Immunotherapy for High-Grade Gliomas

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Introduction

Anti-cancer immunotherapies which activate the patient's own immune system have shown efficacy and specificity in a variety of cancers, promising safer and more effective therapies. FDA approval of the anti-CD20 antibody rituximab for the treatment of lymphoma, the anti-HER2 antibody herceptin for treatment of breast cancer, and breakthrough checkpoint inhibitors such as anti-CTLA4 and anti-PD-1 have validated the field of immunotherapy and herald the start of an immunotherapy age that is revolutionizing cancer treatment [1–3].

The Not-So-Privileged Blood-Brain Barrier

Traditionally, literature describing the central nervous system (CNS) portrays a limited immune response marked by the blood–brain barrier, lack of a conventional lymphatic drainage system, and low levels of T cells, antigen-presenting cells, and major histocompatibility complexes [4]. However, recent findings provide evidence that while CNS entry is limited, there is a fully developed immune response in the brain. These findings include a lymph node-like drainage system which drains CNS antigens from the cerebrospinal fluid into the cervical lymph nodes, thereby facilitating immune surveillance of the CNS [5]. In addition, evidence shows that some immune cells are fully able to migrate into the CNS, where they are involved in diseases such as multiple sclerosis, CNS infections, and are also found in gliomas [4, 6]. In addition to the not-so-privileged blood–brain barrier, angiogenesis around the growing brain tumor leads to deterioration of brain microvasculature, increasing leakage [7]. That is, barrier functions such as tight junctions between the endothelial or transcytosis mechanisms may be relaxed, allowing increased penetration by immune cells [8].

Both the inherent control of the immune system over the brain and the deterioration of the blood–brain barrier during cancer growth warrant the potential of immunotherapy to redirect and activate immune cells that specifically recognize tumor cells within the brain.

Targets for Immunotherapies

The premise of immunotherapy rests on the idea that tumor cells are foreign and that the immune system can be taught to recognize the foreign cells or that a pre-existing immune response can be augmented. In order for such recognition to take place, antigens must be found that identify a specific tumor type and elicit an immune response. Broadly speaking, there are two types

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of immunologic targets, (i) tumor-associated antigens and (ii) tumor-specific antigens.

Tumor-Associated Antigens

Tumor-associated antigens (TAAs) are normal proteins that are overexpressed in tumor cells and can thereby serve to direct the immunologic response. Commonly, these antigens are lineage-differentiation antigens such as colorectal cancer antigens (CEA) [9] and alpha-fetoprotein (AFP) [10]. The major concern is that the expression of these antigens on healthy tissue, even if limited, could lead to autoimmunity if a potent immune response is elicited.

In glioblastoma, several studies have shown the overexpression of numerous proteins that could serve as immunological targets and for some antigens, clinical efficacy has been shown [11, 12]. Numerous studies have tested TAAs as potential immunotherapeutic targets for malignant brain tumors, including survivin, HER2neu, EphA2, EGFR, and telomerase [12–18].

Cancer/testis antigens (CTAs) represent a unique class of TAAs with normal expression restricted to germ cells in the testis but not in adult somatic cells. The melanoma-associated CTAs (MAGE, CAGE) are extensively expressed in a wide range of different cancers [19, 20].

An extensive expression analysis by Freitas et al. analyzed 153 cancer/testis antigens (CTAs), a class of differentiation antigens shown to be variably expressed within GBM tumors, and identified 4 CTAs (ACTL8, CTCFL, OIP5, and XAGE3) uniquely expressed within GBM tumors when compared to normal brain [21].

As with all TAAs, the question remains whether an approach targeting these antigens will yield a therapeutic window that shows efficacy yet limits adverse effects on healthy tissue expressing low levels of the antigen.

Tumor-Specific Antigens

In contrast to TAAs, which are normal proteins upregulated in cancer, tumor-specific antigens (TSAs) arise as mutations of normal proteins during the course of tumor progression and result in antigens that are exclusively expressed on malignant cells, albeit often on a subset of tumor cells. These antigens serve as prime targets for immunotherapies, as possible side effects such as cytotoxicity to healthy tissue are avoided.

In glioblastoma, a number of TSAs have been identified, of which some have already progressed into the clinic. Recent advances in genetic sequencing are rapidly identifying new mutations that identify subgroups of patients expressing a certain histologic type of brain cancer [22]. These neoantigens will need to be tested for their immunogenic potential to determine which can be used to develop future immunotherapies.

Currently, there are two TSAs that are highly prevalent and have shown immunogenicity in numerous studies. EGFRvIII is a conserved mutation of the epidermal growth factor receptor that is seen in approximately 31–50% of patients with GBM as well as in other cancers [23–28]. In those patients positive for EGFRvIII, the mutation is expressed in 37–87% of tumor cells.

