Immunotherapy for Neuro-Oncology

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Abstract

Immunotherapy has changed the landscape of treatment of many solid and hematological malignancies and is at the forefront of cancer breakthroughs. Several circumstances unique to the central nervous system (CNS) such as limited space for an inflammatory response, difficulties with repeated sampling, corticosteroid use for management of cerebral edema, and immunosuppressive mechanisms within the tumor and brain parenchyma have posed challenges in clinical development of immunotherapy for intracranial tumors. Nonetheless, the success of immunotherapy in brain metastases (BMs) from solid cancers such as melanoma and non-small cell lung cancer (NSCLC) proves that the CNS is not an immune-privileged organ and is capable of initiating and regulating immune responses that lead to tumor control. However, the development of immunotherapeutics for the most malignant primary brain tumor, glioblastoma (GBM), has been challenging due to systemic and profound tumor-mediated immunosuppression unique to GBM, intratumoral and intertumoral heterogeneity, low mutation burden, and lack of stably expressed clonal antigens. Here, we review recent advances in the field of immunotherapy for neuro-oncology with a focus on BM and GBM.

Keywords

Glioblastoma · Brain metastases · Checkpoint inhibitors · Immunosuppressive macrophages · Immunotherapy combinations · GBM immune microenvironment · Tumor mutational load · Tumor-infiltrating lymphocytes · Cell therapy · Peptide vaccines · Cell vaccines · Oncolytic viral therapies

Immunosurveillance in the CNS

Early preclinical experiments had demonstrated immunity to skin homografts in mouse brain, cultivating the belief that CNS is an immune-privileged organ [1]. Later, through characterization of immune reactions in multiple sclerosis and encephalitis, the immunologic activity of CNS became apparent [2]. It was only recently discovered that T-cells exist and enter the CNS via lymphatic vessels lining the dural sinuses that connect the CSF to deep cervical lymph nodes [3]. CNS antigens are presented to

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T-cells by antigen-presenting cells (APCs) of the CNS (microglia and dendritic cells) that return to the CNS via perivascular system. The discovery of CNS lymphatic system in the era of immunotherapy advances in cancer was timely and has changed the long-held belief that the CNS is an immune-privileged organ. In addition to trafficking CNS lymphatics, immune cells are able to communicate to the brain parenchyma through a disrupted blood–brain barrier (BBB) as evidenced by gadolinium enhancement on T1-weighted MRI in tumors such as BM and high-grade primary brain tumors.

Immunotherapy for Brain Metastasis

BM is the most common form of intracranial malignancy, and its incidence is on the rise as therapeutic advances are controlling systemic disease leading to longer patient survival [4]. BM occurs as much as ten times more frequently than primary brain tumors occurring in 9–10% of all cancer diagnoses [5]. The incidence has been estimated to be between 11.2 and 14.3 per 100,000 [5]. The three most common primary cancers associated with brain metastasis are lung (20–56%), breast (5–20%), and melanoma (7–16%) [6]. Promising data are emerging on the benefit of checkpoint inhibitors (CPIs) in melanoma and NSCLC brain metastasis [7, 8] suggesting that CNS location of the tumor does not preclude the clinical efficacy of immunotherapy.

CPIs have been at the forefront of immunotherapy advances for the treatment of cancer, and their FDA approvals are on the rise [9]. CPIs are antibodies that bind to T-cell inhibitory signals on T-cells, APC, and tumor cells and stimulate profound immune responses against tumors by activating previously exhausted T-cells and maintaining their effector function. The most widely used CPIs include monoclonal antibodies against CTLA-4 and PD-1 (expressed on T-cells), and PD-L1 (expressed on APCs and tumor cells) [10, 11].

The prognosis of metastatic melanoma was dismal before recent advances in targeted therapy and immunotherapy. One-year overall survival (OS) rate of 25.5% was reported in a 2008 meta-analysis of 42 phase II cooperative group trials in patients with stage IV melanoma [12]. In 2018, there was a report of a 3-year OS rate of 63% in 94 patients with measurable, unresectable stage III or IV melanoma who received ipilimumab (anti-CTLA-4 antibody) and nivolumab (anti-PD-1 antibody) as concurrent therapy in a phase I study [13]. The annual incidence of BM from melanoma is increasing, which may be due to improved survival as a result of novel targeted therapies and immunotherapy for metastatic melanoma and/or more frequent imaging for screening [14]. The current lifetime incidence of BM from metastatic melanoma is estimated to be ≥50% [14, 15]. Conventional treatments such as surgical resection and stereotactic radiotherapy improve local control, but do not impact overall survival. In addition, whole-brain radiation and systemic chemotherapy options (i.e., temozolomide) have limited efficacy for the treatment of melanoma BM [15, 16]. With improved survival of metastatic melanoma patients with the use of CPI, the field moved toward addressing the role of CPI in melanoma with BM.

Initial immunotherapy studies evaluated the combination of CPI and cytotoxic chemotherapy. Di Giacomo and colleagues evaluated the combination of ipilimumab and fotemustine in a single-arm phase II trial of metastatic melanoma that included 20 patients with asymptomatic melanoma BM. In their study, ten patients had complete response (CR), while five had stable disease (SD) with a median progression-free survival (PFS) of 3 months [17]. At a median follow-up of 39.9 months, those with the BM had a 3-year survival rate of 27.8% with a median overall survival (mOS) of 12.7 months [18]. Subsequently, Margolin and colleagues conducted an open-label study of ipilimumab in patients with BM from melanoma. Of the 72 patients in the study, 51 had asymptomatic brain metastases and were not on corticosteroids while 21 had symptomatic BM and were on corticosteroids at the time of receiving ipilimumab. The patients who did not receive corticosteroids had higher response rates of 18% with an OS of 7 months compared to 5%

and an OS of 3.7 months for those who received corticosteroids [19]. The lower response rate and survival in the corticosteroid group might have been because of more advanced disease requiring steroids and/or effect of steroids on CPI efficacy. The above studies were encouraging, but had included patients who had received prior treatment for BM, and therefore, the role of CPI as an upfront treatment for untreated BM was unknown prior to the pivotal study by Tawbi and colleagues.

