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Peptide vaccines for the treatment of glioblastoma

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Abstract Glioblastoma multiforme (GBM) is an extremely malignant brain tumor for which current therapies do little to remedy. Despite aggressive treatment with surgery, radiation therapy, and chemotherapy, tumors inevitably recur as a direct consequence of the infiltrative nature of GBM. The poor prognosis of patients with GBM underscores the clear and urgent need for more precise and potent therapies. Immunotherapy is emerging as a promising means to treat GBM based on the immune system's capacity to mediate tumor-specific cytotoxicity. In this review, we will discuss the use of peptide vaccines for the treatment of GBM. The simplicity of peptide vaccines and their ability to elicit tumor antigen-specific immune responses make them an invaluable tool for the study of brain tumor immunotherapy.

Keywords Glioblastoma · EGFRvIII · Immunotherapy · Glioma · Rindopepimut · Peptide vaccine

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Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant brain tumor affecting adults. The current standard of care for GBM includes maximal tumor resection followed by external beam radiation therapy and temozolomide (TMZ) chemotherapy. However, even with treatment GBM is invariably fatal, with a median survival of approximately 15 months [1]. Due to the imprecise nature of standard of care modalities, healthy peritumoral tissue is subject to collateral damage without complete elimination of the entire tumor cell population. Even with extremely aggressive treatment, such as the removal of an entire cerebral hemisphere [2], GBM is far too invasive to be successfully treated using these methods. Evidenced by the high rate of recurrence following standard of care management, the eradication of the entire malignant cell population is likely critical for the successful and long-term treatment of GBM, given that residual cancer stem cells are competent at repopulating new tumors [3].

In an effort to overcome the limitations of conventional therapies, immunotherapy is being rigorously tested as a means to treat GBM in light of the immune system's capacity for molecular-guided, cell-specific cytotoxicity. Of the various immunotherapeutic modalities that could be used for the treatment of solid tumors, vaccines have garnered considerable support, in part, based on the positive track record of antiviral vaccines, a favorable safety profile, and ease of administration [4]. This review will outline peptide vaccines that are being investigated for the treatment of GBM.

A primer on peptide vaccines

A vaccine is a type of active immunotherapy that provokes the immune system into acquiring long-term immunity

against an antigen of interest. Fundamentally, this activity is prompted by the administration of an immunogen in conjunction with an adjuvant—an immunological stimulator—thereby directing the activation of antigen-specific lymphocytes. Vaccines have traditionally been used in a prophylactic capacity; however, their ability to therapeutically mediate antitumor immunity is now being appreciated. Investigative cancer vaccines take many forms, including autologous/allogeneic tumor cells, tumor lysates, synthetic peptides, proteins, antigen-loaded dendritic cells, "naked" DNA, and recombinant viral vectors [4–6].

Peptide vaccines are comprised of ~8–25 amino acids that encompass an epitope within an antigenic target. To enhance their immunogenicity, these short peptides are often conjugated to a carrier protein, such as keyhole limpet hemocyanin (KLH) and tetanus toxoid. Peptide vaccines are appealing because they are relatively easy to manufacture and store, and they do not require laborious preparations that inconvenience other forms of cancer vaccines. Additionally, peptide vaccines are more chemically defined than alternative vaccine conformations, thus mitigating vaccine-to-vaccine variability [7].

Peptide vaccines are not well suited for all immune responses. In general, secreted and extracellular antigens are targeted by humoral immunity and intracellular antigens by cell-mediated immunity, though there are exceptions. A humoral immune response is initiated though the recognition of a conformational epitope on an antigen by a B cell receptor, which promotes endocytosis and antigen processing. Conformational epitopes are native structures that arise through protein folding and are often composed of discontinuous amino acids. Short peptides do not generally mimic these conformational epitopes and are, therefore, not suitable for these purposes. Cell-mediated immunity, on the other hand, relies on presentation of short peptides in the context of major histocompatibility (MHC) (Fig. 1). Peptide vaccines are ideal for eliciting cell-mediated immunity against an antigen in which there is a known MHC-binding peptide sequence. However, due to the highly polymorphic nature of human MHC genes, not all patients will react similarly to a given epitope [7].

