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Antibody, T-cell and dendritic cell immunotherapy for malignant brain tumors

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Abstract

Modest improvement in brain tumor patient survival has been achieved through advances in surgical, adjuvant radiation and chemotherapeutic strategies. However, these traditional approaches have been unsuccessful in permanently controlling these aggressive tumors, with recurrence being quite common. Hence, there is a need for novel therapeutic approaches that specifically target the molecularly diverse brain tumor cell population. The ability of the immune system to recognize altered tumor cells while avoiding surrounding normal cells offers an enormous advantage over the nonspecific nature of the conventional treatment schemes. Therefore, immunotherapy represents a promising approach that may supplement the standard therapies in eliminating the residual brain tumor cells. This review summarizes different immunotherapeutic approaches currently being tested for malignant brain tumor treatment.

Keywords

brain tumor; dendritic cell; immunotherapy; monoclonal antibody; T cell

Based on National Cancer Institute (NCI) statistics, 22,910 new brain tumor cases and 13,700 brain tumor-related deaths occurred in the USA in 2012. According to the classification scheme developed by Bailey and Cushing in 1926 there are 14 different types of CNS neoplasms that arise from primitive neuro-ectoderm cells arrested at particular stages of development [1]. Currently, there are more than 120 types of brain tumor. WHO classifies brain tumors – based on the cell of origin, morphology and biological behavior – from least aggressive, grade I (benign), to most aggressive, grade IV (malignant). Glioblastoma multiforme (GBM), a WHO grade IV tumor, is the most common primary

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malignant brain tumor of the CNS [2]. Despite rigorous efforts, the overall survival (OS) of GBM patients treated with radiotherapy and concomitant temozolomide at 2 and 5 years is dismal at 27.2 and 9.8%, respectively [3]. Recent clinical trials incorporating the anti-VEGF monoclonal antibody (mAb) bevacizumab has only resulted in modest extensions in survival in recurrent GBM patients [4]. Clearly, there is a dire need for new, and more effective, therapeutic approaches to improve GBM patient survival.

A successful therapeutic regimen for malignant brain tumor treatment depends on targeting and eradicating residual tumor cells after surgical resection of the primary tumor mass. Considering the diffuse nature of glioma, targeting every remaining tumor cell is a daunting task. However, therapeutic approaches that use the body's own immune system to fight the leftover brain tumor cells hold promise. The advantage of immunotherapy for cancer treatment lies in the fact that immune effectors can seek out and destroy tumor cells that are inaccessible to conventional treatment modalities with exquisite specificity and concurrently generate long-term immune surveillance against cancer cells. Development of immunotherapeutics for brain tumors has been hindered by the traditional belief that the CNS is immunologically privileged [5]. This notion is due to several factors: the paucity of organized lymphoid tissue and the traditional lymphatic drainage system within the CNS; the absence of native antigen-presenting cells (APCs) within the brain; the presence of the blood–brain barrier, which is impermeable to antibodies and lymphocytes; MHC antigen expression being undetectable on normal parenchymal cells of the brain; and the extended survival of tissues grafted into the CNS versus those grafted into peripheral sites [6–8]. However, each of these perceived obstacles to immunotherapy has been substantially discounted. Despite the blood–brain barrier, the presence of different types of immune cells within the brain parenchyma during disease processes has been well documented [9,10]. Naive T cells can access the antigens originating from the CNS at the cervical lymph nodes and nasal submucosa through cerebrospinal fluid drainage via the Virchow–Robin perivascular spaces [11]. Activated T cells with CNS tropism then cross the blood–brain barrier where they function as either cytotoxic or helper T cells [12]. Dendritic cells (DCs), the professional APCs, have not been documented in the CNS, but are present in both the choroid plexus and meninges [13]; instead, microglia function as resident APCs within the CNS [14]. Moreover, lower levels of antibody accumulation in the CNS after active or passive peripheral immunization have been well described [15]. From these findings, it is obvious that the CNS is not isolated from immune surveillance. Hence, it would be more appropriate to describe the immune status of the brain as ‘immunologically quiescent’ and it is essential to devise ways to activate the immune system of the brain to mount an effective immune response to destroy tumor cells. In this article, we provide an outline of the various immunotherapeutic strategies that are currently under clinical and preclinical evaluation for malignant brain tumor treatment.

Current immunotherapeutic approaches against malignant brain tumors

Different types of immunological treatments have been employed in the fight against brain tumors, including passive, active and adoptive immunotherapy. Administration of molecules targeting tumor cell-specific antigens in the absence of a host anti-tumor response constitutes passive immunotherapy. Initiating an effective immune response *in vivo*, upon immunization against the cancer cells, is active immunotherapy, and adoptive immunotherapy involves the *ex vivo* expansion and activation of a patient's lymphocytes before returning these effector cells to the tumor-bearing host to fight cancer cells.

mAb therapy

The most extensively employed passive immunotherapy is mAb therapy. mAb therapy targets cancer cells in the absence of the active involvement of the body's immune system.

The development of mAbs against antigens solely expressed on glioma cells or antigen targets that are over-expressed on brain tumor cells [16], compared with those on normal neural tissues, launched the utilization of mAb therapy to kill tumor cells. A summary of naked mAbs and mAbs conjugated to a chemotherapeutic drug, plant or bacterial toxin, or a radionucleotide actively being utilized for cancer treatment is presented in Table 1.

