Special Focus Issue: Cancer vaccine clinical trials

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Novel vaccines for glioblastoma: clinical update and perspective

Glioblastoma is the most common primary brain cancer. Aggressive treatment with surgery, radiation therapy and chemotherapy provides limited overall survival benefit. Glioblastomas have a formidable tumor microenvironment that is hostile to immunological effector cells and these cancers produce profound systemic immunosuppression. However, surgical resection of these tumors creates conditions that favor the use of immunotherapeutic strategies. Therefore, extensive surgical resection, when feasible, will remain part of the equation to provide an environment in which active specific immunotherapy has the greatest chance of working. Toward that end, a number of vaccination protocols are under investigation. Vaccines studied to date have produced cellular and humoral antitumor responses, but unequivocal clinical efficacy has yet to be demonstrated. In addition, focus is shifting toward the prospect of therapies involving vaccines in combination with immune checkpoint inhibitors and other immunomodulatory agents so that effector cells remain active against their targets systemically and within the tumor microenvironment.

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Background

Glioblastoma afflicts about 17,000 new patients per year in the USA [1]. It remains a devastating cancer with relatively modest advances in treatment recorded in recent decades. Despite intensive treatment with surgery, radiation therapy, chemotherapy and biological therapies, most patients die of their disease. Currently, median life expectancy following diagnosis is 14.6 months with aggressive treatment, and 2- and 5-year survival rates are 27.2 and 9.8%, respectively [2–9]. Since the widespread use of temozolomide chemotherapy, more than a decade has passed with little significant improvement in therapy. Recently, the addition of tumor-treating electromagnetic field (TTF) therapy was shown to provide additional survival benefit in one large trial of patients with

newly diagnosed glioblastoma [10]. Use of the tumor-treating electromagnetic field device (Optune, Novocure, Portsmouth, NH, USA) was initially approved by the US FDA for use in patients with recurrent glioblastoma based upon equivalency to physician's choice second-line chemotherapy in such patients [11].

Glioblastoma frequently recurs following initial treatment due largely to the failure of current therapeutic modalities to eradicate residual microscopic disease lying beyond the edge of surgical resection margins. In some patients, surgical resection may be severely limited due to multifocal involvement, the presence of eloquent cerebral tissue or cancer cells that are deeply infiltrative, even at the time of initial diagnosis. Despite many clinical trials, the benefits of conventional radiation therapy and chemotherapy are limited.

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Similarly, targeted molecular therapies designed to attack glioblastoma cells via specific pathways have not produced significant clinical gains as yet. This unfortunate situation, combined with an evolving understanding about the interaction of the immune system and the brain, has stimulated great interest in exploring the usefulness of immunotherapy for patients with malignant gliomas. One aspect of this has been the ongoing investigation of various vaccine strategies designed to stimulate active-specific antitumor immunity.

Immune privilege & glymphatics

Our understanding of the interactions between the CNS and the immune system has grown significantly in recent years. A distinct blood–brain barrier (BBB) separates the circulatory system from the brain and spinal cord. There is now abundant evidence that cellular antitumor immune effectors can traffic into the brain by way of specific molecular interactions with the BBB [12,13]. This interface consists of several actual barriers that regulate interactions between the immune system and the CNS and restrict, or regulate, the transit of drugs, biomolecules, immune cells and cytokines between the two [12,13]. Thus, the immune privilege of the CNS is only relative, and is largely attributable to the BBB's ability to prevent the unregulated exchange of immune cells and antibodies between the brain and the peripheral circulation [12,13]. There is now clear evidence of regulated interaction that permits activated T cells to traffic into the CNS.

Several factors have contributed to the long-held view regarding the immunologically privileged state of the brain. For a long time, it was believed that the CNS had no direct lymphatic drainage. However, recent microanatomical studies have shown the presence of lymphatic channels in the dura mater running in parallel with arterial and venous structures and draining to cervical lymph nodes [12,13]. Moreover, it was also noted that engraftment of tumors was more successful in the brain than in other organs. In addition, the apparent absence of unactivated T lymphocytes in the brain was thought to imply a deficiency in the afferent limb of the immune response allowing malignant glioma cells to evade immune surveillance. It has also meant that expectations for the success of immunologically based antitumor strategies against malignant brain tumors have been low.

There is now a substantial body of data indicating the presence of both afferent and efferent immune pathways in the CNS. Evidence suggests that antigen-presenting cells are present in the brain in the form of microglial cells that express macrophage markers [14,15]. This is supported by the observation that glioma cells genetically modified to express IFN- γ and introduced

into the brain cause direct priming of microglia with subsequent tumor rejection accompanied by CD4⁺ and CD8⁺ tumor cell infiltration [16,17]. While the exact site and mechanism of antigen presentation by APC in the CNS are not yet known, it does appear that the brain is capable of producing an antitumor immune response *via* resident and regional APC. The presence of tumor-infiltrating lymphocytes in malignant gliomas also suggests that specific immune effector cells are capable of invading and killing glioma cells, despite the presence of a BBB [14,18].

Immunosuppression, the tumor microenvironment & tumor heterogeneity

Glioblastoma patients often fail to exhibit delayed skin hypersensitivity reactions and are frequently anergic when their cancer is initially diagnosed [19,20]. It has long been recognized that T cells from glioblastoma patients have impaired responses to antigens and T-cell mitogens with reduced proliferation and IL-2 production [21]. Following surgical removal of a glioma, systemic T-cell responses are partially restored; however, T-cell function declines again with tumor recurrence [20].

