expressing NSCs are visible, interspersed within the tumor mass (inset box), contralateral to their site of inoculation. (C) NSC-mediated delivery of IL-12 in established gliomas induces robust intratumoral T-cell infiltration. Established intracranial murine gliomas were inoculated with IL-12 expressing NSCs. Subsequent immunohistochemical analysis of tumor sections revealed robust infiltration of tumors with CD4<sup>+</sup> (panel 1) and CD8<sup>+</sup> (panel 2 and panel 3) T cells, with numerous aggregates along the tumor/normal tissue boundary (arrows). T designates tumors and N represents normal brain tissue. Panel 3 is a high-power image of the boxed area in panel 2. Magnification = 100 $\times$  for panels 1 and 2 and 400 $\times$  for panel 3. (D) NSCs utilized to deliver TRAIL to intracranial gliomas elicit overwhelming apoptosis in main tumor mass and tumor satellites. Established intracranial human glioma xenografts in mice were treated with intratumoral inoculation of TRAIL-expressing NSCs. Brains were then analyzed histologically for apoptotic activity using a TUNEL assay (red staining). Slides were counterstained with hematoxylin. Panel 1 demonstrates a treated tumor exhibiting almost complete cellular apoptosis in the main tumor mass (T). Panel 2 represents a higher magnification of the boxed area in panel 1, demonstrating specificity of staining in the apoptotic tumor and lack of apoptosis in adjacent normal tissue. Panel 3 illustrates a section from another TUNEL-stained tumor-bearing brain treated with TRAIL-expressing NSCs. Note the highly apoptotic primary tumor mass (T, demarcated by arrowheads). Significant apoptosis is also visible within a tumor satellite (t) at considerable distance from the main tumor mass. Panel 4 is a higher magnification of the boxed area in panel 3, demonstrating specificity of TUNEL staining in the tumor satellite.

Benedetti et al<sup>111</sup> first described the use of IL-4-secreting NSCs for the treatment of experimental rodent gliomas. Although of significant therapeutic benefit, the authors did not demonstrate a strong tropism of their transplanted cells for migrating glioma. More recently, we reported the use of IL-12<sup>112</sup> and TRAIL<sup>113</sup> secreting NSCs in experimental glioma models comprising of intracranial GL26 tumors in C57Bl/6 mice and U343 human glioma xenografts in athymic nude mice, respectively. These studies demonstrated comprehensively that NSCs were able to track glioma cells and exhibited tropism for tumor even when inoculated contralateral to established tumors (Fig 2). NSC-secreted cytokines could be specifically delivered to both the main tumor mass and tumor outgrowths or satellites, resulting in potent antitumor responses. These were evidenced by robust T-cell infiltration leading to enhanced long-term survival with NSC-mediated IL-12 therapy and

the induction of almost complete tumor-cell apoptosis by TRAIL (Fig 3).

Cytokines have proven to be potent mediators of anti-tumor immunity. Overall, the use of cytokines for the treatment of gliomas has produced mixed results. The initial preclinical successes utilizing systemic or local delivery of recombinant cytokines failed to adequately translate into clinical benefit. Subsequently, the development of enhanced delivery systems comprising of cell-based, gene therapy-based or, more recently, NSC-based approaches may circumvent the physiologic and pharmacokinetic limiting factors of earlier strategies.

## Active Immunotherapy to Initiate T-Cell-Mediated Antitumor Immunity

Even though humoral immunity may have therapeutic relevance, substantial evidence has accumulated that implicates cytotoxic T cells as being crucial to the generation of effective antitumor immune responses.<sup>114,115</sup> This recognition spurred the search for treatment modalities that could boost tumor-directed T-cell responses. Although nonspecific cytokine therapy is capable of supporting and enhancing T-cell activity,<sup>116</sup> the generation of effective anti-tumor immunity requires presentation of tumor antigen(s) to naive T cells that then undergo activation and clonal expansion and ultimately exert their cytolytic tumoricidal effector function. Additionally, T-cell activation eventually leads to the generation of memory cells that confer long-lasting antitumor immunity, a cardinal feature of a successful acquired immune response. Adoptive cell transfer of in vitro expanded and sensitized T cells, although successful in some studies at inducing responses in patients with glioma, does not meet the criteria for the true generation of antitumor T-cell immunity, given its lack of specificity and inability to generate memory.

Therefore, the challenge in developing therapeutic models to boost tumor-specific T-cell responses has been in identifying and successfully presenting immunogenically relevant tumor antigens to T cells in vivo. Appropriate tumor-associated antigens (TAAs) have now been described for a variety of cancers. Clinical vaccination paradigms involving the use of TAAs to sensitize T cells against melanoma and prostate carcinoma have produced promising results.<sup>117,118</sup> Although gliomas have been shown to produce some of the melanoma-related TAAs such as gp100, TRP-2, or MAGE-1 in varying degrees,<sup>119</sup> the immunologic significance of this expression remains unclear since the expression of these proteins may be a vestige of the shared neuroectodermal origin of glia and melanocytes and may not have the same quantitative and qualitative distribution in gliomas as they do in melanomas. Therefore, a key limiting factor in the setting of primary malignant brain tumors remains the inability to identify any glioma-specific, immunogenically relevant

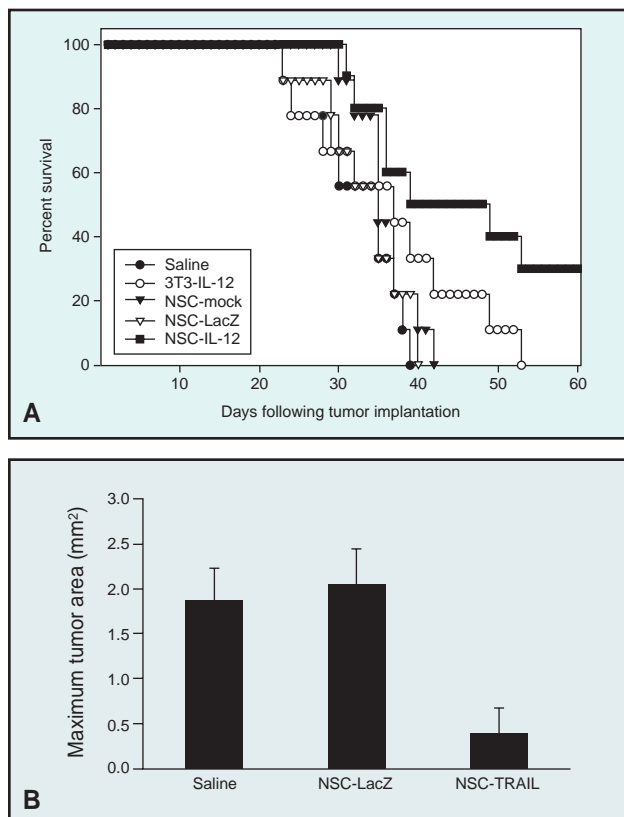


Fig 3A-B. — (A) Mice bearing intracranial GL26 gliomas treated with tumor tropic IL-12-expressing NSCs exhibit significantly prolonged survival compared to nonsecretory NSC-treated controls or to nonmigratory IL-12-expressing fibroblast (3T3)-treated animals. (B) Treatment of established intracranial U343 human glioma xenografts in nude mice with intratumoral administration of tumor-tracking TRAIL-expressing NSCs results in significant inhibition of tumor growth compared to nonsecretory NSC or saline-treated controls. Tumor size was assessed histologically, 1 week following therapeutic intratumoral inoculations. From Ehtesham M, Kabos P, Gutierrez MAR, et al. Induction of glioblastoma apoptosis using neural stem cell-mediated delivery of tumor necrosis factor-related apoptosis-inducing ligand. *Cancer Res.* 2002;62:7170-7174; and 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. Reprinted with permission by the American Association for Cancer Research.