Naked mAb therapy—Malignant glioma is a highly vascularized tumor in which angiogenesis is fueled by several pro-angiogenic growth factors, including VEGF [17]. Bevacizumab (Avastin[®]; Genentech, CA, USA), a humanized mAb targeting VEGF, is the only mAb currently approved by the US FDA for recurrent GBM therapy. Bevacizumab as a single agent [4,18], or in combination with different chemotherapeutics [4,19,20] or with concurrent radiotherapy [21], has been shown to improve 6-month progression-free survival rates by 25–65% in recurrent high-grade glioma (HGG) patients. The precise mode of action of bevacizumab in conjunction with other treatment modalities for glioma therapy is unclear. However, several mechanisms have been described, including binding to VEGF and preventing tumor-associated angiogenesis, sensitizing endothelial cells to radiotherapy, disruption of the perivascular glioma stem cell niche, which is vital for cancer cell maintenance, and normalization of tumor vasculature, thereby promoting efficient drug delivery [22–24].

The EGFR, a member of the ErbB family of receptor tyrosine kinases (RTKs), is frequently overexpressed in brain tumors [25]. Several EGFR-specific mAbs have been generated for targeting EGFR-overexpressing tumors [26]. The anti-EGFR mAb, cetuximab (IMC-C225; Erbitux[®], ImClone Systems, NJ, USA) was tested individually and in combination with bevacizumab and irinotecan for recurrent HGG patients; a median OS of 5–7 months was achieved [27,28]. Interestingly, the response rate with the addition of cetuximab does not seem to be superior compared with that of single-agent bevacizumab (historical data median OS: 9.7 months [29]) or bevacizumab plus irinotecan (historical data median OS: 8.9 months [29]). Promising results with nimotuzumab (h-R3, TheraCIM[®]), a humanized mAb targeting EGFR, by itself or in combination with external beam radiation therapy, have been seen in both adult and pediatric HGG patients [30,31]. In the adult group, the median survival (MS) time for GBM patients was 17.47 months and was not reached in anaplastic astrocytoma (AA) patients. A median OS of 15 (GBM) and 20.2 months (AA) was achieved in a control group that received whole-brain irradiation after surgery at the National Institute of Neurology and Neurosurgery [31]. In the pediatric group, the median OS was extended for responders (10 months) compared with nonresponders (4 months).

The most common *EGFR* deletion mutant, *EGFRvIII*, is generated by the deletion of exons 2–7 from the *EGFR* gene. This deletion produces a constitutively active RTK, with a tumor-specific protein sequence that is extensively expressed in gliomas [32], but not on normal cells. In preclinical studies, a single intratumoral injection of the anti-EGFRvIII mAb Y10 in mice bearing intracranial EGFR-vIII-expressing tumors increased the MS by an average of 286% [33]. Further investigation demonstrated the therapeutic efficacy of Y10 to be Fc receptor dependent [33]. The clinical efficacy of EGFRvIII-specific mAb is yet to be tested. Dual specific mAbs 528 and 806, with specificity for both the EGFR and mutant EGFRvIII proteins expressed on different cell lines, have been well described [34]. Combination therapy with mAbs 528 and 806 demonstrated a significant decrease in the tumor volume of xenografts expressing wild-type EGFR or EGFRvIII [34].

Amplification and overexpression of the RTK PDGFR α has been demonstrated exclusively in the proneural subclass of GBM [35]. IMC-3G3, a human mAb, specifically binds to PDGFR α and blocks PDGF ligand binding and receptor activation [36]. The PDGFR α mAb IMC-3G3 demonstrated significant inhibition of tumor growth; tumor regression was

observed in ten out of 12 IMC-3G3-treated mice in a subcutaneous xenograft model with U118 GBM cell lines [36]. IMC-3G3 is currently being evaluated for the treatment of recurrent GBM patients [201].