A conserved mutation of isocitrate dehydrogenase type 1 (IDH1), which occurs at the critical arginine residue (Arg 132) in the catalytic pocket, results in a neomorphic enzymatic function, genetic instability, and malignant transformation [29]. This mutation, termed IDH1 (R132H), occurs in more than 70% of grade III gliomas and, from a therapeutic viewpoint, represents an ideal candidate for a tumor-specific treatment of malignant glioma [30].

In addition, viral antigens, when upregulated specifically on malignant cells, may also serve as TSAs and have the unique advantage of being intrinsically foreign to the host and thus immunogenic. Therefore, while viruses may not be exclusively restricted to tumor cells, their expression is often undetectable in normal tissue of patients harboring virus-associated cancers. Our laboratory and others have recently shown human cytomegalovirus infection and low-level viral gene expression in malignant glioma [31, 32]. Given the success and safety of cellular immunotherapeutics targeting CMV in

immunocompromised patients, immunodominant CMV antigens such as immediate early 1 (IE1), phosphoprotein 65 (pp65), and glycoprotein B (gB) have been shown to be expressed in GBM tumors and represent possible tumor-specific targets for the development of immunotherapies [33–35].

Antibodies for the Treatment of Intracerebral Malignancies

Monoclonal Antibodies Target Tumor Epitopes

The development of monoclonal antibodies (mAbs) recognizing specific epitopes has been used for the immunological treatment of many diseases, including cancer [36]. By recognizing and binding to specific epitopes, mAbs expose intruding cells and target them for uptake by phagocytic cells of the immune system, such as macrophages and dendritic cells. Furthermore, mAbs can target cellular components, such as secreted proteins, and thereby interfere with cell signaling.

Advances in technology over the past decades have made it possible to produce fully human affinity-matured antibodies via phage display directed evolution, transgenic mice, or mRNA and ribosome display, thereby resolving complications associated with murine antibodies such as human anti-mouse antibody formation and cytokine release syndrome [37–41].

Although antibodies can be found in the central nervous system at physiologic levels, GBM-induced disruptions of the blood–brain barrier facilitate antibody penetration. Several studies have shown that injecting antibodies IV in GBM patients results in significant therapeutic benefit [42–45]. In murine GBM models, an antibody directed against tenascin, a component of the tumor stroma, given systemically was shown to selectively localize to the tumor [42, 43].

The epidermal growth factor receptor (EGFR) is a well-studied and versatile signal transducer that is involved in cell proliferation, differentiation, survival, and metastasis [46]. EGFR is overexpressed in a number of tumors and plays an important role in the development of high-grade gliomas, especially in glioblastoma where it is commonly (40–60% of patients) amplified up to hundreds of gene copies [47, 48]. Anti-EGFR antibodies approved for the treatment of colorectal and head and neck cancers have been shown to inhibit ligand binding, receptor dimerization, and downstream signaling [49]. Sym004, a recently developed anti-EGFR antibody with enhanced effectiveness, is being tested in a phase II trial in recurrent glioblastoma in both patients that failed and did not fail bevacizumab treatment (Table 12.1).

Bispecific Antibodies Redirect and Activate Effector Immune Cells

Various solid tumors show infiltration with T cells and increased T cell infiltration often correlates with a good clinical outcome [50]. T cell infiltration has also been shown in glioma and is increased in high-grade tumors [51]. Substantial evidence suggests that the redirection of these T cells to specifically recognize and kill tumor cells is able to eradicate well-established tumors [52, 53]. Furthermore, clinical data have shown that mABs suffer from major limitations in their mode of action, including alternative Fc glycosylation, leading to suboptimal effector cell interaction, competition with circulating IgG, and activation of inhibitory receptors [54].

Bispecific antibodies (bsABs) are capable of binding two distinct targets and can be used to link T cells to tumor cells. Bispecific T cell engagers (BiTEs) consist of two antibody-derived linked single-chain Fv fragments (scFv) that are translated in tandem. One arm of the BiTE recognizes, for instance, the CD3 epsilon subunit on the T cell and the other arm binds a tumor antigen (Fig. 12.1). Upon binding, the BiTE causes crosslinking between adjacent tumor cells and T cells, regardless of the T cell receptor recognition, leading to T cell activation, synapse formation, and tumor lysis via perforin and granzyme secretion. Following

Table 12.1 Recent clinical trials employing antibody immunotherapy for high-grade gliomas

