

# Tumour Vaccine Approaches for CNS Malignancies

## Progress to Date

*Johnathan D. Ebben, Brandon G. Rocque and John S. Kuo*

Brain Tumor Research Laboratory, Department of Neurological Surgery, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA

### Abstract

Despite the many overall advances in understanding cancer biology and therapeutic development in the last 50 years, most CNS malignancies are still clinically difficult, incurable diseases. Current combinations of aggressive surgical resection, radiation therapy and chemotherapy regimens do not significantly improve long-term patient survival for these cancers. Cancer immunotherapy is a potentially promising new therapeutic strategy that primes a patient's immune system to attack neoplastic cells. We review the preclinical and clinical progress in developing vaccination-based therapy for CNS malignancies to date, including peptide-based vaccinations, dendritic cell-based vaccinations and other potential modalities. Some of the challenges for developing an effective vaccination strategy, such as abnormal immune molecules on glioma cells and abnormal lymphocyte populations within a glioma, are also discussed.

Although significant progress has been made over the last 50 years in the treatment of cancer, these advances have not been reflected across the entire spectrum of malignancies. For patients with CNS cancers, the clinical prognosis remains dismal. The ongoing development of vaccine-based immunotherapies offers a tantalizing new hope for more effective, low-toxicity targeted treatments. A successful vaccine for CNS cancers has been a 'holy grail' in neuro-oncology. Recent clinical trials involving vaccines targeted against glioblastoma multiforme (GBM), a CNS cancer with very poor prognosis, offer promising results. Other trials have inspired additional hope and optimism in the field of cancer immunotherapy. Still, it is important to evaluate the progress of current vaccine and immunotherapy efforts through the prism of past successes and failures of cancer vaccine research and development. This

article seeks to provide an overview of progress in the development of vaccines for CNS tumours, and provide a realistic assessment of advances and remaining challenges in this field. Much of the work to develop a vaccine has been carried out in gliomas and glioma models, as discussed throughout this article. However, as these techniques become validated, it is likely that they will be translated into therapeutic strategies for other CNS cancers. A list of pertinent immunological terms used in this article can be found in table I.<sup>[1]</sup>

### 1. Peptide-Based Vaccines

Peptide-based cancer vaccines represent a major focus of cancer vaccine research that offers exciting clinical possibilities. The underlying premise of peptide-based cancer vaccines is similar to other, more conventional, vaccinations.

**Table 1.** Selected immunological terms defined<sup>[1]</sup>

Term	Definition
APC	Cells that are capable of processing foreign antigen and complexing them with MHC II molecules to present to circulating T cells. These cells are also capable of providing a co-stimulatory signal necessary to activate T cells. Specialized APCs include DC, macrophages and B cells; however, other cells can also serve as APCs
CD4+ T cell	These T cells express the CD4 glycoprotein, which serves as a coreceptor on T cells that are only capable of binding with MHC II expressing cells. The majority of CD4+ cells are known as helper T cells
CD8+ T cell	These T cells express the CD8 glycoprotein, which serves as a coreceptor for T cells that bind to MHC I expressing cells. The majority of CD8+ T-cells are CTLs
Class I MHC	Membrane proteins expressed on virtually all human cells that present antigen to circulating CD8+ T cells
Class II MHC	Membrane proteins that are expressed solely on APCs, which present antigen to CD4+ T cells
Cytokine	Secreted proteins that modulate immune response, primarily by acting on lymphocytes and other immune cells
CTL	A T cell that has the capability to lyse target cells that express antigenic molecules complexed with MHC
DC	Specialized cells that aid in the presentation of antigen to helper T cells
Immunogenicity	The ability of a substance, such as a protein or peptide, to induce an immune response
Interleukin	A subclass of cytokines released primarily by leukocytes
NK cell	Large, cytotoxic lymphocytes that function primarily to target tumour cells, with limited participation in antibody-mediated immunity

**APC**=antigen presenting cell; **CTL**=cytotoxic T lymphocyte; **DC**=dendritic cell; **MHC**=major histocompatibility complex; **NK**=natural killer.