Conjugated mAb therapy

Radioimmunotherapy: By themselves naked antibodies might not always be sufficient to generate a potent anti-tumor response. However, they could be utilized as vehicles for the delivery of a variety of effector molecules to tumor cells. The most commonly utilized conjugates in mAb therapy are radionucleotides. The first reported, and most extensively evaluated, glioma antigen by radioimmunotherapy is TN, which is a large disulfide-linked hexameric extracellular matrix glycoprotein. Among the different TN isoforms, TN-C is extensively expressed in gliomas, with more than 90% of GBM patient specimens demonstrating high levels of TN-C [37]. Among the different anti-TN-C antibodies, BC-2, BC-4 and 81C6 have been introduced in clinical trials in the USA and Europe [38,39]. In a total of 573 GBM patients, a MS of 12.1 and 14.6 months was accomplished with radiotherapy alone or radiotherapy plus temozolomide, respectively, by Stupp *et al.* [3]. However, in a Phase II study, the MS after ^{131}I -labeled BC-2 and BC-4 mAb therapy was 31 months in oligodendroglioma, 23 months in anaplastic oligodendroglioma (AO), more than 46 months in AA and 19 months in GBM [39]. GBM patients with smaller lesions had a better prognosis (MS: 25 months) than those with bulky lesions (MS: 17 months). In a similar study with ^{90}Y -labeled BC-2 and BC-4 mAbs, the MS was 90 months for AA patients, 20 months for GBM patients and 31 months for GBM patients with small tumors [40]. Both the BC-2 and BC-4 mAbs were unsuitable for industrial scale-up due to the production of a nonfunctional light chain. To overcome this difficulty, two additional anti-TN-C mAbs, ST2146 (TN-C epitope similar to BC-4) and ST2485, with greater affinity and immunoreactivity than BC-2 and BC-4, have been developed and evaluated in preclinical models [41]. 81C6 (Neuradiab[®]; Bradmer Pharmaceuticals Inc., ON, Canada) is a murine IgG2b mAb that binds to TN-C. A median OS of 52 weeks was attained in recurrent GBM patients who underwent surgery and ^{125}I -brachytherapy [38]. However, in a Phase II study with 33 newly diagnosed malignant glioma patients (27 GBM, four AA and two AO), administration of 120 mCi of ^{131}I -81C6 followed by conventional radiotherapy and chemotherapy achieved a MS of 87 and 79 weeks among all patients and those with GBM, respectively [42]. In a subsequent Phase II study with 43 recurrent malignant glioma patients (33 GBM, six AA, two AO, one gliosarcoma and one metastatic adenocarcinoma), administration of 100 mCi of ^{131}I -81C6 followed by chemotherapy resulted in an overall MS of 64 and 99 weeks for GBM/glio-sarcoma and AA/AO patients, respectively [38]. A multi-institutional Phase III study randomizing newly diagnosed GBM patients to a regimen of ^{131}I -81C6 radiotherapy/chemotherapy versus standard radiotherapy/chemotherapy has been planned based on these encouraging results.

The EGFR-specific mAb nimotuzumab, labeled with ^{188}Re , was evaluated for toxicity and efficacy in a Phase I clinical trial in 11 recurrent adult HGG patients [43]. Intracavitary administration of 3 mg of nimotuzumab labeled with 10 or 15 mCi of ^{188}Re elicited a beneficial effect in three patients. In the small patient population there was no apparent correlation between EGFR expression and clinical output. The efficacy of boronated polyamidoamine dendrimer cetuximab (BD-C225) and the EGFRvIII-specific mAb L8A4 (BD-L8A4) as combination agents was evaluated in a rat glioma model composed of a mixture of cells expressing either EGFR or EGFRvIII [44]. The mean survival time of tumor-bearing animals that received both BD-C225 and BD-L8A4 was 55 days compared with 38 and 36 days for animals that received BD-C225 or BD-L8A4, respectively.

The high-molecular-weight CSPG is a single-pass type I transmembrane protein expressed either as a 250-kDa glycoprotein or a 450-kDa proteoglycan. CSPG overexpression in gliomas with the mAb Mel-14 is well documented [45]. Intrathecal administration of ^{131}I -labeled mAb Mel-14 F(ab')₂ fragment, in a 46-year-old neoplastic meningitis patient, produced a long-term response, with no indication of the disease 4 years post-treatment [46].

Immunotoxins: The efficacy of plant and bacterial toxins as effector molecules conjugated to either mAbs or antibody fragments has also been extensively evaluated. The transferrin receptor is a transmembrane glycoprotein involved in the cellular uptake of iron from plasma. The over-expression of the transferrin receptor has been observed in GBM biopsy specimens [47]. Several transferrin receptor mAb-derived ricin-based immunotoxins (antihuman transferrin receptor mAb–ricin A-chain and 454A12) demonstrated significant *in vitro* and *in vivo* cytotoxicity against human GBM cell lines [48,49]. However, transferrin–CRM107, a conjugate of human transferrin and full-length diphtheria toxin (CRM107), was more potent than 454A12 in *in vivo* studies [50]. Hence, future studies solely focused on transferrin–CRM107 for GBM therapy.

Full length *Pseudomonas aeruginosa* exotoxinA (PE) consists of a cell-binding domain I at the N-terminus, a translocation and intracellular trafficking domain II (amino acids 253–364) in the middle of the protein, and a catalytically active ADP-ribosylation domain III (amino acids 405–613) at the C-terminus. Activation of full-length PE requires cleavage between amino acids 279 and 280. The truncated form of PE, PE35KDEL, which contains the translocation and ADP-ribosylation domains, and the endoplasmic reticulum retention signal KDEL, and does not require proteolysis for activation, chemically coupled to the EGFRvIII-specific mAbs, L8A4, Y10 and H10 exhibited potent cyto-toxic activity (IC₅₀ in the range of 15–50 pM) against a mutant EGFRvIII-expressing cell line [51]. The anti-tumor efficacy of a single-chain variable fragment (scFv) specific for EGFRvIII, MR1 (mutant receptor), fused to a variant of PE, PE38KDEL (containing domains II and III and the endoplasmic reticulum retention signal KDEL, and requiring proteolysis for activation); MR1–PE38KDEL was tested in a neoplastic meningitis model induced with an EGFRvIII-expressing human glioma cell line U87MG. ΔEGFR [52]. In the MR1–PE38KDEL treatment group, 75% of long-term survivors had an estimated MS of more than 53 days [52]. An affinity-matured variant of MR1, termed MR1-1, with increased affinity to EGFRvIII, was generated and fused to PE38 (containing domains II and III, and requiring proteolysis for activation) to generate MR1-1–PE38. The cytotoxic activity of MR1-1–PE38 was improved in comparison with that of the parental MR1-PE38, against the EGFRvIII-expressing NR6M cell line [53]. A Phase I study with MR1-1–PE38KDEL delivered intracerebrally by convection-enhanced delivery in malignant brain tumor patients is currently ongoing [202].