tumor antigens. Additionally, given the highly aggressive histologic grade of most primary gliomas and the resulting significant cellular heterogeneity, it is unclear whether the definition of a single — or even several — specific antigen(s) would be sufficient to successfully immunize patients against the entire tumor, as has been demonstrated with comparatively indolent neoplasms such as melanoma.<sup>118</sup> In lieu of effective TAAs, vaccination strategies have utilized either irradiated whole tumor cells or dendritic cells (DCs) pulsed with nonspecific tumor-derived peptides/lysate as modalities to present a broad range of tumor antigens to T cells in vivo in the hope that presentation of certain (as of yet unknown) immunogenically relevant tumor antigens might stimulate effective antitumor T-cell immunity.

Initial vaccination strategies for glioma utilized subcutaneous, growth-arrested whole-tumor cell inoculation. The immunogenicity of the vaccinated tumor cells was usually potentiated by engineering the vaccinated tumor cells to secrete immunostimulatory cytokines. Herrlinger et al<sup>7</sup> and Yu et al<sup>20</sup> described preclinical vaccination models utilizing GM-CSF-secreting glioma cells as an effective means of eliciting antitumor immunity and protecting rodents against subsequent tumor rechallenge. An early clinical report of a patient with GMB, Sobol and colleagues<sup>121</sup> reported that vaccination using a mixture of irradiated autologous tumor cells with IL-2-secreting fibroblasts resulted in marked tumor necrosis and an increase in CD8<sup>+</sup> T-cell-mediated antitumor immunity. Subsequently, Holladay et al<sup>122</sup> tested an irradiated autologous tumor vaccine in 15 patients with recurrent gliomas following surgical resection of the main tumor mass. Immunogenicity of the vaccine was enhanced

by mixing tumor cells with bacillus Calmette-Guérin (BCG). This vaccination therapy was combined with intravenous adoptive transfer of predominately CD4<sup>+</sup> T cells that had been expanded in vitro in the presence of irradiated tumor. Additionally, most patients received systemic IL-2 therapy. The authors reported partial responses to therapy in 7 of 15 patients and the development of delayed-type hypersensitivity in all vaccine recipients. More recently, Plautz et al<sup>79</sup> described the use of autologous irradiated tumor cell vaccines admixed with GM-CSF. This study also combined the vaccination paradigm with adoptive T-cell transfer by intravenous infusion of in vitro expanded T cells that were isolated from lymph nodes draining the vaccination site and cultured with staphylococcal enterotoxin. In this study, 4 of 12 vaccinated patients demonstrated partial regression of treated tumor, and the treatment was not associated with any severe adverse effects. Recent evidence has also come to light that tumor-cell based vaccination paradigms may, in fact, lead to accelerated tumor growth secondary to the generation of myeloid suppressor cells.<sup>123,124</sup>

Although promising data has been obtained from tumor-cell based vaccination strategies, the main caveat of this approach centers on the poor inherent antigen-presenting capacity of glioma cells. Despite attempts to augment their APC function by either overexpressing known APC function related surface molecules or by admixing tumor-cell vaccines with known APC stimulating adjuvants, there is now considerable evidence to support the contention that the use of professional APCs to initiate tumor-specific T-cell responses may be a more promising strategy for cancer vaccination. In particular, therapeutic strategies involving DCs, the most potent of the APCs, have

**Characteristics of Glioma Patients Enrolled in a Phase I Clinical Trial Employing Peripheral Tumor-Peptide Pulsed Dendritic Cell Vaccination**

| Patient No. | Tumor Pathology | Age | Prevaccine Therapy | After Vaccination Therapy | Adverse Events | Survival (days) | Time to Progression (days) | Time of Additional Surgery | Intratumoral T Cells | Peripheral Cytotoxic T Lymphocytes |
|-------------|-----------------|-----|--------------------|---------------------------|----------------|-----------------|----------------------------|----------------------------|----------------------|------------------------------------|
| 1           | AA              | 28  | Hydroxyurea        | Poly IC                   |                | 632             |                            |                            |                      | Negative                           |
| 2           | GBM             | 51  |                    | Reoperation               |                | D381            | 259                        | 264                        | Negative             | Positive                           |
| 3           | GBM             | 48  | Gliadel            |                           | Fever, nausea  | 618             |                            |                            |                      |                                    |
| 4           | GBM             | 49  |                    | Reoperation, SRT          |                | D463            | 216                        | 224                        | Negative             | Positive                           |
| 5           | AA              | 30  |                    | Poly IC                   | Lymph nodes    | 550             |                            |                            | Positive             |                                    |
| 6           | GBM             | 59  |                    | Reoperation, SRT          |                | 455             | 105                        | 112                        | Positive             | Positive                           |
| 7           | GBM             | 42  |                    |                           |                | 574             |                            |                            |                      | Pre-existing                       |
| 8           | GBM             | 55  |                    | Reoperation, SRT          |                | 315             | 74/222                     | 81/232                     | Positive             | Pre-existing                       |
| 9           | GBM             | 77  |                    |                           |                | 300             |                            |                            |                      |                                    |

AA = anaplastic astrocytoma  
 Gliadel = intracranial 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea  
 Poly IC = nonspecific immunotherapy  
 SRT = stereotactic radiation therapy  
 D = time of death

From 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. Reprinted with permission by the American Association for Cancer Research.

received increasing attention. Theoretically, DC-mediated antigen presentation should be far more effective than using irradiated tumor cells, as DCs abundantly express many of the co-stimulatory molecules that are essential for appropriate activation of naive T cells. Also, they have the ability to efficiently process and present antigenic peptides in combination with cell-surface MHC.<sup>125</sup>

Following successful reports describing the efficacy of DC-based vaccination in extracranial experimental neoplastic models,<sup>126</sup> Liau and colleagues<sup>5</sup> described the successful treatment of established intracranial gliomas in rats treated with tumor-peptide-pulsed DC vaccination. Subsequently, Yu et al<sup>119</sup> described the use of a DC vaccine in a phase I clinical trial involving patients with newly diagnosed high-grade glioma. DCs cultured out from patients' peripheral blood mononuclear cells (PBMCs) were pulsed ex vivo with autologous tumor cell surface peptides isolated by means of acid-elution. Nine patients (2 with grade 3 astrocytoma and 7 with GBM) received a series of three such intradermal DC vaccinations following surgical resection and external beam radiotherapy (Table). Antitumor cytotoxicity, as determined by exposing PBMCs to autologous tumor targets as part of a JAM assay, was detectable in 4 patients. Radiologic evidence of disease progression was detected in 4 patients, who then underwent reoperation subsequent to the third DC vaccination. In 2 patients, the harvested tissue demonstrated robust infiltration with CD8<sup>+</sup> and CD45RO<sup>+</sup>T cells, which was not apparent in the same patients' tumor specimens resected prior to initiation of the vaccination protocol (Fig 4). Additionally, long-term survival in the study group was compared to survival data from age- and gender-matched controls with similar disease who underwent surgical resection with external beam radiotherapy. The median survival for the study group was 455 days vs 257 days for the control population, indicating that DC vaccination may confer some survival benefit. These data indicated that DC vaccination in malignant brain tumor patients was safe and effective in stimulating antitumor immune responses as assessed by peripheral cytotoxicity assays and intratumoral T-cell infiltration. These encouraging results led to the expansion of this study into phase II trials, which are currently underway.