These vaccines function by introducing small peptides (typically 7–14 amino acids in length) that are immunogenic and expressed by targeted cancer cells. It is hoped that such peptides are processed by host antigen presenting cells (APCs), which travel to the lymph nodes and sensitize circulating T cells, known as cytotoxic T-lymphocyte (CTL) cells, to the target cancer cells (figure 1).<sup>[2]</sup> In the case of CNS cancers, the development of a peptide-based vaccine requires the successful

isolation of an effective immunogenic peptide that can be administered to induce an efficient *in vivo* patient immune response against the cancer. Furthermore, this peptide must be universally expressed by cancer cells, and must be in itself, crucial for tumour survival. Without these factors, tumour cells that do not express the vaccine peptide will be positively selected for. This could be particularly challenging, given the heterogeneous nature of cancers, as well as the inherent difficulty in isolating and identifying a particular peptide that demonstrates both specific restriction to cancer cells and efficiently elicits an appropriate antitumour immune response.

### 1.1 Targeting Epidermal Growth Factor Receptor VIII

Recently, progress has been reported in the development of a vaccine that targets glioma, specifically GBM. The majority of primary GBM cases harbour mutations in the epidermal growth factor receptor (EGFR). In particular, a mutation known as EGFRvIII occurs in about one-half of patients with an EGFR mutation.<sup>[3]</sup> The EGFRvIII mutation arises from an in-frame deletion/fusion that results in constitutively active epidermal growth factor signalling. An EGFRvIII-specific peptide that spans this deletion/fusion has shown promise as an immunogenic antigen.<sup>[4]</sup> This peptide provides a novel target for potential vaccine based therapies with a high degree of specificity to GBM tumour cells. In a phase I clinical trial,<sup>[4]</sup> GBM patients were vaccinated with the EGFRvIII peptide after surgical resection. This approach resulted in median time to progression of 46.9 weeks and overall median survival of 110.8 weeks, yielding similar results to current chemotherapy with temozolomide or carmustine wafers. A phase II trial has recently been suspended<sup>[5]</sup> and concern remains about the universality of this approach to effectively treat GBM tumours because not all GBMs express the EGFRvIII mutation.<sup>[4]</sup>

Intratumoural administration of MR1-1, a specific immune toxin to a murine homologue of the EGFRvIII mutation, has also shown promise. MR1-1 effectively killed EGFRvIII positive

tumour cells and also induced long-term tumour immunity in a mouse model. Importantly, long-term tumour immunity was generated in tumours where not all cells were EGFRvIII positive, although the generation of immunity was dependent on the presence of CD4+ and CD8+ T cells.<sup>[6]</sup> Although additional testing is needed in other models, vaccination against EGFRvIII could potentially be of therapeutic value.

### 1.2 Wilms' Tumour Peptide

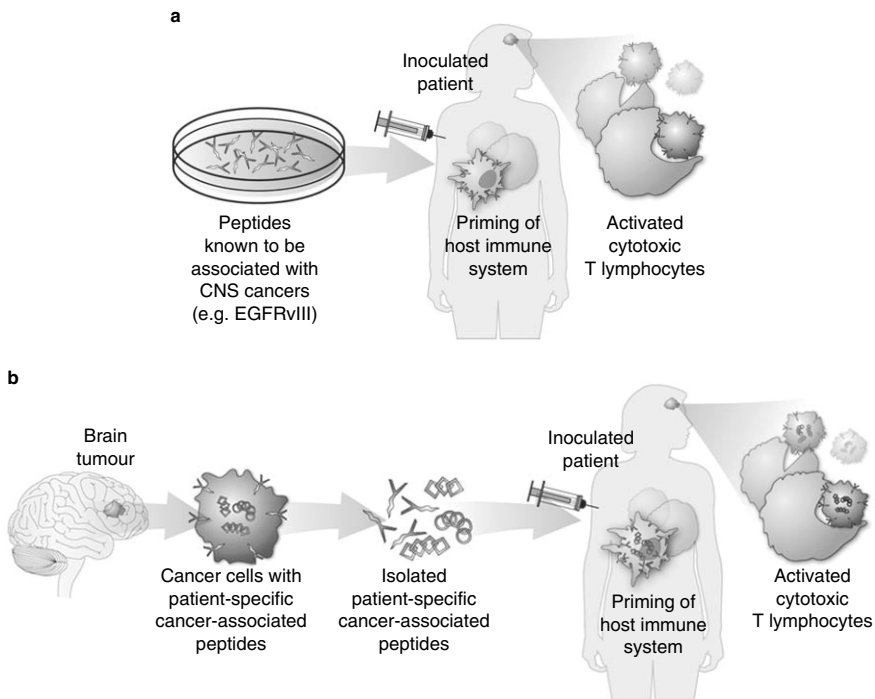
Other tumour-specific antigens have also been identified, which may prove useful in the development of therapeutic vaccines in CNS cancers. Of particular note is the WT-1 peptide produced by Wilms' tumour gene (*WT1*). This protein was first identified in renal cancers, but has since been isolated in a number of haematopoietic cancers and solid tumours. This gene is frequently over-

expressed, and its protein product has also demonstrated immunogenic properties, making it an attractive target for potential vaccines.