Recently, Tawbi and colleagues evaluated the efficacy and safety of nivolumab plus ipilimumab in an open-label, multicenter, phase II study in patients with melanoma who had asymptomatic untreated BM and demonstrated clinically meaningful intracranial efficacy. Fifty-seven percent of patients had intracranial benefit defined as stable disease (SD) for at least 6 months after the initiation of treatment, complete response (CR), or partial response (PR) (26% CR, 30% PR, 2% SD). Therapy with nivolumab plus ipilimumab prevented intracranial progression for more than 6 months in 64% of patients [7]. Similarly, Goldberg and colleagues conducted a nonrandomized phase II trial examining pembrolizumab in patients with untreated or progressive BM from NSCLC and melanoma. They reported responses in 6 and 4 out of 18 patients with NSCLC and 18 patients with melanoma, respectively [8]. The success of CPI in BM from these solid cancers is encouraging to the neuro-oncology community as it indicates that the brain is capable of initiating and regulating immune responses and has raised interest in identifying the role of immunotherapy in malignant primary brain tumors. The above trials of immunotherapy for BMs from solid tumors are summarized in Table 8.1.

Glioblastoma

GBM is the most common malignant brain tumor in adults with mOS of 14.6 months with the current standard of care [20]. The standard of care includes maximal safe resection when possible [21] followed by 60 Gy of radiation administered over 6 weeks (2 Gy per fraction × 30 fractions) with concurrent temozolomide (TMZ) at a dose of 75 mg/m2 administered daily over 6 weeks. This is followed by adjuvant TMZ at 150–200 mg/m2 administered on days 1–5 of 28 days cycles for 6–12 cycles. Despite this multimodality treatment, GBM invariably recurs leading to death with a 2-year survival rate of 26.5% [20].

Preclinical studies of CPI in GBM were promising as increased intratumoral CD8+ T-cells and long-term tumor-free survival were observed in mouse models [22, 23]. However, similar antitumor responses were not seen in a large phase III trial of nivolumab versus bevacizumab in recurrent GBM (n = 1 84, nivolumab; n = 185, bevacizumab) [24]. In addition, there was no survival benefit when nivolumab was added to radiation and temozolomide in newly diagnosed MGMT-unmethylated GBM in a phase III study (CheckMate-498) [25]. The reason for the disparity between preclinical studies and human studies is multifold, including the highly clonal nature of the cell lines used as opposed to clonal heterogeneity in GBM [26] and local and systemic immunosuppression unique to GBM in human. Understanding the mechanisms of immunosuppression in GBM is crucial in our efforts to implement immunotherapeutic approaches for the treatment of this deadly disease.

Immunosuppression in Glioblastoma

Unique local and systemic mechanisms of immunosuppression have posed roadblocks to the clinical development of immunotherapy in GBM.

Several factors contribute to local immunosuppression in GBM: tumor-intrinsic factors, tumor immune microenvironment, and the interaction between the two. GBM cells have intrinsic defects in antigen presentation. Tumor antigen presentation by the HLA class I peptide complex to the activated T-cells is needed for the immune system to recognize and destroy cancer cells. Loss of heterozygosity [27] in HLA class I is frequent in adult GBM and is associated with shorter overall survival [28]. In addition, GBM cells

Table 8.1 Select checkpoint inhibitor clinical trials for brain metastases from solid tumors

Title/Setting	Treatments	Phase	*N*	Outcome	Clinical trial identifier	Reference
Asymptomatic melanoma BM	Ipilimumab and fotemustine	II	20	CR: 10 SD: 5 mPFS: 3 mo mOS: 12.7 mo 3-yr survival rate: 27.8%	NCT01654692	[17, 18]
Symptomatic and asymptomatic melanoma BM	Ipilimumab	II	72	51 asymptomatic BM: RR: 18% mOS: 7 mo 21 symptomatic BM + steroids: RR: 5% mOS: 3.7 mo	NCT00623766	[19]
Untreated melanoma BM	Nivolumab and ipilimumab	II	94	Intracranial benefit: 57% CR: 26% PR: 30% SD > 6 mo: 2%	NCT02320058	[7]
Untreated or progressive BM from NSCLC and melanoma	Pembrolizumab	II	18 per cancer type	RR: 22% in melanoma RR: 33% in NSCLC	NCT02085070	[8]

Abbreviations: *CR* complete response, *BM* brain metastasis, *NSCLC* non-small cell lung cancer, *OS* overall survival, *PFS* progression-free survival, *RR* response rate, and *SD* stable disease

overexpress the T-cell inhibitory ligand, PD-L1 [29], which suppresses T-cell activation via T-cell anergy and apoptosis. GBM tumor cells have also been shown to upregulate immunosuppressive signaling pathways such as signal transducer and activator of transcription 3 (STAT-3) and indoleamine 2,3-dioxygenase (IDO) [30, 31]. In addition to tumor-intrinsic factors, the tumor immune microenvironment plays a pivotal role in GBM immunosuppression. GBM immune microenvironment is filled with immunosuppressive macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T-cells (Treg) [32–34]. Furthermore, the primary APC of the CNS, microglia, and cells capable of spontaneous cytotoxicity, national killer (NK) cells, and monocytic cells are nonfunctional in gliomas [35, 36]. Interaction between tumor and immune cells within the tumor immune microenvironment further contributes to local immunosuppression in GBM. GBM cells overexpress FasL which through its interaction with Fas expressed on T-cells leads to T-cell apoptosis [37]. Similarly, direct interactions between GBM cells and NK cells via atypical HLA molecules suppress NK cell activity [38, 39]. Immunosuppressive soluble factors such as TGF-β [40] and IL-10 [41] released by GBM cells, macrophages, microglia, and Tregs further contribute to local immunosuppression in GBM.

Interestingly, despite being a disease confined to the CNS, GBM imparts profound systemic immune suppression in the host. Total T-cell counts are reduced even in treatment naïve GBM patients [42–44]. Peripheral T-cells are thought to be sequestered in the bone marrow due to decreased surface sphingosine-1-phosphate receptor 1 (S1P1) expression which normally regulates T-cell exit from lymphoid organs and their egression from the bone marrow [44]. GBM patients' peripheral blood contains an abundant monocyte population which inhibits T-cell proliferation and lacks the ability to differentiate into mature dendritic cells (DCs) [45]. In addition, circulating monocytes and macrophages isolated from GBM patients have elevated expression of T-cell inhibitory ligand, PD-L1, and have the ability to suppress activation of cocultured T-cells [46]. The systemic immunosuppression in GBM is further exacerbated by lymphotoxic effects of radiation, TMZ, and corticosteroids [43]. Overall, profound local and systemic immunosuppressive mechanisms in GBM have to be targeted for the successful implementation of immunotherapy in GBM.