In the setting of brain tumors, currently employed treatment strategies utilizing DCs are based on the loading of these cells with tumor-derived proteins ex vivo, as described above. This necessitates the surgical resection of a sufficient quantity of tumor tissue to serve as an antigenic source for DC priming. Therefore, the applicability of current DC vaccination paradigms is limited in situations where open resection of tumor is precluded as a result of surgical inaccessibility. Additionally, recent evidence has demonstrated that a physical interaction between DCs and tumor cells may be fundamental for the induction of therapeutically effective immunity<sup>127</sup> and that DCs are capable of processing apoptotic tumor cells

to induce CTL activity.<sup>128,129</sup> Given these factors, we hypothesized that the intratumoral administration of DCs in the context of radiotherapy to induce tumor cell death may prove a feasible strategy to enhance in vivo glioma-specific antigen presentation to the immune system. This methodology would bypass the cumbersome process of ex vivo DCs pulsing with tumor antigens and would circumvent the requirement of resecting tumor tissue

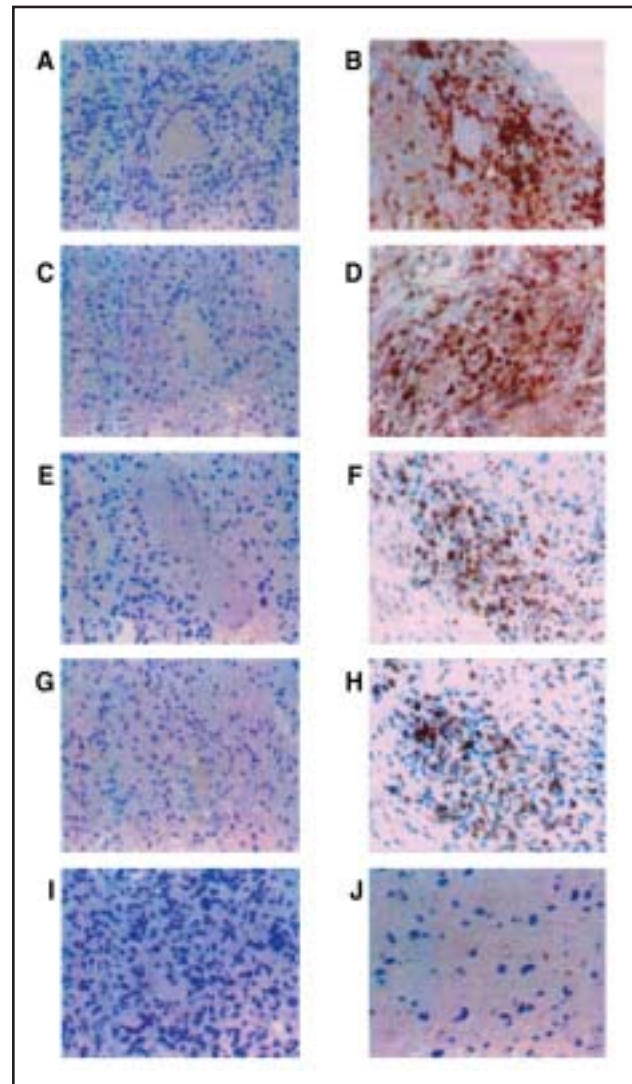


Fig 4A-J. — Surgical tumor specimens from glioma patients enrolled in a phase I clinical trial employing peripheral tumor-peptide pulsed DC vaccination (see Table for patient characteristics). Immunohistochemical characterization of infiltrating cells in intracranial tumor at first surgery, before vaccination (left column) and at reoperation, after vaccination (right column). (A) CD45RO<sup>+</sup> memory T-cell staining before vaccination in patient 8. (B) CD45RO<sup>+</sup> staining after vaccination in patient 8. (C) Patient 8 before vaccination CD8<sup>+</sup> cells. (D) Patient 8 after vaccination CD8<sup>+</sup> cells. (E) Patient 6 before vaccination CD45RO<sup>+</sup> cells. (F) Patient 6 after vaccination CD45RO<sup>+</sup> cells. (G) Patient 6 before vaccination CD8<sup>+</sup> cells. (H) Patient 6 after vaccination CD8<sup>+</sup> cells. (I) Control patient CD8<sup>+</sup> cells at first operation. (J) Control patient CD8<sup>+</sup> cells at reoperation (magnification = 400×). From 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. Reprinted with permission by the American Association for Cancer Research.

before vaccination therapy. Therefore, we designed and tested a novel therapy involving the direct inoculation of immature bone marrow-derived DCs into established intracranial gliomas in rats.<sup>37</sup> Implanted gliomas had been partially irradiated to simulate neoplastic cell death associated with clinical radiotherapy and to provide an appropriate antigenic source for DC priming. We found that intracranially inoculated DCs could drain to deep cervical lymph nodes and elicit systemic antitumor cytotoxic immunity. This was associated with robust intratumoral T-cell infiltration, inhibition of glioma growth, and prolongation in survival compared to monocyte treated controls (Fig 5). These results indicate that placing immature DCs

in direct contact with partially apoptotic tumor may serve as an effective means of stimulating antitumor immunity, eventually leading to tumor rejection and immune memory. In particular, this strategy may be of particular benefit in patients with surgically inaccessible brain tumors who can be treated with radiotherapy. Kikuchi and colleagues<sup>130</sup> also recently described a similar intracranial DC vaccination strategy employing the inoculation of immature DC into partially irradiated intracranial gliomas, with encouraging results.

As part of a strategy to improve DC-mediated tumor antigen presentation by means of enhancing tumor cell-APC interaction, Akasaki et al<sup>131</sup> vaccinated DC-glioma fusion cells in intracranial glioma-bearing mice. This therapy significantly inhibited intracranial glioma growth and resulted in markedly enhanced survival in treated animals. These results were accompanied by a strong increase in specific antitumor CTL activity. Subsequently, Kikuchi and colleagues<sup>132</sup> employed this methodology in a phase I clinical trial. Eight patients (5 with GBM, 2 with grade 3 astrocytoma, and 1 with anaplastic oligodendroglioma) were treated with a series of between 3 and 7 peripheral intradermal vaccinations of DC-autologous glioma fusion cells. No significant increase in tumor-specific CTL activity was noted, and only minor and temporary responses to therapy were detected in 2 patients who subsequently developed progressive disease. The authors speculated that the disappointing results seen in clinical use of their therapy may have resulted from a change in the profile of tumor-expressed immunogenic antigens during their in vitro glioma cell culture process, rendering their fusion vaccines ineffective against the residual primary or recurrent tumor cells in vivo. Conceivably, this was not a factor in their earlier preclinical study,<sup>131</sup> which utilized a uniform immortalized glioma cell line not subject to the same degree of in vitro selection pressures as the primary glioma explants used in their clinical trial.