Glioblastomas produce a variety of substances that suppress antitumor immunity. Much glioma-derived immunosuppression is associated with TGF- β 2 produced by the tumor and by glioma-derived T-cell suppressive factor (G-TsF). Downregulation of TGF- β expression by antisense methodologies in rat 9L glioma cells enhances tumor cell immunogenicity, prolongs survival and can lead to tumor eradication in that model [22]. While it has not been fully characterized, G-TsF is probably identical to TGF- β 2 [23]. TGF- β 2/G-TsF inhibits proliferation and IL-2 production by T cells from healthy individuals [24]. Consequently, antisense-mediated inhibition of TGF- β 2 expression improves the survival of 9L tumor-bearing rats vaccinated with irradiated 9L glioma cells [25].

Glioblastomas also display many other defects in local antitumor immunity. These include decreased expression of IL-12, IFN- γ and TNF- α , as well as increased expression of IL-4, IL-5, IL-6 and IL-10 [26]. In turn, IL-10 expression may lead to downregulation of MHC class II expression [27,28]. Expression of Fas and Fas ligand has also been detected in glioma cells where they may contribute to local immunosuppression [29,30]. Similarly, the co-stimulatory molecule CD80 (B7.1), which is a ligand of CTLA-4 is frequently downregulated by glioma cells [19]. Hypoxia may also induce immunosuppression through STAT-3 signaling mediated by VEGF and HIF-1 α [31].

CD8⁺ cells from glioblastoma patients have reduced expression of CD28 co-stimulatory molecule, defective

IL-2 α receptor subunit expression and reduced phosphorylation of CD3 ζ T-cell receptor chains [32–34]. Other members of the IL-2 family are also downregulated [16]. Collectively, these and other alterations probably exert important effects on both local and systemic cellular immune function and may be responsible for apoptosis and anergy of immunologic effector cells in glioblastomas [14,35].

More recently, the expression of indoleamine 2,3-dioxygenase 1 (IDO-1), a tryptophan-catabolizing enzyme has been hypothesized to adversely affect the glioma microenvironment. IDO is frequently expressed in glioblastomas where it appears to modulate tumor-infiltrating Treg cells. Specifically, tryptophan metabolites inhibit CD8⁺ function and enhance CD4⁺/CD25⁺/Foxp3⁺ Treg function. IDO is expressed in glioblastomas, which accumulate significant numbers of Treg cells. Natural Tregs and inducible Tregs complement each other's action by maintaining tolerance to self-antigens, suppressing autoimmunity and enabling effective immune responses to nonself antigens. IDO expression promotes the accumulation of Tregs in glioblastomas; whereas, IDO deficiency decreases Treg accumulation and enhances T-cell-mediated antitumor effects [36,37].

Thus, the tumor microenvironment in a glioblastoma has the potential to be extremely hostile to immune effector cells. The tumor stroma contains a complex milieu of glial, endothelial and white blood cells that create a highly immunosuppressive setting. Such molecules as TGF- β 2, metabolites of tryptophan metabolism and other molecules can have potent effects on T-cell function that may render fully armed-specific CD8⁺ killer T cells wholly ineffective. Shifting the balance within the microenvironment from one that is tolerant of tumor cell growth to one that supports immunologically mediated tumor cell lysis is likely to be important for the development of clinically effective immunotherapy for glioblastoma.

Further complicating the issue of the tumor microenvironment is the heterogeneity (multiform nature) of glioblastoma itself. Recently identified genetic alterations have led to a pending revision of WHO tumor-grading criteria into a more biologically based classification system for glioblastoma. This is also reflected by the fact that there are at least four different types of glioblastoma, based upon findings derived from The Cancer Genome Atlas (TCGA). As a result, proneural, neural, classical and mesenchymal glioblastoma variants have now been characterized, along with different survival curves, responses to intense treatment and corresponding molecular pathways and common mutations [38–41]. Each of these could also be accompanied by different changes in the immunological environment

that could render immune effector cells either more or less active by differing mechanisms. In addition, it has yet to be determined whether the microenvironment in areas of the brain that have undergone microscopic infiltration of tumor cells is substantially different from that of the bulk tumor itself. Such differences could provide yet one more source of heterogeneity that could complicate evaluation of vaccine-mediated immunotherapeutic strategies for this cancer.

The survival of patients with glioblastoma is clearly and directly linked to the extent of initial surgical resection [42]. Given that fact, maximum safe surgical resection is likely to remain an important component in the comprehensive management of glioblastoma for the foreseeable future. As noted, it was also recognized years ago that surgical resection of gliomas leads to significant improvement in systemic immunologic function. One implication of this observation is that vaccine therapy may be most effective when used in the early postoperative period following gross total, or at least near-total, surgical tumor resection. For these reasons, many clinical vaccine trials exclude patients with bulk disease, precisely because of the known immunosuppressive features of glioblastoma. Presumably, resection of bulk tumor may affect the local concentration of immunosuppressive factors that render effector cells anergic at their intended site of action.

Specific vaccine approaches

Numerous clinical trials of peptide vaccines have been carried out since the first such study of MART-1 two decades ago [43]. Early generations of peptide vaccines were composed of one or more HLA class I-restricted peptides of a single HLA type. A number of vaccine strategies are currently under development (Table 1). More recent approaches include: class I-restricted peptide cocktails; multi-peptide vaccines, including helper peptides; long peptide vaccines; peptide mimics; personalized peptide vaccines; and peptide-, RNA- or tumor lysate-loaded dendritic cell vaccines. Currently, there are a relatively small but growing number of studies focused on vaccine therapy for glioblastoma and also a number of combination strategies of vaccines plus immunomodulatory agents.

