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Immunotherapeutic Peptide Vaccine Approaches to Glioma

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ABSTRACT

Heretofore, there are no FDA-approved immunotherapeutics for malignant gliomas despite many novel therapies currently in different stages of clinical trials. Malignant gliomas are immunosuppressive tumors and are difficult for immune effector cells to infiltrate the tumor sites in the central nervous system. This inefficiency results in median survival of about only two years with a few long-term survivors. Recent clinical trials of vaccine-based immunotherapies against malignant gliomas have demonstrated encouraging results in enhancing progression-free survival and overall survival of patients. The vaccine-based treatments include peptide and heat-shock proteins, dendritic cell-based vaccines, as well as viral-based immunotherapy. In this review, we will focus on recent clinical trials of neoantigen peptide vaccines on gliomas, the delivery routes of such peptide vaccines, their adjuvants, clinical challenges, and its future strategies, respectively.

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Abbreviations: Glioblastoma multiforme, GBM; Major histocompatibility complexes, MHCs; Human leukocyte antigens, HLAs; Immune Epitope Database, IEDB; Polyinosinic-polycytidylic acid and poly-L-lysine, Poly-ICLC; Toll-like receptor 3, TLR3; Melanoma differentiation-associated protein 5, MDA5; Dendritic cells, DCs; New York esophageal squamous cell carcinoma-1, NY-ESO-1; Overlapping long peptide, OLP; Granulocyte-macrophage colony-stimulating factor, GM-CSF; Natural killer, NK; Interleukin-12, IL-12; Interferon alpha or gamma, TNF-α or IFN-γ; CpG oligodeoxynucleotides, CpG-ODNs; Antigen-presenting cells, APCs; Langerhans cells, LCs; Wilms tumor 1, WT1; Neutral liposome combined with monophosphoryl lipid A and Quillaja saponaria fraction 21, LMQ; Cytotoxic T lymphocytes, CTLs; Brevican, BCAN; Condroitin sulfate proteoglycan 4, CSPG4; Fatty acid-binding protein 7, FABP7; Insulin-like growth factor 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; Baculoviral IAP repeat-containing 5, BIRC5; Vascular endothelial growth factor, VEGF; Diffuse midline glioma, DMG; Mutated isocitrate dehydrogenase 1, IDH1; Epidermal growth factor receptor class III variant, EGFRvIII

Introduction

In general, glioma refers to a tumor arising from the glial group of cells within the brain that provide support functions to neurons throughout the brain. There are four grades of glioma. Pilocytic astrocytomas are considered to be a Grade I, it is rare and typically occurs in children, but is relatively benign. Low-grade glioma which is Grade II typically occurs in adults and is typically a benign tumor. Grade III is malignant glioma from anaplastic cells, and Grade IV is glioblastoma multiforme (GBM), which is the most aggressive one. Each grade has different types of proliferating cells and different treatment strategies. A glioblastoma is a grade IV glioma, which is the most aggressive form. This means that all glioblastomas are gliomas, but not all gliomas are glioblastomas. Malignant glioma is the most common and most aggressive primary brain cancer in adults. Despite advances in surgery, radiation, and chemotherapy, the prognosis of patients with malignant glioma is still very poor with a median survival of 14.6 months and two-year median survival of 27% according to the report of Glioblastoma and malignant astrocytoma from the American Brain Tumor Association.

Currently, patients with malignant glioma do not sufficiently respond to recent breakthrough therapies of immune checkpoint blockade [1-3]. The traditional treatment for malignancies involves surgery, chemotherapy, radiotherapy, along with targeted treatment. In particular, for malignant giomas like glioblastoma, surgical resection, chemotherapy with temozolomide, as well as radiotherapy, has improved the median survival time. Targeted therapy, after the addition of bevacizumab and everolimus to the standard of care, has comparatively improved the quality of life of patients with glioblastoma [4]. Therefore, the development of a novel therapeutic modality is urgently needed. Recent reports are demonstrating that systemic immunotherapy using dendritic cells, or a peptide vaccine can induce an anti-glioma immune response. Peptide vaccine immunotherapy strategies appear promising as an

approach to successfully induce an antitumor immune response and prolong survival in patients with glioma without major side effects. Consequently, peptide vaccine immunotherapy could be a new treatment modality for patients with brain tumors.

Neoantigen Peptide Design

After the whole-exome sequencing, different bioinformatics algorithms are used to identify and predict the affinity of neoantigens to major histocompatibility complexes (MHCs). One of the tumor vaccines targeting neoantigens is the synthetic peptide vaccine. Currently, hundreds of clinical trials have demonstrated the safety and efficacy of such peptide vaccines.

Neoantigen design is a critical method of identifying peptides from tumor biomarkers or mutations for an effective antitumor T-cell response. To overcome the weakness of current neoantigen prediction methods focusing on the binding between human leukocyte antigens (HLAs) and peptides, which is insufficient for high-confidence neoantigen prediction, several computational models have been developed. A study applied deep learning to a large HLA peptide and genomic dataset from various human tumors to create a computational model of antigen presentation for neoantigen prediction. They named this model EDGE and showed that this model increases the positive predictive value of HLA antigen prediction by up to nine-fold. They applied EDGE to enable the identification of neoantigens and neoantigen-reactive T cells using routine clinical specimens and small numbers of synthetic peptides for the most common HLA alleles [5]. EDGE could enable an improved ability to develop neoantigen-targeted immunotherapies for cancer patients. A new study applied deep learning techniques to predict neoantigens considering both the possibility of HLA-peptide binding (binding model) and the potential immunogenicity (immunogenicity model) of the peptide-HLA complex (pHLA) [6]. The binding model achieves comparable performance with other well-acknowledged tools on the latest Immune Epitope Database (IEDB) benchmark datasets and an independent mass spectrometry dataset. The immunogenicity model could significantly improve the prediction precision of neoantigens. Further application of our method to the mutations with pre-existing T-cell responses indicates its feasibility in clinical application. The DeepHLApan is freely available at https://github.com/jiujiezz/deephlapan and http://biopharm.zju.edu.cn/deephlapan.

