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REVIEW

Improving vaccine efficacy against malignant glioma

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ABSTRACT

The effective treatment of adult and pediatric malignant glioma is a significant clinical challenge. In adults, glioblastoma (GBM) accounts for the majority of malignant glioma diagnoses with a median survival of 14.6 mo. In children, malignant glioma accounts for 20% of primary CNS tumors with a median survival of less than 1 y. Here, we discuss vaccine treatment for children diagnosed with malignant glioma, through targeting EphA2, IL-13Rα2 and/or histone H3 K27M, while in adults, treatments with RINTEGA, Prophage Series G-100 and dendritic cells are explored. We conclude by proposing new strategies that are built on current vaccine technologies and improved upon with novel combinatorial approaches.

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Introduction

Malignant glioma

Primary brain tumors have an annual incidence of ~5 in 100,000 adults.[1-3] Within the United States, it is estimated that there will be 24,790 newly diagnosed malignant brain cancer patients in the year 2016.[4] Glioblastoma (GBM) is the most common primary malignant central nervous system (CNS) tumor in adults, accounting for ~54% of all malignant glioma diagnoses.[5,6] Despite the current standard of care regimen including maximum surgical resection, radiotherapy (RT) and chemotherapy, median overall survival (OS) remains at 14.6 mo with less than 26% of patients surviving at 2 y post-diagnosis.[7-9] In the absence of therapy, OS is limited to 30–35 weeks.[10-13] The poor outcome for GBM patients is largely due to the molecular and cellular heterogeneity of the cancer, which equips the tumor with multiple strategies for adapting to and overcoming the effects of therapy.[14]

Pediatric high-grade glioma (HGG) is clinically and biologically distinct from adult glioma. However, similar to adult GBM, these tumors are a major contributor toward cancer-related morbidity and mortality in infants, children, and adolescents, with long-term survival rates of only 10–15%.[15] Pediatric HGG is found throughout the CNS, with those tumors localizing to the ventral pons of the brainstem possessing a particularly devastating prognosis. Commonly referred to as diffuse intrinsic pontine glioma (DIPG), these highly malignant tumors primarily affect young children with a peak incidence at 6 y of age and possess a high mortality rate when compared among all childhood solid cancers. Children diagnosed with DIPG possess a median survival of 9 mo and virtually all patients die within 2 y. Immunotherapy has been proposed as an approach for treating both pediatric and adult glioma. Here, we review targeted vaccination approaches for these tumors and discuss strategies for enhancing future therapeutic efficacy.

Immunosuppression

While the cellular composition and molecular profile of GBM varies, the immunosuppressive microenvironment is a consistent feature of these tumors. The accumulation of tumor-infiltrating myeloid-derived suppressor cells [16,17] and regulatory T cells (Treg; $CD4^+CD25^+FoxP3^+$),[18,19] the presence of IDO1,[20,21] interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), collectively contribute to the suppression of normal tumor surveillance.[22-24] Additionally, PD-L1, a ligand highly expressed by GBM-infiltrating macrophages [25] and GBM cells,[26] interacts with PD-1 on cytotoxic T cells, further contributing toward immunoevasion of antitumor immunity. Similarly, CTLA-4, a molecule constitutively expressed by Tregs, suppresses T cell cytotoxic activity,[27] and is another mediator of immunotolerance.[28,29] Beyond the immediate microenvironment of GBM, systemic lymphopenia is the result of cytotoxic therapy and coincident with a decreased expression of HLA-ABC, HLA-DR, CD86, ICAM-1 and TNFRII on peripheral blood monocytes.[30]

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Decreased MHC expression on antigen-presenting cells (APCs) in lymphopenic patients further limits GBM-specific T cell activity and function. Therefore, the immunosuppressive properties of malignant glioma may well act synergistically with cytotoxic therapies to compromise the patient's immune-mediated antitumor response, with these combined effects providing additional impetus for testing numerous immune checkpoint blockade strategies in ongoing clinical trials.[31]

Much less is known about immunosuppressive mechanisms underlying malignant glioma in children, but this is an active area of preclinical research, currently ongoing within and external to our group. With advanced techniques that spare critical brain function during tumor biopsy becoming more common, in addition to the preclinical models that have recently been developed, more information about the novel immunosuppressive nature of pediatric HGG is likely to significantly increase during the next several years.