Checkpoint Inhibitors for the Treatment of GBM

PD-1/PD-L1 Inhibitors

PD-1/PD-L1 axis inhibitors are among the best-studied CPIs in GBM. Responses to anti-PD-1 antibodies, nivolumab and pembrolizumab, have been described in cases of GBM with high mutation burden. Examples include a case report of durable response to nivolumab in two siblings with biallelic mismatch repair deficiency with recurrent multifocal GBM [47] and successful use of pembrolizumab in a patient with germline POLE deficiency and GBM metastatic to the spine [48]. High mutational load and mismatch repair deficiency are known markers of response to CPI in a number of solid tumors [49], but these molecular characteristics are only found in a minority of GBM patients [50] and their associations with clinical response to CPI is unproven. The relevance of hypermutation and response to CPI in GBM is currently being tested in a clinical trial of pembrolizumab in patients with recurrent malignant glioma with a hypermutator phenotype (NCT02658279).

Completed trials of CPI in GBM have been summarized in Table 8.2. CheckMate 143 (NCT 02017717) was the first large randomized trial of PD-1 inhibitors in GBM where nivolumab was compared with bevacizumab in recurrent GBM at first relapse (n = 184, nivolumab; n = 185, bevacizumab). Preliminary results reported as an abstract at the World Federation of Neuro-Oncology Societies meeting in 2017 reported no difference in overall survival [24]. An exploratory phase I cohort within CheckMate 143 assessed nivolumab monotherapy (n = 10) versusvs. nivolumab plus ipilimumab (n = 30). Adverse events leading to discontinuation

Table 8.2 Completed checkpoint inhibitors clinical trials for GBM

Title/setting	Treatments	Phase	*N*	Outcome	Clinical trial identifier number	Reference
CheckMate 143 Recurrent GBM	NIVO versus BEV	III	369	mOS 9.8 mo vs. 10 mo similar use of corticosteroids in above cohorts	NCT02017717	[24]
CheckMate 143 Recurrent GBM	Cohort 1B NIVO +/− IPI	I	40	Adverse event profile superior in NIVO monotherapy than in combination arms	NCT02017717	[51]
CheckMate 143 ND GBM	Cohort 1C: NIVO+TMZ + RT - amp;gt; TMZ (MGMT methylated and unmethylated)	I	55	Neurological adverse events were similar to other trials without immunotherapy	NCT02017717	[52]
	Cohort 1D: NIVO+RT - amp;gt; TMZ (MGMT unmethylated)	I	58			
Recurrent GBM	Pembro versus pembro + BEV	II	80	PFS-6 6.7% versus 26%	NCT02337491	[53]
Recurrent GBM	Neoadjuvant pembro versus adjuvant pembro	II	35	mOS 13.7 mo versus 7.5 mo mPFS 3.3 versus 2.4	NCT02852655	[57]
Recurrent GBM	Neoadjuvant nivo	II	30	mOS 7.3 mo mPFS 4.1 mo	NCT02550249	[58]
Recurrent GBM	Neoadjuvant pembro	II	15	mPFS: 7 mo mOS: not reached at median follow-up of 12 mo 1-yr survival rate: 72%	NCT02337686	[33]

Abbreviations: *BEV* bevacizumab, *GBM* glioblastoma, IPI ipilimumab, *ND* newly diagnosed, *NIVO* nivolumab, *OS* median overall survival, *Pembro* pembrolizumab, *PFS* progression-free survival, *RT* radiation, and *TMZ* temozolomide

occurred more commonly in patients receiving dual immunotherapy [51]. Therefore, the combination therapy with nivolumab and ipilimumab is not being further pursued at this time.

Recurrent GBM is a highly resistant tumor, and therefore, the implementation of CPI clinical trials in the newly diagnosed setting has been pursued. An exploratory cohort of CheckMate 143 assessed the safety and tolerability of nivolumab in combination with radiation +/− TMZ in patients with newly diagnosed GBM and found a similar neurological adverse event as in other trials without CPI in the newly diagnosed setting [52]. However, a phase III trial of nivolumab plus radiation versus. temozolomide plus radiation in MGMT-unmethylated GBM demonstrated no survival benefit [25]. Another phase III trial of nivolumab in combination with radiation and TMZ (standard of care) in MGMT-methylated GBM is currently ongoing (NCT02667587).

Similar to nivolumab, pembrolizumab was shown to have limited monotherapy activity in recurrent GBM. Early results of a phase II study of pembrolizumab or pembrolizumab plus bevacizumab in recurrent GBM at first or second relapse demonstrated that patients receiving bevacizumab had superior PFS at 6 months (26%), as expected given pseudoresponse seen on MRI with bevacizumab. However, PFS6 for pembrolizumab only patients was similar to historical controls for recurrent GBM (6.7%) [53]. In this study, the combination of bevacizumab and pembrolizumab was well tolerated.

Until recently, PD-1 inhibition was mainly used as adjuvant treatment in GBM trials. However, recent successes with the use of neoadjuvant PD-1 blockade in melanoma [54, 55] and respectable lung cancer [56] have raised interest in the use of anti-PD-1 in the neoadjuvant setting with the goal to alter GBM immune microenvironment. Cloughesy and colleagues recently reported on the success of neoadjuvant pembrolizumab in recurrent GBM [57]. They randomized 35 recurrent GBM patients to receive neoadjuvant pembrolizumab followed by surgery and subsequent pembrolizumab monotherapy versus. adjuvant pembrolizumab. They reported a survival benefit in the neoadjuvant versus the adjuvant group (13.7 months vs. 7.5 months; hazard ratio 0.39 neoadjuvant/adjuvant; $P = 0.04$). Treatment with neoadjuvant pembrolizumab was associated with upregulation of T-cells and interferon-γ-related gene expression and downregulation of cell cycle–related genes. These results are encouraging with the caveat that the study was powered for tissue analysis and not survival. Similarly, Schalper and colleagues performed a single-arm phase II clinical trial (NCT02550249) in which they tested a presurgical dose of nivolumab followed by postsurgical nivolumab and demonstrated enhanced expression of chemokine transcripts, higher immune cell infiltration, and augmented TCR clonal diversity among tumor-infiltrative T-cells in resected tumor tissue [58]. In another single-arm neoadjuvant study by de Groot and colleagues, neoadjuvant pembrolizumab was tested in 15 patients with recurrent GBM where mPFS was 7 months and mOS was not reached at median follow-up of 12 months with an estimated 1-year OS rate of 72% (95% CI: 52–99.6%) at the time of reporting the results [33]. GBM tissue treated with pembrolizumab was found to be poorly infiltrated with T-cells and was enriched with distinct CD68+ populations consistent with an immunosuppressive tumor microenvironment. The ability of neoadjuvant PD-1 blockade to alter the tumor immune landscape has challenged the previous dogma that minimum tumor burden is required for effective immune therapy.