GPNMB is a type I transmembrane protein that is highly expressed in GBM patient samples [54]. An affinity-matured GPNMB-specific mutant scFv clone, F6V, fused to PE38 (F6V–PE38), exhibited significant cytotoxicity (IC₅₀: 8 pM) and anti-tumor activity against malignant glioma cells [55]. In addition to the Mel-14 mAb discussed above, the CSPG tumor antigen is also recognized by the mAb 9.2.27. Intratumoral infusion of the 9.2.27–PE molecule extended the survival of nude rats with established human glioma xenografts from 30 to 90% [56]. Currently, our group is working on the construction of a recombinant immunotoxin, Mel-14–PE38KDEL and 9.2.27–PE38KDEL, by fusing the Mel-14 scFv and 9.2.27 scFv to PE38KDEL to generate immunotoxins for targeted therapy of GBM expressing CSPG [57].

Despite offering improved specificity over current treatment modalities, there are several limitations inherent to mAb-based targeted therapy that have to be overcome to achieve

clinical success of brain tumor therapy. Importantly, a sufficient amount of therapeutic molecules has to reach the tumor site to generate an effective anti-tumor response. Hence, mAbs have been administered locoregionally into the surgical cavity after tumor resection. This technique offers the availability of high concentrations of therapeutic agents at the tumor site, while minimizing nonspecific toxicity to peripheral normal tissues. However, slow diffusion of mAbs from the resection cavity and high interstitial pressure inside the tumor opposing mAb diffusion results in nonuniform distribution of the therapeutic moiety within the residual tumor, resulting in ineffective therapy. To overcome the diffusion barrier, novel convection-enhanced delivery approaches of directly delivering therapeutic drugs into brain tumors are being utilized. Convection-enhanced delivery involves the continuous infusion of therapeutic agents through intracranial catheters together with a pressure gradient to attain a higher concentration of drug distribution to the target tumor tissue. Furthermore, the development of human antimouse antibodies against mouse mAbs has been described [39]. The generation of human antimouse antibodies might not be favorable for the repeated use of the mAb therapy. In order to reduce immunogenicity, recombinant strategies have been utilized to create chimeric mouse/human antibodies, with the variable epitope-binding region of mouse mAbs inserted into a human immunoglobulin framework. Finally, GBM is characterized by extensive heterogeneity both at the cellular and molecular levels. Hence, a combination therapy targeting multiple antigens might be efficacious.

Genetically engineered T-cell receptor-based adoptive immunotherapy

T-cell receptor-modified lymphocytes—Adoptive T-cell transfer (ACT) immunotherapy was initially demonstrated to be effective in treating patients with cancer by the Rosenberg group at the NCI, using tumor-infiltrating lymphocytes grown from resected melanoma tumors, expanded *ex vivo* and reinfused into patients [58]. While this marked a major advance in ACT immunotherapy, the process was laborious and not all patients had resectable tumors that produced effective tumor-infiltrating lymphocytes. In addition, this approach was largely limited to melanoma, as few other cancer types were known to generate tumor-infiltrating lymphocytes. To overcome these problems, the same group then demonstrated that patient T cells could be genetically modified to express a cloned T-cell receptor (TCR) recognizing a melanoma antigen (MART-1) and result clinically in tumor regression [59], and that selecting increasingly high-affinity TCRs resulted in both increased anti-tumor activity and autoimmune toxicity in patient tissues bearing the shared antigen [60]. Following this work, patients with synovial cell sarcomas and colorectal cancer were given autologous T cells genetically engineered to express a TCR recognizing the tumor-associated shared antigens NY-ESO-1 and CEA. This was the first use of TCR-engineered ACT to treat tumors other than melanoma [61,62].

Chimeric antigen receptor-modified lymphocytes—While beneficial to some patients, these TCR-based ACT trials were restricted by the need to match the HLA type in the patient, so only *HLA-A*02* patients could be treated. To overcome this limitation, several groups focused on using chimeric antigen receptors (CARs) to generate non-HLA-restricted tumor-reactive T cells. Initially introduced over 20 years ago by Eshhar *et al.*, CARs combine the recognition specificity of an antibody with T-cell signaling through the CD3 ζ -chain [63]. In recent years, advances in technology and methodology have allowed for rapid advancement of CAR therapy. Preclinically, CARs have shown tremendous anti-tumor efficacy when redirected against ovarian cancer [64], lymphoma [65], breast cancer [66] and brain cancer [67–72] as an antiangiogenic agent against VEGF-R2 [73].