NIH clinical trial	Type	Phase	Eligibility criteria	Primary outcome	Dosing regimen	Treatment groups
NCT02311920	Checkpt	1	Newly diagnosed GBM	Maximum safe dose	In addition to TMZ, Arm I receives anti-CTLA4 Ab ipilimumab once every 4 weeks (4 doses) followed every 3 months (4 doses), Arm II receives anti-PD1 Ab nivolumab once every 2 weeks (32 doses), and Arm III receives both Abs	Arm I: TMZ + Ipi Arm II: TMZ + Nivo Arm III: TMZ + Ipi + Nivo
NCT02526017	mAb + checkpt	1	GBM	Safety, RD, efficacy (ORR)	Arm I will receive anti-CSF1R Ab FPA008 every 2 weeks. Arm II will receive 3 mg/kg nivolumab every 2 weeks in addition to dose-escalated FPA008 every 2 weeks. Arm III uses MTD determined in Arm II and expands patient group	Arm I: mAb Arm II: mAB dose escalation + Nivo Arm III: mAB + Nivo expansion group
NCT02521090	BiTE	1/2	Recurrent and refractory GBM	Toxicity (phase I), OS (phase II)	Phase I: patients receive anti-EGFR-CD3 BiTE armed T cells IT twice weekly for 4 weeks. Phase II: patients continue to receive BiTE armed T cells IV twice weekly for 2 weeks	Single-arm
NCT02423343	Small molecule + checkpt	1/2	GBM	MTD of galunisertib combined with Nivolumab	In Arm I escalating doses of TGFβR1 kinase small molecule inhibitor galunisertib are given daily for 14 days of each 4 week cycle in combination with nivolumab every 2 weeks. Arm II will use same treatment regimen with MTD galunisertib	Cohort A: dose escalation Cohort B: expansion group
NCT02530502	Checkpt	1/2	Newly diagnosed GBM	Phase I: DLT Phase II: PFS	Focal RT over 42 days followed by TMZ on days 1–42 and anti-PD1 antibody pembrolizumab on days 1, 22, and 43 for each course for up to 6 courses	Single-arm
NCT01952769	Checkpt	1/2	Diffuse intrinsic pontine glioma	Treatment-related toxicity	Anti-PD1 antibody given biweekly. Cohort A receives two doses with increasing concentration. Cohort B concentration varies between patients	Cohort A: 2 doses Cohort B: doses of different concentration

(continued)

Table 12.1 (continued)

NIH clinical trial	Type	Phase	Eligibility criteria	Primary outcome	Dosing regimen	Treatment groups
NCT02337686	Checkpt	2	Recurrent GBM	PFS6 and immune effector:Treg ratio	Anti-PD-1 antibody pembrolizumab given once every 3 weeks (2 doses) before surgery and once every 3 weeks thereafter	Single-arm
NCT02337491	Checkpt + mAB	2	Recurrent GBM	PFS6 and MTD	Not described	Cohort A: pembrolizumab + bevacizumab Cohort B: pembrolizumab
NCT01149850	mAb	2	Newly diagnosed GBM	Overall survival	Anti-VEGF antibody bevacizumab every 2 weeks	Single-arm
NCT02540161	mAb	2	Recurrent GBM	PFS6	Anti-EGFR antibody Sym004 given every 2 weeks at 18 mg/kg	Cohort A: non-bevacizumab failures Cohort B: bevacizumab failures

Ab antibody; *BiTE* bispecific T cell engager; *Checkpt* immune checkpoint modulator; *DLT* dose-limiting toxicity; *GBM* glioblastoma; *Ipi* ipilimumab; *IT* intrathecal; *mAb* monoclonal antibody; *Nivo* nivolumab; *OS* overall survival; *MTD* maximum tolerated dose; *PFS* progression-free survival; *PFS6* 6-month progression-free survival; *ORR* objective response rate; *RD* recommended dose; *TMZ* temozolomide; *Treg* regulatory T cell