Antigenic targets

Glioma expresses a number of antigenic targets, including tumor-associated antigens (TAAs) that are not a direct result of mutagenic events, such as interleukin-13 receptor α 2 (IL-13Rα2). Additionally, they can also express tumor-specific antigens (TSAs) that are the result of mutant protein expression, such as epidermal growth factor receptor variant III (EGFRvIII). The select overexpression of wild-type epitopes, as well as the unique expression of mutant epitopes, has provided the solid foundation for vaccination approaches aimed at malignant glioma treatment.[32-51] Recent work has helped distinguish pediatric HGG from adult GBM by characterizing unique epigenetic alterations that are exclusive to brain tumors in children.[35] One classic example is the Lys27Met (K27M) missense mutation in genes encoding histone 3 isoforms, often found in midline malignant glioma and in up to 80% of DIPG patients [36-38]; providing a novel tumor-specific vaccination target.

Vaccines for treatment of adult malignant glioma

RINTEGA/Rindopepimut

Whereas GBM is known to express several mutant proteins, EGFRvIII is the only TSA currently being investigated as a vaccine target in patients diagnosed with GBM (Fig. 1). EGFRvIII is the result of an in-frame deletion of 801 nucleotides (exons 2–7) of the wild-type gene. The mutation manifests as a shortened protein containing a novel glycine residue at the exon 1–8 in-frame junction.[52] The mutant epitope is presented in the extracellular space, with the transmembrane and cytoplasmic portions of the altered receptor left intact. The occurrence of EGFRvIII in GBM is almost always in the context of corresponding mutant gene amplification, resulting in a high level of expression.[53] In a preclinical GBM model, the ectopic expression of EGFRvIII caused increased tumor growth, following subcutaneous and intracranial engraftment of modified cells.[54] Therapeutically, mice bearing established tumors and treated with the combination of rindopepimut, which consists of the EGFRvIII junction sequence conjugated to keyhole limpet hemocyanin (KLH) and complete Freund's adjuvant, showed an average survival increase of >120 d ($p = 0.014$): a 173% gain when compared to vehicle-treated mice.[55] Clinically, the presence of EGFRvIII is independently prognostic for decreased OS[56-59] Accordingly, Phase I and II clinical trials treating newly diagnosed GBM patients with RINTEGA, the tradename for rindopepimut, found an increase in median OS when compared to historical controls and was well tolerated (Table 1).[60,61] ACTIII (n = 65), the largest of the Phase II studies utilizing RINTEGA, demonstrated a PFS of 12.3 mo and median OS of 24.6 mo in GBM patients.[62] Recently, ACTIV, the first Phase III study investigating the benefits of RINTEGA in newly diagnosed GBM patients, was ended in accordance with a recommendation by the trial's independent Data Safety and Monitoring Board which concluded that the study would not reach statistical significance for OS.[63] Notably, 43% of vaccine-treated patients showed evidence of a humoral response to EGFRvIII. Furthermore, at the time of tumor regrowth following treatment, 82% of the recurrent GBM demonstrated loss of EGFRvIII expression, suggesting that EGFRvIII-positive GBM evades the antitumor-mediated effects of RINTEGA by suppressing the expression of EGFRvIII.[59]

Prophage series G-100/HSPPC-96

Prophage series G-100 is a clinical vaccine utilizing heat shock protein peptide complex 96 (HSPPC-96). The HSPPC-96 treatment strategy relies on heat shock protein (HSP) family member gp96 interactions with intracellular peptides in tumor and tumor-associated APCs. In 1986, Srivastava *et al.*, demonstrated that tumor-derived gp96 facilitates intrinsic immunogenicity as a proof-of-concept vaccine in a model of fibrosarcoma [64] leading to priming of $CD8^+$ [65] and $CD4^+$ T cells [66] in wild-type Balb/c mice as a result of APC presentation of tumor-specific peptides by MHC I and II, respectively.[67] In clinical trials for treating GBM, HSPPC-96-peptide complex is isolated from a patient's tumor, and then used as an autologous vaccine in treating the same patient.[68] Based on the ability to induce a presumably multi-epitope specific immune response against a patient's resected tumor, HSPPC-96 vaccination is considered to be a form of personalized medicine.[69] A preclinical model for HSPPC-96 vaccination in GBM does not yet exist, although this is an active area of investigation by our group.