Pembrolizumab is currently being tested in the newly diagnosed setting in combination with radiation plus TMZ as monotherapy (NCT02530502). In addition to the above PD-1 inhibitors, 2 PD-L1 inhibitors, atezolizumab and durvalumab, are currently being tested in newly diagnosed GBM patients (NCT03174197 and NCT02336165, respectively).

CTLA-4 Axis Inhibitors

Dual immunotherapy targeting both PD-1/PD-L1 and CTLA-4 pathways has been more successful than monotherapy in melanoma [59]. However, higher rates of adverse events were seen when dual therapy was used in CheckMate 143 GBM

trial [53]. Several combinatorial therapies with CPI and other forms of immunotherapy are going, and dual CTLA-4 and PD-1/PD-L1 blockade are currently being proposed.

Why Is Checkpoint Inhibition More Effective in BM Than in GBM?

The differences between the effectiveness of CPI in brain metastasis and GBM likely lie in low mutation burden in GBM, the overwhelming impact of GBM on local and systemic immunosuppression, and the infiltrative nature of GBM tumor within the brain parenchyma.

Strong associations between clinical response and high mutation burden and/or PD-L1 expression have been described in melanoma and NSCLC, but it is not yet clear how these factors contribute to intracranial responses seen with CPI in the brain metastasis from these solid tumors [7, 8]. Tumor mutation load, which is associated with abundance of antigens and neoantigens leading to increased immunogenicity, is lower in GBM in comparison to cancer types in which CPIs are highly active [60], GBM has a higher expression of the T-cell inhibitory ligand, PD-L1, than BM [61]; however, the role of PD-L1 as a marker of response to CPI in GBM is not clear. Another key difference is that GBM is among the most immunosuppressive of solid tumors despite confinement to the intracranial compartment [62]. In fact, GBM utilizes a variety of immune suppressive mechanisms to prevent its immune detection and eradication [63]. These immunosuppressive mechanisms include infiltration of GBM microenvironment by immunosuppressive T-cells (regulatory T-cells) and macrophages [64] and release of immunosuppressive soluble factors such as TGF-ß and IL-10 [63]. In addition to local immune suppression, systemic immune suppression has been described in GBM patients even prior to the start of radiation and chemotherapy [44]. Local and systemic immunosuppressive mechanisms in GBM are described in detail in section "Introduction".

In addition, GBM tumor cells infiltrate the brain parenchyma and disseminate while in BM, the infiltrative growth is not seen, and parenchymal metastases remain in the perivascular space [65]. The infiltrative nature of GBM is a barrier to the success of drug delivery. Therapeutic monoclonal antibodies in particular tend to accumulate in the necrotic center which has a disrupted BBB rather than the infiltrative edge which has a more intact BBB [66]. Since GBM cells are highly infiltrative with single cells shown to migrate into regions distant from the initial tumor mass, the disease has an extremely high propensity for recurrence making it more challenging for immunotherapy to be as successful [67].

Vaccines

The fundamental notion behind cancer vaccine strategies is the induction of antitumor immune responses that mediate tumor regression through a targeted cytotoxic T-cell effect while sparing normal tissue. Peptide vaccines and cell vaccines comprise the two major types. Peptide vaccines take advantage of tumor-specific antigens which are proteins encoded by mutant genes in the tumor to induce an immune response against the tumor cells. Cell vaccines comprise autologous or allogenic immune cells that trigger antitumor immune responses.

Peptide Vaccines

EGFRvIII (type III epidermal growth factor receptor mutation) is expressed in 20–30% of patients with GBM and has been targeted for treatment of GBM via pharmacological inhibition and a peptide vaccine. EGFRvIII is formed due to the deletion of exons 2-7 of EGFR resulting in an extracellular truncation of EGFR allowing it to be constitutively active in the absence of ligand [68]. The EGFRvIII targeting vaccine PEP-3-KLH (keyhole limpet hemocyanin) (rindopepimut) was studied in a large multicenter, double-arm phase III clinical trial, ACT IV [69]. Seven hundred patients with newly diagnosed GBM were enrolled into two arms: PEP-3-KLH plus TMZ versus KLH plus TMZ (control arm). Though PEP-3-KLH exhibited sufficient safety in the study, it failed to provide a survival benefit. There was no difference in the mOS of patients who received the vaccine compared to

the control group for patients with minimum residual disease (MRD) and all intention-to-treat (ITT) patients (PEP-3-KLH vs. control: MRD: 20.1 months vs. 20 months; ITT: 17.4 months vs. 17.4 months). Interestingly, a post hoc analysis revealed that patients with bulky disease had a survival benefit from PET-3-KLH with a 2-year OS rate of 30% versus 19% for the control arm (P = 0.029) [69]. This finding challenged the dogma that a minimum tumor burden is required for effective immunotherapy. The unsatisfactory efficacy results of the ACT IV phase III trial ended the development of EGFRvIII-targeted peptide vaccines. Remarkably, evidence of loss of EGFRvIII expression was noted in about 60% of the small subset of patients with tumor tissue available at recurrence, although this may be a general evolutionary phenomenon that may have occurred independent of EGFRvIII-targeted vaccination. The lack of stability of EGFRvIII expression may preclude its use as a molecular target for treatment in GBM. GBM is a heterogeneous tumor, and the selection of one molecular target of immunotherapy like EGFRvIII might be insufficient. This may especially be the case if its expression is not stable and not ubiquitous which means that multipeptide vaccines against several targets and non-peptides with higher immunogenicity are likely needed.

Mutations in isocitrate dehydrogenase (IDH) exist in about 80% of low-grade gliomas affecting multiple pathways and metabolisms [70]. The most common of such mutations is the R123H mutation in IDH1 which accounts for approximately 70% of all IDH mutations [70]. Typically, GBM tumors that evolve from low-grade glioma harbor IDH1 mutations while only a small fraction of primary GBM cases harbor mutations in IDH1 [71]. Schumacher and colleagues demonstrated that IDH1 (R132H) contains an immunogenic epitope suitable for mutation-specific vaccination and developed a 15-amino-acid polypeptide targeting IDH1 R132H [72]. They found that peptides encompassing the mutated region were presented on major histocompatibility complexes (MHC) class II and induced mutation-specific CD4+ responses. In a mouse model, IDH1 peptide vaccines were shown to promote improved survival leading to intratumoral downregulation of TGF-β2 and IL-10 and upregulation of granzyme-b, IFN-γ, and perforin-1 [73]. Platten and colleagues tested a mutation-specific peptide vaccine targeting IDH1R132H in patients with newly diagnosed anaplastic astrocytoma and GBM with IDH1R132H mutations in a phase I trial. The trial demonstrated safety and immunogenicity [74]. Currently, an ongoing phase I clinical trial investigates the IDH1 peptide vaccine in recurrent low-grade gliomas (NCT02193347).