In summary, the use of a treatment strategy to boost antitumor T-cell responses may prove to be the most effective immunomodulatory treatment modality for glioma. The key to the therapeutic success of this strategy will lie in eliciting an antiglioma immune response of sufficient potency and specificity in order to eliminate all residual tumor burden remaining following primary surgical resection and radiotherapy or chemotherapy. Based on the demonstrated ability of DCs to powerfully initiate specific activation of naive T cells, the use of professional APCs such as DCs to present tumor proteins in vivo may be the most appropriate way to stimulate tumoricidal T-cell responses. In addition, the identification of a panel of immunologically relevant TAAs specific for glioma may make the process more effective. However, even in the absence of clearly defined TAAs, optimizing the delivery methodology for DCs by facilitating tumor cell-APC interaction in vivo may still allow for the generation of potent antitumor T-cell responses.

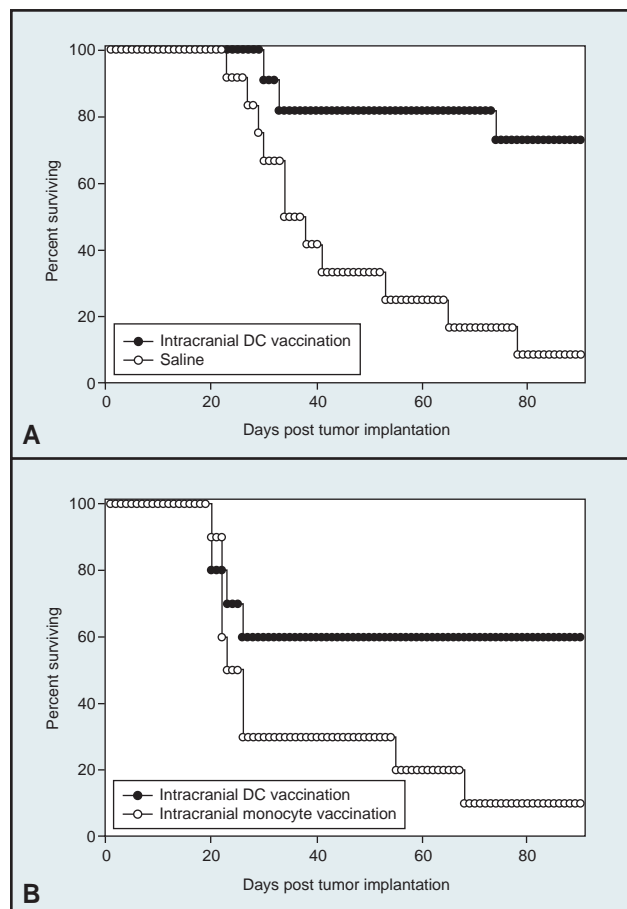


Fig 5A-B. — In vivo intracranial dendritic cell (DC) vaccination prolongs survival in 9L glioma-bearing rats. Partially irradiated 9L gliomas were either (A) co-inoculated or (B) injected 2 days following implantation with freshly cultured DC or either (A) saline or (B) monocytes. All treatments were followed 2 weeks later with a second intracranial vaccination comprising a mixture of irradiated tumor cells with freshly cultured DC or either (A) saline or (B) monocytes. Kaplan-Meier survival curves illustrate that glioma-bearing rats treated with DC either (A) at the time of tumor implantation or (B) 2 days later demonstrated prolonged survival compared to either (A) saline or (B) monocyte inoculated controls. All DC-inoculated survivors from (B) were rechallenged with a second intracranial inoculation of 9L glioma cells 4 months following the initial tumor implantation. Long-term survivors rejected the tumor rechallenge in contrast to 6 naive animals that were also implanted with tumor, all of whom died. From Ehtesham M, Kabos P, Gutierrez MA, et al. Intratumoral dendritic cell vaccination elicits potent tumoricidal immunity against malignant glioma in rats. *J Immunother.* 2003;26:107-116. Reprinted with permission by Lippincott Williams & Wilkins. <http://www.lww.com>.

## Future Directions and Challenges

Given the poor prognoses associated with high-grade intracranial gliomas, there is an urgent need for the development of therapies that can improve clinical survival rates. In this regard, the development of immunotherapeutic treatment strategies for gliomas is an attractive option, given the potential of the immune system to serve as a powerful means of eliminating residual neoplastic infiltrates following primary surgery, radiotherapy, and/or chemotherapy. However, the field is faced with significant challenges, including the need to further understand the complex immunosuppressive mechanisms employed by gliomas to blunt endogenous immune recognition and clearance. The identification of certain important chemokines such as TGF- $\beta$  that are involved in this process has led to the development of various treatment strategies (exemplified by TGF- $\beta$  antisense therapy<sup>135</sup>) focused on overcoming tumor-induced immune defects. It has become clear that glioma-associated immune suppression is mediated by a plethora of secreted and tumor cell-surface expressed factors. A better understanding of these mediators and their relevant biological mechanisms may eventually allow for more comprehensive and better-targeted therapies.

An additional factor limiting the effectiveness of serologic and tumor-specific vaccination protocols has been the difficulty in isolating true glioma-specific tumor antigen(s). The identification of EGFRvIII<sup>57</sup> and, more recently, a mutated form of the alpha 2 chain of the IL-13 receptor<sup>134</sup> on gliomas has been encouraging. However, the high degree of cellular heterogeneity within these tumors makes it unclear how relevant these antigens will be for effective use as immunotherapeutic targets. Additionally, gliomas have been shown to express certain melanoma-associated antigens in various degrees.<sup>119</sup> Again, the immunologic significance of this expression, as well as the extent to which it can be relied on as a tumor-specific trait for vaccine development, is unknown and untested. It is encouraging to note that Okano and colleagues<sup>135</sup> recently identified a MHC class I-restricted CTL epitope within the mutated alpha 2 IL-13 receptor chain found in gliomas. This may indicate that the use of this antigen may yet hold promise as an immunologic target. Recently, however, a novel concept termed *immunoediting* has been developed to describe the ability of small, immunotherapy-resistant populations of tumor cells within a heterogeneous neoplasm to expand preferentially in response to selection pressures applied by endogenous immune surveillance or targeted immunotherapy.<sup>9</sup> This implies that the specific immunotherapeutic targeting of one or several immunogenic antigens in a heterogenic glioma cell population may effectively select out for neoplastic population negative for those particular traits, thereby preventing any significant impact on long-term tumor burden or survival. This concept has potentially important ramifications and could indicate that the use of whole tumor

lysate/peptides may indeed be beneficial in comparison to the use of specific-tumor antigens.