A notable limitation to the HSPPC-96 approach for treating GBM is the requirement for a minimum of 7 g resected tumor tissue. Therefore, ~35–40% of all GBM patients do not qualify for autologous HSPPC-96 vaccination due to insufficient resected tumor (Table 2).[70,71] Nonetheless, a Phase II study of newly diagnosed GBM patients (n = 46), whose resected tumors were of appropriate mass, received Prophage Series G-100 and experienced PFS of 17.8 mo and median OS of 23.3 mo: both representing substantial improvements when compared to historical control values.[72] Moreover, a phase II trial of recurrent GBM patients treated with HSPPC-96 yielded results showing PFS of 19.1 weeks and median OS of 42.6 weeks (n = 46). These values also represent substantial increases relative to historical controls (PFS of 9 weeks and an OS of 35 weeks). Interestingly, patients diagnosed with lymphopenia

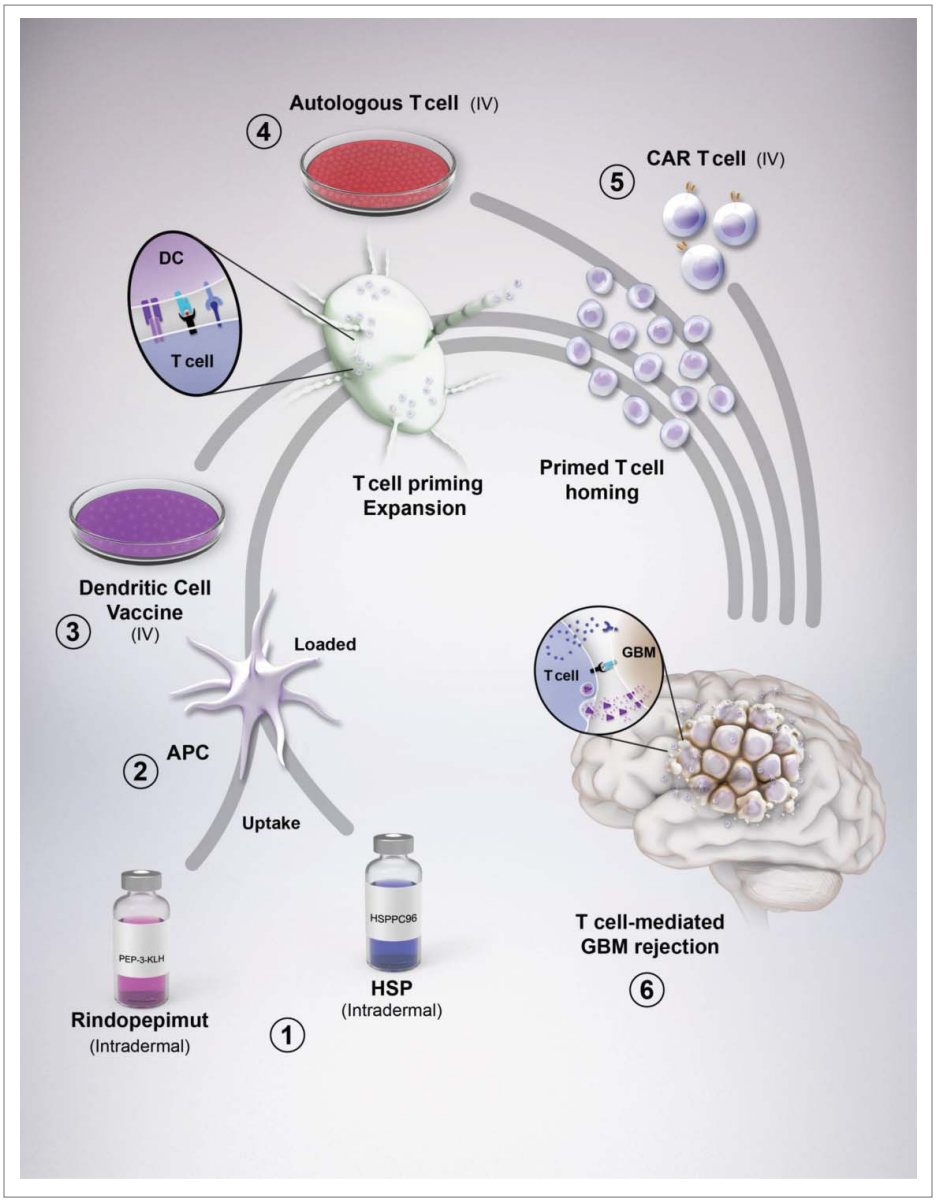

Figure 1. Glioblastoma (GBM) vaccines and their interaction with immunity. (1) Rindopepimut (RINTEGA), a synthetic peptide vaccine targeted at the EGFRvIII mutation, and HSPPC-96 (Prophage), an autologous-derived complex consisting of heat shock proteins complexed with GBM antigens, are intradermally injected for uptake by resident dendritic cells (DC). (2) The vaccines are co-administered with adjuvants, such as GM-CSF and keyhole limpet hemocyanin (with respect to RINTEGA) for stimulating DC uptake, antigen processing and upregulation of costimulatory molecules. (3) Peripheral blood mononuclear cells can be isolated from patient blood (not shown), expanded in culture, primed with autologous patient-derived GBM tumor lysate, followed by intravenous transfer back into the patient, bypassing the need for dermal injection of tumor antigens. Regardless of whether DCs are primed and loaded in culture, or a cutaneous route, DC drain to lymphoid tissue for subsequent T cell activation and expansion. (4) Similar to PBMC isolation for DC preparation, autologous T cells can also be isolated from this pool. These cells can be (re-)stimulated with plate-bound anti-human CD3ε, without anti-CD28, as to only (re-)engage the experience T cells that might respond to TAAs. This CD3ε-targeted stimulation can be further boosted by adding in cytokines that favor a proinflammatory/antitumor T cell subset such as IL-2, IL-12 and IL-15. (5) An alternative to simple autologous T cell isolation, (re-)priming and expansion, is by engineering the T cell to be highly specific to a GBM-expressed antigen, in the form of a chimeric antigen receptor-expressing T cell. This step provides a high level of stringency and targeting for all T cell adoptively transferred into the GBM patient. Ultimately, the goal of all vaccines is to eventually cause GBM-specific T cells to infiltrate the tumor and elicit immune-mediated rejection.