A phase II clinical trial has been performed using the WT-1 peptide for vaccination of patients with recurrent GBM.<sup>[7]</sup> Twenty-one patients with WT1/HLA-A\*2402 (where HLA is human leukocyte antigen) positive recurrent GBM, in whom standard therapy had failed, were treated with intradermal injections of WT-1 peptide weekly for at least 12 weeks. Two patients showed partial response, ten patients had stable disease and disease progressed in nine patients. No adverse effects of vaccination were noted.

### 1.3 Identifying Novel Antigens

In order for peptide-based vaccines to be effective, a vast repertoire of tumour-specific



**Fig. 1.** (a) Specific peptides known to be associated with CNS cancers are isolated and given to the patient via intradermal injection. This results in activation of CD4+ and CD8+ T cells in the host immune system. Activated CD8+ cytotoxic T cells then recognize tumour-associated antigens and attack tumour cells. (b) Cancer cells from a patient are cultured and patient-specific cancer peptides are isolated. These peptides are capable of provoking an immune response. Immunogenic peptides are then introduced into the patient via intradermal injection, again activating tumour-specific CD4+ and CD8+ T cells. **EGFRvIII** = epidermal growth factor receptor mutation.

antigens must be developed. To achieve that goal, teams have worked to identify additional antigens that might serve as novel targets for CNS tumours. In the case of paediatric glioma, three promising targets were recently identified, EphA2, interleukin (IL)-13R- $\alpha$ 2 and survivin. These proteins were each differentially expressed in tumour tissue, although their expression levels did not correlate with tumour grade.<sup>[8]</sup> The continual refinement and development of new tumour antigens is an essential component of the peptide-based vaccine development process because of the molecular heterogeneity of tumours. The inherent molecular heterogeneity of tumours between patients has been one of the major challenges that hinder effective implementation of peptide-based therapeutic strategies. With the development of a more expansive library of target antigens, there is hope that individualized, or personalized, peptide-based vaccines can be developed. The development of these vaccines would involve exposing dendritic cells (DCs) [highly effective APCs] to multiple tumour antigens, to evaluate their immunogenicity on a patient-by-patient basis *ex vivo* in an effort to identify a particular immunogenic cocktail that will be most effective for the given patient's tumour.<sup>[9]</sup> The strategy of designing a personalized peptide-based vaccine has been applied with varying success in a number of other cancer types. Early studies have shown that this technique shows promise in increasing the efficacy of peptide-based vaccines in the case of malignant gliomas. In one study, peripheral blood mononuclear cells (PBMCs) were cultured from a patient with a glioma and exposed to a library of previously identified glioma antigens. The four antigens that produced the strongest immune response were then isolated and injected, enhancing the overall immune response of the participants.<sup>[10]</sup> While this is not a vaccine, in the sense that it does not induce active immunity, it does boost a patient's pre-existing tumour immunity and could be used in conjunction with a true vaccine.

Another set of potentially fruitful targets is tumour angiogenesis-associated antigens. In a phase I trial, Okaji et al.<sup>[11]</sup> vaccinated six patients

with recurrent malignant brain tumours with human umbilical vein endothelial cells. Magnetic resonance imaging (MRI) showed partial or complete resolution of enhancing cancer masses in three patients. This may represent a promising technique. However, the observed decreased contrast enhancement on MRI may be a direct effect of immune targeting of tumour vasculature, rather than an immune response against the tumour proper. More research is needed to determine whether this strategy promotes improved patient survival.

Table II provides an overview of selected phase I and phase II clinical trials of CNS cancer vaccines.