Immunotherapeutic targets

Tumors express an array of tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) that can be exploited as immunotherapeutic targets. TSAs are unique to tumor cells and derive from genetic mutations or defective post-transcriptional/translational processing. TAAs are aberrantly overexpressed proteins (e.g. EGFR) or ectopically-expressed fetal-development (i.e. oncofetal), germ line-restricted (i.e. cancer/testis), and differentiation-associated proteins. TSAs arguably serve as better vaccine targets compared to TAAs because of the potential expression of TAAs on healthy cells. Immunological reactivity to normal, "self" antigens is prevented by various tolerance mechanisms, including the establishment of immunosuppressive regulatory T cells (T_{regs}) against "self" epitopes, the clonal deletion of "self" reactive lymphocytes, and the induction of "self" reactive lymphocytes into a state of unresponsiveness known as anergy. Consequently, the efficacy of a TAA-targeting vaccine may be compromised as a result of the deletion or suppression of TAA-cognate lymphocytes. Furthermore, because the immune system boasts potent cytotoxic potential, autoimmunity is a major concern when targeting antigens present on healthy cells. This is exemplified by studies demonstrating that experimental autoimmune encephalomyelitis can be induced through immunizations with myelin basic protein, myelin oligodendrocyte glycoprotein, or myelin proteolipid protein [8].

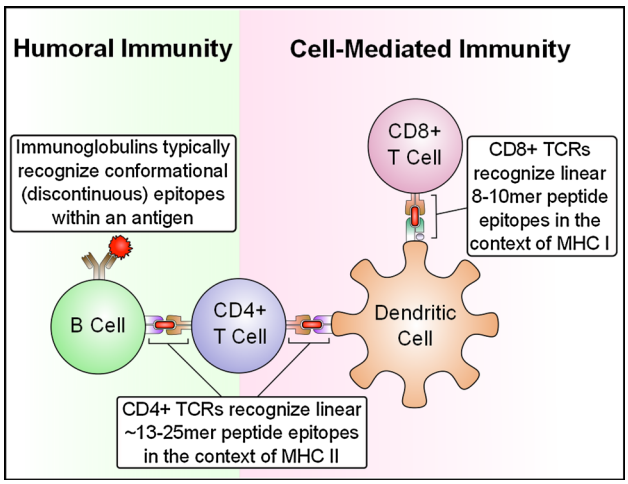

Fig. 1 Proteinaceous-antigen presentation and recognition by cells of the adaptive immune system (graphic created using Inkscape 0.48)

Peptide vaccines for GBM

GBM tumors exhibit profound genomic, transcriptomic, and proteomic alterations that may be exploited for the purposes of immunotherapy. Proteins frequently mutated or atypically expressed in GBM include EGFR, NF1, PDGFRA, PTEN, TERT, RB1, TP53, IDH1, PIK3CA and PIK3R1 [9]. Human cytomegalovirus (hCMV) antigens have also been shown to be uniquely present in GBM tumor cells [10]. Despite the tens of thousands of tumor antigens discovered in GBM [11], only a few have been pursued as targets for peptide vaccines due to the lack of conservation among GBM patients.

EGFRvIII vaccine: rindopepimut

Present in ~10–64 % of adult GBMs, EGFRvIII is a ligand-independent, constitutively active splice variant of EGFR that has been shown to enhance tumor growth and chemoresistance [9, 12, 13]. The coding sequence for EGFRvIII, residing primarily on episomal bodies, encodes a transcript devoid of exons 2-7 [14]. Consequently, a novel glycine is introduced into the amino acid sequence at the junction of exons 1 and 8 [15]. In light of its oncogenic function, tumor-specific expression, and distinctiveness from wild-type EGFR, EGFRvIII was quickly recognized as an ideal candidate for immunotherapeutic targeting.

Rindopepimut is a peptide vaccine composed of a 14mer peptide spanning the EGFRvIII-specific exon junction site conjugated to the carrier protein KLH. Initially, rindopepimut was used as an immunogen for the development of EGFRvIII-specific antibodies, which were shown to mediate effective antitumor responses against EGFRvIII-positive tumor cells when used in a passive immunotherapeutic capacity [16]. Mice vaccinated with rindopepimut, in combination with Freund's complete adjuvant or Freund's incomplete adjuvant plus GM-CSF, developed an EGFRvIII-specific humoral response that was capable of suppressing tumor growth and statistically enhancing median survival following intracerebral challenge with EGFRvIII-positive tumor cells. However, despite a few long term survivors, several mice succumbed to EGFRvIII-negative escape tumors [17]. This phenomenon, known as antigen escape, would later be revealed in clinical trials as one of the most critical impediments to long term effective treatment with rindopepimut.