EGFRvIII

Amplification and overexpression of the EGFR occur frequently in glioblastoma [44,45]. In addition to overexpression of the wild-type EGFR, some tumors express mutant EGFR species arising from specific rearrangements between introns [46–50]. These EGFR mutants are often constitutively autophosphorylated and oncogenic. A number of them contain either deletion or duplication of specific sets of exons correspond-

Table 1. Representative peptide vaccine trials.

Study	Vaccine	Phase	Participants	Protocol groups
NCT02149225 [123]	APVAC1, APVAC2	I	NDG	APVAC1 (5–10 stock peptides) + poly-ICLC + sargramostim APVAC2 (individualized peptides) + poly-ICLC + sargramostim
NCT02510950 [83]	Personalized neoantigen long peptide vaccine	0	NDG	Hiltonol + poly-ICLC
NCT01222221 [124]	Multipeptide vaccine (IMA950)	I	NDG HLA-A*02	IMA950 + Stupp
NCT02455557 [63]	Survivin vaccine (SurVaxM)	II	NDG HLA-A*02, A*-03, A*-11, A*-24	SurVaxM peptide vaccine + Montanide + sargramostim + Stupp
NCT01920191 [78]	Multipeptide vaccine (MA950)	I/II	NDG HLA-A*02	IMA950 + poly-ICLC + Stupp protocol
NCT00905060 [58]	HSPPC96	II	NDG	Heat-shock protein–peptide complex vaccine + Stupp
NCT01814813 [60]	HSPPC96 +/- bevacizumab	II	Resectable RG	1. Vaccine + bevacizumab 2. Bevacizumab alone
NCT00643097 [125]	PEP-3 vaccine	II	EGFRvIII	1. PEP-3 + sargramostim postradiation 2. PEP-3 + sargramostim, postradiation, subsequent high-dose TMZ 3. PEP-3 + sargramostim, postradiation, low-dose TMZ
NCT02122822 [126]	GP96	I	NDG	GP96 peptide complex from autologous tumor cells
NCT02287428 [82]	NeoVax	I	NDG Unmethylated MGMT (O-6-Methylguanine-DNA-methyltransferase) promoter	Radiotherapy + recovery period, no TMZ NeoAntigen vaccine
NCT00069940 [127]	Telomerase peptide vaccine	I	NDG, DIPG or gliosarcoma	HSPPC96 with sargramostim

Current clinical trials for glioma are listed by National Clinical Trial (NCT) assignment. Vaccine name, phase, target and general composition are listed.
Data taken from: <http://clinicaltrials.gov>
DIPG: Diffuse interstitial pontine glioma; GB: Glioblastoma; NDG: Newly diagnosed glioblastoma; RG: Recurrent glioblastoma; TMZ: Temozolomide.

ing in some cases to discrete functional domains of the molecule. One deletion mutation (EGFRvIII) is present in up to 30% of glioblastomas [51,52]. This particular mutation is almost exclusively seen in the ‘classical’ subtype of glioblastoma defined by TCGA project [40].

The EGFRvIII protein has been of substantial interest to the neuro-oncology field because it contains a unique epitope encoded by the mutant gene that should permit highly specific targeting of glioblastoma cells [53]. The EGFRvIII vaccine known as Rintega (CDX-110, rindopepimut) contains a synthetic peptide that matches a unique portion of the EGFRvIII molecule encoded by the mutant gene in which intron recombination has led to an internal deletion of sequence [50].

Since its introduction, a number of clinical trials

of Rintega have been conducted, including a recently completed, prospective, randomized Phase III trial in patients with newly diagnosed EGFRvIII-expressing glioblastoma. In four separate prior glioblastoma trials, ACTIVATE [54], ACT II [55], ACT III [51] and ReACT [56,57], Rintega given in combination with temozolomide showed no serious toxicity. The randomized Phase II ReACT trial in recurrent glioblastoma tested Rintega in combination with bevacizumab. Preliminary analysis suggested a survival benefit to patients receiving combination therapy. In this study, a small number of patients with bulk disease appeared to benefit from vaccine therapy, when combined with Avastin [56,57] had an immune response. In the single-arm Phase II studies, comparison to an historical or ‘matched’ group of glioblastoma patients treated with

standard therapy was used to gauge potential clinical efficacy. Such historical controls would have included glioblastomas from each of the different TCGA-defined glioblastoma types; whereas, treated patients with the EGFRvIII marker would have come almost exclusively from the 'classical' TCGA subtype.

Although, Rintega showed great promise in Phase II studies, results of the recent Phase III trial (ACT IV) in patients with newly diagnosed glioblastoma demonstrated the importance of evaluating cancer vaccines in randomized studies and point to the potential for bias in single-arm studies. The ACT IV trial evaluated 745 patients with newly diagnosed glioblastoma who were randomized to receive standard therapy (surgery, radiation and temozolomide, plus keyhole limpet hemocyanin [KLH] carrier protein), or standard therapy plus Rintega (KLH-conjugated EGFRvIII peptide). Final data analysis of this study demonstrated that Rintega-treated patients fared slightly worse, with median overall survival of 20.4 months compared with 21.1 months for the control arm [58].