With regard to active vaccination protocols, it has become clear that the use of professional APCs, such as DCs, is key for potentiating effective antitumor T-cell responses. In order to refine DC-based immunotherapy for glioma, it will be important to optimize the route of delivery for DC vaccines. Clinical use of peripheral DC therapy has been encouraging.<sup>119</sup> However, recent reports indicate that intraglioma administration of DCs is also beneficial<sup>137,130</sup> and may be particularly relevant in situations where sufficient quantities of autologous tumor cannot be surgically harvested. Additionally, the methodology of DC antigen loading will also have to be optimized, with either *ex vivo* tumor lysate/peptide pulsing or the uptake of apoptotic tumor cells *in vivo* following tumor irradiation. The ability of DC to activate tumoricidal T-cell responses may also depend on other factors governing the state of DC maturation and activation. It is well known that immature DCs are more effective at uptake of tumor antigen and mature DCs are better at presenting processed antigen to T cells.<sup>125</sup> However, recent evidence indicates that glioma-associated immunosuppressive cues may skew the DC maturation process so as to inhibit their ability to support Th1-mediated T-cell activity (Y. Akasaki, MD, unpublished data, 2004). A better understanding of DC biology in the setting of glioma-induced immunosuppression, the optimization of route of vaccination, and the methodology of DC priming will be key in the development of better active vaccination paradigms.

## Conclusions

Given the complex and divergent mechanisms with which gliomas evade immune clearance, an effective treatment paradigm for malignant gliomas may eventually require a multifaceted approach combining two or more different immunotherapeutic strategies. Such scenarios may involve the use of local cytokine gene therapy to enhance glioma-cell immunogenicity in conjunction with DC-based active vaccination to stimulate systemic tumoricidal T-cell immunity. Additionally, given the heterogeneity of this disease process and the potential risk of immunoediting out a selected, treatment-refractory tumor cell population, the concurrent use of multiple modalities that target disparate tumor characteristics may prove to be of greatest therapeutic relevance.

## References

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. Surawicz TS, Davis F, Freels S, et al. Brain tumor survival: results from the National Cancer Data Base. *J Neurooncol.* 1998;40:151-160.

3. Kelly KA, Kirkwood JM, Kapp DS. Glioblastoma multiforme: pathology, natural history and treatment. *Cancer Treat Rev.* 1984;11:1-26.
4. Ehtesham M, Samoto K, Kabos P, et al. Treatment of intracranial glioma with in situ interferon-gamma and tumor necrosis factor-alpha gene transfer. *Cancer Gene Ther.* 2002;9:925-934.
5. Liao LM, Black KL, Prins RM, et al. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg.* 1999;90:1115-1124.
6. Liu Y, Ehtesham M, Samoto K, et al. In situ adenoviral interleukin 12 gene transfer confers potent and long-lasting cytotoxic immunity in glioma. *Cancer Gene Ther.* 2002;9:9-15.
7. Herrlinger U, Kramm CM, Johnston KM, et al. Vaccination for experimental gliomas using GM-CSF-transduced glioma cells. *Cancer Gene Ther.* 1997;4:345-352.
8. Flueren G. Immune surveillance. In: Delves P, ed. *Encyclopedia of Immunology.* San Diego, Calif: Academic Press Limited; 1998: 1243-1247.
9. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoeediting: from immunosurveillance to tumor escape. *Nat Immunol.* 2002;3:991-998.
10. Brooks WH, Netsky MG, Normansell DE, et al. Depressed cell-mediated immunity in patients with primary intracranial tumors: characterization of a humoral immunosuppressive factor. *J Exp Med.* 1972;136:1631-1647.
11. Young HF, Sakalas R, Kaplan AM. Inhibition of cell-mediated immunity in patients with brain tumors. *Surg Neurol.* 1976;5:19-23.
12. Elliott LH, Brooks WH, Roszman TL. Activation of immunoregulatory lymphocytes obtained from patients with malignant gliomas. *J Neurosurg.* 1987;67:231-236.
13. Elliott LH, Brooks WH, Roszman TL. Cytokinetic basis for the impaired activation of lymphocytes from patients with primary intracranial tumors. *J Immunol.* 1984;132:1208-1215.
14. Morford LA, Elliott LH, Carlson SL, et al. T cell receptor-mediated signaling is defective in T cells obtained from patients with primary intracranial tumors. *J Immunol.* 1997;159:4415-4425.
15. Bhoneley MK, Mehra RD, Mehra NK, et al. Imbalances in T cell subpopulations in human gliomas. *J Neurosurg.* 1988;68:589-593.
16. Roszman TL, Brooks WH. Immunobiology of primary intracranial tumours. III. Demonstration of a qualitative lymphocyte abnormality in patients with primary brain tumours. *Clin Exp Immunol.* 1980;39:395-402.
17. Brooks WH, Roszman TL, Mahaley MS, et al. Immunobiology of primary intracranial tumours. II. Analysis of lymphocyte subpopulations in patients with primary brain tumours. *Clin Exp Immunol.* 1977;29:61-66.
18. Roszman TL, Brooks WH, Steele C, et al. Pokeweed mitogen-induced immunoglobulin secretion by peripheral blood lymphocytes from patients with primary intracranial tumors. Characterization of T helper and B cell function. *J Immunol.* 1985;134: 1545-1550.
19. Mahaley MS, Jr, Brooks WH, Roszman TL, et al. Immunobiology of primary intracranial tumors. Part I: studies of the cellular and humoral general immune competence of brain-tumor patients. *J Neurosurg.* 1977;46:467-476.
20. Yang T, Witham TF, Villa L, et al. Glioma-associated hyaluronan induces apoptosis in dendritic cells via inducible nitric oxide synthase: implications for the use of dendritic cells for therapy of gliomas. *Cancer Res.* 2002;62:2583-2591.
21. Kikuchi T, Abe T, Ohno T. Effects of glioma cells on maturation of dendritic cells. *J Neurooncol.* 2002;58:125-130.
22. Wrann M, Bodmer S, de Martin R, et al. T cell suppressor factor from human glioblastoma cells is a 12.5-kd protein closely related to transforming growth factor-beta. *Embo J.* 1987;6:1633-1636.
23. Bodmer S, Strommer K, Frei K, et al. Immunosuppression and transforming growth factor-beta in glioblastoma: preferential production of transforming growth factor-beta 2. *J Immunol.* 1989;143: 3222-3229.
24. Castelli MG, Chiabrando C, Fanelli R, et al. Prostaglandin and thromboxane synthesis by human intracranial tumors. *Cancer Res.* 1989;49:1505-1508.
25. Couldwell WT, Dore-Duffy P, Apuzzo ML, et al. Malignant glioma modulation of immune function: relative contribution of different soluble factors. *J Neuroimmunol.* 1991;33:89-96.
26. Fredman P, Mansson JE, Dellheden B, et al. Expression of the GM1-species, [NeuN]-GM1, in a case of human glioma. *Neurochem Res.* 1999;24:275-279.
27. Kawai K, Takahashi H, Watarai S, et al. Occurrence of ganglioside GD3 in neoplastic astrocytes: an immunocytochemical study in humans. *Virchows Arch.* 1999;434:201-205.
28. Nitta T, Hishii M, Sato K, et al. Selective expression of interleukin-10 gene within glioblastoma multiforme. *Brain Res.* 1994;649: 122-128.
29. Huettner C, Czub S, Kerkau S, et al. Interleukin 10 is expressed in human gliomas in vivo and increases glioma cell proliferation and motility in vitro. *Anticancer Res.* 1997;17:3217-3224.
30. Hishii M, Nitta T, Ishida H, et al. Human glioma-derived interleukin-10 inhibits antitumor immune responses in vitro. *Neurosurgery.* 1995;37:1160-1166; discussion 1166-1167.
31. Zou JP, Morford LA, Choungnet C, et al. Human glioma-induced immunosuppression involves soluble factor(s) that alters monocyte cytokine profile and surface markers. *J Immunol.* 1999;162: 4882-4892.
32. Akbasak A, Oldfield EH, Saris SC. Expression and modulation of major histocompatibility antigens on murine primary brain tumor in vitro. *J Neurosurg.* 1991;75:922-929.
33. Roussel E, Gingras MC, Grimm EA, et al. Predominance of a type 2 intratumoural immune response in fresh tumour-infiltrating lymphocytes from human gliomas. *Clin Exp Immunol.* 1996;105: 344-352.
34. Saas P, Walker PR, Hahne M, et al. Fas ligand expression by astrocytoma in vivo: maintaining immune privilege in the brain? *J Clin Invest.* 1997;99:1173-1178.
35. Yu JS, Lee PK, Ehtesham M, et al. Intratumoral T-cell subsets and endothelial fas ligand expression in brain tumors. *J Neurooncol.* 2003;64:55-61.
36. Plautz GE, Touhalisky JE, Shu S. Treatment of murine gliomas by adoptive transfer of ex vivo activated tumor-draining lymph node cells. *Cell Immunol.* 1997;178:101-107.
37. Ehtesham M, Kabos P, Gutierrez MA, et al. Intratumoral dendritic cell vaccination elicits potent tumoricidal immunity against malignant glioma in rats. *J Immunother.* 2003;26:107-116.
38. Wikstrand CJ, Cokgor I, Sampson JH, et al. Monoclonal antibody therapy of human gliomas: current status and future approaches. *Cancer Metastasis Rev.* 1999;18:451-464.
39. Day ED, Lassiter S, Woodhall B, et al. The localization of radioantibodies in human brain tumors. I. Preliminary exploration. *Cancer Res.* 1965;25:773-778.
40. Mahaley MS Jr, Mahaley JL, Day ED. The localization of radioantibodies in human brain tumors. II. Radioautography. *Cancer Res.* 1965;25:779-793.
41. Patel K, Kiely F, Phimister E, et al. The 200/220 kDa antigen recognized by monoclonal antibody (Mab) UJ127.11 on neural tissues and tumors is the human L1 adhesion molecule. *Hybridoma.* 1991;10:481-491.
42. Hopkins K, Papanastassiou V, Kemshead JT. The treatment of patients with recurrent malignant gliomas with intratumoral radio-immunoconjugates. *Recent Results Cancer Res.* 1996;141: 159-175.
43. Bigner DD, Brown M, Coleman RE, et al. Phase I studies of treatment of malignant gliomas and neoplastic meningitis with <sup>131</sup>I-radiolabeled monoclonal antibodies anti-tenascin 81C6 and anti-chondroitin proteoglycan sulfate Me1-14 F (ab')<sub>2</sub>: a preliminary report. *J Neurooncol.* 1995;24:109-122.
44. Riva P, Arista A, Franceschi G, et al. Local treatment of malignant gliomas by direct infusion of specific monoclonal antibodies labeled with <sup>131</sup>I: comparison of the results obtained in recurrent and newly diagnosed tumors. *Cancer Res.* 1995;55:5952s-5956s.
45. Kalofonos HP, Pawlikowska TR, Hemingway A, et al. Antibody guided diagnosis and therapy of brain gliomas using radiolabeled monoclonal antibodies against epidermal growth factor receptor and placental alkaline phosphatase. *J Nucl Med.* 1989;30:1636-1645.
46. Brady LW, Miyamoto C, Woo DV, et al. Malignant astrocytomas treat-