To address the challenges of developing peptide vaccines against one antigen, the development of the latest peptide vaccines for brain tumors has now moved toward personalized multipeptide vaccines with activity against several targets. GBM-specific peptide vaccine, IMA950, was developed to target 11 tumor-associated peptides identified on HLA surface receptors in primary human GBM tissue [75]. Rampling and colleagues conducted a phase I trial of IMA950 and found that 20 of the 40 evaluable patients were multi tumor-associated peptide (TUMAP) responders which exceeded their primary endpoint of multi-TUMAP responses in at least 30% of patients [75]. Similarly, a phase I/II trial testing IMA950 adjuvanted with poly-ICLC in HA-A2 + glioma patients observed CD8+ T-cell responses to a single or multiple peptides in 63.2% and 36.8% of patients, respectively [76].

In addition, Keskin and colleagues have demonstrated that the use of multi-epitope, personalized neoantigen vaccination is feasible in GBM despite its relatively low mutation load and immunologically "cold" tumor microenvironment [77]. They conducted a phase I/Ib trial involving ten patients with newly diagnosed GBM. Neoantigens were identified in each individual patient by comparing whole-exome sequencing data from the surgically resected tumor to that of matched normal cells [77]. For each patient vaccine, a pool of 7–20 peptides were selected as actionable neoepitopes predicted to bind to the HLA class I molecules of each patient. The vaccine was safe with no serious adverse side effects. Patients who received corticosteroids to treat side effects did not have a

T-cell response to vaccination. However, the two patients that did not receive dexamethasone had strong antitumor immune responses generating neoantigen-specific T-cells that were able to cross the blood–brain barrier and traffic to the tumor in the brain. The T-cells comprised of both CD8+ and CD4+ T-cells enriched in a memory phenotype [78]. Clonal expansion of neoantigen-reactive T-cells was seen in the tumor identical to circulating T-cells. These correlative results are encouraging, but need to be interpreted with caution as responses were only seen in two patients. These responses were seen in patients who were not on steroids emphasizing the judicious use of steroids in immunotherapy trials.

Similarly, Hilf and colleagues used a similar multi-epitope-based personalized vaccine strategy, but targeted both neoantigens and unmutated tumor-specific antigens to increase the number of actionable epitopes. In this phase I study, 15 patients were enrolled by the multicenter initiative Glioma Actively Personalized Vaccine Consortium (GAPVAC), and two types of vaccines were tested [79]. The results of microarray analysis of the patient transcriptome and mass spectrometry analysis of their HLA immunopeptidome determined the composition of both vaccines. The patients were first vaccinated with APVAC1 which is a pool of nine unmutated peptides derived from a premanufactured library of non-mutated antigens that are overrepresented in GBM tumors. The second vaccine, APVAC2, was preferentially targeted against mutated neoantigens, and if no neoantigens were identified in a patient, then the vaccine was targeted against non-mutated antigens that were not present in the premade library. Both of these vaccines were safe and generated T-cell responses against the proteins in the vaccine with APVAC1 inducing a sustained CD8+ T-cell response and APVAC2 inducing both CD4+ and CD8+ T-cell responses [79]. There is a favorable mOS in this study of 29 months, which suggests a potential clinical benefit compared with historical controls. These two recent first-in-human phase I studies of personalized neoantigen vaccines for patients with GBM have demonstrated that “cold tumors” with a low mutational burden can be infiltrated with antigen-specific T-cells through personalized vaccines.

Another approach in the peptide vaccine has been the development of heat shock protein (HSP) vaccines. HSPs function as intracellular chaperones and have been shown to be involved in the activation of both innate and adaptive immune systems. HSPs are involved in protein folding, protein stabilization, peptide loading onto MHC class I molecules, tumor initiation, and proliferation [80]. Akin to GAPVAC, HSP vaccines do not just target one antigen but rather target a mechanism that is implicated in tumor-specific antigen presentation in GBM. HSP–peptide complexes (HSPPCs) mediate endocytosis and trigger immune responses to tumor-antigenic peptides by antigen presentation [81]. Bloch and colleagues conducted a first phase II clinical trial investigating the HSPCC-96 vaccine in recurrent GBM after gross total resection and administered the vaccine every week for 4 weeks and then every 2 weeks until tumor recurrence. Following the treatment, mOS was 42.6 weeks (95% CI: 34.7–50.5) and OS rate at 12 months was 29.3% (95% CI: 16.6–45.7). The toxicity of the vaccine was also minimal with a single grade 3 event related to the vaccine [82]. Completed peptide and cell vaccine trials are summarized in Table 8.3. Overall, the generation of peptide vaccines for glioma has been feasible with correlative studies indicating biological activity. However, sustained clinical benefit has not been observed indicating that the degree of immune activation may not be sufficient for meaningful clinical response. Combinatorial immunotherapy approaches may aid in improving immune stimulation and clinical benefit.

Cell Vaccines

In addition to peptide vaccines, cell-based vaccines using DCs have been of particular interest in GBM. DCs are the most potent APC of the immune system. In order to produce autologous DC vaccines, DCs are first isolated from the patient, loaded with the tumor antigen, matured via exposure to cytokines, and then reinjected into the patients’ body. The very first report of a DC vaccine used in GBM was by Liau and