The designed neoantigen peptides can be either long, short, or amphiphilic peptides (palmitoylated peptides). Amphiphilic antigen constructs such as palmitoylated peptides were shown to be better immunogens than long peptide constructs, which now are in vogue in the clinic [7]. In general, neoantigen peptides typically elicit weak CD8 T-cell responses, the strategies of peptide vaccine adjuvant selections and delivery routes are very important to enhance the immunogenicity of these peptides.

Adjuvants

1) Poly-ICLC

Poly-ICLC is a synthetic immunostimulant, an agonist for Toll-like receptor 3 (TLR3) and melanoma differentiation-associated protein 5 (MDA5). It is polyinosinic-polycytidylic acid (poly I:C) mixed with the stabilizers carboxymethylcellulose and polylysine. It can be used as an adjuvant for immunization due to its capability of activation of classical DCs to express high levels of IL-12 and type I IFN to promote Th1 polarization [8,9]. Studies in humanized mice models have validated the significance of Poly-ICLC as a potent adjuvant for driving DC-induced inflammation and activation of antigen-specific cytotoxic T cells [10]. Although several clinical trials in various advanced solid tumors have demonstrated that repetitive injection of poly-ICLC alone, in the absence of exogenous tumor-specific antigens, elicits an antitumor immune response, the injection of poly-ICLC alongside overlapping long peptides (OLP) from tumor-specific antigens into patients with ovarian cancer induce both humoral and cellular immune responses [11-13]. Moreover, A Phase I/II clinical trial study with 19 patients using Poly-ICLC as an adjuvant with multi-peptide IMA950 administrated separately or together [14]. The study provides evidence that both Poly-ICLC and multi-peptides should be mixed before administration to get multi-peptides CD8 T-cell and sustained T helper (Th1) CD4 T-cell responses. In the study, for the first 6 patients, the multi-peptides were given intradermally and poly-ICLC intramuscularly in close vicinity that showed no peptide-specific CD4 T-cell response at any time point in any patient. After protocol amendment, IMA950 and Poly-ICLC were mixed and injected subcutaneously, then multipeptide CD8 and sustained T helper 1 CD4 T cell responses were observed.

2) Montanides

Montanides (Montanide ISA 51 VG and ISA 720 VG) are adjuvants composed of mineral or metabolizable oil combined with a surfactant system designed to make a water-in-oil emulsion. Presumably, Montanide is important to protect and be effective in the slow-release of antigens, and therefore increase immunogenicity. Studies have demonstrated that Montanide and Poly-ICLC possess distinct and cooperative effects for the induction of antigen-specific Th1 cells and integrated immune responses [15,16]. In a phase-I clinical study of NY-ESO-1 overlapping long peptide (OLP) vaccine, vaccination with OLP alone did not induce measurable humoral or cellular immune responses. Whereas OLP in Montanide induced NY- ESO-1–specific CD4+ T cells along with inconsistent or transient antibody and CD8+ T-cell responses. The inclusion of poly-ICLC significantly induced integrated immune responses consisting of OLP-specific CD4+ T cells, CD8+ T cells, and antibody responses in nearly all patients. Importantly, though not a primary endpoint, OLP, and poly-ICLC in Montanide, significantly prolonged progression-free survival in patients who had NY-ESO-1 expressing tumors as compared with those who had tumors with no NY-ESO-1 expression. The syngenetic effect of Poly-ICLC and Montanide has been further confirmed in another phase I/II clinical trial using the full-length protein of NY-ESO-1 in patients with high-risk melanoma [15,16]. The combination of the protein antigen with Poly-ICLC and Montanide enhances antigen-specific T cell avidity and more pronounced CD8+ T cell response.

3) Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)

GM-CSF has been regulatory approved for stimulating recovery of granulocytes and monocytes in neutropenic patients who have received chemotherapy. In recent years, there has been interest in GM-CSF as anticancer immunotherapy because of its protean effects on the immune system [17,18]. In addition to its proliferative and differentiation effects on cells of myelomonocytic lineage, GM-CSF increases NK activity, expansion of DC populations, IL-12 production of DC, and attracts DC to vaccination sites in the body, thus increases DC-mediated cellular responses to tumor cells, activates and enhances monocyte cytotoxicity and secretion of IFN-γ as well as TNF-α in cancer patients. However, based on two large randomized trials, GM-CSF is not considered effective as a monotherapy for treating melanoma. Most likely, the nonspecific immune-stimulating effects induced by systemic administration of GM-CSF are not sufficient to enhance existing local immune responses [19,20].

There is a strong rationale for GM-CSF as a vaccine adjuvant, but GM-CSF appears to provide immune-enhancing effects when it is admixed with DC loaded *ex vivo* with autologous tumor antigens, or when the cytolytic virus that secretes GM-CSF is injected indirectly into tumors [21]. GM-CSF as an adjuvant in many clinical trials has been dosed in different ways including admixing with vaccine, injection adjacent to the vaccination site and repeated injections at distant subcutaneous sites. In a large trial with 435 melanoma patients, subjects were randomized to four groups: GM-CSF with peptide vaccine, GM-CSF alone, peptide vaccine alone, and control group, respectively [20]. In this study, GM-CSF was injected subcutaneously for days 1 to 14 of each 28 days cycle. Peptide vaccine was emulsified with Montanide and injected subcutaneously into 3 different sites on days 1 and 15 of cycle one and then on day 1 of subsequent cycles. In the two groups namely vaccine with GM-CSF and vaccine without GM-CSF as an adjuvant, there was no difference in overall survival between the two groups.