at the time of vaccination were associated with a poor survival outcome.[69]

Dendritic cells (DCs)

DCs are immunological sentinels that respond to tissue injury, inflammatory stimuli and/or changes of cellular homeostasis, such as hypoxia, acidity or osmolarity. DCs internalize, process and present antigens to T cells that facilitate epitope-specific immune responses.[73,74] DCs can be expanded *in vitro*, for subsequent administration to cancer patients, using a variety of methods that include the isolation of circulating monocytes or bone-marrow-derived precursor cells that can differentiate, *ex vivo*, and become DCs.[75-79] Pre-clinically, DCs treated with murine GL261 glioma lysates have been administered to C57BL6 mice, at one week post-intracranial injection of GL261 cells, with DC administration resulting in a reduction of tumor growth: 78.5 mm^3 (control) to 39.9 mm^3. An alternative approach has utilized DCs treated with a tumor extract-cationic liposome complex (synthetic small unilamellar vesicles), which

Table 1. Clinical efficacy of vaccines for patients with newly diagnosed adult GBM or pediatric DIPG. *Trial closed ahead of stated objectives.

Newly diagnosed adult GBM							
Therapeutic mediator(s)	Percent eligible	Trial (Phase)		n	PFS (weeks)	OS (weeks)	References
Current standard (resection, radiation, temodar)					29.6	62.6	
RINTEGA (Rindopepimut)	27–67	ACTII (II)		22	65.6	104.6	[61]
		ACTIII (II)		65	52.7	105.4	NCT00458601
		ACTIVATE (II)		18	60.9	105.4	NCT00643097
		ACTIV (III)	Control Arm	700		91.7	[119]
			Rintega Arm			88.6	
Prophage series G-100 (HSPPC-96)	60–65	Prophage series G-100 (II)		46	76.3	99.9	NCT00905060
DCs	60–65	Tumor lysate (I)		12	66.4	100.2	[82]
	100	RT and TMZ with DCs (PGE2 and TNFα) (I)		11	40.7	120	[85]
	60–65	ICT-107 (I)		16	72.4	164.6	[83]
		ICT-107 (II)	Placebo	43	9	16.7	NCT01280552
			ICT-107	81	11.2	18.3	
T cells	100	CAR T cell (I)			Ongoing	Ongoing	NCT01454596
Recurrent adult GBM							
Current standard (currently no effective therapy)					9	35	
RINTEGA (Rindopepimut)	27–67	ReACT			Ongoing	Ongoing	NCT01498328
Prophage series G-100	60–65	Phase I		12		47	[120]
		Phase II		41	19.1	42.6	[69]
Pediatric malignant gliomas							
Current standard (radiation)					13–26	30.8–60.8	
Peptide based		Phase I		14		55.2	[86]
DC		Phase I		33	19	59	[88]
		Autologous lysate pulsed DCs (I)		3		144.7	[87]
HSPPC-96		Phase I			Ongoing	Ongoing	NCT02722512

results in a dramatic decrease in tumor volume relative to the control group of mice ($p < 0.01$).[80] Similarly, in a rat glioma model, vaccination with bone-marrow-derived DCs, pulsed with acid-eluted peptides from syngeneic cells, results in an increased median OS from 16 (control) to 35 d ($p = 0.027$).[81]