- ed with iodine-125 labeled monoclonal antibody 425 against epidermal growth factor receptor: a phase II trial. *Int J Radiat Oncol Biol Phys.* 1992;22:225-230.
47. Sampson JH, Crotty LE, Lee S, et al. Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors. *Proc Natl Acad Sci U S A.* 2000;97:7503-7508.
  48. Wikstrand CJ, Fung KM, Trojanowski JQ, et al. Immunohistochemistry and antigens of diagnostic significance. In: Bigner DD, McLendon RE, Bruner JM, eds. *Russell and Rubinstein's Pathology of the Nervous System.* 6th ed. New York, NY: Oxford University Press; 1998:251-304.
  49. Riva P, Arista A, Tison V, et al. Intralesional radioimmunotherapy of malignant gliomas: an effective treatment in recurrent tumors. *Cancer.* 1994;73:1076-1082.
  50. Riva P, Arista A, Sturiale C, et al. Glioblastoma therapy by direct intralesional administration of I-131 radioiodine labeled antineascin antibodies. *Cell Biophys.* 1994;24-25:37-43.
  51. 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.
  52. Riva P, Franceschi G, Frattarelli M, et al. Loco-regional radioimmunotherapy of high-grade malignant gliomas using specific monoclonal antibodies labeled with 90Y: a phase I study. *Clin Cancer Res.* 1999;5:3275s-3280s.
  53. Riva P, Franceschi G, Frattarelli M, et al. 131I radioconjugated antibodies for the locoregional radioimmunotherapy of high-grade malignant glioma: phase I and II study. *Acta Oncol.* 1999;38:351-359.
  54. Cokgor I, Akabani G, Kuan CT, et al. Phase I trial results of iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with newly diagnosed malignant gliomas. *J Clin Oncol.* 2000;18:3862-3872.
  55. Bigner DD, Brown MT, Friedman AH, et al. Iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with recurrent malignant gliomas: phase I trial results. *J Clin Oncol.* 1998;16:2202-2212.
  56. Reardon DA, Akabani G, Coleman RE, et al. Phase II trial of murine (131I)-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J Clin Oncol.* 2002;20:1389-1397.
  57. Wikstrand CJ, Reist CJ, Archer GE, et al. The class III variant of the epidermal growth factor receptor (EGFRvIII): characterization and utilization as an immunotherapeutic target. *J Neurovirol.* 1998;4:148-158.
  58. Miyamoto CT, Brady LW, Rackover MA, et al. The use of epidermal growth factor receptor-425 monoclonal antibodies radiolabeled with iodine-125 in the adjuvant treatment of patients with high grade gliomas of the brain. *Recent Results Cancer Res.* 1996;141:183-192.
  59. Crombet T, Torres O, Rodriguez V, et al. Phase I clinical evaluation of a neutralizing monoclonal antibody against epidermal growth factor receptor in advanced brain tumor patients: preliminary study. *Hybridoma.* 2001;20:131-136.
  60. Trouillas P, Lapras C. Active immunotherapy of cerebral tumor: 20 cases [in French]. *Neurochirurgie.* 1970;16:143-170.
  61. Takakura K, Matsutani M, Koyama Y, et al. Treatment of malignant brain tumors by the intrathecal administration of BAR (EUdR-antimetabolite-radiation) [in Japanese]. *No To Shinkei.* 1972;24:585-593.
  62. Young H, Kaplan A, Regelson W. Immunotherapy with autologous white cell infusions ("lymphocytes") in the treatment of recurrent glioblastoma multiforme: a preliminary report. *Cancer.* 1977;40:1037-1044.
  63. Neuwelt EA, Clark K, Kirkpatrick JB, et al. Clinical studies of intrathecal autologous lymphocyte infusions in patients with malignant glioma: a toxicity study. *Ann Neurol.* 1978;4:307-312.
  64. Vaquero J, Martinez R, Barbolla L, et al. Intrathecal injection of autologous leucocytes in glioblastoma: circulatory dynamics within the subarachnoid space and clinical results. *Acta Neurochir (Wien).* 1987;89:37-42.
  65. Vaquero J, Martinez R, de Haro J, et al. Adoptive immunotherapy in glioblastoma multiforme: experience with the use of intrathecal infusions of autologous leukocytes [in Spanish]. *Arch Neurobiol (Madr).* 1987;50:183-190.
  66. Vaquero J, Martinez R, Oya S, et al. Intratumoural injection of autologous lymphocytes plus human lymphoblastoid interferon for the treatment of glioblastoma. *Acta Neurochir (Wien).* 1989;98:35-41.
  67. Vaquero J, Martinez R, Ramiro J, et al. Immunotherapy of glioblastoma with intratumoural administration of autologous lymphocytes and human lymphoblastoid interferon: a further clinical study. *Acta Neurochir (Wien).* 1991;109:42-45.
  68. Hiserodt JC, Vujanovic NL, Reynolds CW, et al. Studies on lymphokine activated killer cells in the rat: analysis of precursor and effector cell phenotype and relationship to natural killer cells. *Prog Clin Biol Res.* 1987;244:137-146.
  69. Kruse CA, Merchant RE. Cellular therapy of brain tumors. In: Kornblith PL, Walker MD, eds. *Advances in Neuro-Oncology II.* Armonk, NY: Futura; 1997:487-504.
  70. Hayes RL, Koslow M, Hiesiger EM, et al. Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. *Cancer.* 1995;76:840-852.
  71. Merchant RE, Grant AJ, Merchant LH, et al. Adoptive immunotherapy for recurrent glioblastoma multiforme using lymphokine activated killer cells and recombinant interleukin-2. *Cancer.* 1988;62:665-671.
  72. Barba D, Saris SC, Holder C, et al. Intratumoral LAK cell and interleukin-2 therapy of human gliomas. *J Neurosurg.* 1989;70:175-182.
  73. Saris SC, Spiess P, Lieberman DM, et al. Treatment of murine primary brain tumors with systemic interleukin-2 and tumor-infiltrating lymphocytes. *J Neurosurg.* 1992;76:513-519.
  74. Quattrocchi KB, Miller CH, Cush S, et al. Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. *J Neurooncol.* 1999;45:141-157.
  75. Merchant RE, Baldwin NG, Rice CD, et al. Adoptive immunotherapy of malignant glioma using tumor-sensitized T lymphocytes. *Neurol Res.* 1997;19:145-152.
  76. Wahl WL, Sussman JJ, Shu S, et al. Adoptive immunotherapy of murine intracerebral tumors with anti-CD3/interleukin-2-activated tumor-draining lymph node cells. *J Immunother Embasis Tumor Immunol.* 1994;15:242-250.
  77. Kitahara T, Watanabe O, Yamaura A, et al. Establishment of interleukin 2 dependent cytotoxic T lymphocyte cell line specific for autologous brain tumor and its intracranial administration for therapy of the tumor. *J Neurooncol.* 1987;4:329-336.
  78. Plautz GE, Shu S. Systemic T-cell immunotherapy for brain tumors. In: Liau LM, Becker DP, Cloughesy TF, et al, eds. *Brain Tumor Immunotherapy.* Totowa, NJ: Humana Press; 2001:133-148.
  79. Plautz GE, Miller DW, Barnett GH, et al. T cell adoptive immunotherapy of newly diagnosed gliomas. *Clin Cancer Res.* 2000;6:2209-2218.
  80. Kruse CA, Schiltz PM, Bellgrau D, et al. Intracranial administrations of single or multiple source allogeneic cytotoxic T lymphocytes: chronic therapy for primary brain tumors. *J Neurooncol.* 1994;19:161-168.
  81. Kruse CA, Cepeda L, Owens B, et al. Treatment of recurrent glioma with intracavitary alloreactive cytotoxic T lymphocytes and interleukin-2. *Cancer Immunol Immunother.* 1997;45:77-87.
  82. 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 906-907.
  83. Kikuchi T, Joki T, Saitoh S, et al. Anti-tumor activity of interleukin-2-producing tumor cells and recombinant interleukin 12 against mouse glioma cells located in the central nervous system. *Int J Cancer.* 1999;80:425-430.
  84. 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.