In another Phase III tiral with 815 patients, GM-CSF was used to promote the numbers and functions of DCs, the melanoma multiple antigen peptides in Montanide was used to promote antigen specific CD8+ T cell response. Most patients treated with GM-CSF developed neutralizing antibodies, which were associated with improved surviva, perhaps these patients were more immune competent. GM-CSF does not increase the rate of antigen-specific responses. In the trials of GM-CSF admixed with peptide vaccines or peptide vaccine-loaded DCs, evidence that GM-CSF affects the enhanced immunity appeared more favorable [22-24]. In a randomized trial in patients with metastatic melanoma, DCs loaded with autologous tumor antigens from irradiated self-renewing cancer cells engineered to express GM-CSF, were associated with a 50% 5-year survival rate in a single arm-trial. Dillman et al [26] reported another randomized trial that was associated with more than doubling of median survival and the rate of 3-year survival, along with a 70% reduction in the risk of death when GM-CSF was admixed with DC-loaded autologous tumor antigens from irradiated autologous cancer cells. Therefore, admixing GM-CSF with DCs that are preloaded with tumor antigens may increase the immune-enhancing effects by direct effects on the DCs and indirect effects from the local inflammatory response induced by GM-CSF [25,26].

4) CpG Oligodeoxynucleotides

CpG oligodeoxynucleotides (CpG-ODNs) are short, single-stranded, synthetic DNA molecules that contain a cytosine "C" followed by a guanine "G" nitrogenous base. The "p" refers to the phosphodiester backbone of DNA, however, some ODNs have a modified phosphorothioate backbone. When these CpG motifs are unmethylated, they act as immunostimulants trigger cells expressing Toll-like receptor-9 (TLR-9) to mount an innate immune response characterized by the production of Th1 and proinflammatory cytokines. Thus, CpG-ODNs are used as vaccine adjuvants to improve the function of professional antigen-presenting cells (APCs and boost the generation of humoral and cellular vaccine-specific immune responses). Clinical trials demonstrate that CpG ODNs have a good safety profile and increase the immunogenicity of coadministered vaccines [27]. In a trial, 28 patients were placed into treatment groups as follows: WT1 peptide alone, WT peptide with GM-CSF and WT1 peptide with CpG-ODN. WT1 peptide emulsified with Montanide and dosed intradermally every week for eight weeks. The disease control rate of the groups treated with WT1 peptide alone, with combinatorial use of GM-CSF, and with combinatorial use of CpG-ODN, in the initial two months was 20%, 25%, and 60%, respectively. Therefore, the study concludes that the addition of GM-CSF or CpG-ODN to the WT1 peptide vaccine for patients with solid malignancy was safe and improved the effectiveness of the clinical response [28].

Vaccine Delivery Routes

Major delivery methods of neoantigen vaccination include intradermal as well as subcutaneous routes of injection, intramuscular route of injection, and intravenous infusion.

1) Intradermal and Subcutaneous Route

The antigen-presenting cells (APCs) include dendritic cells, monocytes, and macrophages that reside in the skin of the body. Skin APCs are endowed with antigen sensing, processing, and presenting machinery and play key roles in initiating, modulating, and resolving cutaneous inflammation. Most importantly, skin APCs can travel in between skin and blood circulation, patrol within the skin, and migrate through the lymphatic system to draining lymph nodes. Intradermal inoculation of a vaccine targeting dermal APCs mobilizes both the cellular and humoral immune responses [9]. Langerhans cells (LCs) are a specialized subset of dendritic cells that account for approximately 3-5% of all epidermal cells in the skin. The recruitment of LCs after vaccination through intradermal administration is essential for the induction of antigen-specific CD8 T- cells [29]. In a recent phase I clinical trial, transcutaneous immunization through open hair follicles, which allows the targeting of follicular LCs, preferentially induces CD8 T- cells, whereas the intramuscular route cannot [30-32].

2) Intramuscular Route

In a phase I/II clinical trial of the multi-peptide IMA950/poly-ICLC vaccine in adult malignant astrocytoma patient, among 13 patients seven received subcutaneous and six patients received intramuscular injections, respectively [14]. The peptides IMA950 and poly-ICLC were mixed and injected at a single site. In this study, it was not able to determine an optimal route of vaccination with regard to the elicitation of both CD4 and CD8 T-cell responses, since higher multi-peptide CD8 T-cell responses were observed in the intramuscular group, and higher multi-peptide CD4 T-cell responses were observed in the subcutaneous group, respectively. In another study, while comparing intramuscular and subcutaneous vaccination of a recombinant leptospirosis vaccine rLigAc formulated with liposome-based adjuvant (LMQ), it has been demonstrated that intramuscular vaccination did not only elicit faster antibody production but also protected from kidney damage following leptospiral infection with better performance than subcutaneous immunization. However, both tested routes did not influence protective efficacy in terms of survival rate and the level of renal colonization [33]. In another phase II clincal trial of a novel amyloid beta synthetic peptide vaccine UB-311 for Alzheimer's disease (AD), the vaccine comprises two amyloid beta targeting peptides, each peptide synthetically linked to different helper T-cell peptide epitopes and formulated in an alum-containing Th2-biased solution, mixed with polyanionic CpG-ODN to form stable immunostimulatory complexes of micro-size particulates. Patients with mild-moderate AD were immunized by intramuscular route with 3 doses of UB-311 at weeks 0, 4 and 12, and monitored unitl week 48. UB-311 induced a 100% responder rate, and a slow rate of increase in AD Assessment Scale-Cognitive Subscale from baseline to week 48 was observed in the subgroup of mild AD patients compared with the moderate AD subgroup, indicating that UB-311 may have a potential of cognition improvement in patients with early stage of AD [34].