Rindopepimut was shown to be generally well-tolerated with minimal adverse effects in a Phase I trial, known as VICTORI. In this study, newly diagnosed GBM patients were vaccinated with rindopepimut-pulsed, monocyte-derived dendritic cells. Immunological monitoring suggested that most patients developed an EGFRvIII-specific immune response [18]. To determine the safety and efficacy of rindopepimut as a peptide vaccine for patients with EGFRvIII-positive GBM, three Phase II trials were initiated. In all three trials—ACTIVATE, ACT II and ACT III—rindopepimut was administered with adjuvant GM-CSF [19–21]. ACT II and ACT III evaluated rindopepimut/GM-CSF vaccination in conjunction with TMZ maintenance therapy based on earlier reports showing that TMZ-induced lymphopenia enhanced immunotherapeutic efficacy [22, 23]. The results from these trials further confirmed the safety of rindopepimut and demonstrated a statistical increase in median progression-free (PFS) and overall survival (OS) in vaccinated patients, compared to a standard of care treated cohort (PFS = 6.4 months, OS = 15.2 months) [19–21]. Consistent with histological data from preclinical investigations, most patients unfortunately succumbed to recurrent tumors that were devoid of EGFRvIII expression. (See Table 1 for more information on rindopepimut clinical trials).

Under the auspices of Celldex Therapeutics, Inc., enrollment is currently open for two rindopepimut clinical trials: ReACT and ACT IV. ReACT is a non-pivotal Phase II trial for patients with recurrent GBM, and will include a group of relapsed patients that is refractory to treatment with bevacizumab (anti-VEGF monoclonal antibody). In addition to receiving bevacizumab, patients will be vaccinated with either rindopepimut/GM-CSF or KLH. ACT IV is a Phase III trial that will be conducted at over 200 locations world-wide. Patients will receive either rindopepimut/GM-CSF or KLH, as well as TMZ maintenance therapy at the standard dose.

IDH1 R132H vaccine

Isocitrate dehydrogenase 1 (IDH1) is a cytosolic enzyme that is frequently mutated in gliomas [24]. The IDH1 R132H mutation, present in 5–12 % of GBMs, is typically associated with secondary GBMs that affect young adults [9, 24]. The R132H amino acid substitution alters the catalytic site, preventing IDH1 from catalyzing the $NADP^+$ dependent oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG) [25]. Alternatively, IDH1 R132H consumes NADPH and αKG to produce the oncometabolite 2-hydroxyglutrate (2HG) [25], which has been shown to perturb protein and DNA methylation [26]. Wild-type IDH1 also performs NADPH/CO_2-dependent reductive carboxylation of αKG to isocitrate (reverse reaction)—a reaction that is implicated in fatty acid and cholesterol biosynthetic pathways [27]. Lipogenic dysregulation promoted by the inability of IDH1 R132H to execute this function may underlie IDH1 R132H's association with less aggressive, secondary GBMs [27, 28].

A recent preclinical study demonstrated that vaccination of MHC-humanized (i.e. HLA-A*0201 HLA-DRA*0101 HLA-DRB1*0101) mice with a peptide vaccine representing amino acids 123-142 (p123-142) of IDH1 R132H was capable of suppressing the growth of an IDH1 R132H-positive sarcoma. Consistent with the identification of MHC class II epitopes within p123-142, p123-142 vaccinated mice possessed IDH1 R132H-reactive, IFN-γ-producing CD4 + T cells. Supporting the contribution of this arm of immunity, CD4 depletion diminished vaccine-mediated tumor suppression [29]. Though these results are encouraging, further studies are needed to determine whether an IDH1 R132H vaccine is effective in the context of a glioma, which do not generally express MHC II [30].