There are several possible explanations for the failure of Rintega to show efficacy in ACT IV and important lessons to be learned. Patients in ACT IV were treated with surgery, radiation therapy and temozolomide, plus either Rintega or KLH alone. It has been demonstrated that the classical TCGA group of glioblastoma patients has substantially greater benefit from treatment with chemotherapy (i.e., temozolomide) than is the case in the proneural, neural or mesenchymal groups [40]. Consequently, since EGFRvIII expression was essential for inclusion in each of the prior Phase II trials, there would have been a disproportionate number of classical glioblastomas with inherently better response to intensive chemotherapy with temozolomide. Thus, the expression of particular target antigens may be associated with inherent differences in either survival, or response to other prior or concomitant therapies, making the specific effects of immunotherapy difficult to tease out.

In one trial, it was also reported that patients treated with Rintega had markedly reduced expression of the EGFRvIII target antigen at the time of tumor recurrence. This was hypothesized to reflect selection pressure by the vaccine effector cells with overgrowth of nonexpressing tumor cells and possible immune escape at recurrence [51]. Subsequently, it has been reported that recurrent glioblastomas have reduced EGFRvIII expression, even in the absence of specific anti-EGFRvIII therapy, much as glioblastoma cells lose EGFRvIII expression when established in cell culture [59]. Although Rintega is no longer being developed clinically, one study (ExCeL) of EGFRvIII chimeric anti-

gen receptor T cells for newly diagnosed glioblastoma is currently ongoing (NCT02664363) [60].

Heat-shock protein vaccines

Antigens are taken up by dendritic cells, processed by the proteasome, and loaded onto MHC class I molecules for presentation to T cells. Dendritic cells (DCs) also receive danger signals, by way of Toll-like receptors, and stimulate cytokine secretion and co-stimulatory molecule expression promoting cellular immune responses. Heat-shock proteins (HSPs) carry an antigenic representation of the cells from which they are derived, act as adjuvants and promote antigen uptake by DCs. HSPs act as chaperones for intracellular proteins to prevent aggregation and support transport. In particular, HSP90 plays a significant role as chaperone to antigenic peptides in presentation [61]. Antigens that are chaperoned by HSP undergo MHC class I-restricted presentation to specific T cells, eliciting CD8⁺ cytotoxic T lymphocyte (CTL) responses [62].

HSPPC-96 is a dendritic cell vaccine derived from autologous tumor-derived HSP-peptide complex. Crane *et al.* evaluated vaccination with HSPPC-96 complexed with several glioblastoma-associated antigens in patients with recurrent glioblastoma. A majority of patients had infiltration of their tumors by CD4, CD8 and CD56 IFN- γ -positive cells, suggesting a specific immunologic response to vaccination. Eleven patients had positive immune responses based on testing of cells from peripheral blood. Of the responders, median survival was 47 weeks compared with 16 weeks in a single nonresponder [63].

NCT00905060 [64] was a single-arm, Phase II trial evaluating safety, immune response and survival in patients treated with HSPPC-96 following failure of standard therapy for newly diagnosed glioblastoma. In this study, 41 patients underwent gross total resection of recurrent glioblastoma and received a median of six doses of HSPPC-96 vaccine. Following treatment, 90.2% of patients were alive at 6 months and 29.3% were alive at 12 months [65]. Median overall survival was 42.6 weeks. Patients who had lymphopenia prior to beginning vaccine therapy had shorter overall survival, raising the question of how prior temozolomide treatment may affect the response to vaccines in the setting of recurrent disease. NCT01814813 [66] is a randomized Phase II trial designed to study HSPPC-96 vaccine therapy, with or without the angiogenesis inhibitor bevacizumab, in patients with recurrent glioblastoma with $\geq 90\%$ surgical resection.

Survivin

Survivin belongs to the inhibitor of apoptosis protein (IAP) family and has been identified as a specific cancer

marker. It is a targetable antigen in glioblastoma and it is currently being studied in several clinical trials. The immune system of cancer patients is often primed to recognize survivin as suggested by the presence of low-level antibody production and existing T-cell responses in some cancer patients [67,68]. These observations signify that the immune systems of some cancer patients have been exposed to this antigen and that it is at least weakly immunogenic [67]. This raises the possibility that vaccines targeting survivin and other tumor antigens may restimulate or redirect a memory response, rather than initiating a *de novo* response.

SurVaxM is a synthetic survivin peptide conjugate with greatly enhanced binding to HLA-A*02 molecules. SurVaxM incorporates a long peptide carrying multiple CTL epitopes within and an antigen-specific CD4⁺ T-cell stimulus for cytokine support without a helper peptide. The SurVaxM vaccine was recently tested in a Phase I clinical trial of recurrent malignant glioma patients and is currently being studied (NCT02455557) [69] in combination with standard therapy in patients with newly diagnosed glioblastoma. Of potential significance, downregulation of the target antigen as a possible avenue of immunologic escape during vaccine therapy is predicted to lead to tumor-cell apoptosis [70,71].

Human cytomegalovirus proteins

Several laboratories have reported the presence of human cytomegalovirus (HCMV) proteins and RNA species in human glioblastomas [72,73]. DNA and RNA sequences and peptides derived from cytomegalovirus (CMV), including IE1 and pp65, have been detected in malignant gliomas by several laboratories using PCR. Some laboratories have reported difficulty in confirming these results, leaving the possible role of HCMV in gliomagenesis unsettled at the present time [74,75]. Nevertheless, although HCMV is not thought to be an oncogenic virus in the classical sense, latent CMV infection may provide specific viral antigenic targets for immunotherapy against glioblastoma.

The Valcyte Treatment of Glioblastoma Patients (VIGAS) trial initially reported on an exploratory group of 22 patients with glioblastoma and low-grade CMV infection that received >6 months of treatment with valganciclovir, resulting in 50% 2-year overall survival compared with 20.6% in the standard care group (median overall survival of 24.1 vs 13.7 months) [76].