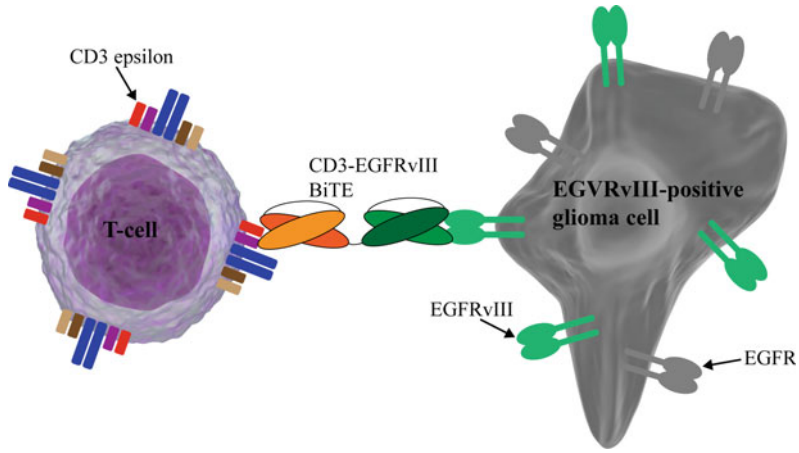

Fig. 12.1 BiTE mode of action. The anti-CD3-EGFRvIII bispecific T cell engager (BiTE) is able to bind the CD3 epsilon subunit of the T cell receptor with one of its single-chain Fv (scFv) fragments and EGFRvIII on the glioma cell with the other scFv fragment. This leads to spatially restricted crosslinking and activation of the T cell, resulting in T cell-mediated tumor cell cytotoxicity via synapse formation and the release of perforin and granzyme

BiTE-mediated tumor cell lysis, the T cells proliferate, express surface activation markers, and undergo serial rounds of killing [53, 55–57]. Furthermore, since crosslinking depends on binding to CD3 epsilon, T cell subsets implicated with tumor progression, such as Tregs, are also activated to lyse tumor cells [58, 59].

Since T cell activation requires physical linking to a tumor antigen, the immune activation is spatially and temporally restricted and highly specific for the chosen antigen. Furthermore, the small size of the BiTE results in a short half-life that allows quick regulation of antibody-mediated toxicity [60].

A recent clinical trial aims to treat patients with recurrent or refractory glioblastoma with a bispecific antibody made by the heteroconjugation of anti-EGFR and anti-CD3 antibody. Autologous activated T cells are loaded with the anti-EGFR-CD3 BiTE and injected intravenously into the patient with the goal of increasing T cell-mediated cytotoxicity toward tumor expressing EGFR [61]. The aim in this trial will be to determine whether a therapeutic window exists that will allow cell killing of EGFR over-expressing tumor cells without afflicting normal tissue (Table 12.1).

Our laboratory recently developed a BiTE produced by the heteroconjugation of an anti-EGFRvIII and anti-CD3 antibody. Experiments in mice show that systemic administration of the BiTE activates T cells in mice, resulting in extended survival and durable complete cures at rates of up to 75% [62]. Given the tumor specificity of the EGFRvIII antigen, treatment of patients with this antibody may have fewer side effects and increased efficacy.

Immune Checkpoint Modulators

The growth of a tumor is marked by significant changes to the microenvironment, leading to cancer-associated immunosuppression. This means that despite the presence of tumor-specific endogenous T cells, tumors escape destruction by upregulating inhibitory ligands that bind to inhibitory receptors on T cells, secretion of inhibitory cytokines (including TGF-beta and IL-10), and other mechanisms. This immunosuppression is particularly pronounced in glioma patients and leads to T cell dysfunction and an increase in the regulatory T cell phenotype [63–66].

Novel strategies for dealing with tumor-associated immunosuppression are the development of antagonistic mABs which block inhibitory ligands, such as CTLA-4, PD-1 and PD-L1, and agonistic mABs that stimulate the immune response by binding agonistic cell surface molecules, such as OX40 and 4-1BB (Fig. 12.2). Recent advances, in particular the FDA approval of the nivolumab–ipilimumab combination for the treatment of metastatic melanoma, highlight the powerful effect and curative potential of immune checkpoint modulators [67].

Using anti-CTLA4 antibodies, our laboratory was able to show that systemic CTLA-4 blockade leads to long-term survival in 80% of treated mice with established gliomas without eliciting experimental allergic encephalomyelitis. Furthermore, treatment resulted in the recovery of normal $CD4^+$ T cell counts and proliferative capacity and also suppressed increases in $CD4^+$ CD25+ Foxp3+ GITR+ regulatory T cell fractions [68].

The first clinical trials with anti-CTLA4 and anti-PD-1 antibodies have recently begun for the treatment of newly diagnosed and recurrent GBM and are being tested alone or in combination with other checkpoint modulators, small molecules, and mAbs (Table 12.1). In one study comparable to the recent approval of ipilimumab–nivolumab combination for melanoma, anti-CTLA4 and anti-PD-1 are being tested separately or in combination in a three-armed study in patients with newly diagnosed GBM (Table 12.1).

However, even though trials using checkpoint inhibitors and agonists or combinations thereof have shown unprecedented potential for treating various cancer types, only a certain percentage of patients respond and toxicities are significant [3, 69]. The reasons for this are still unclear but are likely to also occur in GBM, emphasizing the need for in-depth diagnosis and hinting at the future of personalized medicine where certain checkpoint modulators or combinations thereof are prescribed based on patient-specific cancer and genetic traits.