Clinical trials utilizing CARs have targeted a variety of antigens and malignancies, including GD2 in neuroblastoma [74], carbonic anhydrase IX in renal cell carcinoma [75], HER2/ERBB2-expressing metastatic cancer [76], and CD19 and CD20 in B-cell malignancies [77–

79]. Targeting GD2 showed promising tumor necrosis or regression in half of the tested subjects and targeting CD19 resulted in over half of the patients with progressive malignancy obtaining remission; however, in two of the aforementioned trials, severe adverse events were reported, including systemic B-cell aplasia, and the death of a patient in the trial targeting HER2. These events were due to the expression of the targeted antigen on normal tissues and the subsequent potent cytotoxicity of the CAR-bearing T cells at these nontumor sites. Thus, in CAR-based immunotherapy, the target antigen must be carefully selected to reduce the risk of life-threatening toxicity from activated CAR-transduced T cells. This is especially true in the treatment of brain tumors, as toxicity to surrounding eloquent normal tissues would be disastrous.

CAR treatment of brain tumors—Early CAR work targeting brain tumors conducted by the Jensen group focused on the use of an IL-13 zetakine CAR to target IL13R α 2, which is preferentially expressed on GBM brain tumors [68]. They showed in a murine xenograft model that injecting intratumoral zetakine CAR-expressing T cells could eliminate 5-day established orthotopic human glioma tumors in immune-compromised mice. The same group has since implemented clinical zetakine CAR ACT trials for patients with GBM [71], and demonstrated trafficking of these cells into the site of tumors within 2 h following intracranial injection [80], although full clinical trial results have not been published. Kong *et al.* are continuing in this line of work, having recently generated a mutated IL-13 zetakine CAR with improved targeting specificity for the tumor-expressing IL13R α 2 over IL13R α 1, which can be expressed on normal tissues [72].

The Gottschalk group recently published work using patient T cells engineered to express a CAR targeted against HER2/ERBB2 or EphA2 that were delivered intratumorally to treat autologous orthotopic tumors in immune-compromised mice [67,70]. Overexpressed in a number of cancers, HER2 is a shared tumor-associated antigen, and this work successfully demonstrates its expression in primary GBM xenografts. This work holds promise for potential clinical translation; however, the fatality reported in the NCI HER2 CAR clinical trial for patients with melanoma suggests the need for extreme caution, as HER2 is also expressed on normal tissues in the body. It is possible that a ‘window’ of treatment exists, either a using lowered dosage of T cells, or changing to a direct intratumoral route of CAR administration, may be sufficient to bypass overt toxicity in systemic tissues. The Gottschalk group at Baylor (TX, USA) is currently enrolling patients with recurrent GBM for a HER2/ERBB2 CAR clinical trial that starts treatment at a very low dose and implements a graduated dosing schedule to determine safety [203]. EphA2 may be a more promising tumor-associated antigen as it has been shown to confer tumorigenic properties and is highly expressed by GBM tumors [70].

EGFRvIII as a CAR target in GBM—Ideally, CAR treatment for brain tumors would target an absolutely tumor-specific antigen that is not expressed in any normal tissue. Described earlier, GBM does express such an antigen in the tumorigenic EGFRvIII mutation, which is expressed as a unique epitope on the surface of up to half of GBMs. With well-defined mAbs to this protein, several groups, including ours, are currently pursuing the use of EGFRvIII CARs in preclinical models [69,81,82]. It has been demonstrated that the EGFRvIII antibody single-chain variable regions from murine-based 3C10 [82], MR1 [81] and the humanized 139 [69] mAbs can successfully convert T cells to recognize and destroy EGFRvIII-expressing tumor cell lines and CD133⁺ glioma stem cells derived from primary GBM, both *in vitro* and *in vivo* in xenogeneic murine models. Early preclinical results with this CAR are encouraging and promising for rapid translation into clinical trials for patients with GBM. Currently, the Rosenberg group at the NCI is enrolling patients for a clinical trial [204] to treat those with recurrent GBM with the 139 mAb-based EGFRvIII CAR [69].

DC therapy

DCs are the professional APCs of the immune system, and, as such, are responsible for the initiation and regulation of adaptive immune responses to viruses, bacteria and tumors [83,84]. The discovery of DCs, and development of the capacity to generate large numbers of these cells through *ex vivo* culture, has fundamentally changed the approaches to active cancer immunotherapy [85]. Almost all current immunization strategies in cancer immunotherapy are directed toward the efficient loading of DCs with tumor-derived antigens and the proper activation of these antigen-loaded DCs to initiate potent anti-tumor immune responses. This can be accomplished through *in vivo* immunization strategies that direct tumor antigens to host DCs *in situ*, or through the *ex vivo* expansion and loading of DCs with tumor antigens and the return of these antigen-pulsed DCs to patients with cancer. DC vaccines have been utilized as a therapeutic strategy for multiple cancers, including melanoma, renal cell carcinoma, lymphoma, colorectal cancer, prostate cancer, and pediatric and adult brain cancers [86]. A current search of the website clinicaltrials.gov lists 346 studies involving DC vaccines for cancer (search terms: 'dendritic cell' and 'cancer') and 125 active clinical trials. DC vaccines have shown considerable promise in the treatment of advanced cancers with several randomized and placebo-controlled Phase III trials underway, including a Phase III clinical trial of DC vaccines for newly diagnosed GBM [205].