85. Yu JS, Wei MX, Chiocca EA, et al. Treatment of glioma by engineered interleukin 4-secreting cells. *Cancer Res.* 1993;53:3125-3128.
86. Packer RJ, Prados M, Phillips P, et al. Treatment of children with newly diagnosed brain stem gliomas with intravenous recombinant beta-interferon and hyperfractionated radiation therapy: a Childrens Cancer Group phase I/II study. *Cancer.* 1996;77:2150-2156.
87. Nagai M, Arai T. Clinical effect of interferon in malignant brain tumours. *Neurosurg Rev.* 1984;7:55-64.
88. Piguet V, Carrel S, de Tribolet N. Modulation of HLA-DR expression and growth inhibition on glioma cells: heterogenous responses to IFN-gamma. In: Walker MD, Thomas DGT, eds. *Biology of Brain Tumors.* Boston, Mass: Nijhoff; 1986.
89. Jean WC, Spellman SR, Wallenfriedman MA, et al. Interleukin-12-based immunotherapy against rat 9L glioma. *Neurosurgery.* 1998;42:850-856; discussion 856-857.
90. Saleh M, Jonas NK, Wiegman A, et al. The treatment of established intracranial tumors by in situ retroviral IFN-gamma transfer. *Gene Ther.* 2000;7:1715-1724.
91. Lee J, Hampl M, Albert P, Fine HA. Antitumor activity and prolonged expression from a TRAIL-expressing adenoviral vector. *Neoplasia.* 2002;4:312-323.
92. Staba MJ, Mauceri HJ, Kufe DW, et al. Adenoviral TNF-alpha gene therapy and radiation damage tumor vasculature in a human malignant glioma xenograft. *Gene Ther.* 1998;5:293-300.
93. Baher AG, Andres ML, Folz-Holbeck J, et al. A model using radiation and plasmid-mediated tumor necrosis factor-alpha gene therapy for treatment of glioblastomas. *Anticancer Res.* 1999;19:2917-2924.
94. Hao C, Beguinot F, Condorelli G, et al. Induction and intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis in human malignant glioma cells. *Cancer Res.* 2001;61:1162-1170.
95. Fathallah-Shaykh HM, Zhao LJ, Kafrouni AI, et al. Gene transfer of IFN-gamma into established brain tumors represses growth by antiangiogenesis. *J Immunol.* 2000;164:217-222.
96. Zou JP, Yamamoto N, Fujii T, et al. Systemic administration of rIL-12 induces complete tumor regression and protective immunity: response is correlated with a striking reversal of suppressed IFN-gamma production by anti-tumor T cells. *Int Immunol.* 1995;7:1135-1145.
97. Thomas GR, Chen Z, Enamorado I, et al. IL-12- and IL-2-induced tumor regression in a new murine model of oral squamous-cell carcinoma is promoted by expression of the CD80 co-stimulatory molecule and interferon-gamma. *Int J Cancer.* 2000;86:368-374.
98. Laut VM. Pharmacological elements in clinical application of synthetic peptides. *Fundam Clin Pharmacol.* 2000;14:425-442.
99. Leonard JP, Sherman ML, Fisher GL, et al. Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood.* 1997;90:2541-2548.
100. Inge TH, Hoover SK, Susskind BM, et al. Inhibition of tumor-specific cytotoxic T-lymphocyte responses by transforming growth factor beta 1. *Cancer Res.* 1992;52:1386-1392.
101. Kuppner MC, Hamou MF, Sawamura Y, et al. Inhibition of lymphocyte function by glioblastoma-derived transforming growth factor beta 2. *J Neurosurg.* 1989;71:211-217.
102. Siepl C, Bodmer S, Frei K, et al. The glioblastoma-derived T cell suppressor factor/transforming growth factor-beta 2 inhibits T cell growth without affecting the interaction of interleukin 2 with its receptor. *Eur J Immunol.* 1988;18:593-600.
103. Merchant RE, Ellison MD, Young HF. Immunotherapy for malignant glioma using human recombinant interleukin-2 and activated autologous lymphocytes: a review of pre-clinical and clinical investigations. *J Neurooncol.* 1990;8:173-188.
104. Merchant RE, McVicar DW, Merchant LH, et al. Treatment of recurrent malignant glioma by repeated intracerebral injections of human recombinant interleukin-2 alone or in combination with systemic interferon-alpha: results of a phase I clinical trial. *J Neurooncol.* 1992;12:75-83.
105. Brandes AA, Scelzi E, Zampieri P, et al. Phase II trial with BCNU plus alpha-interferon in patients with recurrent high-grade gliomas. *Am J Clin Oncol.* 1997;20:364-367.
106. Buckner JC, Brown LD, Kugler JW, et al. Phase II evaluation of recombinant interferon alpha and BCNU in recurrent glioma. *J Neurosurg.* 1995;82:430-435.
107. Rajkumar SV, Buckner JC, Schomberg PJ, et al. Phase I evaluation of radiation combined with recombinant interferon alpha-2a and BCNU for patients with high-grade glioma. *Int J Radiat Oncol Biol Phys.* 1998;40:297-302.
108. Yung WK, Steck PA, Kelleher PJ, et al. Growth inhibitory effect of recombinant alpha and beta interferon on human glioma cells. *J Neurooncol.* 1987;5:323-330.
109. Yung WK, Prados M, Levin VA, et al. Intravenous recombinant interferon beta in patients with recurrent malignant gliomas: a phase I/II study. *J Clin Oncol.* 1991;9:1945-1949.
110. Jadas MR, Chen Y, Boldaji MT, et al. Human U251MG glioma cells expressing the membrane form of macrophage colony-stimulating factor (mM-CSF) are killed by human monocytes in vitro and are rejected within immunodeficient mice via paraptosis that is associated with increased expression of three different heat shock proteins. *Cancer Gene Ther.* 2003;10:411-420.
111. Benedetti S, Pirola B, Pollo B, et al. Gene therapy of experimental brain tumors using neural progenitor cells. *Nat Med.* 2000;6:447-450.
112. 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.
113. Ehtesham M, Kabos P, Gutierrez MA, et al. Induction of glioblastoma apoptosis using neural stem cell-mediated delivery of tumor necrosis factor-related apoptosis-inducing ligand. *Cancer Res.* 2002;62:7170-7174.
114. Dermime S, Armstrong A, Hawkins RE, et al. Cancer vaccines and immunotherapy. *Br Med Bull.* 2002;62:149-162.
115. Holladay FP, Heitz T, Wood GW. Antitumor activity against established intracerebral gliomas exhibited by cytotoxic T lymphocytes, but not by lymphokine-activated killer cells. *J Neurosurg.* 1992;77:757-762.
116. Glick RP, Lichtor T, Cohen EP. Cytokine-based immuno-gene therapy for brain tumors. In: Liao LM, Becker DP, Cloughesy TF, et al, eds. *Brain Tumor Immunotherapy.* Totowa, NJ: Humana Press; 2001: 273-288.
117. Tjoa BA, Simmons SJ, Bowes VA, et al. Evaluation of phase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides. *Prostate.* 1998;36:39-44.
118. Nestle FO, Aljagic S, Gilliet M, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med.* 1998;4:328-332.
119. 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.
120. Yu JS, Burwick JA, Dranoff G, et al. Gene therapy for metastatic brain tumors by vaccination with granulocyte-macrophage colony-stimulating factor-transduced tumor cells. *Hum Gene Ther.* 1997;8:1065-1072.
121. Sobol RE, Fakhrai H, Shawler D, et al. Interleukin-2 gene therapy in a patient with glioblastoma. *Gene Ther.* 1995;2:164-167.
122. Holladay FP, Heitz-Turner T, Bayer WL, et al. Autologous tumor cell vaccination combined with adoptive cellular immunotherapy in patients with grade III/IV astrocytoma. *J Neurooncol.* 1996;27:179-189.
123. Graf MR, Prins RM, Hawkins WT, et al. Irradiated tumor cell vaccine for treatment of an established glioma. I. Successful treatment with combined radiotherapy and cellular vaccination. *Cancer Immunol Immunother.* 2002;51:179-189.
124. Prins RM, Scott GP, Merchant RE, et al. Irradiated tumor cell vaccine for treatment of an established glioma. II. Expansion of myeloid suppressor cells that promote tumor progression. *Cancer Immunol Immunother.* 2002;51:190-199.
125. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* 1998;392:245-252.
126. Mayordomo JI, Zorina T, Storkus WJ, et al. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit pro-