## 2. Dendritic Cell-Based Vaccination

Another strategy used to induce an immune response to CNS malignancy involves vaccination with patient DCs that have been treated with various tumour components. DCs are specialized APCs. In their immature state, they show high ability to capture and process antigens of all types. After antigen capture, they mature, lose antigen-capturing ability and become excellent APCs. DCs express high concentrations of major histocompatibility complex (MHC) class I and II molecules on their surface, which are necessary components to effectively present antigen and to activate both CD8+ and CD4+ T cells.<sup>[23]</sup>

Several methods have been reported for priming or charging DCs with tumour antigens, including pulsing cells with tumour peptides as discussed in section 1, fusing tumour cells with DCs and loading DCs with tumour lysate, apoptotic tumour cells or tumour messenger RNA.<sup>[24]</sup> Fusion of DCs and tumour cells results in hybrid cells containing fused cytoplasm with two separate, independently active nuclei (figure 2).<sup>[25]</sup> This could theoretically result in tumour genes being transcribed in the tumour nucleus and presented on the cell surface by the DC component. A phase I clinical trial showed no adverse effects with this approach,<sup>[26]</sup> and a second trial with coadministration of IL-12 showed partial radiographic response in 3 of 12 patients.<sup>[18]</sup>

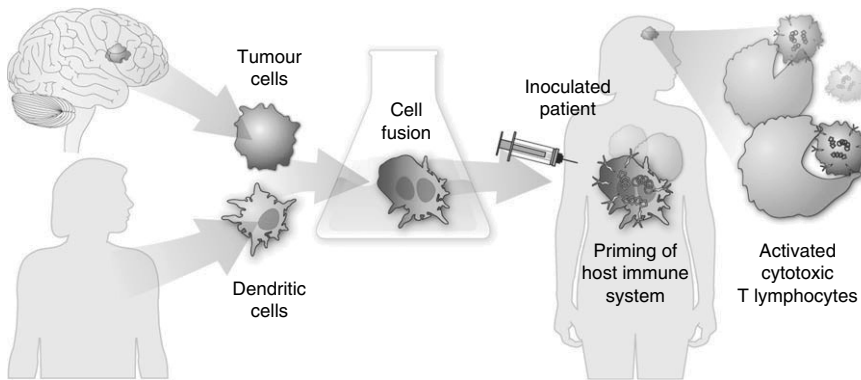
**Table II.** Selected clinical trials of vaccination for CNS malignancies

Study (year)	Trial phase	Patient population	Treatment	Results
De Vleeschouwer et al. <sup>[12]</sup> (2008)	II	53 patients with recurrent GBM	Differing schedules of DC/tumour lysate vaccine	Trend towards better PFS in patients with faster vaccination schedule
Izumoto et al. <sup>[7]</sup> (2008)	II	21 patients with <i>WT1</i> positive recurrent GBM	WT-1 peptide intradermal injection for 12 wk or more	2 patients with partial response, 10 with stable disease; median PFS 20 wk
Okada et al. <sup>[13]</sup> (2007)	I	5 patients with newly diagnosed GBM, after GTR and radiation therapy	2 injections of IL-4 transfected fibroblasts and type-I DCs pulsed with autologous tumour lysate	No improvement in PFS. Treatment well tolerated
Walker et al. <sup>[14]</sup> (2008)	I	13 patients with AA or GBM	Vaccination with autologous DCs previously co-cultured with autologous tumour	No adverse events noted. PFS not reported
Okaji et al. <sup>[11]</sup> (2008)	I	6 patients with recurrent malignant brain cancer	HUVEC vaccination	3 patients showed partial or complete decrease of enhancement on MRI
Ishikawa et al. <sup>[15]</sup> (2007)	I	12 patients with GBM, 8 recurrent, 4 residual primary	Intradermal injection of autologous formalin-fixed tumour tissue	1 complete response, 1 partial response, 7 progressive disease. PFS 10.7 mo
Yajima et al. <sup>[10]</sup> (2005)	I	8 patients with AA, 17 with GBM	Vaccination with peptides found to provoke reaction in patient monocytes	5 partial response, 8 stable disease, 8 progressive disease; PFS in GBM subset 622 days
Liau et al. <sup>[16]</sup> (2005)	I	7 newly diagnosed GBM, 5 recurrent GBM	Vaccination with autologous DCs, pulsed with tumour lysate	PFS 15.5 mo
Yamanaka et al. <sup>[17]</sup> (2005)	I/II	6 patients with AA, 18 with GBM, all recurrent	Intradermal or intratumoural and intradermal injection of tumour lysate-pulsed DCs	PFS of GBM patients 480 days. Trend toward better survival in patients with intratumoural injection
Kikuchi et al. <sup>[18]</sup> (2004)	II	15 patients with malignant glioma	Vaccination with fused autologous DCs and tumour cells and recombinant IL-12	4 patients with reduction in size of tumour on MRI by 50%
Steiner et al. <sup>[19]</sup> (2004)	I	23 patients with GBM treated with GTR and radiation	Autologous tumour cells grown in culture, infected with NDV, then irradiated and injected	PFS 40 wk
Caruso et al. <sup>[20]</sup> (2004)	I	7 paediatric patients with GBM, AA, medulloblastoma or ependymoma	Tumour RNA-pulsed autologous DCs	2 patients with stable disease, 1 clinical response
Yu et al. <sup>[21]</sup> (2004)	I	14 patients with GBM or AA	Tumour lysate-pulsed DCs	Significant CD8+ T cells found in tissue at repeat resection. PFS of GBM patients 133 wk
Schneider et al. <sup>[22]</sup> (2001)	I	11 patients with GBM, post radiation and GTR	Vaccinated with autologous tumour cells, infected with NDV	No significant change in survival. No significant adverse effects