Clinically, newly diagnosed GBM patients (n = 12) treated with autologous DCs and pulsed with acid-eluted tumor peptides demonstrates a PFS of 15.5 mo and median OS of 23.4 mo. In 4/12 patients, survival is >30 mo and tumors isolated at recurrence show robust CD3 T cell infiltration when compared to corresponding untreated tumor obtained at the time of initial surgery. In contrast, 4 of 12 patients that succumbed to tumor within 12 mo post-treatment initiation show decreased T cell infiltration of recurrent tumor, suggesting that T cell exclusion was an important determinant of therapeutic outcome.[82] Another Phase I trial studying newly diagnosed GBM patients (n = 16) treated with DCs pulsed with HER2/neu, TRP-2, AIM-2, MAGE1 and IL13Rα2 antigens (ICT-107; Immunocellular Therapeutics Ltd.) yielded results showing PFS of 16.9 mo and median OS of 38.4 mo.[83] In a recent randomized Phase II study of ICT-107 treatment in newly diagnosed GBM patients (n = 124), median PFS is 11.2 mo and median OS is 18.3 mo when compared to a PFS and OS of 9 mo ($p = 0.01$) and 16.7 mo, respectively, in patients treated with control dendritic cells.[84] A Phase III study for ICT-107 is currently recruiting patients (NCT02546102). In yet another Phase II trial, GBM patients (n = 11) treated with radiation and temozolomide (TMZ), followed by vaccination with autologous tumor lysate-loaded DCs primed with PGE_2 and TNF-α had a PFS of 9.5 mo and median OS of 28 mo. The frequency of

Table 2. Factors that limit patient selection for vaccine therapy.

Therapeutic mediators	Vaccine	Limiting factors

RINTEGA (Rindopepimut)	Synthetic peptide	• Requires EGFRvIII expression
		  • Expressed in 27–67% of GBMs
		  • Leads to EGFRvIII negative recurrent GBM[121]

Prophage series G-100 (HSPPC-96)	Tumor lysate isolation	• Requires 7 g of patient-resected tumor
		  • Not all patients are surgical candidates
		  • Not all GBM tumors are large enough for vaccine production[120]

DCs	Tumor-lysate pulsed DCs	• Patient must be a surgical candidate • Normal bone marrow function • 2 weeks without radiation therapy[82]
	Lysate-pulsed DCs (PGE2 and TNFα)	• Patient must be a surgical candidate • Yield must be $\geq 8 \times 10^7$ tumor cells • Adequate hepatic and renal function[85]
	Synthetic peptide-pulsed DCs	• Gross total resection >95% • Presence of at least one of six antigens[83]
T Cells	CAR T cells	• Requires expression of novel antigen[122] • In this case EGFRvIII

$CD4^+$ T cells in post-vaccination tumor tissue was significantly increased ($p = 0.004$) relative to pre-vaccination, whereas the frequency of $CD8^+$ T cells was not significantly changed.[85] Notably, a number of Phase II DC vaccine trials are ongoing, including studies whereby DCs are treated with: autogenic glioma stem-like cells (A2B5+) (NCT01567202), CMV RNA plus tetanus-diptheria toxoid (NCT02465268) and autologous tumor lysate plus resiquimod or adjuvant poly-ICLC (NCT01204684).

Vaccines for treatment of pediatric malignant glioma

Relative to vaccine attempts in the setting of adult GBM, analogous pursuits have been modest with respect to treating children diagnosed with malignant glioma. In a Phase I trial of newly diagnosed DIPG (n = 26), a peptide vaccine against the glioma-associated antigens, EphA2, IL-13Rα2 and survivin were targeted. In addition to safety aspects of the study, which were satisfactory in avoiding grade III or higher systemic toxicities, patients had an OS of 55.2 weeks, representing a substantial increase over historical control levels of 39–43 weeks. Inclusion in this trial required patients with HLA-A2-positive status and minimal or no dexamethasone usage at the time of enrollment.[86] In a separate Phase I trial of newly diagnosed patients with HGG between the ages of 1 and 18, autologous tumor lysate-pulsed DCs were generated for 3/9 enrolled patients with 2 of the DC-treated individuals still alive at 40 and 51 mo post-surgery, respectively.[87] Another Phase I trial using DCs treated with tumor lysate in 33 malignant glioma patients showed an average PFS of 19 weeks and OS of 59 weeks, with 7 patients surviving at the time of publication.[88] A Phase I study utilizing the HSPPC-96 vaccine for treatment of pediatric malignant glioma recently opened at the Ann and Robert H. Lurie Children's Hospital of Chicago (NCT02722512). Also notable is an effort to leverage the presence of H3K27M mutations found in the high percentage of midline malignant glioma cases in the soon to open H3K27M peptide vaccine trial (S. Mueller, personal communication). Although no Phase II studies have reported results using vaccines in pediatric patients, preliminary results that address safety and tolerability indicate a high level of feasibility in the pediatric cohort with HGG.