DCs have been shown in numerous studies to be the most potent activators of *de novo* and recall responses in B and T lymphocytes and, therefore, are regarded as one of the most promising biological entities capable of realizing the potential benefits of cancer immunotherapy. Approaches to loading DCs with tumor antigens have included pulsing with peptides or recombinant proteins, fusion with whole tumor cells or pulsing with tumor extracts, transfection with defined antigens using DNA or viral vectors, and pulsing with mRNA encoding tumor-associated antigens. At one time, there was considerable concern that the active immunization against CNS tumors would result in intolerable autoimmune or inflammatory toxicities [87]. However, the safety and potential efficacy of DC vaccines for the treatment of malignant glioma has now been demonstrated in a number of reported and ongoing clinical trials [88–91]. In 2001, Yu *et al.* reported a demonstrable increase in tumor-specific cytotoxicity in four out of seven testable patients who received DCs pulsed with MHC-I peptides eluted from the surface of autologous glioma cells [91]. Two patients that underwent surgical resection of recurrent tumors demonstrated robust CD8⁺ and memory (CD45RO⁺) T-cell infiltrates in tumor areas. This study was limited in assessing efficacy owing to the small sample size, but established the safety and feasibility of DC vaccination in patients with malignant gliomas. A number of subsequent DC-based clinical trials have been reported, most consisting of small Phase I and II trials in patients with HGGs, and most employing the use of DCs pulsed with unfractionated tumor antigens, either as autologous tumor cell lysates or peptide elution derived from tumor cells [92–94]. A few studies have examined the targeting of specific tumor-associated antigens through pulsing of DCs with defined peptides or transfection of autologous DCs with antigen-encoding RNA [95–97].

In a Phase I study at Duke University (NC, USA), 16 patients with malignant gliomas received intradermal (id.) immunizations with autologous DCs pulsed with a peptide spanning the mutated region of the EGFRvIII mutation conjugated to keyhole limpet hemocyanin (KLH) [98]. The patients were adults with malignant gliomas who had undergone gross total tumor resection and radiotherapy. Patients underwent leukapheresis to remove autologous peripheral blood mononuclear cells, which were differentiated into immature DCs using GM-CSF and IL-4. The monocyte-derived DCs were pulsed with the peptide conjugate and matured in a cocktail of TNF- α , IL-1 β and IL-6, and then delivered to the patients in three biweekly id. injections. All 16 patients tolerated the treatment with no adverse events and 13 patients were available for evaluation of immunologic responses to

the vaccine. While no patient exhibited baseline delayed-type hypersensitivity to KLH or EGFR-vIII prior to vaccination, 13 out of 13 (100%) showed postvaccine delayed-type hypersensitivity responses to KLH and five out of 13 (38.5%) demonstrated vaccine-induced immunity to the EGFRvIII peptide. An *in vitro* proliferative response using peripheral blood mononuclear cells pulsed with antigen showed responses in 12 out of 13 (92.3%) patients to KLH and ten out of 13 (76.9%) patients to EGFRvIII.

While most patients enrolled did not have radiographically evident tumors after resection and radiation, two patients (one AA and one GBM) with residual disease experienced a nearly complete response following completion of vaccination. For patients with GBM, the MS time of this trial was 25.6 months, which compares favorably with reported historical medians of 12.1 and 14.6 months in patients with newly diagnosed GBM receiving radiation alone, and radiation with concomitant and adjuvant temozolomide [3]. Although caution must be exercised in interpretation of clinical data from single-arm trials of small numbers of patients, these findings suggest that autologous mature DCs loaded with the tumor-specific antigen EGFRvIII are safe and may induce a beneficial immunologic response in patients with malignant gliomas. Current vaccine approaches targeting the EGFRvIII mutation have utilized the peptide vaccine alone conjugated to KLH as a carrier protein, along with the adjuvant GM-CSF [99]. These studies have demonstrated that the capacity to elicit strong immunologic responses against the EGFRvIII mutation produced promising clinical responses and demonstrated loss of the target antigen in patients suffering from relapsed disease [100]. These results and other ongoing studies suggest that peptide-based vaccines have significant biological and clinical activity alone in human patients, and warrant development and exploration independent of a DC-based platform [101]. These studies also highlight the need to target multiple antigens in GBM for long-term control owing to the heterogeneity in expression that is inherent in these tumors.