**AA** = anaplastic (WHO grade III) astrocytoma; **DC** = dendritic cell; **GBM** = glioblastoma multiforme (WHO grade IV astrocytoma); **GTR** = gross total resection; **HUVEC** = human umbilical vein endothelial cells; **IL** = interleukin; **MRI** = magnetic resonance imaging; **NDV** = Newcastle disease virus; **PFS** = progression-free survival; **WT1** = Wilm's tumour gene.

Several other techniques using DCs have also demonstrated potential efficacy. Loading of DCs with tumour antigen *ex vivo* followed by vaccination of the patient with the loaded cells has also shown potential as a method of overcoming

chemotherapy resistance, a serious problem that is frequently associated with higher grade gliomas and GBM.<sup>[27]</sup> DCs loaded with tyrosinase-related protein-2 antigen, which is associated with tumour cells expressing chemoresistance,



**Fig. 2.** Fusion of dendritic cells and tumour cells. Patient tumour cells and patient dendritic cells are cultured together *in vitro*. Their cytoplasmic membranes are destabilized to allow the two types of cells to fuse into a hybrid cell. The fused hybrid cells are introduced into the patient via intradermal injection. Tumour antigens are presented on the hybrid cell surface and stimulate activation of host CD4+ and CD8+ T cells that specifically recognize and are active against tumour cells.

not only led to antibody- and cell-mediated responses, but also to a re-sensitization of tumour cells previously resistant to certain classes of chemotherapeutics, including the standard anti-GBM drug temozolomide.<sup>[28]</sup>

A similar approach has been used with PBMCs, involving co-culture of autologous tumour cells with PBMCs under conditions that promote immune activation (addition of IL-1, IL-2, IL-4 and IL-6 to tissue culture media). Intratumoural injection of these cells in recurrent gliomas showed a 50% tumour response rate in preliminary studies, suggesting that the culture media and exposure to tumour antigens caused an immune response *ex vivo*, which could then be propagated and expanded *in vivo*. This also suggests that further research into this technique is warranted.<sup>[29]</sup>

### 3. Viral Vaccination Strategies

In an effort to expand the library of target antigens available, a novel technique has been developed in the case of glioma. Intratumoural vaccination with herpes simplex virus (HSV) has been demonstrated to generate an immune response by CTLs. After intratumoural injection of HSV into a mouse glioma model, a team of scientists isolated activated CTLs and performed gene expression profile analysis in an effort to find specific tumour antigens that were recog-

nized by activated CTLs.<sup>[30]</sup> A new glioma-specific immunogenic antigen was thereby identified and isolated for study in future therapeutic strategies.

Using a similar technique, tumour was obtained from 23 patients and infected with Newcastle disease virus *ex vivo*. The tumour cells were then irradiated and returned to the patient via subcutaneous injection on a set schedule. Treated patients had a longer time to progression and median survival than those not receiving this therapy. Observation of resected tissue from patients with recurrent tumour revealed that many CD8+ T cells had migrated into the tumour, suggesting that the vaccine induced an anti-tumour immune response.<sup>[19]</sup>

Other groups have also shown that many herpes-like viruses may be associated with gliomas, and GBM in particular. Human cytomegalovirus (hCMV) DNA was successfully isolated from the peripheral blood of 80% of newly diagnosed, sampled GBM patients. In healthy control subjects, no hCMV DNA was found in peripheral blood, including in a majority of controls who were seropositive for CMV. Furthermore, among GBM patients, >90% were found to have detectable hCMV nucleic acids and proteins within tumours, yet not within surrounding brain tissue.<sup>[31]</sup> Furthermore, a recent report makes note of a patient who received a vaccination with DCs, pulsed with autologous tumour lysate, who



immediately developed a CMV-specific immune response.<sup>[32]</sup> This discovery, although controversial and disputed by some, could provide an interesting new target for vaccination against viral antigens associated with gliomas.