3) Intravenous Route

Peptide vaccines are used to elicit cytotoxic T lymphocytes (CTLs) in the clinic. They have routinely been administered in the same manner through the intravenous route as vaccines designed to induce antibody responses, which are injected subcutaneously and in many instances using Freund's adjuvant. An improved peptide vaccination strategy was reported to be capable of generating vast CD4T- cell responses by intravenous administration of combining synthetic peptides with TLR agonists and OX40/CD40 co-stimulation [35]. This strategy was efficient in overcoming immune tolerance to a self-tumor-associated antigen and generated significant antitumor effects in a mouse model of malignant melanoma. Peptide vaccines and poly-ICLC adjuvants administered via the intravenous route of immunization resulted in substantially higher CTL responses as compared to conventional subcutaneous and intramuscular injections and an improved antitumor effect observed in the mouse model [7]. The studies clearly demonstrated that intravenous delivery can generate the best antitumor effects whereas the intramuscular injection was superior to subcutaneous injection.

4) Intranasal Route

In addition to the above routes of peptide vaccine delivery, the intranasal delivery of certain formulated peptides has also been reported to be effective. A strategy for simple and rapid packaging of peptide antigens into pH-responsive nanoparticles with endosomal escape activity was described [36] . Electrostatically-stabilized polyplex nanoparticles can increase and prolong antigen uptake, facilitating presentation on MHC-I molecules expressed by dendritic cells, resulting in enhanced activation of CD8+ T-cells. Using an intranasal immunization route, nanoparticle vaccines inhibit the formation of lung metastases in a murine melanoma model [36]. Additionally, the vaccines strongly synergize with the adjuvant alpha-galactosylceramide (alpha-GalCer) in stimulating robust CD8+ T- cell responses, significantly increasing survival time in mice with established melanoma tumors.

Peptide Vaccine Therapies for Gliomas

Cancer peptide vaccines are a bunch of promising cancer immunotherapeutics that can induce cancer-specific CTLs in tumors. The peptides are designed based on tumor biomarkers or mutations to bind to MHC class I and MHC class II. The promising results from the two most recent phase I clinical trials published in Nature showed that personalized peptide vaccination to newly diagnosed glioblastoma patients generates tumor-reactive T-cells which can infiltrate glioblastomas and make tumor cells potentially susceptible to further immunotherapeutic approaches [1,37]. Compared to the personalized vaccine, the development of a universal neoantigen vaccine for one or more common types of HLA phenotype could potentially treat a number of more related malignant gliomas. Based on recent publications from clinical trials, the following peptide vaccines represent promising non-personalized neoantigen vaccines for malignant gliomas.

1) IMA950 Peptide Vaccine

IMA950 is a neoantigen peptide vaccine focusing on inducing a broad spectrum of T-cell responses against a variety of tumor-relevant antigens expressed by glioblastoma multiforme (GBM) cells. It results in decreased tumor growth of GBM. The neoantigens comprise of 11 synthetic tumor-associated peptides (TUMAPs) isolated from more than 30 primary human GBM specimens. Nine of the TUMAPs bind to the human leukocyte antigen (HLA) class I allele A*02 expressed by ~ 45% of the Caucasian population. Two TUMAPs bind to various HLA class II alleles expressed by the majority of patients. Therefore, cytotoxic T-lymphocyte (CTL), as well as T helper responses, are expected to arise from vaccination with IMA950. The IMA950 peptide vaccine was derived from the following tumor antigens: brevican (BCAN); chondroitin sulfate proteoglycan 4 (CSPG4); fatty acid-binding protein 7, brain (FABP7); insulin-like growth factor 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; baculoviral IAP repeat-containing 5 (BIRC5); and hepatitis B virus core antigen. The selected peptide set was further supported by the detected spontaneous antigen-specific Tcell responses to one or more of the IMA950 antigens in 100% and 71% of grade II and grade III glioma patients, respectively. The study was conducted on a total of 27 patients [38,39]. These patients displayed Tcell responses of better quality (higher frequency, broader epitope targeting) than patients with glioblastoma or grade IV glioma. Detection of spontaneous T-cell responses to the IMA950 antigens shows that these antigens are relevant for tumor targeting. Tumor targeting will be best achieved by combining IMA950 with CD4 epitopes such as the IDH1R132H peptide.

IMA950 vaccination increases the likelihood of a multi-clonal, highly specific T-cell response against tumor cells leading to the decreased likelihood of immune evasion by the tumor through down-regulation of target antigens. A two-cohort phase I clinical trial of IMA950 was first performed by the Rampling R group in patients with newly diagnosed glioblastoma in addition to standard chemoradiotherapy and adjuvant temozolomide. Patients were HLA-A*02-positive and had undergone tumor resection. Vaccination comprised 11 intradermal injections with IMA950 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) over 24 weeks, beginning 7 to 14 days prior to initiation of chemoradiotherapy (Cohort 1) or 7 days after chemoradiotherapy (Cohort 2) [40]. Forty-five patients were recruited. Of 40 evaluable patients, 36 were TUMAP responders and 20 were multi-TUMAP responders, with no significant differences between cohorts. No effect of pretreatment on Treg levels after IMA950 immunogenicity was observed, and steroids did not affect TUMAP responses. Progression-Free Survival (PFS) rates were 74% at 6 months and 31% at 9 months, respectively. The IMA950 plus GM-CSF was well-tolerated with the primary immunogenicity endpoint of observing multi-TUMAP responses in at least 30% of patients. In a recent phase I/II trial, the IMA950 peptide vaccine was combined with adjuvant poly-ICLC to dose glioblastoma (n=16) and grade III astrocytoma (n=3) patients [14]. After optimization of vaccine formulation, multi-peptide CD8 and sustained T helper 1 CD4 T-cell responses were observed. For the entire cohort, CD8 T-cell responses to single or multiple peptides were observed in 63.2% and 36.8% of patients, respectively. Median overall survival was 19 months for glioblastoma patients. The results of the early clinical trial of IMA950 were encouraging. The strategy of IMA950 vaccination such as adjuvant and formulation needs to be further optimized to achieve better clinical efficacy.