Vaccinations for Tumor Control

The goal of vaccination is to sensitize the immune system against a target antigen and thereby elicit a potent and specific immune response that includes a memory response to the target. While vaccination has been used to successfully prevent and eradicate numerous diseases such as polio, tetanus, and typhoid,

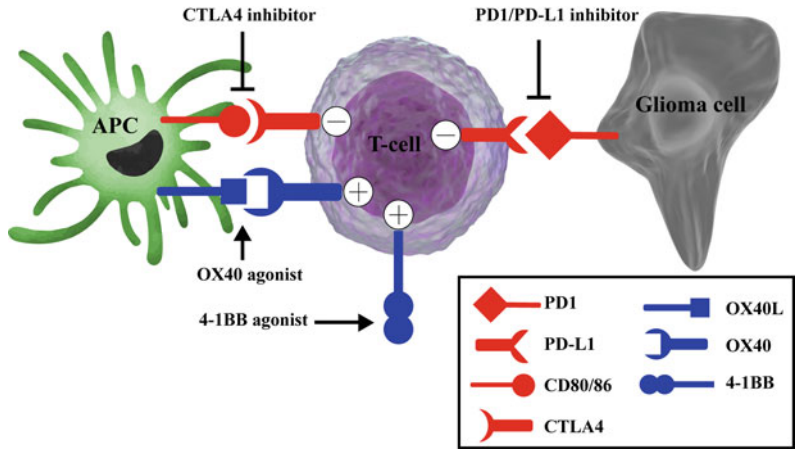

Fig. 12.2 Immune checkpoint modulators. Monoclonal antibodies directed against the immune checkpoint inhibitors CTLA4 and PD1/PD-L1 are used to prevent downregulation of T cell activity and show high potential in GBM. OX40 and 4-1BB are agonistic molecules that, when bound by an antibody, stimulate T cell activity. Both mechanisms lead to a broad upregulation of immune cell activity. *APC*, antigen-presenting cell

anti-tumor vaccinations have not shown the same efficacy and a lot of research is currently ongoing in this field.

Peptides

The major determinant for peptide vaccine-mediated immunogenicity is antigen choice. TAAs, given their expression on normal cells, usually elicit a subdued immune response due to central tolerance. On the other hand, TSAs, given their exclusive presentation on tumor cells, generally elicit a robust immune response similar to the immune response seen against antigens of infectious diseases.

The advantage of TAAs is their high frequency of expression in gliomas, making it possible to give most patients off-the-shelf synthetic tumor antigen peptides. Furthermore, by giving patients a cocktail of peptides, a broader immune response targeting multiple tumor subsets can be elicited. In contrast, TSAs are unique to the tumor and thereby peptides from these antigens may result in a highly tumor-focused immune response.

The mutated protein EGFRvIII, as discussed previously, represents an ideal target for anti-tumor immunotherapy. Our laboratory constructed a 13-amino-acid peptide spanning the vIII mutation and conjugated it to keyhole limpet hemocyanin (KLH). A phase II clinical trial showed that patients with EGFRvIII-positive newly diagnosed GBM, when vaccinated with rindopepimut, the EGFRvIII peptide, had a median survival of 26 months compared with the control historical cohort, which had a median survival of 15 months [70]. These positive results led to the start of a currently ongoing phase III clinical trial with the EGFRvIII peptide vaccine (Table 12.2).

However, given the heterogeneous nature of malignant brain tumor and peptide HLA restrictions, the drawback of single peptide vaccinations is that they may only be effective in a percentage of patients, and in the case of tumor-specific peptides only in the subset of patients expressing the mutated peptide. Trials are ongoing to determine whether combinations of multiple peptides will result in clinically effective peptide vaccination strategies (Table 12.2). Furthermore, increased research on neoantigens, antigens that spontaneously arise in individuals during the course of tumor progression, may lead to personalized solutions in which a patient's tumor is sequenced after resection and peptide vaccinations are constructed based on the mutanome. Even though major challenges remain, such as locating immunogenic mutations and quickly constructing immunogenic peptides, clinical trials employing a personalized peptide pool approach have commenced (Table 12.2).

Whole Tumor Lysate

Whole tumor lysate can be used as a source of antigen and has the advantage of providing a tumor-specific repertoire of all potentially immunogenic epitopes. The rich repertoire of tumor-associated antigens contains epitopes for both CD8+ and CD4+ T cells, which is important as the parallel presentation of MHC Class I and II antigens could result in a stronger anti-tumor response and boost CD8+ T cell memory [71]. The use of tumor lysate and its encompassing antigen repertoire could also eliminate the time-consuming task of discovering strongly immunogenic antigens.