Table 8.3 Select vaccine clinical trials for GBM

Title/setting	Treatments	Phase	*N*	Outcome	Clinical trial identifier	Reference
ACT IV ND GBM	TMZ + rindopepimut-KLH versus KLH	III	745	MRD mOS: 20.1 months versus 20 months	NCT01480479	[69]
NOA-16 ND GBM and AA (IDH1R132H-mutated)	IDH1 peptide vaccine	I	32	Demonstrated safety and immunogenicity	NCT02454634	[74]
IMA950 ND GBM	GBM multipeptide vaccine IMA950	I	40	Well tolerated with multi-TUMAP responses in at least 30%	NCT01222221	[75]
IMA950 ND GBM and AA HLA-A2 +	IMA950/poly-ICLC vaccine	I/II	GBM = 16 AA = 3	Safe and well tolerated mOS 19 mo for GBM CD8+ T-cell response to multipeptides: 36.8%	NCT01920191	[76]
GAPVAC ND GBM	APVAC1 vaccine plus Poly-ICLC and GM-CSF APVAC2 vaccine plus Poly-ICLC and GM-CSF	I	16	Safe with mOS of 29 mo	NCT02149225	[79]
GP96 heat shock protein–peptide complex vaccine Recurrent GBM	HSPPC-96	I/II	41	mPFS 19.1 weeks mOS 42.6 weeks	NCT00293423	[82]
HGG-2006 ND GBM	DC-based tumor vaccination	I/II	77	mPFS 10.4 months mOS 18.3 months more severe than that of other DC vaccine studies	2006–002881-20	[84]
DCVax-L ND GBM	Adjuvant TMZ plus DCVax-L versus adjuvant TMZ	III	2:1 DCVax-*L* = 232 Control = 99	mOS 23.1 (90% of the ITT received DCVax-L) 2-yr survival rate: 46.2% 3-yr survival rate: 25.4%	NCT00045968	[85]

Abbreviations: *AA* anaplastic astrocytoma, *DC* dendritic cells, *GBM* glioblastoma, *HGG* high-grade glioma, *HSPPC* heat shock protein–peptide complex, *IDH* isocitrate dehydrogenase, *ITT* intention-to-treat, *KLH* keyhole limpet hemocyanin, *MRD* minimal residual disease, *ND* newly diagnosed, *OS* overall survival, *PFS* progression-free survival, and *TMZ* temozolomide

colleagues in 2000, where they treated a patient with recurrent brainstem GBM with autologous DCs pulsed with allogeneic MHC-I matched tumor peptides. A measurable cellular immune response to the allogeneic GBM peptides was seen as demonstrated by increased T-cell infiltration within the intracranial tumor site in the biopsy sample obtained following vaccination. However, improved survival was not observed [83].

On a larger scale, Ardon and colleagues treated 77 patients with newly diagnosed GBM with an autologous DC vaccine. They integrated the vaccination into the Stupp regimen and found a median PFS and OS of 10.4 and 18.3 months, respectively. However, the adverse events were more severe than that of other DC vaccine studies with 38 serious adverse events found in 30 patients and 19 hematological adverse events in 18 patients [84].

Liau and colleagues conducted a phase III trial evaluating the addition of DCVax-L, an autologous tumor lysate-pulsed DC vaccine, to standard therapy for newly diagnosed GBM [85]. In their study, patients were randomized to TMZ plus DCVax-L or TMZ and placebo after surgery and chemoradiotherapy. The primary endpoint was PFS while the secondary endpoint was OS. The median OS was 23.1 months from surgery for the intent-to-treat population with nearly 90% of the ITT population receiving DCVax-L. The 2- and 3-year survival rates were 46.2 and 25.4%, respectively. The addition of DCVax-L to standard therapy is feasible and safe, and may extend survival. Generating DC vaccines that are engineered to target numerous tumor antigens specific to a patient's tumor or to target a common antigen presented by most tumors is time and resource demanding.

Cell Therapy

Another form of immunotherapy is active transfer of immune cells such as CAR T-cells and NK cells to the donor to leverage their antitumor activity. The main challenges in development of cell therapy in GBM are the intracranial location of the tumor, determining the most efficacious route of cell delivery (intravenous vs. intrathecal), and identification of a universal cell surface antigens to target.

CAR T-Cells

Chimeric antigen receptor (CAR) T-cells are engineered T-cells that target a specific target on the tumor cells and mount T-cell-mediated antitumor responses [86]. CAR T-cell therapies are at the forefront of immunotherapy approaches for the treatment of highly clonal neoplasms such as lymphoma and leukemia [87]. Aside from ubiquitously expressing monoclonal antigens, the location of the tumor cells (peripheral blood) make hematological malignancies perfect candidates for CAR T-cell therapies.

CAR T-cell therapies have not been as successful in solid tumors [88]; however, a case report of success in GBM has been promising and has raised interest in the generation of CAR T-cells in GBM. Brown and colleagues treated a 50-year-old male with multifocal GBM with intracavitary injections of IL13Rα2-targeted CAR T-cells into a right temporo-occipital lesion through a catheter placed within the resection cavity [89]. Local tumor control was achieved, but meanwhile, the tumor grew in the leptomeningeal spinal space and the patient received treatments via an intrathecal catheter placed in the lateral ventricles. Complete remission of the spinal tumors and the intracranial tumors were achieved with intrathecal administration of IL13Rα2-targeted CAR T-cells, which was sustained for 7.5 months. The cause of tumor recurrence was thought to be due to decreased expression of IL13Rα2 based on preliminary analysis. This case report best exemplifies the barriers in the successful use of CAR T-cells in GBM: lack of stably expressed antigens and identifying an effective route of administration. The effectiveness of IL13Rα2 CAR T-cells can be attributed to the CSF location of cancer cells and the ease of delivery of CAR T-cells in the intrathecal compartment.

In addition to IL13Rα2, CAR T-cells targeting EGFRvIII and HER2 have been evaluated in clinical trials [90, 91]. O'Rourke and colleagues

treated ten recurrent GBM patients with EGFRvIII mutation with EGFRvIII CAR infusions. They demonstrated transient expansion of CART-EGFRvIII cells in peripheral blood of all patients and increased expression of inhibitory molecules and Treg infiltration in five out of seven patients with available post-treatment tissue. However, despite the promising correlative outcome, mOS of the patients was not improved [90]. Ahmed and colleagues generated HER2-specific T-cells using HER2 positive autologous GBM cells in 2010 and demonstrated their antitumor efficacy in autologous GBM xenografts in the brain of severe combined immunodeficient mice. Phase I trial of HER2 CAR T-cells in recurrent GBM is currently ongoing (NCT03389230).

Several factors contribute to lack of response to CAR T-cells in GBM including lack of stably expressed antigens, intratumoral heterogeneity, impaired CAR T-cell proliferation in a hypoxic environment, and an immunosuppressive microenvironment which leads to ineffectiveness of CAR T-cells. Efforts in altering the tumor microenvironment have focused on combinatorial immunotherapy approaches. For example, increased levels of PD-1 expression on transduced anti-HER2 CD8+ T-cells following antigen-specific stimulation with anti-PD-L1+ tumor cells in mice have been described [92], and combination of EGFRvIII CAR T-cells with pembrolizumab is currently being evaluated in newly diagnosed GBM (NCT03726515).