2) VEGFR Peptide Vaccine

The expression of vascular endothelial growth factor (VEGF)-A/VAGF receptors (VEGFRs) and signaling plays a pivotal role in the tumor angiogenesis and the development of the immunosuppressive tumor microenvironment in glioblastomas. Vascular endothelial growth factor receptors (VEGFRs)-specific cytotoxic T lymphocytes (CTLs) can kill both tumor vessel cells and tumor cells expressing VEGFRs.

A pilot study to evaluate the safety and immunogenicity of VEGFRs (VEGFR1 and VEGFR2) peptide vaccine in patients

with recurrent/progressive high-grade gliomas was reported [41]. The peptide vaccine was emulsified in Montanide and admisistered subcutaneously close to an axillary or inguinal lymph node and dosed weekly for eight weeks. T-lymphocyte responses against VEGFR epitopes were found in the peripheral blood mononuclear cells in 87.5% of patients. Two patients achieved progression-free status lasting at least 6 months and the other two patients with recurrent GBM demonstrated stable disease status.

Another exploratory clinical study of the VEGFRs peptide vaccine was conducted in seven patients with progressive NF2-derived schwannomas [42]. The peptide vaccine was emulsified with Montanide and injected subcutaneously at infra-axillary and inguinal lymph nodes four times for the first week and then four times monthly (a total of eight t imes vaccination). Results showed that hearing along with enhancement of word recognition scores was observed to improve in two out of five diagnosed patients (40%) as determined by international guidelines. Tumor volume reductions of ≥20% were observed in two patients, including one in whom VEGF antibody (bevacizumab) had not been effective. Both VEGFR1- and VEGFR2-specific CTLs were induced in six patients. Surgery was performed after vaccination in two patients, and significant reductions in the expression of VEGFRs in schwannomas were observed. This clinical study demonstrates the safety and preliminary efficacy of VEGFRs peptide vaccination in patients with NF2-derived schwannomas. The CTLs induced by the vaccination can directly kill a wide variety of cells associated with tumor growth, including tumor vessels, tumor cells, and immunosuppressive cells, Treg expressing VEGFR1, and/or 2. A further clinical trial was performed to evaluate the synergistic activity of the combination of VEGFRs peptide vaccination along with temozolomide (TMZ)-based chemoradiotherapy for patients with primary glioblastomas [43]. The peptide vaccine was again emulcified with Montanede and injected subcutaneously for a total of 14 times vaccination. The disappearance of the radiographically enhanced lesion was observed in two patients after the vaccination, whereas the histopathological findings of pre- and post-vaccination specimens demonstrated that tumor vessels showed negative or slight VEGFRs expressions after the vaccination along with most endothelial cells, which were covered with PDGFR-β-positive pericytes. Thus, the study suggested a preliminary synergistic effect when administered with TMZ in grade IV gliomas.

3) WT1 Peptide Vaccine

Wilms' tumor 1 protein (WT1) possesses oncogenic functions and is expressed in various kinds of malignancies suggesting WT1 as one of the most promising cancer antigens. The WT1 was shown to be highly immunogenic in cancer patients. WT1 peptides that could induce WT1-specific CTLs (WT1 CTL peptides) were identified, and vaccination of cancer patients with these WT1 CTL peptides induced immunological responses led to clinical responses such as solid tumor shrinkage, a decrease in leukemia cells, and reduction of M-protein, which is a biomarker of multiple myeloma. It is noteworthy that injection with a "single" kind of WT1 peptide elicited a strong immunological response to induce a clinical response, indicating that the WT1 peptide vaccine has therapeutic potential. There is an increasing number of reports mentioning WT1 peptide vaccinations giving affirmative and successful treatment of cancer patients, not only for adults but also for childhood malignancies. Strategies for further improvement in the efficacy of therapy, including the combined use of chemotherapy drugs, molecular-target-based drugs, or WT1 helper peptides, are being proposed. WT1 peptide vaccination in an "adjuvant setting" should be considered a promising treatment to protect against progression or relapse of malignancies in the case of patients with minimal residual disease.

Diffuse midline glioma (DMG) in children is a highly aggressive, malignant brain tumor.DMG is fatal when relapsed. An encouraging case report of a pediatric patient who had DMG that regrew after chemoradiotherapy and underwent WT1 peptide vaccination (formulated with Montanide and injected intradermally) was published [44]. A 13-year-old Japanese boy was diagnosed with DMG, which is a grade IV glioma. The patient underwent radiotherapy and chemotherapy with TMZ. After three cycles of chemotherapy, the tumor regrew and translated into deteriorated clinical manifestations. Immunohistochemically, the H3.3K27M mutation in the biopsy specimen was confirmed and the specimen was positive for WT1 protein. The patient received a WT1-specific peptide vaccination because of having HLA-A*24:02. Consequently, his quality of life dramatically improved, so much so that the patient became capable of conducting nearly normal daily activities at weeks 8 to 12 after vaccination. MRI at week 8 of vaccination revealed an obvious reduction of the tumor. Furthermore, the beta-methasone dose could be reduced successively without deteriorating clinical manifestations. The pediatric patient exhibited an encouraging clinical evolution as manifested by stable disease status.