- protective and therapeutic antitumor immunity. *Nat Med.* 1995;1:1297-1302.
127. Celluzzi CM, Falo LD Jr. Physical interaction between dendritic cells and tumor cells results in an immunogen that induces protective and therapeutic tumor rejection. *J Immunol.* 1998;160:3081-3085.
  128. Albert ML, Pearce SF, Francisco LM, et al. Immature dendritic cells phagocytose apoptotic cells via alpha5beta1 and CD36, and cross-present antigens to cytotoxic T lymphocytes. *J Exp Med.* 1998;188:1359-1368.
  129. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature.* 1998;392:86-89.
  130. Kikuchi T, Akasaki Y, Abe T, et al. Intratumoral injection of dendritic and irradiated glioma cells induces anti-tumor effects in a mouse brain tumor model. *Cancer Immunol Immunother.* 2002;51:424-430.
  131. Akasaki Y, Kikuchi T, Homma S, et al. Antitumor effect of immunizations with fusions of dendritic and glioma cells in a mouse brain tumor model. *J Immunother.* 2001;24:106-113.
  132. Kikuchi T, Akasaki Y, Irie M, et al. Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. *Cancer Immunol Immunother.* 2001;50:337-344.
  133. Fakhrai H, Gramatikova S, Safaei R. Downregulation of transforming growth factor Beta as therapeutic approach for brain tumors. In: Liao LM, Becker DP, Cloughesy TF, et al, eds. *Brain Tumor Immunotherapy.* Totowa, NJ: Humana Press; 2001:289-306.
  134. Mintz A, Gibo DM, Slagle-Webb B, et al. IL-13Ralpha2 is a glioma-restricted receptor for interleukin-13. *Neoplasia.* 2002;4:388-399.
  135. Okano F, Storkus WJ, Chambers WH, et al. Identification of a novel HLA-A\*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain. *Clin Cancer Res.* 2002;8:2851-2855.