#### 4. Heat-Shock Proteins as a Vaccination Target

While the identification of a broad spectrum of tumour antigens is certainly valuable, new techniques may also provide ways for clinicians to tailor each vaccine to an individual patient. Autologous heat-shock protein-peptide complexes offer the potential to allow for unique and personalized treatment for each patient. Heat-shock proteins (HSPs) are believed to play an important role in the chaperoning antigens within cells during the antigen presentation process. It is theorized that while each patient may have unique tumour antigens, the targeting of autologous HSPs that are coupled with unique tumour antigens offers a method of targeting cancer cells in a specific way without the need to identify specific and differentially expressed tumour antigens.<sup>[33,34]</sup> This technique allows clinicians to take into account the heterogeneity of cancer with regard to each individual patient, rather than relying on a library of targets that may not be complete or which may not contain relevant antigen targets that are most effective for individual patients.<sup>[35,36]</sup> Studies of HSP vaccination in chronic myeloid leukaemia patients demonstrated some promise. The administration of HSP 70 was found to effectively increase the number of active natural killer (NK) cells by stimulating the expression of a NK activating ligand on DCs.<sup>[37]</sup> This carries important implications because of the vital role of NK cells in antitumour immune responses.

#### 5. Hurdles to Effective Vaccination

While there has been much progress made in terms of vaccines, there are significant drawbacks and technical hurdles that could still hinder these therapies. Chief among these are the frequent lack of MHC molecules on the surface of glioma

cells.<sup>[24]</sup> Peptide-based vaccines function through the use of APCs that sensitize and activate a CTL response by presenting antigen on MHC I. Because CTLs are MHC I restricted, antigens that are presented in other ways fail to provoke an immune response. In glioblastoma, it was documented that MHC I is absent in approximately 50% of patients.<sup>[38]</sup> And furthermore, it was observed that HLA-G, an aberrant form of MHC I that suppresses both NK and CTL activity, may be over expressed, resulting in compromised immune activity.<sup>[39]</sup> Taken together, glioma-mediated immunosuppression, due to abnormal MHC I, prevents CTL or NK cells from effectively targeting cancer cells.<sup>[40,41]</sup> In addition, data suggest that a small subset of glioma cancer stem cells (cells with stem-like properties of self-renewal, multipotent differentiation and tumour initiation efficiency) also may not express MHC I.<sup>[41]</sup> This could be an important hurdle to overcome in the development of cancer vaccines because it is theorized that cancer stem cells may be the cellular origin of recurrent gliomas and have been implicated in many cancers with a dismal prognosis. In contrast to work with gliomas, studies with medulloblastoma tumour lines showed that both CD133+ (a marker of cancer stem cells) and CD133- cells are susceptible to attack by NK cells.<sup>[42]</sup>

Additional work has shown that gliomas mediate patient immunosuppression that may hinder the efficacy of an immune response. High levels of IL-10, as well as tumour growth factor- $\beta$ 2 signalling in the vicinity of tumours may lead to a bias in T-cell differentiation towards the T helper-2 ( $T_H2$ ) cell subtype. In addition to the observation that quantitative levels of immune cells observed near tumours are typically depressed, a larger fraction of those cells are regulatory T cells ( $T_H2$  phenotype) known for their ability to further depress the immune response.<sup>[43]</sup>

Finally, in testing new cancer therapies, particularly for glioma, patients eligible for experimental therapies are often the ones with recurrent or progressive disease. These patients are likely to have a significant burden of unresectable tumour, and/or have recurrent tumours composed of cells resistant to previous therapies. It is possible that

the observed response to new cancer therapies (such as vaccines) in this patient population would not represent the response that one might see in a newly diagnosed patient.

## 6. CNS Cancer Vaccines: New Hope

An effective vaccine would be a potent addition to our current therapeutic arsenal against CNS cancers. Significant progress has been made that offers new hope and unprecedented therapeutic opportunities. However, it is important to realize that much additional work lies ahead before an effective CNS tumour vaccine is validated for clinical use.