Tumor lysates can either be obtained from autologous tumor cells, which are taken from the patient, or from an allogenic cell line. Autologous tumor cells are only useful in patient-specific anti-tumor immunotherapies while allogenic tumor cells can be stored at cell banks and vaccines can be created en masse at GMP facilities [72]. Given alone, tumor lysates are administered with a strong adjuvant hapten to provoke a strong inflammatory response and increase their immunogenicity. In a murine glioma model, a CpG-tumor lysate vaccine given subcutaneously had a cure rate of up to 55% and showed significantly longer survival times than tumor lysate or CpG alone. Given their potential to be immunosuppressive, an alternative approach, discussed below, is to create dendritic

Table 12.2 Recent clinical trials employing vaccination immunotherapy for high-grade gliomas

NIH clinical trial	Type	Phase	Eligibility criteria	Primary outcome	Dosing regimen	Treatment groups
NCT02510950	Peptide (personalized)	0	Newly diagnosed glioblastoma	Safety and tolerability, feasibility of creating vaccine	Neoantigen-specific long peptide vaccine + poly-ICLC given on cycle 1 day 1 of maintenance TMZ, then on days 3, 5, 8, 15, 22, followed by maintenance on day 22 of subsequent cycles	Single-arm
NCT02454634	Peptide	1	IDH1R132H mutated grade III–IV gliomas	Safety and tolerability (RLT), immunogenicity of IDH1 peptide	20-mer peptide with IDH1(R132H) mutation given 8 times every 2 or 4 weeks	Single-arm
NCT02287428	Peptide	1	MGMT unmethylated, newly diagnosed glioblastoma	# of adverse events, # of patients with >10 actionable peptides	Injections with personalized peptide pool (NeoVax) with 5 priming and 2 boost doses over 7 months	Single-arm
NCT02149225	Peptide (personalized)	1	Newly diagnosed glioblastoma patients	Safety study (# of AEs and SEAs), frequency of CD8 T cell specific for peptides	5–10 peptides (individually assembled, APVAC1) plus Poly-ICLC and GM-CSF given 11 times over 22 weeks. GM-CSF given along first 6 vaccinations. 2 peptides synthesized de novo (APVAC2) and given 6 months after enrollment 8 times within 10 weeks	Single-arm
NCT02049489	DC loaded with peptide antigens	1	Recurrent GBM	Safety study	At least 4 doses of DCs loaded with CD133 peptides given followed by additional vaccines for maintenance	Single-arm
NCT02010606	DC loaded with lysate	1	Newly diagnosed or recurrent GBM	Safety, adverse events, treatment-related toxicities	Autologous DCs loaded with lysate from allogeneic GBM stem-like cell line given once every week for 4 weeks followed by once every 8 weeks	Cohort A: newly diagnosed GBM Cohort B: recurrent GBM
NCT01491893	Virus	1	Recurrent supratentorial GBM	Maximum tolerated dose or optimal dose	Genetically recombinant, non-pathogenic poliovirus:rhinovirus chimera with tumor-specific conditional replication phenotype (PVSRIPO) given directly into tumor during biopsy	Single-arm
NCT01967758	Virus	1	Treated and recurrent WHO	Maximum tolerated dose	Live attenuated strain of *L. monocytogenes* expressing EGFRvII and NY = ESO-1 antigens	Arm I: low dose

(continued)

Table 12.2 (continued)