Improving vaccine efficacy

Combinatorial approaches

Whereas vaccines aim to induce tumor-specific immune responses, effective immunotherapy against cancer requires the co-treatment against tumor-induced immune evasion. In patients diagnosed with GBM, as well as other cancers, spontaneous T cell infiltration has been associated with improved survival.[89-94] However, the basis for this relationship has been difficult to describe comprehensively. One possibility is that the necrotic release of DNA from tumor cells leads to activation of the stimulator of interferon genes (STING) pathway, providing a mechanism for T cell recruitment to tumor.[95] However, in malignancies with potent and active immunoevasive mechanisms, T cell infiltration, alone, is unlikely to change patient outcome.[96] New strategies that engage STING, while simultaneously inhibiting a tumor's immunosuppressive activity, may help to recruit vaccine-conditioned cytotoxic T cells from the periphery to CNS, thereby promoting more effective tumor rejection that results in greater patient survival.

Future clinical studies should be designed to provide patients with multiple therapies to address the immunosuppressive phenotype present in adult GBM and pediatric HGG, while also aiming to improve T cell infiltration and T-cell-mediated killing of tumor cells. Although the optimal timing for administration of each treatment type (RT, chemotherapy, immunotherapy, vaccination, etc.) still requires further investigation. As shown in Fig. 2, it may be beneficial to treat malignant glioma with radio-/chemotherapy to (1) release TSA, (2) increase inflammatory cues responsible for immune cell recruitment and (3) facilitate the signals required for APC uptake and maturation. Therefore, vaccination in combination with standard of care therapies may promote a more effective antitumor T cell response with the benefit of improved survival.

Adoptive T cell therapy

An additional immunotherapeutic approach that negates the problems associated with suboptimal T cell activation in patients, is the *ex vivo* preparation of activated autologous T cells. Similarly, T cells can be engineered to express chimeric antigen receptors (CAR) specific to tumor antigens, while co-expressing genes that confer resistance to tumor-induced immune inhibitory signals.[97] One such approach involves the fusion of intracellular γ or ζ subunits of the immunoglobulin or T-cell receptor (TCR) to the variable domain of the high-affinity monoclonal antibody, specific to the TAA.[98] This strategy facilitates T cell activation through interaction of the chimeric TCR with the antigen on the surface of the tumor cell, overcoming the T cell's inability to recognize GBM cells with insufficient levels of MHC I/II for effective antigen presentation.[99] Given the ability to rapidly generate CAR T cells in ~2 weeks, preparation of adequate GBM-specific T cell levels can be achieved within reasonable time for therapeutic utilization.[100] Currently, an ongoing clinical trial evaluating the safety and PFS in newly diagnosed GBM patients treated with CAR T cells engineered to target EGFRvIII has been announced but is not yet recruiting patients. (NCT02664363)

A novel strategy for generating high-affinity tumor-reactive T cells against autologous patient malignancy utilizes humanized mice. These mice gain their name by combining severely immunodeficient NOD-SCID-IL-2Rγ^{null} (NSG) hosts, modified for constitutive expression of human stem cell factor (SCF), granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-3 (IL-3) (SGM3) transgenes, with engrafted human fetal thymus and fetal liver-derived hematopoietic stem cells (BLT).[101-103] These mice support the reconstitution of a human immune system [104] and can be used as hosts for patient tumor and immune system engraftment, followed by immune checkpoint blockade (anti-human CTLA-4, PD-(L)1 and/or IDO1 inhibition) to activate and expand a tumor-specific T cell response. Although current studies are aimed at optimizing mouse models for human cancers, in principle, memory lymphocytes could be isolated from the systemic immune cell repertoire of these mice, expanded *in vitro*, and adoptively transferred back into the patient for therapeutic benefit. Although it is possible that select T cells may also respond