Simultaneous induction of tumor antigen-specific cytotoxic T lymphocytes (CTLs) and helper T lymphocytes (HTLs) is required for an optimal anti-tumor immune response. A peptide containing MHC class I and II epitopes could induce an effective CD4 and CD8 T-cell response [45]. A phase I clinical trial of a "cocktail" vaccine of WT1 MHC class I and II peptides for patients with recurrent malignant gliomas was performed, the cocktail vaccine was formulated with Montanide and dosed intradermally. The immunological responses, as well as survival data, were assessed. Fourteen HLA-A*24:02-positive patients with recurrent malignant glioma were enrolled [46]. Eleven of the fourteen patients completed WT1 vaccination for six weeks, while three patients dropped out earlier due to disease progression. Six of the 14 patients had stable disease status at six weeks. Median OS and one-year overall survival (OS) rates were 24.7 weeks and 36%, respectively. In another clinical trial with WT1 peptide-pulsed dendritic cell (DC) vaccine, five out of seven treated glioma patients had stable disease clinical response, and two out of five patients had stable disease with improved neurological findings showing tumor shrinkage in magnetic resonance imaging [47]. This study of WT1-pulsed DC vaccination therapy demonstrated safety, improved immunogenicity, and feasibility in the management of relapsed malignant gliomas. WT1332, a 16-mer WT1-derived helper peptide, induce HTLs in an HLA class II-restricted manner and enhance the induction of WT1-specific CTLs in vitro. Fujiki F et al. demonstrated a striking difference in WT1-specific T cell responses between WT1 CTL + WT1 helper peptide and WT1 CTL peptide vaccines in patients with recurrent glioma [48]. WT1-specific CTLs were more strongly induced in the patients who were co-vaccinated with WT1 killer and helper peptide vaccine (WT1 CTL + WT1 helper peptides) compared to those who were immunized with WT1 CTL vaccine alone. Importantly, a clear correlation was obtained between WT1-specific CTL and WT1332-specific HTL responses. These results demonstrated the advantage of the WT1 helper peptide vaccine for the enhancement of WT1-specific CTL induction by WT1 CTL peptide.

A phase II clinical trial to investigate the safety and clinical responses of immunotherapy targeting the WT1 in patients with recurrent glioblastoma (GBM) was reported [49]. Twenty-one

patients with WT1/HLA-A*2402–positive recurrent GBM were included in phase II clinical study of WT1 vaccine therapy. In all patients, the tumors were resistant to standard therapy. Patients received intradermal injections of a modified 9-mer WT1 peptide every week for 12 weeks. The clinical responses showed partial response in 2 patients, stable disease status in 10 patients, and progressive disease in 9 patients. The overall response rate was 9.5%, and the disease control rate was 57.1% as a clinical response. This group further investigated the immunological microenvironment in glioma tissues before and after WT1 peptide vaccine treatment [50]. Paired tissue samples were obtained from 20 malignant glioma patients who had received the WT1 peptide vaccine for more than 3 months and experienced tumor progression confirmed radiographically and/or clinically, during vaccination. They discovered that the expression of WT1 and HLA class I antigens in the tumor cells significantly decreased after vaccination. Maintenance of WT1 expression, which is the target molecule of immunotherapy, in tumor cells during the vaccination period was significantly associated with a longer progression-free and overall survival. A high expression of HLA class I antigens and low CD4+/CD8+ tumor-infiltrating lymphocytes (TIL) ratio in pre-vaccination specimens, were also associated during the prognosis, which is good.

4) Survivin Peptide Vaccine

Survivin is an anti-apoptotic protein that is highly expressed in many cancers, including malignant gliomas. Preclinical studies established that the conjugated survivin peptide mimic called SurVaxM (SVN53-67/M57-KLH) could stimulate an anti-tumor immune response against murine glioma in vivo, as well as human glioma cells ex vivo. A clinical study was further conducted to test the safety, immunogenicity, and clinical effects of the vaccine, recurrent malignant glioma patients whose tumors were survivin-positive, and who had either HLA-A*02 or HLA-A*03 MHC class I allele-positivity, were given subcutaneous injections of SurVaxM in Montanide and Sargramostim (GM-CSF) adjuvant at 2-weeks interval. SurVaxM was well tolerated, and six of eight immunologically evaluable patients developed both cellular and humoral immune responses to the vaccine [51]. The vaccine also stimulated HLA-A*02, HLA-A*03, and HLA-A*24 restricted T cell responses. Three patients maintained a partial clinical response or stable disease status for more than 6 months. Median progression-free survival was 17.6 weeks, and median overall survival was 86.6 weeks from study entry with seven out of nine patients(77.7%) surviving more than 12 months.

5) Other Glioma Biomarker Peptide Vaccines

Mutation-specific vaccines have become increasingly important in glioma immunotherapy. Mutated isocitrate dehydrogenase 1 (IDH1) defines a molecularly distinct subtype of diffuse glioma. The most common IDH1 mutation in gliomas affects amino acid position 132 as IDH1(R132H), which harbors a shared clonal neoepitope that is presented on major histocompatibility complex (MHC) class II. An IDH1 (R132H)-specific peptide vaccine (IDH1-vac) induces specific therapeutic T helper cell responses that are effective against IDH1 (R132H)+ tumors in syngeneic MHC-humanized mice[52,53]. A multicentric single-arm, open-label, first-in-humans phase I trial that was carried out in 33 patients with newly diagnosed WHO grade 3 and 4 IDH1 (R132H)+ astrocytomas was conducted [54]. The IDH1 vaccine was emulsified in Montanide and administered subcutaneously in combination with topical imiquimod (5%, Aldara). Vaccine-induced immune responses were observed in 93.3% of patients across multiple MHC alleles. Three-year progression-free and death-free rates were 63% and 84%, respectively. Patients with immune responses showed a two-year progression-free rate of 82%. Two patients without an immune response showed tumor progression within two years of the first diagnosis. A mutation-specificity score that incorporates the duration and level of vaccine-induced IDH1 (R132H)-specific T-cell responses was associated with the intratumoral presentation of the IDH1 (R132H) neoantigen in pre-treatment tumor tissue. There was a high frequency of pseudoprogression, which indicates intratumoral inflammatory reactions. Pseudoprogression is defined based on areas of contrast, seeming like tumor progressing regions radiologically in the tissue examination. Such pseudoprogression was associated with increased vaccine-induced peripheral T-cell responses.