Okada *et al.* reported a Phase I/II trial to evaluate the safety and immunogenicity of vaccination with autologous DCs matured with IL-1 β , TNF- α , IFN- α , IFN- γ and polyinosinic-polycytidylic acid (called α -type 1 polarized DCs or α DC1), and loaded with peptides encoding for the HLA-A2⁺-restricted peptides from several tumor antigens (EphA2, IL13R α 2, YKL-40 and gp100) in patients with recurrent gliomas [102]. The vaccines were delivered intranodally along with weekly intramuscular injections of poly-ICLC. Twenty two patients (13 GBM, four AA, three AO and one anaplastic oligoastrocytoma) received between one and four vaccinations in the reported study. Immunologic responses to at least one of the four antigens was observed in 58% of patients with peripheral blood samples also showing an increase in type 1 cytokines and chemokines. One patient with recurrent GBM demonstrated a sustained complete response after vaccination. The investigators reported a correlation between IL-12 production in the α DC1 vaccines and clinical responses. Median time to progression for patients with GBM was 4 months and median OS was 12 months. While matched historical data are not available, these results are comparable with data analyzing the impact of bevacizumab in patients with recurrent GBM (median time to progression: 6.1 months; and MS: 9.3 months) [103]. This study underscores the fact that the maturation of DCs and various adjuvant preparations administered with these cells are one of the many facets of DC biology that can be exploited to potentially enhance the biologic activity and clinical efficacy of DC vaccines. DCs can respond to various inflammatory stimuli and cues within the microenvironment that alter their migratory behavior, antigen processing machinery, costimulatory molecule expression and cytokine secretion [104]. Each of these factors can dramatically alter the biological outcome of DC-lymphocyte interactions *in vivo*, such that potent immunostimulatory responses are induced or attenuating immunosuppressive signals are delivered. It is unknown at this time which method of differentiating, loading and maturing DCs may be the

most effective in eliciting effective anti-tumor immunity in humans, as well as which adjuvant(s) may be most effectively deployed with DC vaccines.

In addition to interacting with effector lymphocytes to elicit immunologic rejection responses, DCs also interact with host regulatory T cells (Tregs) and can induce the activation and expansion of these potentially immunosuppressive T-cell populations [105]. Patients with malignant gliomas exhibit elevated levels of Tregs that contribute significantly to the immunosuppressive phenotype observed in these patients, probably limiting the effectiveness of immunotherapy in these patients [106]. Successful strategies that deplete or inactivate Tregs will probably have a major impact on DC-based therapies and other immunotherapeutic interventions. We have shown in a pilot study of patients with newly diagnosed GBM that a mAb recognizing the high-affinity IL2R α (CD25), which is constitutively expressed on Tregs, could be used to deplete peripheral Tregs without impairing host effector T cells [107]. After completion of surgical resection and concomitant radiation and temozolomide therapy, six patients received a single, intravenous infusion of a humanized anti-CD25 mAb (daclizumab), along with the first of four id. injections of autologous DCs electroporated with RNA encoding for pp65, a human cyto-megalovirus antigen that is frequently expressed in GBM. Anti-CD25 mAb therapy resulted in an approximately 50% depletion of Tregs measured in the peripheral blood without impairment of non-Treg compartments or prevention of expansion of antigen-specific T-cell responses after vaccination. This pilot study is clearly too small to make assessments of clinical efficacy; however, it is of interest to note that three out of the six patients experienced progression-free survival exceeding 24 months and the MS for this cohort exceeds 36 months. Unfortunately, daclizumab was recently withdrawn from the market by the manufacturer, but another anti-CD25 mAb, basiliximab, is clinically available and may have utility in Treg depletion strategies.

Clinical studies to date employing DC vaccines for the treatment of malignant brain tumors have clearly demonstrated the feasibility, safety and biological activity of these cancer vaccines. Clinical results, although preliminary, are also encouraging in that these vaccines may prolong survival in patients with GBM. Relatively few randomized studies comparing DC-based therapy with other treatment modalities have been published, although a recently published Phase II trial in patients with newly diagnosed GBM, comparing standard chemoradiation therapy (n = 16 patients) with chemoradiation plus DC vaccines (n = 18 patients), reported a survival benefit in patients receiving DC therapy over the comparator arm (median OS: 31.9 vs 15 months; p < 0.002) [108]. This study was limited by the fact that treatment for recurrent disease differed significantly between the two arms, confounding the interpretation of OS differences, and there was no observed difference in progression-free survival between the two arms. Nonetheless, this suggests that DC vaccine therapy may be truly beneficial in this patient population.

Confirmation of clinical efficacy in larger trials is certainly warranted and a limited number of randomized Phase II and III DC vaccine studies in patients with GBM is underway [205,206]. Of considerable promise in enhancing the efficacy of DC vaccination platforms is the potential to combine these treatments with a number of immune response modifiers that have shown considerable clinical efficacy in patients with advanced cancer. For example, ipilimumab, a mAb that blocks the immune checkpoint receptor CTLA-4, has shown considerable clinical efficacy in patients with advanced cancers and was recently approved for the treatment of meta-static melanoma [109–112]. While clinical studies examining the efficacy of ipilimumab in patients with GBM have not been reported, preclinical studies have demonstrated safety of CTLA-4 blockade in an orthotopic glioma model and significantly enhanced efficacy when combined with DC vaccination targeting glioma antigens [112]. Furthermore, other mAb blockade strategies, such as disruption of the PD-1–

PD-L1 axis, are showing considerable anti-tumor activity in ongoing clinical trials and are logical candidates for combinatorial treatment strategies with active vaccination platforms [113–115]. Other innovative approaches to improving the efficacy of DC vaccines may include the incorporation of reagents that can overcome potent immunosuppressive pathways known to be operative in patients with GBM. Such examples are indoleamine 2,3-dioxygenase inhibitors [116] and STAT3 inhibitors [117], which have demonstrated capacity to overcome immunosuppressive pathways utilized by Tregs and myeloid-derived suppressor cells. Combinatorial and mechanistic investigations in preclinical glioma models to determine the best way to combine these emerging clinical agents with established DC vaccine platforms are warranted in order to advance rationally designed trials into the clinical arena.