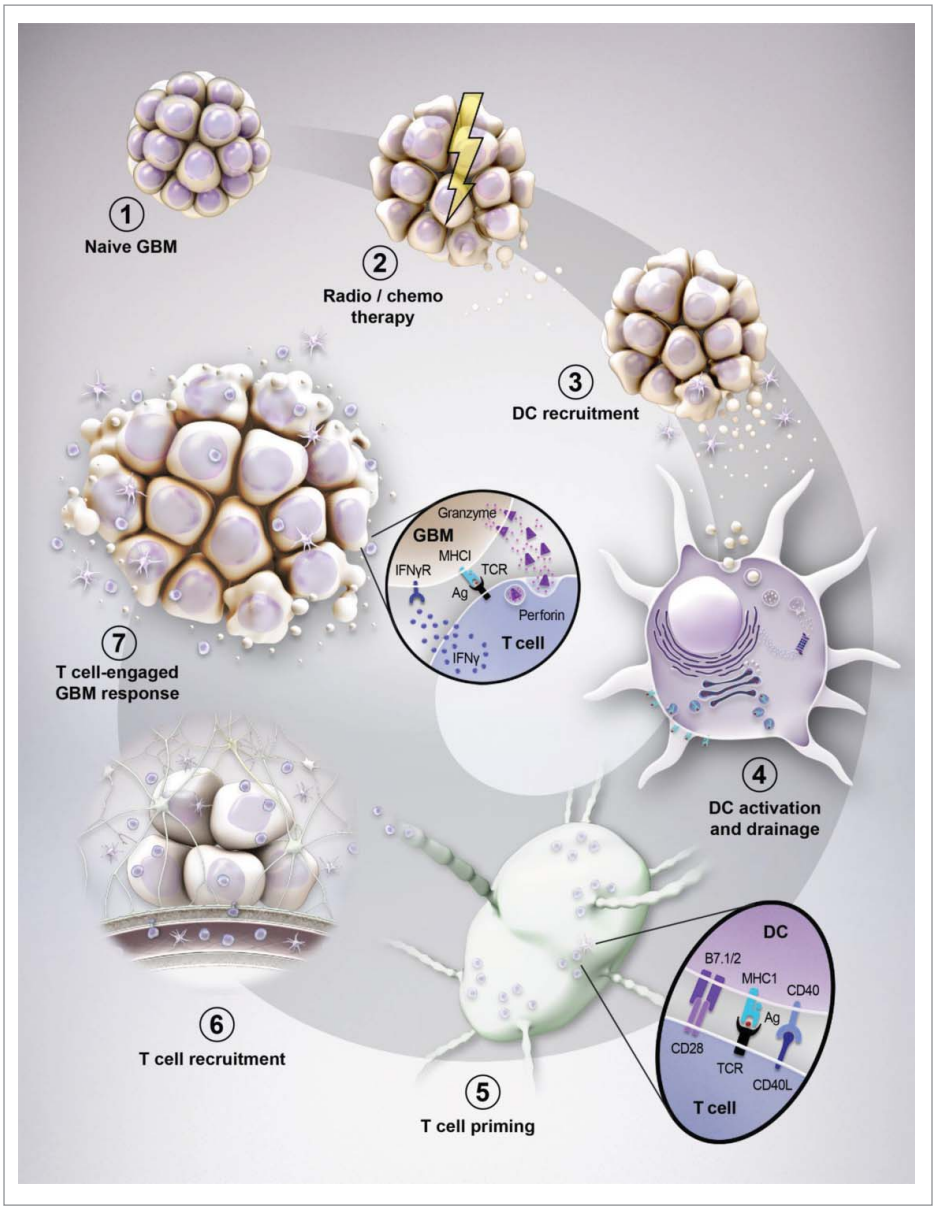

Figure 2. Evolution of brain tumor immunity. (1) The naive (newly diagnosed) brain tumor flourishes in the immunosuppressive environment of the central nervous system and is relatively non-immunogenic. (2) Standard of care for patients with brain tumors causes cancer cell death and the subsequent release of antigens. (3) The inflammation caused by cancer cell death triggers the recruitment of dendritic cells to the brain tumor microenvironment. (4) CD8α dendritic cells (DC) engulf the cancer-associated antigens and utilize the cross-presentation pathway to facilitate the loading of those epitopes onto major histocompatibility complex (MHC) I molecules. The CD8α-loaded DC subsequently emigrate out of the tumor microenvironment while simultaneously increasing costimulatory molecules that facilitate the future productive interaction(s) of naive $CD8^+$ (and $CD4^+$ T cells through MHCII) T cells. (5) After immigration into the lymph node, DC-mediated T cell priming and expansion occurs through MHC/antigen (Ag) expressed by DC and a high affinity T-cell receptor (TCR) expressed by T cells. (6) T cells that are now activated and specific to tumor-associated antigens then emigrate from the lymphoid tissue, back into the circulation. (7) To successfully penetrate the brain tumor, T cells must first come into contact with selectins and integrins (not shown) that facilitate the attachment to inflamed endothelium. Upon successful adhesion, T cells extravasate through the endothelial basement membrane, followed by the perivascular space and eventually through the parenchymal basement membrane whereby they can now come into direct contact with the CNS-resident tumor cells. (8) Productive T cell-mediated GBM rejection occurs when lymphocytes are reactivated by (re-)stimulation of T cell-expressed TCR with MHC/Ag expressed by brain tumor cells. This interaction facilitates the robust production and release of proinflammatory, interferon-gamma (IFNγ), in addition to the release of the pore forming complex, perforin, which facilitates the passive transfection of granzyme molecules (serine proteases) that cleave tumor-expressing procaspases into active molecules that trigger apoptosis.

to mouse antigens, the predicted multiclonality of the T cell response to human antigens presented by human MHC is expected to supersede those T cells not directed toward relevant targets. Also, it is expected that mouse antigens will not be expressed in human patients, further diminishing this concern. However, these considerations will be necessary to address, should a humanized mouse bearing autologous immune system and tumor be utilized in this regard. This highly novel approach would also be considered an adaptation to, 'personalized medicine'.