Table 1 Summary of rindopepimut clinical trials

	Participants	Treatment arm(s)	Median PFS[a]	Median OS[a]	Outcome
Phase I					
VICTORI [18]	12 newly diagnosed patients w/GBM	I.d. injections with up to 10^6 autologous, monocyte-derived DCs pulsed with 500 μg rindopepimut	10.2 months (95 % CI 5.7–12.6)	22.8 months (95 % CI 17.5–29.0)	
Phase II					
ACTIVATE [19]	18 newly diagnosed patients w/EGFRvIII + GBM	I.d. injections with 500 μg rindopepimut/150 μg GM-CSF	14.2 months (95 % CI 9.9–17.6)*	26 months (95 % CI 21.0–47.7)***	11 of 13 relapsed tumors were EGFRvIII-negative. Two surviving participants
ACT II [20]	22 newly diagnosed patients w/EGFRvIII + GBM	I.d. injections with 500 μg rindopepimut/150 μg GM-CSF + STD[b] maintenance TMZ after 3rd vaccination	15.2 months (95 % CI 11.0–18.5)**	23.6 months (95 % CI 18.5–33.1)**	11 of 12 relapsed tumors were EGFRvIII-negative. No survival advantage between STD[b] and DI[c] maintenance TMZ cohorts. One surviving participant
		I.d. injections with 500 μg rindopepimut/150 μg GM-CSF + DI[c] maintenance TMZ after 3rd vaccination			
ACT III [21]	65 newly diagnosed patients w/EGFRvIII + GBM	I.d. injections with 500 μg rindopepimut/150 μg GM-CSF + STD[b] maintenance TMZ after 3rd vaccination	12.3 months**	24.6 months***	4 of 6 relapsed tumors were EGFRvIII-negative
ReACT	~170 patients with EGFRvIII + , recurrent GBM	I.d. injections with 500 μg rindopepimut/150 μg GM-CSF + 10 mg/kg bevacizumab (bevacizumab naïve participants)			Trial is currently recruiting participants
		I.d. injections with 100 μg KLH + 10 mg/kg (bevacizumab naïve participants)			
		I.d. injections with 500 μg rindopepimut/150 μg GM-CSF + 10 mg/kg bevacizumab (bevacizumab refractory participants)			
Phase III					
ACT IV	~700 newly diagnosed patients w/EGFRvIII + GBM	I.d. injections with 500 μg rindopepimut/150 μg GM-CSF + STD[b] maintenance TMZ after 3rd vaccination			Trial is currently recruiting participants

* $p < 0.05$, rindopepimut treated cohort(s) versus standard of care treated cohort (contemporaneous with ACTIVATE)

** $p < 0.01$, rindopepimut treated cohort(s) versus standard of care treated cohort (contemporaneous with ACTIVATE)

*** $p < 0.001$, rindopepimut treated cohort(s) versus standard of care treated cohort (contemporaneous with ACTIVATE)

[a] from date of histological diagnosis

[b] STD: 200 mg/m^2 TMZ for the first 5 days of a 28-day cycle

[c] DI: 100 mg/m^2 TMZ for the first 21 days of a 28-day cycle

I.d. intradermal, *DCs* dendritic cells, *GM-CSF* granulocyte macrophage colony stimulating factor, *KLH* keyhole limpet hemocyanin, *TMZ* temozolomide, *STD* standard TMZ therapy, *DI* dose-intensified TMZ therapy, *PFS* progression-free survival, *OS* overall survival, *CI* confidence interval

CMV vaccine: PEP-CMV

Human cytomegalovirus (hCMV) is a common herpes virus that causes life threatening disease in infants and immunocompromised individuals. Approximately 50–80 % of healthy individuals have been exposed to CMV, though primary infection is generally asymptomatic. Once the immune system suppresses the initial infection, CMV becomes latent, commonly using myeloid cells as a reservoir [31].

Though their role and origin remains controversial, several hCMV proteins have been detected in GBM specimens and not in normal tissues [32]. These proteins include IE1, US28, pp65, gB, HCMV IL-10, and pp28 [32]. Due to their exclusivity in tumor cells, these antigens have been exploited as immunotherapeutic targets. A peptide cocktail containing class I and class II restricted epitopes from CMV antigens, known as PEP-CMV, will soon be investigated in a clinical trial known as PERFORMANCE.

Multiple GBM antigen vaccines

Due to the high rate of relapse encountered by vaccination against a single tumor antigen, multiple tumor antigen-targeting vaccines are likely required to combat the vast antigenic heterogeneity present among cells within a tumor population. The German company immatics Biotechnologies GmbH is currently investigating an 11 peptide GBM vaccine—known as IMA950—that targets multiple high frequency tumor antigens, which were revealed through mass spectrometric analysis of MHC-complexed peptides from 30 primary human GBM specimens. Nine of the peptides bind to the common MHC I allele HLA-A*02, and two of the peptides bind to various HLA-DR (MHC II) alleles [33]. A Phase I trial conducted in the UK recently met two primary endpoints for safety and immunogenicity, demonstrating that $\sim$90 % of the patients responded to the vaccine. An additional multiple tumor antigen-targeting GBM vaccine, known as SL-701 by Stemline Therapeutics Inc., will soon begin Phase II testing based on the recent acceptance of an IND.