## Acknowledgments

We acknowledge Betsy True of the University of Wisconsin Media Solutions for expert assistance in creating illustrations. This work was partially supported by a Young Clinician Investigator Award from the American Association of Neurological Surgeons Neurosurgery Research and Education Foundation, and funding from the University of Wisconsin-Madison, Madison, WI, USA. The authors have no conflicts of interest that are directly relevant to the content of this review.

## References

- Kindt TJ, Goldsby RA, Osborne BA. *Kuby immunology*. 6th ed. New York: W.H. Freeman and Company, 2007
- Yamanaka R, Itoh K. Peptide-based immunotherapeutic approaches to glioma: a review. *Expert Opin Biol Ther* 2007 May; 7 (5): 645-9
- Pelloski CE, Ballman KV, Furth AF, et al. Epidermal growth factor receptor variant III status defines clinically distinct subtypes of glioblastoma. *J Clin Oncol* 2007 Jun 1; 25 (16): 2288-94
- Sampson JH, Archer GE, Mitchell DA, et al. Tumor-specific immunotherapy targeting the EGFRvIII mutation in patients with malignant glioma. *Semin Immunol* 2008 Oct; 20 (5): 267-75
- M.D. Anderson Cancer Center. An immunotherapy vaccine against grade IV brain tumors [ClinicalTrials.gov identifier NCT00090597]. US National Institutes of Health, ClinicalTrials.gov [online]. Available from URL: <http://www.clinicaltrials.gov> [Accessed 2009 Feb 10]
- Ochiai H, Archer GE, Herndon JE, et al. EGFRvIII-targeted immunotoxin induces antitumor immunity that is inhibited in the absence of CD4+ and CD8+ T cells. *Cancer Immunol Immunother* 2008 Jan; 57 (1): 115-21
- Izumoto S, Tsuboi A, Oka Y, et al. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. *J Neurosurg* 2008 May; 108 (5): 963-71
- Okada H, Low KL, Kohanbash G, et al. Expression of glioma-associated antigens in pediatric brain stem and non-brain stem gliomas. *J Neurooncol* 2008 Jul; 88 (3): 245-50
- Itoh K, Yamada A. Personalized peptide vaccines: a new therapeutic modality for cancer. *Cancer Sci* 2006 Oct; 97 (10): 970-6
- Yajima N, Yamanaka R, Mine T, et al. Immunologic evaluation of personalized peptide vaccination for patients with advanced malignant glioma. *Clin Cancer Res* 2005 Aug 15; 11 (16): 5900-11
- Okaji Y, Tsuno NH, Tanaka M, et al. Pilot study of anti-angiogenic vaccine using fixed whole endothelium in patients with progressive malignancy after failure of conventional therapy. *Eur J Cancer* 2008 Feb; 44 (3): 383-90
- De Vleeschouwer S, Fieuids S, Rutkowski S, et al. Post-operative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. *Clin Cancer Res* 2008 May; 14 (10): 3098-104
- Okada H, Lieberman FS, Walter KA, et al. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of patients with malignant gliomas. *J Transl Med* 2007 Dec; 5: 10
- Walker DG, Laherty R, Tomlinson FH, et al. Results of a phase I dendritic cell vaccine trial for malignant astrocytoma: potential interaction with adjuvant chemotherapy. *J Clin Neurosci* 2008 Feb; 15 (2): 114-21
- Ishikawa E, Tsuboi K, Yamamoto T, et al. Clinical trial of autologous formalin-fixed tumor vaccine for glioblastoma multiforme patients. *Cancer Sci* 2007 Aug; 98 (8): 1226-33
- Liau LM, Prins RM, Kiertscher SM, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. *Clin Cancer Res* 2005 Aug 1; 11 (15): 5515-25
- Yamanaka R, Homma J, Yajima N, et al. Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. *Clin Cancer Res* 2005 Jun 1; 11 (11): 4160-7
- Kikuchi T, Akasaki Y, Abe T, et al. Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. *J Immunother* 2004 Nov-Dec; 27 (6): 452-9
- Steiner HH, Bonsanto MM, Beckhove P, et al. Antitumor vaccination of patients with glioblastoma multiforme: a pilot study to assess feasibility, safety, and clinical benefit. *J Clin Oncol* 2004 Nov; 22 (21): 4272-81
- Caruso DA, Orme LM, Neale AM, et al. Results of a phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. *Neuro Oncol* 2004 Jul; 6 (3): 236-46
- Yu JS, Liu G, Ying H, et al. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 2004 Jul 15; 64 (14): 4973-9
- Schneider T, Gerhards R, Kirches E, et al. Preliminary results of active specific immunization with modified tumor cell vaccine in glioblastoma multiforme. *J Neurooncol* 2001; 53 (1): 39-46



23. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998 Mar 19; 392 (6673): 245-52
24. Steinman RM, Dhodapkar M. Active immunization against cancer with dendritic cells: the near future. *Int J Cancer* 2001 Nov; 94 (4): 459-73
25. Koido S, Ohana M, Liu C, et al. Dendritic cells fused with human cancer cells: morphology, antigen expression, and T cell stimulation. *Clin Immunol* 2004 Dec; 113 (3): 261-9
26. 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 Sep; 50 (7): 337-44
27. Liu GT, Black KL, Yu JS. Sensitization of malignant glioma to chemotherapy through dendritic cell vaccination. *Expert Rev Vaccines* 2006 Apr; 5 (2): 233-47
28. Liu G, Akasaki Y, Khong HT, et al. Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. *Oncogene* 2005 Aug 4; 24 (33): 5226-34
29. Tsuboi K, Saijo K, Ishikawa E, et al. Effects of local injection of *ex vivo* expanded autologous tumor-specific T lymphocytes in cases with recurrent malignant gliomas. *Clin Cancer Res* 2003 Aug 15; 9 (9): 3294-302
30. Iizuka Y, Kojima H, Kobata T, et al. Identification of a glioma antigen, GARC-1, using cytotoxic T lymphocytes induced by HSV cancer vaccine. *Int J Cancer* 2006 Feb 15; 118 (4): 942-9
31. Mitchell DA, Xie W, Schmittling R, et al. Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. *Neuro Oncol* 2008 Feb; 10 (1): 10-8
32. Prins RM, Cloughesy TF, Liau LM. Cytomegalovirus immunity after vaccination with autologous glioblastoma lysate. *N Engl J Med* 2008 Jul 31, 2008; 359 (5): 539-51
33. Przepiorka D, Srivastava PK. Heat shock protein-peptide complexes as immunotherapy for human cancer. *Mol Med Today* 1998 Nov; 4 (11): 478-84
34. Li Z. Priming of T cells by heat shock protein-peptide complexes as the basis of tumor vaccines. *Semin Immunol* 1997 Oct; 9 (5): 315-22
35. Hoos A, Levey DL, Lewis JJ. Autologous heat shock protein-peptide complexes for vaccination against cancer: from bench to bedside. *Dev Biol (Basel)* 2004; 116: 109-15
36. Amato RJ. Heat-shock protein-peptide complex-96 for the treatment of cancer. *Expert Opin Biol Ther* 2007 Aug; 7 (8): 1267-73
37. Qiao Y, Liu B, Li Z. Activation of NK cells by extracellular heat shock protein 70 through induction of NKG2D ligands on dendritic cells. *Cancer Immun* 2008; 8: 12
38. Facchetti A, Nano R, Zelini P, et al. Human leukocyte antigen and antigen processing machinery component defects in astrocytic tumors. *Clin Cancer Res* 2005 Dec; 11 (23): 8304-11
39. Wiendl H, Mitsdoerffer M, Weller M. Hide-and-seek in the brain: a role for HLA-G mediating immune privilege for glioma cells. *Semin Cancer Biol* 2003 Oct; 13 (5): 343-51
40. Seliger B, Cabrera T, Garrido F, et al. HLA class I antigen abnormalities and immune escape by malignant cells. *Semin Cancer Biol* 2002 Feb; 12 (1): 3-13
41. Aptsiauri N, Cabrera T, Mendez R, et al. Role of altered expression of HLA class I molecules in cancer progression. *Adv Exp Med Biol* 2007; 601: 123-31
42. Castriconi R, Dondero A, Negri F, et al. Both CD133+ and CD133- medulloblastoma cell lines express ligands for triggering NK receptors and are susceptible to NK-mediated cytotoxicity. *Eur J Immunol* 2007 Nov; 37 (11): 3190-6
43. Fecci PE, Mitchell DA, Whitesides JF, et al. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res* 2006 Mar 15; 66 (6): 3294-302

---

Correspondence: Dr *John S. Kuo*, Department of Neurological Surgery, School of Medicine and Public Health, University of Wisconsin, K3/803 Clinical Science Center, Mail Code 8660, 600 Highland Ave., Madison, WI 53792, USA.  
E-mail: J.Kuo@neurosurg.wisc.edu