NK Cells

Decades of failed targeted therapy approaches in GBM and recent failures in immunotherapy targeting specific antigens (checkpoint inhibitors, vaccine peptides, and CAR T-cells) indicate that alternative strategies that are not dependent on tumor antigen presentation are needed in GBM. One such approach would be to leverage the innate immune system which is able to destruct tumor cells without the need for antigen presentation. NK cells are large lymphocytes of the innate immune system capable of lysing infected cells directly via secreting granules and granzymes or via antibody-dependent cellular cytotoxicity [93].

NK cells for the treatment of solid tumors have shown promise [94]. Autologous NK cells have been used in early clinical trials for the treatment of gliomas via a combination of focal and intravenous injections without severe neurological toxicity [95]; however, the generation of autologous NK cells from individual patients is time-consuming and only attainable in specialized centers. Therefore, there has been interest in the generation of allogeneic over-the-shelf. NK cells obtained from cord blood and placenta. Similar to CAR T-cells, the route of administration of NK cells is debated and will be tested in upcoming NK cell trials within our institution. NK cells for the treatment of pediatric medulloblastoma via posterior fossa are currently ongoing at MD Anderson Cancer Center (NCT02271711).

Oncolytic Viral Therapies

Oncolytic viruses have been the subject of intense investigation for the treatment of cancer. Initially, the mechanism of action of oncolytic viruses was thought to be due to direct tumor lysis and cytotoxicity [96]. With the discovery of profound immunosuppression and immune escape by tumor cells, it became apparent that oncolytic viruses may release pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) molecules that alter the tumor immune microenvironment. It is now known that viral infection of tumor cells induces inflammation within the tumor via T-cell priming and facilitates the recognition of cellular antigens by the host immune system [97]. The antitumor effect of viral therapy is likely driven by both cytotoxicity and adaptive immune responses. Several oncolytic viruses have been studied in GBM including polio-, retro-, adeno-, measles, and herpes viruses, and many virus therapy trials in GBM are in early stages. Here, we describe three selected advanced clinical trials of viral therapy in GBM: PVSRIPO (poliovirus), Toca 511 (retrovirus), and DNX2401 (adenovirus). The summary of these trials can be found in Table 8.4.

Table 8.4 Select virus therapy clinical trials for GBM

Title/setting	Route of delivery	Phase	*N*	Outcome	Clinical trial identifier number	Reference
Polio virus (PVSRIPO) Recurrent GBM	Convection-enhanced delivery	I	61	OS rate: 21% at 24 and 36 months	NCT01491893	[98]
Retrovirus Toca 511 (vocimagene amiretrorepvec) Recurrent GBM	Injection of virus into the resection cavity	I	45	mOS: 13.6 mo	NCT02414165	[100]
Adenovirus DNX-2401 Recurrent GBM	Injection of virus into the tumor	I	37	OS rate: 20% at 72 months	NCT00805376	[101]

The recombinant oncolytic poliovirus, PVSRIPO, is a genetically engineered form of poliovirus Sabin type 1 with attenuated neurovirulence. PVSRIPO received breakthrough therapy designation from FDA in 2016 for a phase I study in recurrent GBM (NCT01491893). The results of this trial were published in 2018 by Desjardin and colleagues [98]. They treated 61 patients with recurrent GBM in a dose-escalation study via intratumoral infusion by convection-enhanced delivery. One dose-limiting toxic effect (grade IV intracranial hemorrhage immediately after catheter removal) was observed at dose level number 5 and dose level-1 was selected as the phase 2 dose (5.0×10^7 $TCID_{50}$). The overall survival rate was 21% at 24 months and 36 months. Safety results indicated that the neurovirulence potential of poliovirus was effectively eliminated in PVSRIPO.

Toca 511 is a non-lytic retrovirus and has been engineered to preferentially kill tumor cells by encoding a modified yeast cytosine deaminase that converts the prodrug 5-fluorocytosine (5-FC) to the potent anticancer drug, 5-fluorouracil (5-FU), in an infected tumor cell [99]. In a phase I open-label study, Cloughesy and colleagues treated 45 patients with recurrent or progressive high-grade glioma undergoing resection with intracavitary injections of Toca 511 followed by IV injection of Toca FC, an extended-release form of prodrug 5-FC [100]. Infected cells convert the prodrug 5-FC to 5-FU which leads to cell death via cytosine deaminase that is otherwise not present in normal noninfected humans cells. Toca 511 and Toca FC were well tolerated and demonstrated OS of 13.6 months (95% confidence interval, 10.8–20.0) and OS rate of 29.1% at 2 years. A phase II/III study of this approach is currently ongoing.

DNX-2401 is an oncolytic adenovirus that achieves tumor cell targeting through a 24-base deletion of E1A and insertion of an Arg–Gly–Asp (RGD) motif onto a viral capsid protein. In a phase I trial of DNX-2401 administered via intratumoral injection in recurrent malignant gliomas, 20% of patients were alive >3 years after treatment of their recurrent GBM [101]. Molecular profiling of pre- and post-treated tissue showed tumor infiltration by CD4+ and CD8+ T-cells and reduction of TIM-3 expression indicating that DNX-2401 may be able to overcome some features of T-cell exhaustion. Given immune-mediated anti-glioma response elicited by DNX-2401, it is currently being assessed in a phase I/II clinical trial in combination with pembrolizumab (NCT02798406).

The significance of the survival rate of about 20–30% at 2 years seen in the above viral trials has been questioned [102]. Retrospective analysis and literature review have shown similar survival rates in patients enrolled in other non-viral therapy trials [102, 103]. The patients with longer survival seem to possess favorable biological and/or demographic features [102]. Larger randomized trials that stratify for the favorable diagnostic features, such as IDH mutation and MGMT status, are needed to

determine the efficacy of viral therapy monotherapy and in combination with CPIs.

Combinatorial Approaches

CPIs have been the backbone of immunotherapies in various solid cancers. However, their ineffectiveness in phase III trials in GBM as monotherapy has led to combinatorial immunotherapy trials that combine CPI with other forms of immunotherapy in order to overcome the profound immunosuppression in GBM and increase antitumor effects of CPI. Combinatorial trials have focused on approaches to overcome the potential mechanism of resistance to CPI in GBM including lack of T-cell infiltration, impaired T-cell activation, and augmenting BBB penetration.