NIH clinical trial	Type	Phase	Eligibility criteria	Primary outcome	Dosing regimen	Treatment groups
			grade III/IV astrocytomas		(ADU-623) given on day 0, 21, 42, 63 (Arm I: 3E7 cfu, Arm II: 3E8 cfu, Arm III: 3E9 cfu)	Arm II: medium dose Arm III: high dose
NCT02649582	DC loaded with RNA	1/2	Newly diagnosed, histologically verified GBM	Overall survival	WT1 mRNA loaded DC vaccine given weekly for 3 weeks followed by maintenance vaccine on day 21 of every TMZ cycle	Single-arm
NCT01567202	DC loaded with lysate	2	Histologically confirmed GBM	Overall survival	8-10E6 DCs loaded with autogeneic glioma stem-like cell-associated antigens given once a week for 6 weeks	Triple-blind Arm I: DCs Arm II: placebo
NCT02366728	DC loaded with RNA	2	Newly diagnosed GBM	Overall survival	CMV pp65-loaded DC vaccines #1–3 given every two weeks followed by vaccine #4 (only Arms I–II). Td given to all during vaccine #1. Arm III patients receive basiliximab 1 week before vaccine #1–2. Before vaccine #4, Arm I receives unloaded DCs, Arm II–III Td dose	Arm I: unloaded DCs + loaded DCs Arm II: Td + loaded DCs Arm III: Td + loaded DCs + basiliximab
NCT02455557	Peptide	2	Survivin-positive GBM	Progression-free survival	Survivin-mimic peptide vaccine (SurVaxM) 1–2 weeks after chemoradiation followed by doses every 2 weeks (total of 4 doses) followed by doses every 12 weeks	Single-arm
NCT01480479	Peptide	3	Newly diagnosed EGFRvIII-positive GBM	Overall survival	EGFRvIII peptide rindopepimut or placebo given ID two times in month 1 followed by monthly injections	Arm I: rindopepimut Arm II: placebo
NCT00045968	DC loaded with lysate	3	Newly diagnosed GBM	PFS	Autologous dendritic cells pulsed with tumor lysate antigen, called DCVax-L, or autologous PBMCs (placebo) given ID twice daily on days 0, 10, 20, and at weeks 8, 16, 32, 48, 72, 96, and 120	Arm I: DCVax-L Arm II: placebo

AE adverse event; *cfu* colony-forming units; *CMV* cytomegalovirus; *DC* dendritic cell; *GBM* glioblastoma; *ID* intradermal; *lysate* tumor cell lysate; *PBMC* peripheral blood mononuclear cells; *RLT* regimen limiting toxicity; *SAE* serious adverse event; *Td* tetanus toxoid; *TMZ* temozolomide; *PFS* progression-free survival

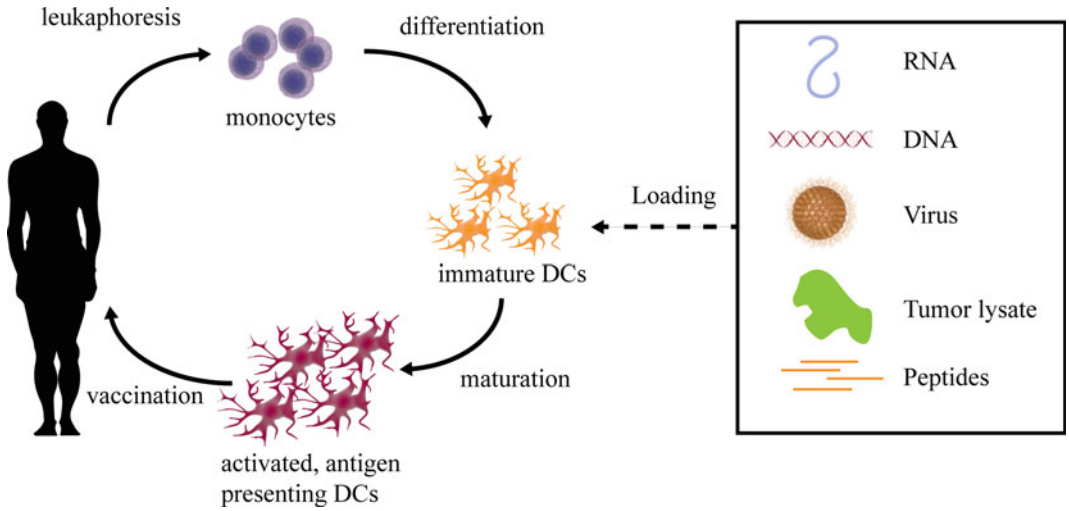

Fig. 12.3 Dendritic cell vaccine production. Patients first undergo leukapheresis to isolate PBMCs, followed by a period of differentiation to obtain immature dendritic cells (DCs). These cells are then loaded with antigens in the form of RNA, DNA, viral vectors, tumor lysate, or peptides. The DCs endogenously process the antigen and present it on their MHC molecules and, after a maturation step, the DCs are reinfused into the patient where they home to the lymph node and activate a tumor-specific immune response

cell vaccinations by pulsing dendritic cells with tumor lysate [73].

Dendritic Cells

Dendritic cells (DCs), with their powerful antigen-presenting function and unique ability to activate naïve T cells, form a crucial link between the innate and adaptive immune system. As sentinel members of the innate immune system, DCs scavenge for foreign antigens (PAMPs) and in response release cytokines. As members of the adaptive immune response, DCs take up pathogenic antigens, process them internally, and present them on their cell surface, thereby activating naïve, effector, and memory T cells and B cells, as well as maintaining tolerance against self-antigens [74]. In fact, DCs are described as the most potent endogenous activators of de novo T cell and B cell responses [75].