Future areas for development in DC-based vaccines for malignant brain tumors should include applicability to tumor types other than malignant gliomas, optimization of DC biology including migratory and costimulatory properties, and the manipulation of the host and tumor microenvironments to alleviate immunosuppressive mechanisms. With the initiation of Phase III cancer vaccine trials for GBM; the FDA approval of immunomodulatory agents and cancer vaccines for other cancers, such as melanoma and prostate cancer [118]; and the large number of ongoing clinical studies of DC vaccines in patients with brain tumors, it is likely that we will see DC-based therapy for malignant brain tumors take its place among standard adjuvant therapies in the near future.

Conclusion

Immunotherapy for brain tumors used to be a nonspecific therapy that was successful only in a small group of responders. Currently, it is evolving as a highly specific, targeted therapy against brain tumor antigens. The anti-tumor efficacy of the antibodies and *ex vivo* activated lymphocytes targeting brain tumor-specific antigens has been clearly demonstrated in several individual preclinical and clinical studies. However, given the complex and highly heterogeneous nature of this malignant disease, an effective therapeutic regimen for brain tumors will require a multifaceted strategy involving a combination of immunotherapeutic approaches.

Future perspective

We have gained tremendous insight into basic brain tumor biology over the past two decades. Understanding of the molecular and cellular heterogeneity inherent to brain tumors has emphasized the formidable therapeutic challenges ahead. Breakthroughs, such as detailed molecular characterization, classification of malignant gliomas into specific subgroups and identification of specific subpopulations of tumor cells with stem cell-like properties that are resistant to therapy, have paved the way for rationally designed, efficacious individualized targeted therapies that could supplement currently available treatment options for brain tumor patients. As the field of brain tumor therapy continues to advance, in the future we will see personalized forms of treatment involving a combination of different therapeutic approaches targeting diverse molecular characteristics specific for individual brain tumor patients.

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Papers of special note have been highlighted as:

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- ▪ of considerable interest

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Executive summary

Background

- Immunotherapy for brain tumors involves antibodies, antibody fragments, T cells and dendritic cells (DCs).

Monoclonal antibody therapy

- EGFR, EGFRvIII, tenascin, PDGFR α and CSPG are being clinically evaluated for antibody-based therapy, while bevacizumab, targeting VEGF, is approved by the US FDA for recurrent glioblastoma multiforme (GBM) therapy.
- Immunotoxins targeting brain tumor antigens delivered directly to the tumor site by convection-enhanced drug delivery are being tested in both preclinical and clinical settings
- Future success of antibody-based clinical trials depends on selection of patients expressing target antigen, real-time monitoring of drug delivery and optimizing catheter position during convection-enhanced delivery.

Chimeric antigen receptor-based therapy

- Genetically modified T cells targeting IL13R α 2 and EGFRvIII are being evaluated for GBM treatment.

DC therapy

- DC vaccines have been shown to be safe, feasible and potentially efficacious in numerous Phase I/II GBM clinical trials.
- Overcoming immunosuppressive mechanisms, such as those imparted by regulatory T cells, will probably enhance the efficacy of DC vaccines significantly.
- Further understanding of the biology of DC maturation will allow for the optimization of protocols to generate large numbers of immunostimulatory DCs for vaccine preparation.

Table 1

Monoclonal antibodies and antibody fragments under investigation for brain tumor therapy.

Ab	Antigen targeted	Ab format	Development stage	Ref.
Bevacizumab (Avastin®)	VEGF	Naked	Approved by the US FDA for recurrent GBM therapy	[4,18–21]
Cetuximab (IMC-C225, Erbitux®)	EGFR	Naked Conjugated to BD	Phase II clinical trial Preclinical	[27,28] [44]
Nimotuzumab (h-R3, TheraCIM®)	EGFR	Naked Conjugated to radionuclide	Phase I/II clinical trial Phase I clinical trial	[30,31] [43]
Y10	EGFRvIII	Naked Conjugated to PE	Preclinical	[33,50]
L8A4	EGFRvIII	Conjugated to BD Conjugated to PE	Preclinical	[44,50]
MR1-1	EGFRvIII	Conjugated to PE	Phase I clinical trial	[202]
528, 806	EGFR/EGFRvIII	Naked	Preclinical	[34]
IMC-3G3	PDGFR α	Naked	Phase II clinical trial	[201]
BC-2, BC-4, 81C6 (Neuradiab®)	Tenascin	Conjugated to radionuclide	Phase II clinical trial	[38–40,42]
Mel-14 F(ab') ₂	CSPG	Conjugated to radionuclide	Phase I clinical trial	[46]
9.2.27	CSPG	Conjugated to PE	Preclinical	[55]
454A12	TR	Conjugated to ricin toxin	Preclinical	[49]
F6V	GPNMB	Conjugated to PE	Preclinical	[54]

Ab: Antibody; BD: Boronated polyamidoamine dendrimer; GBM: Glioblastoma multiforme; PE: *Pseudomonas aeruginosa* exotoxin A; TR: Transferrin receptor.