A subsequent randomized study involving 42 glioblastoma patients who had ≥90% resection of their tumors was conducted [76]. Patients were randomized to receive standard therapy, or standard therapy plus oral valganciclovir. After 6 months of treatment, there was no significant difference in tumor volume between the

two groups. Patients not receiving valganciclovir were allowed to crossover and those who had been receiving the drug continued on it. Median overall survival was similar in both groups. Thus, the potential role of antiviral drug therapy for HCMV-associated gliomas will require further study before widespread adoption.

At least one group has begun development of tumor vaccine strategies for targeting HCMV proteins in an effort to treat glioblastoma [77]. This is based, in part on a demonstration that patient-derived CMV pp65-specific T cells are capable of killing autologous glioblastoma cells [77]. A randomized Phase II clinical trial is being conducted to test how well CMV pp65 RNA-pulsed autologous dendritic cell vaccine and tetanus/diphtheria toxoid helper vaccine work in treating patients with newly diagnosed glioblastoma (NCT02366728) [78]. Tetanus toxoid upregulates the CCL3 recall response and is thought to enhance immunogenicity [79]. In this glioblastoma vaccine study, DCs loaded with CMV-pp65 mRNA are used with tetanus antigen preconditioning with the intent to improve lymph-node homing and efficacy of DC.

Basiliximab is a chimeric-humanized monoclonal antibody to the α -chain (CD25) of the IL-2 receptor on activated T cells that is used to prevent transplant rejection. In NCT02366728 [78], basiliximab is being studied to see if it augments vaccine action by selectively depleting CD25⁺ Treg cells [80]. Agents such as basiliximab might help to enhance the antitumor action of vaccines provided that they do not adversely affect CD8⁺ and CD4⁺ antitumor effector cells, which may also have CD25 on their surface during IL-2-dependent expansion. In this regard, differential effects of this antibody may be schedule dependent.

Wilm's tumor protein-1

Wilm's tumor protein-1 (WT-1) has been shown to be a potential regulator of glioma growth [81]. In one Phase II study involving 21 patients with recurrent glioblastoma and co-expression of WT-1 and HLA-A*24, investigators studied a modified 9-mer HLA-A*2402-restricted, WT-1 peptide vaccine given by intradermal injection [82]. Median progression-free survival (PFS) was 20.0 weeks, and the 6-month PFS rate was 33.3%. Currently, NCT02498665 [83] is a Phase I dose-escalation study of DSP-7888 in patients with advanced cancers, including glioblastoma. DSP-7888 is a vaccine that contains peptides that induce WT1-specific CTLs and helper support.

Multipeptide vaccines

IMA950 is a vaccine that contains multiple tumor-associated peptides (TUMAPs) that are naturally presented in human glioblastoma tissue. Peptides in IMA950

are derived from: brevican (BCAN); chondroitin sulfate proteoglycan 4 (CSPG4); fatty acid-binding protein 7 (FABP7); IGF-2 mRNA-binding protein 3 (IGF2BP3); neuroligin 4, X-linked (NLGN4X); neuronal cell adhesion molecule (NRCAM); protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1); tenascin C (TNC); Met proto-oncogene (MET); and baculoviral inhibitor of apoptosis protein repeat-containing 5 (BIRC5; survivin). A Phase I trial of IMA950 with GM-CSF in 45 patients was completed in 2014 and PFS at 6 months was 74%. Of 40 evaluable patients, 36 (90%) were TUMAP responders, and 20 (50%) were multi-TUMAP (more than or equal to two T-cell responses) [84]. A Phase I/II trial (NCT01920191) [85] of IMA950 plus the adjuvant poly-ICLC (Hiltonol) in combination with temozolomide in newly diagnosed HLA-A*02-positive glioblastoma patients is being conducted.

NCT02078648 [86] is a Phase I/II study of SL-701 (Stemline Therapeutics, Inc., NY, USA), a multivalent class I-restricted, peptide vaccine and bevacizumab in patients with recurrent glioblastoma. SL-701 employs a cocktail of class I-restricted peptides combined with a linear carbohydrate-peptide construct based on the 13 amino acid pan-DR epitope (PADRE), which is a nonspecific T-helper peptide.

Personalized peptide vaccines

Defining truly tumor-specific targets for glioblastoma and other cancers, to which the immune system is not tolerized, has been a challenge. EGFRvIII provides an example of one such highly specific cancer target. Brown *et al.* identified a large number of neoepitopes in human cancers and correlative data suggesting that a higher mutational burden is associated with greater infiltration of tumors by tumor-infiltrating lymphocyte, and better clinical outcomes [87]. In addition, it appears that the presence of neoepitopes with higher binding to specific HLA molecules may be associated with better clinical outcome [88]. Similarly, the affinity of a peptide for its cognate HLA molecule is predictive of that peptide's ability to elicit a CD8⁺ T-cell response. Thus, some neuro-oncology groups have focused on defining neoepitope-based vaccines for glioblastoma.

NCT02287428 [89] is a study designed to test a personalized neoantigen cancer vaccine (NeoVax) in patients with newly diagnosed glioblastoma with unmethylated MGMT promoters. The vaccine consists of a panel of individual tumor-specific peptides combined with the immunoadjuvant poly-ICLC following completion of radiation therapy. Of note, participants in this study will not receive temozolomide since the unmethylated MGMT promoter is predictive

of limited benefit from that drug. Thus, chemotherapy-associated lymphopenia should not be apparent in this study. Similarly, NCT02510950 [90] is a Phase I study designed to test another neoepitope-based personalized vaccine in patients with newly diagnosed glioblastoma. In this study, synthetic long peptides are combined with poly-ICLC.