Oncolytic viral therapies described above are thought to induce tumor T-cell infiltration, and combinatorial trials with CPI are currently ongoing with DNX2401 (NCT02798406) and an inducible adenoviral vector engineered to express hIL-12 (Ad-RTS-hIL-12) (NCT03636477). In addition, active transfer of CAR T-cells is thought to overcome the lack of T-cell infiltration within GBM tumor microenvironment, and combinatorial trials of CAR T-EGFRvIII and pembrolizumab are currently ongoing (NCT03726515). Another approach to increase intratumoral T-cells is vaccination with DCs [104–106]. Trials of DC vaccines in combination with anti-PD-1 therapy in recurrent GBM are currently ongoing (NCT02529072 and NCT03014804).

Other efforts to alter the GBM microenvironment have focused on overcoming impaired T-cell activation via inhibition of immune-modulating enzymes (IDO1) and cytokines (TGF-β, CSF-1) and immune cell surface molecules (LAG-3).

Indoleamine 2,3-dioxygenase I (IDO1) is the rate-limiting enzyme in conversion of tryptophan into kyneurenine and its by-products [107]. Elevated IDO1 expression is thought to down-regulate T-cell activity via depletion of tryptophan and induces T-cell apoptosis via increased levels of kyneurenine and its by-products [108]. Two IDO1 inhibitors, epacadostat (ECHO-204) and INT230–6 (IT-01), are currently in phase I/II clinical trials in combination with nivolumab for advanced cancers to include recurrent GBMs (NCT02327078 and NCT03058289, respectively).

Transforming growth factor-β (TGF-β) is among the most well-established immunosuppressive soluble factors released by GBM cells, TAMs, Tregs, and microglia within the GBM microenvironment [22]. In addition to its role in immunosuppression, TGF-β activates genes that are involved in proliferation, invasion, angiogenesis, and glioma stemness. Multiple TGF-β compounds have been used as monotherapy for the treatment of gliomas including anti-sense oligonucleotides targeting soluble extracellular TGF-βII [109], TGF-β receptor sequestering soluble TGF-β (GC1008) [110], and TGF-βI receptor kinase inhibitor (galunisertib/LY2157299) [111]. These agents have not been shown to be efficacious in treatment of recurrent GBM as monotherapy when compared with chemotherapy [109, 110]. Their lack of effectiveness maybe due to differential expression of TGF-β and the relevance of a particular isoform during GBM evolution. A recent study on differential expression and clinical significance of TGF-β isoforms in GBM suggests that TGF-β expression and its correlation to survival outcome are more relevant in the newly diagnosed setting and that TGF-βI, and not TGF-βII, is the dominant isoform [112]. Galunisertib, a small molecular inhibitor of TGF-β receptor kinase I, is being combined with nivolumab in a phase I/II trial in recurrent GBM (NCT 02423343) in order to prime the tumor microenvironment to augment CPI effectiveness.

Another growth factor that has been implicated in GBM immunosuppressive microenvironment is colony stimulating factor-1 ligand (CSF-1). CSF-1 ligand interaction with its receptor (CSF-1R) has been shown to induce generation of immunosuppressive M2 macrophages and enhances glioma cell progression [113]. Similar to TGF-β inhibitor monotherapy trials, the CSF-1R and KIT inhibitor, PLX3397, did not show efficacy in recurrent GBM despite its ability to readily cross the BBB [114]. Combinatorial

trials of CSF-1R in combination with two PD-1 antibodies, spartalizumab and nivolumab, are currently ongoing in two distinct trials in advanced cancers to include gliomas (NCT02829723 and NCT02526017).

Lymphocyte-associated globulin-3 (LAG-3) is a surface molecule expressed on activated T-cells, B-cells, and NK cells [115], and was shown to be present in perivascular niche of the tumor in six of nine of human GBM samples tested [116]. In preclinical mouse models, dual anti-PD-1 and anti-LAG-3 was superior to either treatment alone in improving survival of glioblastoma bearing mice [116]. A phase I/II study of nivolumab with anti-LAG3 antibody or urelumab in recurrent GBM is currently ongoing (NCT02658981). Urelumab is a fully humanized IgG4 monoclonal antibody targeting CD137 or 4-1BB, an inducible receptor-like protein expressed in both cytotoxic and T-helper cells, which upon cross-linking with anti-CD3-stimulated T-cells results in enhancement of T-cell proliferation [117].

CPIs are also being tested in combination with blood–brain barrier (BBB) disruption methods with the goal to increase the exposure of intratumoral antigens to immune cells and their access to tumor microenvironment. The phase I and II trials of pembrolizumab in combination with MRI-guided laser ablation (MLA) in recurrent GBM are currently enrolling patients (NCT02311582).

Continued efforts at stepwise multimodality immunotherapy strategies are needed to overcome immunosuppressive mechanisms in GBM for successful implementation of immunotherapy in GBM.

Conclusion

Immunotherapy advances in solid cancers such as melanoma and NSCLC are promising and raise the interest in implementing immunotherapy for the treatment of GBM. CPIs have been at the forefront of immunotherapy advances in various solid cancers; however, phase III clinical trials of CPI in GBM have been disappointing. Window-of-opportunity trials of CPIs in recurrent GBM have been instrumental in improving our understanding of the GBM microenvironment, potential reasons for lack of clinical efficacy, and a potential novel mechanism to enhance the efficacy of these agents through a neoadjuvant approach. Through these studies, we have learned that the GBM microenvironment lacks cytotoxic T-cells and contains abundant immunosuppressive macrophages and myeloid-derived suppressor cells. Current combinatorial immunotherapy trials aim to overcome the immunosuppressive GBM microenvironment via approaches that address lack of T-cell infiltration (oncolytic viral therapies, vaccine peptides, dendritic cell vaccines, and CAR T-cells), lack of success with targeting one antigen in GBM (GAPVAC vaccine and NK cells), increase in T-cell activation (antibodies against T-cell stimulatory ligands and pro-inflammatory cytokines), and maintenance of T-cell activation (CPI and TGF-B inhibition). Given the success of immunotherapy for the treatment of BM from melanoma and NSCLC, we now know that successful treatment of intracranial neoplasms with CPI is possible and that the CNS location of GBM does not preclude antitumor immune responses. Continued efforts at conducting well-designed window-of-opportunity clinical trials with a focus on successful activation and maintenance of tumor-specific responses are needed to improve the clinical development of immunotherapy in GBM.

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