Immunopotentiation using adjuvants

Adjuvants are compounds that enhance immunogenicity. The delivery of an antigen in the absence of an adjuvant generally leads to tolerance; therefore, adjuvants are an essential component of vaccine strategies. Peptide vaccines for glioblastoma, such as rindopepimut and PEP-CMV, are typically resuspended in water or saline and delivered with the adjuvant GM-CSF, which has been shown to recruit and activate antigen presenting cells [34]. One adjuvant that is showing great promise with regard to cancer vaccines is dendritic cells—dubbed "nature's adjuvant." The utility of dendritic cells as an adjuvant derives from their capacity to provide the costimulation and immunostimulatory molecules needed to prime naïve T cells. Numerous studies have shown that autologous dendritic cells pulsed with peptides or tumor lysate are capable of inducing powerful antitumor responses and are well tolerated in human subjects (reviewed in [35]). Furthermore, because many traditional adjuvants exert their effects on dendritic cells, dendritic cells may be treated with adjuvants ex vivo prior to vaccination, thereby minimizing the risk of toxicity associated with the direct delivery of adjuvants into a patient. Because adjuvants play an integral role in the immune response, meeting the demand for effective, yet safe, adjuvants will be necessary for the progression of cancer vaccines as a feasible treatment strategy.

Overcoming the challenges of tumor vaccines

Though cancer vaccines are demonstrably safe and effective, studies have shown that they are rarely curative. GBM tumors have evolved certain mechanisms that allow them to evade immunological attack, and these defense mechanisms, by their very nature, impede the effectiveness of cancer vaccines. For example, GBM cancer stem cells and migrating glioma cells have been shown to lack expression of MHC molecules [36, 37]. This behavior precludes antigen presentation and prevents tumor antigen-cognate lymphocytes from recognizing tumor cells in an MHC-dependent manner. GBM tumors also mediate profound immunosuppression. GBM-associated cancer stem cells produce an arsenal of immunosuppressive molecules including PD-L1, an inhibitor of T cell proliferation, and the T_{reg}-inducing cytokine TGF-β [38]. Furthermore, host-induced immunosuppression at the GBM tumor microenvironment is coordinated by regulatory T cells (T_{regs}), myeloid-derived suppressor cells, and type 2 microglia. These cells have been shown to repress effector T cell activity through direct cell-to-cell contact or by secreting immunosuppressive mediators [38, 39]. The contribution of these mechanisms to immunotherapeutic recalcitrance has been demonstrated by studies showing that their inhibition or manipulation leads to more effective antitumor immunity [40–42]. However, uncovering clinically applicable modalities that safely, specifically, and potently target these mechanisms remains a challenge.

Current tumor vaccines are also encumbered by the vast antigenic heterogeneity displayed among tumor cells. Both EGFRvIII and IDH1 R132H frequently exhibit heterogeneous expression within GBM tumors [43, 44]. Consequently, vaccination strategies targeting a single tumor

antigen commonly lead to the outgrowth of antigen-loss escape variants, exemplified in clinical trials with rindopepimut. Due to the effort and cost associated with vaccine development and clinical evaluation, peptide vaccines are generally only practical for targeting common tumor antigens, and exhaustive molecular examination of numerous GBM specimens has revealed that only a few immunotherapeutically-feasible tumor antigens are widespread and conserved [11, 45]. To cope with the unique antigenic landscape of an individual tumor, dendritic cells—the most potent antigen presenting cells—offer a powerful platform for creating personalized and multivalent vaccines tailored to an individual patient's tumor. Methods for loading dendritic cells with tumor antigen include total tumor RNA transfection, fusion to tumor cells, and pulsation with apoptotic tumor cells, tumor lysate, or synthetic peptides. Several dendritic cell vaccines for the treatment of GBM are currently under investigation in clinical trials and have been reviewed elsewhere [6, 46, 47].

Conclusion

GBM is a devastating disease, for which very few treatment options exist. Therapies that are traditionally used to treat GBM are invasive, damaging to healthy tissue, and do not provide long term relief; therefore, there is a clear and urgent need for safer, more selective antitumor modalities. Immunotherapy is emerging as a promising means to treat malignant brain tumors. Particularly, cancer vaccines are proving to be a minimally-invasive immunotherapeutic strategy that is safe, selective, and sympathetic towards the delicate nature of the central nervous system. The use of peptide vaccines to target individual tumor antigens is a logical approach to easily and effectively elicit antitumor immunity; however, the resilience of cancer is reaffirmed by the high incidence of antigen escape that generally follows vaccination. Future cancer vaccines will likely employ a personalized approach to target a vast array of unique tumor antigens—a seemingly overwhelming task for a peptide-based approach. Despite the apparent limitations of peptide vaccines, they remain an essential tool for the elucidation of brain tumor immunotherapy.

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