Dendritic cell vaccines

Peptide-based, cell-free vaccines generally employ either one or a small number of antigens. The use of dendritic cells offers the potential to deliver a large number of tumor antigens and a number of DC-based vaccines are currently under investigation (Table 2). One can create cellular vaccines using either synthetic or tumor-derived peptides, RNA or crude tumor lysate. In some respects, the epitope specificity of a particular region of a tumor antigen may be lost with lysate-based strategies leading to immunodominance of particular antigens, which may not be ideal targets [91].

ICT-107 is an autologous dendritic-cell vaccine pulsed with immunogenic peptides from tumor antigens, including: HER2, TRP-2, gp100, MAGE 1, IL13R α 2 and AIM-2. In a single Phase I study, ICT-107 was tested in 21 glioma patients, including 17 with newly diagnosed glioblastoma. Median PFS in the newly diagnosed glioblastoma patients was 16.9 months, and median overall survival was 38.4 months [92]. Following these initially promising results, NCT01280552 [93] was conducted as a double-blind, randomized Phase II trial of ICT-107, which was compared with autologous unpulsed dendritic cells in patients with newly diagnosed glioblastoma following surgical resection and chemoradiation in 124 patients. The study, which included HLA-A01 and HLA-A02 patients, showed that those who received ICT-107 survived 2 months longer than those in the control arm, a difference that was not statistically significant [94]. NCT02546102 [95] is a Phase III trial of ICT-107 in patients with newly diagnosed glioblastoma designed as a randomized, double-blind, placebo-controlled study restricted to HLA-A2+ subjects to focus upon responder characteristics.

NCT01967758 [96] is a Phase I study designed to test safety and immunogenicity of ADU-623, a live-attenuated *Listeria monocytogenes* strain expressing an EGFRvIII-NY-ESO-1, antigen-containing vaccine in patients with recurrent malignant gliomas. Patients in successive cohorts will receive ADU-623 in escalating doses by intravenous infusion, followed by a 3-day course of antibiotics.

HGG-2006 is a lysate-loaded, DC vaccine that has been tested in patients with newly diagnosed glioblastoma following radiation with chemotherapy. In

Table 2. Representative cellular vaccine trials.

Study	Vaccine	Phase	Participants	Protocol groups
NCT02366728 [72]	DC pulsed with Human CMV pp65 mRNA	II	NDG	1. Unpulsed DC with ¹¹¹ In-label for migration study + TMZ 2. DC tetanus/diphtheria-toxoid (Td) preconditioning before 4th vaccination + TMZ 3. Basiliximab before vaccines 1 and 2 + Td + TMZ
NCT00639639 [128]	Human CMV pp65-LAMP (Loop mediated isothermal amplification) mRNA-pulsed DCs	I	NDG	with Stupp 1) CMV ALT-IV + CMV-DC 2) CMV-DC 3) Unloaded DC skin prep + ¹¹¹ In-labeled CMV DC 4) Tetanus toxoid skin prep prior to CMV DC
NCT02010606 [129]	DC pulsed with glioblastoma multiforme stem cell-like line (allogeneic)	I	NDG + RG	NDG – DC with Stupp RG – DC + bevacizumab (bev) if bev. naive
NCT01957956 [130]	DC – tumor lysate pulsed	I		
NCT02546102 [87]	ICT-107 peptide loaded	III	NDG	with Stupp
NCT02049489 [93]	ICT 121 Peptide – CD133 antigen	I	RG	
NCT01808820 [131]	DC/tumor lysate	I	Recurrent high-grade glioma (HGG)	DC vaccine + Imiquimod No treatment 1 month prior to initiation
NCT01902771 [132]	DC/tumor lysate	I	Any CNS tumor relapse	No treatment 1 month prior to initiation
NCT02649582 [133]	Addit-GLIO WT-1 mRNA load	I/II	NDG	with Stupp protocol
NCT01213407 [134]	GBMVax/Trivax (ATL)	II	NDG	with Stupp
NCT01567202 [135]	DC + glioma stem-like cell-associated antigens	II	NDG	+ Stupp Active vs placebo
NCT02465268 [136]	ATTAC-II CMV RNA-pulsed DC with TD toxoid vaccine	II	NDG	+ Stupp 1) Pp65 DC vaccine 2) Unloaded DC vaccine 3) No vaccine (placebo)
NCT01204684 [137]	DC ATL +/- poly-ICLC	II	NDG or recurrent HGG	1) ATL-DC + placebo prep 2) ATL-DC + resiquimod prep 3) ATL-DC + poly-ICLC adjuvant
NCT01280552 [85]	ICT 107 Peptide loaded DC	II	NDG	+ Stupp
NCT00045968 [91]	DCVax-L	III	NDG	DC vs placebo
NCT00890032 [138]	Brain tumor stem cell (BTSC) mRNA-loaded DC	I	RG	

Current clinical trials for glioma are listed by NCT assignments. Vaccine name, phase, target and general composition are listed.

Data taken from: <http://clinicaltrials.gov>

CMV: Cytomegalovirus; GB: Glioblastoma; NDG: Newly diagnosed glioblastoma; RG: Recurrent glioblastoma; TMZ: Temozolomide; WT-1: Wilm's tumor protein-1.

the initial pilot study, six of seven patients showed an increase in CD8⁺CD25⁺ cells. Five patients had tumor antigen-reactive T cells following their fourth vaccination, which persisted through three cycles of temozolomide [97]. Subsequently, HGG-2006 was tested in a Phase I/II study of 77 patients with newly

diagnosed glioblastoma. In that trial (EudraCT 2006–002881–20), PFS at 6 months was 70.1% and median overall survival was 18.3 months [98].