For highly aggressive midline gliomas, a recurrent point mutation in the histone-3 gene H3F3A causes an amino acid change from lysine to methionine at position 27 (K27M). A peptide vaccine against K27M-mutant histone-3 can induce effective, mutation-specific, cytotoxic T-cell- and T-helper-1-cell-mediated immune responses in an MHC-humanized mouse model [55]. By proving an immunologically effective presentation of the driver mutation H3K27M on MHC class II in human H3K27M-mutant gliomas, the data provide a basis for the further clinical development of vaccine-based or cell-based immunotherapeutic approaches targeting H3K27M.

In addition to the H3K27M mutated histone-3-gene encoding biomarker antigens as peptide vaccines for gliomas, other biomarker peptide vaccines can also be considered as vaccines for glioma immunotherapy. An immunohistochemistry analysis was conduct using antibodies specific for EphA2, IL-13Rα2, Survivin, and WT1 on paraffin-embedded specimens from 19 pediatric and 13 adult ependymomas [56]. In the 19 pediatric cases, 18 (95 %) demonstrated positive staining for EphA2, 16 (84 %) for IL-13Rα2, 18 (95 %) for Survivin, and only 7 (37 %) for WT1. Only three of the 19 cases were positive for two or fewer tumor-associated antigens (TAAs); whereas 16 out of 19 cases were positive for three or more TAAs. Pediatric and adult ependymomas frequently express EphA2, IL-13Rα2, and Survivin. This provides the basis for the utilization of an established multiple peptide vaccine for ependymoma in a clinical trial setting.

The epidermal growth factor receptor class III variant (EGFRvIII), a constitutively activated mutant of the wild-type tyrosine kinase, is present in a substantial proportion of malignant gliomas and other human cancers. This receptor variant consists of an in-frame deletion, the translation of which produces an extracellular junction with a novel glycine residue, flanked by amino acid sequences that are not typically adjacent in the normal protein. Following vaccination, potent, redirected cellular and humoral immunity against cancer cells expressing the specific mutant receptor without significant toxicity were observed [57]. Thus, the corresponding therapeutic outcomes observed in these studies lend credence to the potential role of peptide-based vaccination strategies among emerging antitumor immunotherapies in patients with malignant gliomas. Another study on mice model emphasized the usefulness of peptide vaccine Y6-pepVIII in combination with drugs in improving mean survival in treatment of glioblastomas [58]. Dozens of malignant glioma peptide vaccine therapies have been studied in the clinical settings, different strategies have also been used in the vaccination including the addition of adjuvants, chemo drug, and combination with checkpoint inhibitors and/or immune cell agonists such as anti-CD27 antibody promoting T cell response. A summary of completed and currently ongoing clinical trials of glioma vaccine therapies can be found in Table 1 and Table 2.

Summary and Future Perspectives

Malignant glioma remains one of the most studied tumors in the context of cancer-associated immunosuppression. Understanding specific biomarkers such as neoantigens for appropriate patient selection as well as tumor progression is necessary for the implementation of immunotherapy for malignant gliomas and a better prognosis. Neoantigens are basically in the form of peptides and proteins that arise from mutations within a tumor cell. Effective antitumor immunotherapies for malignant glioma depend on the selection of biomarker neoantigens and overcome the immunosuppression and specific CTL response against tumor cells. Regarding peptide antigens, there are two broad categories as tumor associated peptide antigens and tumor specific antigens, which needs to be the target molecules for developing vaccines. Although the desirable efficacy has yet to be achieved for any of the glioma-specific peptide vaccines, they are currently in clinical development, having the definitive hope for the future peptide vaccine immunotherapy for malignant gliomas as precision approaches. Especially, it appears most encouraging for the combination with immune checkpoint inhibitors or other approaches to overcome the profound immunosuppression of this disease as well as to demonstrate that active immunotherapy to control the growth of malignant brain cancer.

Table 1: Completed peptide vaccine clinical trials for glioma

Peptide vaccines	Adjuvant and/or intervention	Conditions	Phase	Trial Number	Sponsor
IDH1 peptides	Topical imiquimod (Aldara)	IDH1R132H-mutated Grade III-IV Gliomas	I	NCT02454634	National Center for Tumor Diseases, Heidelberg
Peptides pulsed DC vaccine	Poly-ICLC	Malignant glioma	I/II	NCT00766753	University of Pittsburgh/Oncovir Inc
GAA/TT-peptides	Poly-ICLC	Astrocytoma, oligo-Astrocytoma, Glioma	I	NCT00795457	University of Pittsburgh (Dr. Ian F. Pollack)
HLA-A2-Restricted glioma antigen-peptides	Poly-ICLC	Recurrent GBM	I	NCT00874861	Ian F. Pollack, M.D., University of Pittsburgh
glioma-associated antigen peptide-pulsed autologous dendritic cell vaccine		Brain and Central Nervous System Tumors	I	NCT00612001	Jonsson Comprehensive Cancer Center
Survivin SVN53-67/M57-KLH peptide	GM-CSF, Montanide	Malignant glioma	I	NCT01250470	Roswell Park Cancer Institute
GP96 Heat Shock Protein-Peptide Complex vaccine		Brain and Central Nervous System Tumors	I/II	NCT00293423	University of California, San Francisco
Multivalent glioma-associated antigen peptides	Poly-ICLC	Recurrent GBM	I/II	NCT02078648	Stemline Therapeutics, Inc.
PEP-3-KLH (EGFRvIII-KLH)	N/A	Malignant Neoplasms of Brain	I	NCT00626015	ohn Sampson, Duke University
APVAC1 and APVAC2 Peptides	Poly-ICLC and GM-CSF	GBM	I	NCT02149225	immatics Biotechnologies GmbH
IMA950 (multipeptide vaccine)	GM-CSF	GBM	I	NCT01222221	Cancer Research UK
DEC-205/NY-ESO-1 Fusion Protein CDX-1401	N/A	NY-ESO-1 Positive cancer		NCT01522820	Roswell Park Cancer Institute
IMA950	Poly-ICLC	GBM	I/II	NCT01920191	University Hospital, Geneva
ADU-623, a Live-attenuated Listeria Monocytogenes Strain (ΔactA/ΔinlB) Expressing the EGFRvIII-NY-ESO-1 vaccine	N/A	Brain tumor	I	NCT01967758	Providence Health & Services
Telomerase: 540-548 peptide vaccine	GM-CSF	Brain tumor or Sarcoma	I	NCT00069940	Dana-Farber Cancer Institute
Rindopepimut (CDX-110) EGFRvIII peptide-KLH	GM-CSF	EGFRvIII-Positive Glioblastoma	II	NCT01498328	Celldex Therapeutics
Rindopepimut (CDX-110) EGFRvIII peptide-KLH	GM-CSF	GBM	III	NCT01480479	Celldex Therapeutics
DSP-7888 (WT1 protein-derived peptide vaccine		Advanced malignancies including GBM	I	NCT02498665	Sumitomo Dainippon Pharma Oncology, Inc