Conclusion

Early vaccine-based clinical trials have demonstrated promising results, though questions and concerns remain with respect to the durability of therapeutic efficacy and ultimate benefit from

such cancer treatments. Recent data reporting disappointing results from the Phase III study of RINTEGA highlights the necessity for cautious optimism of early phase clinical trials that are limited to single arm approaches with small numbers of enrolled patients. This design has several restrictions that include a possible placebo effect, as well as the evolving standard of care that may incrementally increase in efficacy over time.[105]

Conceptually, an attractive antigenic target for GBM treatment is the human cytomegalovirus (CMV), first reported to be expressed by GBM in 2002.[48] Since that initial study, a growing body of literature implicating CMV as a factor present in GBM has grown substantially.[49-51] Notably, a Phase I clinical assessment of CMV-specific adoptive T cell therapy demonstrated PFS during the study period (175, 462, 1010, and 1447 d) in 4/10 GBM patients.[106] Additionally, a Phase I randomized trial in newly diagnosed GBM (n = 12) whereby the vaccine site was pre-conditioned with tetanus/diphtheria (Td) toxoid and then vaccinated with CMV pp65 RNA-pulsed DCs, showed a median PFS of 10.8 mo and a median OS of 18.5 mo; similar to patients treated with standard of care in this study.[107]

Similar to targeting EGFRvIII, independent groups have developed vaccines against mutant isocitrate dehydrogenase 1 (mIDH1).[108,109] This mutation occurs in 12% of total GBM patients, but is expressed prolifically in low-grade glioma (II and III). Interestingly, the presence of mIDH1 expression is associated with a favorable prognosis of GBM patients with a median OS of 3.8 y when compared to 1.1 y for GBM patients presenting with wild-type IDH1 ($p < 0.001$).[110] Given that mIDH1 expression is associated with extended survival in GBM patients, the rationale for targeting this mutation and potentially selecting for a more aggressive GBM phenotype should be thoroughly considered.

In addition to targeting mutant peptide sequences, it is important to consider that cancer cells possess altered cellular surfaces with distinct carbohydrate modifications of cell membrane components.[111-113] One glycosylation pattern, O-linked N-acetylgalactosamine (Tn antigen), has been shown to be selectively expressed in GBM,[114] breast cancer,[115] metastatic melanoma,[116] as well as stomach, colon and pancreatic cancer.[117] Brooks *et al.* demonstrated that targeting this carbohydrate moiety can result in striking tumor specificity.[118] Further study of unique GBM posttranslational modifications that occur on the surface of the tumor cells may well reveal additional targets with vaccination potential.

There are some aspects of vaccine therapy which are unique to pediatric HGG. While adult GBM most often develops in the cerebral hemispheres, lending to neurosurgeons' ability to remove a significant amount of tumor en bloc for vaccine development, pediatric malignant glioma is often unresectable and only small amounts of tumor are possible to obtain during biopsy. The currently open HSPPC-96 vaccine trial will help to clarify the minimum of amount of tumor necessary for suitable vaccine development. Efforts directed against known TAAs that are available 'off-the-shelf' are attractive for pediatric patients. However, identification of appropriate antigens is still a challenge given the molecularly heterogeneity of histologically similar pediatric HGG and the relatively low mutational rate, when compared to adult GBM.

As vaccination therapies for patients with malignant glioma continue to be tested and refined, discussion(s) of how best to integrate standard-of-care therapy and other novel approaches will likely dominate in the future. The efficacy of combinatorial multi-modal treatments that include vaccine-induced immune responses will be influenced, in-part, by the timing of each administered modality. For instance, concurrent cytotoxic and vaccine regimens may substantially boost overall immune-mediated efficacy and OS, but at the cost of inducing significant and long-lasting adverse side effects in patients. Thus, one of the most significant hurdles going forward is how best to minimize immunotherapeutic-induced toxicity, without disabling therapeutic efficacy and immunological responsiveness. Toward this goal, increasing the study of humanized immunocompetent mice bearing HLA-matched intracranial adult and pediatric malignant glioma may prove especially informative.

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No potential conflicts of interest were disclosed.

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