DCVax[®]-L is a dendritic-cell vaccine that is loaded with antigens from the patient's glioblastoma. One Phase I/II clinical trial of DCVax-L examined 20

newly diagnosed and 19 recurrent glioma (including glioblastoma) patients who received the vaccine plus standard care treatment. In this study, the median time to tumor recurrence was approximately 2 years. NCT00045968 [99] is a subsequent ongoing randomized Phase III study designed to determine the efficacy of DCVax-L in patients with newly diagnosed glioblastoma following gross total, or near-total, surgical resection. Patients receive standard of care, including radiation and temozolomide therapy and vaccine as an intradermal injection. Patients randomized to the placebo arm may receive vaccine in a cross-over arm at the time of tumor progression. DCVax-L has been reviewed extensively elsewhere [100].

Vaccines against cancer stem cells

Cancer stem cells (CSCs) in glioblastoma are phenotypically similar to normal neural stem cells. They express CD133 and other genes characteristic of neural stem cells. Glioblastoma CSCs possess the capacity for self-renewal and form polyclonal tumors in nude mice. CD133-positive, CSCs are resistant to chemotherapy. One trial of ICT-107 showed a decrease of CD133⁺ tumor cells in immunized patients who progressed while on study [92]. A subsequent agent, ICT-121, is designed to provide immunotherapy directed at CD133-expressing cells in glioblastoma using an autologous dendritic-cell vaccine. In a Phase I study (NCT02049489) [101], HLA-A*02 patients with recurrent glioblastoma are being treated with DC pulsed with purified CD133 peptides.

Complementary strategies

Helper support

The generation of an effective cellular immune response requires the activation of both CD8⁺ lymphocytes and specific CD4⁺ helper cells. To activate a CD4⁺ T-cell response, target antigens must be presented by MHC class II molecules [102]. Once CD4⁺ cells have been activated, they proliferate and produce cytokines (e.g., IFN- γ , IL-2 and IL-4) in support of the associated CD8⁺ immune response [103,104]. These cytokines are essential to provide a fully activated CD8⁺ antitumor CTL response [105]. The presence of MHC class II-restricted CD4⁺ T cells that are specific for tumor-associated antigens has been recognized to be an important element for providing essential helper factors in eliciting and sustaining cytotoxic CD8⁺ responses against tumors [106,107]. It is becoming clearer that attempts to maximize the immune response to tumors need to include efforts to elicit CD4⁺ T-helper cell activity. A number of the approaches highlighted above take this concept into account.

Immunologic tolerance & mimics

T-cell clones with the capacity to be activated by self proteins are frequently preserved following negative selection of higher affinity, self-recognizing clones in the thymus [108]. These potentially self-reactive cells remain tolerized under normal conditions [109]. Altered peptide ligands (mimics) can provide a way to break tolerance to the natural self-epitope [110,111]. Studies have shown that altered peptide ligands generated by substituting certain amino acids within a peptide epitope can markedly alter immune responses. This strategy may be used to increase the affinity of the peptide for MHC class I molecules via alterations in binding anchor residues [112–115]. Effects of this manipulation can range from the induction of T-cell receptor antagonism, to T-cell anergy, to enhancement of T-cell responses [116–118]. Mimics can be used to enhance the immunogenicity of tumor-associated antigens in vaccine design [119–123]. For example, SurvaxM contains a mimic that enhances binding to MHC class I molecules leading to greater immunogenicity than the wild-type survivin homolog. Importantly, the immune response generated must retain cross-reactivity to the wild-type protein expressed by tumor cells to be effective.

Combination therapy with immunomodulators

Standard therapy for glioblastoma consists of surgery, radiation therapy and chemotherapy with temozolomide. The alkylating agent temozolomide causes lymphopenia in a large percentage of treated patients; however, concern that concomitant temozolomide could attenuate the response to cancer vaccines seems to be unfounded. Some evidence suggests that temozolomide may even enhance cellular antitumor responses by having selective action against Treg cells in mice [124]. Similarly, low-dose temozolomide has been shown to reduce Tregs in melanoma patients [125]. However, the effect of temozolomide on Tregs may be highly schedule dependent [126]. As a result of these findings, a number of vaccine trials for patients with newly diagnosed glioblastoma have combined the vaccines with temozolomide in the upfront treatment of this disease. It remains to be determined whether the potentially selective action of temozolomide against Tregs is sufficiently great to mitigate the general lymphopenia that the drug also produces.

IDO inhibitors might provide additional benefit to glioblastoma patients whose tumors express high levels of IDO. There are a number of IDO inhibitors under development. Currently, the IDO-1 inhibitor indoximod is being assessed in combination with temozolomide (NCT02052648) [127] in glioblastoma patients. It will be of interest to see whether IDO inhibitors might

enhance the clinical effects of specific glioblastoma vaccines in appropriate patient groups.