Table 2: Ongoing peptide vaccine clinical trials for glioma

Peptide vaccines	Adjuvant and/or intervention	Conditions	Phase	Trial Number	Sponsor
H3K27M	Imiquimod (5%) and Anti-PDL1 antibody (Tecentriq)	H3-mutated gliomas	I	NCT04808245	German Cancer Research Center
H3.3-K27M peptides	Poly-ICLC	Diffuse intrinsic pontine Glioma (DIPG)	I	NCT04749641	Tiantan Hospital, Beijing
IDH1R132H	Montanide and Checkpoint inhibitor anti-PDL1 antibody (Avelumab)	Progressive diffuse glioma	I	NCT03893903	German Cancer Research Center
H3K27M	Poly-ICLC, Montanide and PD1 inhibitor Nivolumab	Diffuse intrinsic pontine glioma (DIPG) and HLA-A2+H3K27M positive gliomas	I/II	NCT02960230	UCSF (Dr. Sabine Mueller)
Personalized peptide vaccine	N/A	Malignant Glioma	I	NCT04943718	Xuanwu Hospital, Beijing
HLA-A2 restricted glioma antigen	Poly-ICLC	Pediatric gliomas	I	NCT01130077	University of Pittsburgh
HLA-A2 restricted glioma antigen	Poly-ICLC	Low-grade glioma	II	NCT02358187	University of Pittsburgh (Dr. Ian F. Pollack)
Neoantigen pulsed DC vaccine	N/A	DIPG and GBM	I	NCT03914768	Shenzhen Geno-Immune Medical Institute
IDH1 Peptides	GM-CSF and Montanide	Recurrent Grade II Glioma (RESIST)	I	NCT02193347	Gary Archer Ph.D., Duke University
IMA950	Poly-ICLC and Anti-CD27 antibody (Varlilumab)	Low-grade glioma	I	NCT02924038	Nicholas Butowski, University of California, San Francisco
IMA950	Poly-ICLC	Relapsing Glioblastoma	I/II	NCT03665545	University Hospital, Geneva
Multi peptide vaccine	Pam3Cys-GDPKHPKSF (XS15) immunomodulator and Montanide.	Newly diagnosed Glioblastoma	I	NCT04842513	University Hospital Tuebingen
Tumor antigen peptide	Imiquimod	Recurrent Ependymomas in Children	I	NCT01795313	
rHSC-DIPGVax (neoantigen heat shock protein containing 16 peptides)	PD1 inhibitor (Dalstilimab) and anti-CTLA-4 antibody (Zalifrelimab)	DIPG and diffuse midline glioma (DMG)	I	NCT04943848	Ann & Robert H Lurie Children's Hospital of Chicago
UCPVax: Telomerase (TERT) -derived universal cancer peptide vaccine	Montanide	Glioblastoma	I/II	NCT04280848	Centre Hospitalier Universitaire de Besancon
A mutation-derived tumor antigen (MTA-based vaccine)	Poly-ICLC and device Tumor Treating Fields (TTFields)	Glioblastoma	I	NCT03223103	

SurVaxM (SVN53-67/M57-KLH)	Montanide	Children Progressive or Relapsed Medulloblastoma, High-Grade glioma, Ependymoma and Newly Diagnosed Diffuse Intrinsic Pontine glioma	I	NCT04978727	Pediatric Brain Tumor Consortium
VBI-1901 CMV antigen gB and pp65	GM-CSF	GBM	I/II	NCT03382977	VBI Vaccine Inc.
Neoantigen peptides pulsed DCs	N/A	GBM	I	NCT04968366	Yang Zhang, Beijing Tiantan Hospital
Personalized neoantigen	N/A	GBM	I	NCT02287428	Dana-Farber Cancer Institute
HSPPC-96 (Heat shock protein-peptide complex 96)	N/A	GBM	II	NCT01814813	Alliance for Clinical Trials in Oncology
EO2401 (Innovative cancer peptides)	N/A	GBM	I/II	NCT04116658	Enterome
SVN53-67/M57-KLH Peptide vaccine	Montanide	GBM	II	NCT02455557	Roswell Park Cancer Institute
HLA-A2 restricted synthetic tumor antigen	Imiquimod	Ependymomas	I	NCT01795313	Ian F. Pollack, M.D., University of Pittsburgh
DSP-7888 (WT1 protein-derived peptide vaccine	With or without Bevacizumab (Avastin)	GBM	III	NCT03149003	Sumitomo Dainippon Pharma Oncology, Inc

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