DC vaccination in GBM is based on the premise that patient-derived DCs can be generated *ex vivo*, stimulated to present immunogenic antigen, and reinfused into the patient where the cells will activate the adaptive immune response to destroy malignant cells (Fig. 12.3). Tumor-specific stimulation can be achieved by loading DCs with tumor cell lysate, peptides, viral vectors, DNA, or RNA [76–82].

In addition to loading DCs with the optimal tumor antigen, numerous components of the DC vaccine production process are undergoing investigation to produce potent immune responses. DCs can be matured *in vitro* to amplify the immune response using adjuvants or pro-inflammatory molecules. Though the optimal DC maturation is still under investigation, the current "gold standard" is a cytokine cocktail containing GM-CSF, IL-4, TNF-α, IL-1β, IL-6, and, in some instances, prostaglandin E2 (PGE2) [83, 84]. Subsequently, cytokines and chemokines have been used as adjuvants to increase antigen presentation and boost T cell expansion. Specifically, GM-CSF has been the most frequently used adjuvant and has shown efficacy in various systemic cancers and experimental brain tumors [85]. The therapeutic mechanism of GM-CSF involves the paracrine-mediated local release of GM-CSF at the vaccine/tumor antigen

presentation interface and the resulting recruitment and activation of APCs [86]. These APCs consequently prime CD8+ and CD4+ T cells which recognize the tumor antigen, infiltrate the tumor cells, and lead to tumor regression [87].

Our laboratory has shown clinical efficacy in treating GBM patients with mRNA-transfected DCs [88]. RNA-transfected DCs have the major advantage that this approach is applicable to a wide range of patients as RNA can be amplified from a small number of tumor cells, meaning very little tumor sample is needed to prepare the therapy. In terms of safety, stimulating DCs with mRNA poses no risk of integration and is therefore a transient therapy, as compared to viral or DNA vectors [74]. In a recent randomized clinical trial, our group generated a dendritic cell vaccine using pp65 mRNA for treating glioblastoma (NCT00639639). Given its high and specific expression in glioblastoma, this viral antigen is ideal for eliciting a specific tumor response. By pre-conditioning patients with tetanus/diphtheria toxoid, lymph node homing and efficacy of the tumor antigen-specific DCs as well as patient survival was significantly increased [88]. A confirmatory double-blinded clinical trial is now testing the effects of tetanus preconditioning on survival in patients with newly diagnosed GBM (Table 12.2).

Alternatively, DCs can be pulsed with whole tumor lysate, which has a number of (theoretical) advantages over peptide loading, including the availability of the full repertoire of tumor-associated antigens, thereby allowing the DCs to "choose" the immunogenic antigen, and increasing the patient-response rate. Using autologous tumor cell lysate from each patient to load the DCs could represent an important step toward personalized medicine in the treatment of GBM. The DCVax-L vaccine (autologous dendritic cells pulsed with autologous tumor cell lysate) showed a 3-year overall survival rate, 2.5 times the usual period of survival, in a phase I/II clinical trial in newly diagnosed GBM, extended survival by 5 months or more for recurrent GBM, and is currently being tested in a blinded randomized phase III trial (Table 12.2) [89–91].

Considerations for the Future

Despite years of dedicated research, diagnosis with malignant gliomas, especially glioblastoma, remains a death sentence and places a heavy burden upon society. With a median survival of 15–17 months, traditional tumor treatments for GBM are of limited use and the need for directed therapy is dire. Recent developments in the field of immunotherapy, such as the peptide vaccine rindopepimut and the dendritic cell vaccine DCVax-L, have seen significant increases in overall survival and give hope that immunotherapy will play a major role in the treatment of malignant gliomas in the upcoming years.

The recent stunning success of checkpoint modulators, particularly the FDA approval of nivolumab–ipilimumab combination for treating metastatic melanoma, further validates immunotherapeutic approaches and is driving a number of ongoing clinical trials testing checkpoint inhibitors alone or in combination in high-grade glioma patients. However, issues such as serious toxicities and the large fraction of non-responders seen in other tumors will need to be addressed in glioma treatment.

Ultimately, long-term treatment of malignant gliomas may require approaches that combine traditional cancer therapies with various immunotherapeutics that serve to activate a tumor-specific immune response and maintain a tumor-suppressive milieu. The optimal combination of treatments could include peptides, mAbs, checkpoint modulators, and loaded DCs as well as activated immune cells and viral vectors and may require patient-specific personalization based on glioma subgroups, heterogeneity profiling, genetic sequencing, and current immune cell counts. Clinical trials testing such extensive combination approaches will need to

use high-powered multi-armed approaches to discern therapeutic efficacy.

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