Nivolumab is a human monoclonal antibody that targets the programmed death-1 (PD-1) receptor, which is expressed by activated B and T cells. Binding of PD-1 to its ligands, PDL-1 and PDL-2, results in the downregulation of lymphocyte activation. Inhibition of this interaction promotes antigen-specific T-cell responses to both foreign antigens and self-antigens. Thus, autoimmunity is a known risk of such drugs. NCT02529072 [128] is a study of nivolumab with pp65 CMV DC vaccine for recurrent malignant brain tumors (AVERT). This two-arm randomized trial is designed to evaluate nivolumab in combination with DC vaccination for the treatment of bevacizumab-naïve subjects with recurrent, resectable malignant gliomas, including glioblastoma. Patients are randomized to one of two treatments. Group I will receive nivolumab monotherapy and Group II will receive nivolumab plus DC vaccine therapy. So far, only a limited number of immune checkpoint inhibitors have been studied clinically, primarily those that inhibit the anti-CTLA-4 and the PD-1/PDL-1 axis. A number of additional possible sites of intervention exist [129] and it remains to be seen which of these might prove most beneficial for combination with vaccine therapy.

Clinical response criteria

Interpretation of response to immunotherapy in brain tumors can be complicated. Response Assessment in Neuro-Oncology (RANO) criteria have been developed for evaluating response to chemotherapy in clinical neuro-oncology trials. Potential inflammatory responses have been observed in some immunotherapy trials and the MRI findings in such instances can be difficult to distinguish from tumor progression. In addition, pseudoprogression which is seen in as much as 25% of patients with newly diagnosed glioblastoma in response to radiation therapy can further complicate the conduct of clinical trials and interpretation of disease response or progression. Toward that end, immunoRANO criteria are under development in an effort to help guide the field in the assessment of tumor progression in clinical protocol design and execution [130].

Conclusion

Vaccines have the potential to provide effective tumor treatment with limited toxicity or serious adverse events. A number of glioblastoma vaccines are under active investigation in single-arm and randomized Phase II

trials and at least one has recently been evaluated in a major Phase III trial. Most vaccine studies in glioblastoma patients are being conducted in the upfront setting of newly diagnosed glioblastoma in recognition of the likelihood that immunosuppressive bulk tumor may limit response to vaccines and create a hostile tumor microenvironment for cellular effectors. In glioblastoma patients with unmethylated MGMT, trial design may include deferral of temozolomide chemotherapy since it may not provide significant survival benefit in this group of patients. Consideration should be given to potential bias in single-arm Phase II trials that draw comparisons to historical controls. Such factors as TCGA class, extent of tumor resection, IDH-1 mutational status and MGMT promoter methylation can have significant effects on survival and may confound interpretation.

Future perspective

Combination drug strategies designed to mitigate systemic and local immunosuppression may help to improve the results beyond those seen to date with standalone vaccine therapy. Also, given the concomitant problems of local immunosuppression and the heterogeneity of tumor cells within a typical human glioblastoma, the combination of multivalent personalized peptide or DC vaccines and tailored immunomodulation would appear to be promising avenues for future investigation.

Although uncommon in glioblastoma, high mutational burden does occur in a subset of patients, particularly those with germline DNA repair defects. Tabori and co-authors found very significant radiologic response in two glioblastoma patients with hypermutated glioblastomas who received nivolumab [131]. This particular group of patients may benefit significantly with PD-1/PD-L1 inhibitor treatment. Similarly, combination strategies targeting tumor antigens in combination with immune checkpoint blockade may lead to significant clinical benefit for glioblastoma patients.

Financial & competing interests disclosure

RA Fenstermaker and MJ Ciesielski are co-inventors on patents regarding SurVaxM and are co-founders of MimiVax, LLC, which has licensed such patents from Roswell Park Cancer Institute. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Overview

- Glioblastoma afflicts about 17,000 new patients per year in the USA.
- Median life expectancy is 14.6 months with aggressive treatment.
- Recent developments in immunotherapy highlight inroads in this disease.

Immune privilege

- Cellular immune effectors can traffic into the brain by way of specific molecular interactions with the blood–brain barrier.
- Microanatomical studies reveal lymphatic channels in the dura mater running with drainage to cervical lymph nodes.

The glioma microenvironment

- T cells from glioblastoma patients have impaired immune responses.
- Glioblastomas display many other defects in local antitumor immunity.
- CD8⁺ cells from glioblastoma patients have impaired function.
- Indoleamine 2,3-dioxygenase 1 expression by glioma cells adversely affects the glioma microenvironment.

Specific vaccine approaches

- Vaccine strategies include:
 - Class I-restricted peptide cocktails
 - Multipeptide vaccines, including helper peptides
 - Long peptide vaccines
 - Peptide mimics
 - Personalized (neoantigen) peptide vaccines
 - Peptide, RNA or tumor lysate dendritic-cell vaccines

Complementary strategies

- The generation of optimal cellular antitumor immune responses requires activation of both CD8⁺ lymphocytes and specific CD4⁺ helper support, which many previous class I-restricted vaccines could not accomplish.
- Immunomodulating agents and/or checkpoint inhibitors may reduce the effect of immunosuppression when used in combination with vaccines.

Clinical response criteria

- ImmunoRANO criteria are under development in an effort to help guide the field in the assessment of tumor progression in clinical protocol design and execution.

Future perspective

- Vaccines have the potential to provide effective tumor treatment with limited toxicity or serious adverse events.
- Combination drug strategies designed to mitigate systemic and local immunosuppression may help to improve the results beyond those seen to date with stand-alone vaccine therapy.
- Increasingly, vaccines will be combined with targeted agents that modulate the microenvironment in a ‘tip of the spear’ approach to obtain more clinically significant effects.
- Vaccine studies may help point the way toward targets for adoptive T cell or targets.
- Synthetic vaccines might provide lower cost strategies making more affordable combinations with immune checkpoint inhibitors and other modulators of the microenvironment.

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