Journal of Neuro-Oncology (2021) 151:375–391
https://doi.org/10.1007/s11060-020-03481-0

TOPIC REVIEW

Combination immunotherapy strategies for glioblastoma

Hok Yee Chan[1] · John Choi[1] · Christina Jackson[1] · Michael Lim[1]

Received: 14 January 2020 / Accepted: 1 April 2020

Abstract

Introduction Despite recent advances in treatment for a number of cancers with immune checkpoint blockade (ICB), immunotherapy has had limited efficacy in glioblastoma (GBM). The recent multi-centered CheckMate 143 trial in first time recurrent GBM and the Checkmate 498 trial in newly diagnosed unmethylated GBM showed that antibodies against programmed cell death protein 1 (PD-1) failed to improve overall survival in patients with GBM. Recent preclinical and clinical studies have explored combining ICB with several other therapies including additional ICB against alternative checkpoint molecules, activation of costimulatory checkpoint molecules such as 4-1BB, radiation-induced tumor cell lysis and immunogenic recruitment, local chemotherapy, neoadjuvant ICB therapy, and myeloid cell reactivation.

Methods We have reviewed the literature on ICB seminal to the progression of several preclinical studies and clinical trials in order to provide a compendium of the current state of combination immunotherapy for GBM. For ongoing clinical trials without associated publications, we searched clinicaltrials.gov for ongoing studies using the keywords, "GBM" and "glioblastoma", as well as names of checkpoint molecules.

Results Recent trends from clinical trials demonstrate that despite a variety of different combination strategies involving ICB, GBM remains largely elusive to current immunotherapies. There is a discordance of survival outcomes between GBM pre-clinical models and clinical trials, likely due to the heterogeneity of GBM in patients as well as other adaptive immune mechanisms not otherwise represented in murine models. However, in clinical studies, neoadjuvant ICB in GBM was found to diversify the T cell receptor (TCR) repertoire and increase chemokine mRNA transcripts when comparing pre- and post-surgical time points. Moreover, an increase in peripheral and tumor-infiltrating lymphocyte (TIL) clonotypes were also observed when comparing adjuvant and neoadjuvant cohorts.

Discussion Despite the lack of clinical survival benefit, immune modulation was observed in multiple different combination strategies for GBM in both preclinical and clinical studies, indicating that ICB combination therapy results in a significant immunological impact on the tumor microenvironment.

Keywords Glioblastoma · Cancer · Immunotherapy · Checkpoint · Combination

Introduction

Since the introduction of William Coley's toxins for bone and soft-tissue sarcomas over a hundred years ago, the success and barriers to cancer immunotherapy have been well summarized by the Delphic maxim—"Know thyself." The immune system is inherently founded on the principle of distinguishing self from non-self. Inhibitory checkpoint molecules are expressed when T cells are activated, which also serves as a channel for the body to regulate immune response. Self-antigens that are targeted by T cells express checkpoint inhibitor ligands that can induce T cell inactivation. Cancers have developed resistance against immune responses by co-opting this principle to obscure themselves as "self".

Therapeutic antagonism against checkpoint molecules such as Programmed cell death protein-1 (PD-1) and Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have seen varying degrees of success in treating several cancers including melanoma and non-small cell lung cancer [1–4]. However, the transposition of these same immune

Hok Yee Chan and John Choi contributed equally to the work.

✉ Michael Lim
mlim3@jhmi.edu

[1] Department of Neurosurgery, Johns Hopkins School of Medicine, Baltimore 21231, USA

checkpoint blockade (ICB) strategies to glioblastoma (GBM) has had limited efficacy [5].

Current standard of care for GBM involves the Stupp protocol, which includes maximal surgical resection and chemoradiation [6, 7]. Despite treatment, prognosis for patients diagnosed with GBM remains poor [8]. The recent multi-centered trial CheckMate 143 explored the potential of using ICB as a new therapy for GBM. The phase III trial showed that therapy with antibodies against PD-1 failed to reach its primary endpoint of improving median overall survival in patients. [9] However, while less than 10% of GBM patients responded to Nivolumab, patients who did respond to immunotherapy had more durable responses than standard of care [9].

Several lessons were gained from the findings of the CheckMate 143 trial. The basis of ICB relies on the premise that the body is able to recognize tumor cells as foreign and subsequently mount T cell responses against these tumor antigens [10]. The relative low response rate demonstrated that the conventional strategy of depending on ICB to shift GBM from self-back to non-self is insufficient as a primary therapy. GBM is an immunologically "cold" or nonresponsive tumor, with both intrinsic resistance to the immune system due to low quality neoantigens, poor antigen presentation/priming, and relatively low mutational burden, as well as extrinsic resistance from an immunosuppressive tumor microenvironment (TME) [5]. Of note, in comparison to other solid tumors, GBM has relatively few tumor infiltrating lymphocytes (TILs). In addition, the small number of TILs exhibit high expression of multiple inhibitory checkpoint markers indicative of an exhaustive state. The GBM TME is also characterized by high numbers of myeloid cells, which can be usurped by the tumor to perform pro-tumor functions [11, 12].

However, the fact that there were more durable responses within the responder arm of the CheckMate 143 trial as well as emerging preclinical data that has elucidated some of the mechanisms of immune response in cold tumors like GBM still show promise for treatments that incorporate ICB [13, 14]. In this review, we focus on studies that have looked into combination treatments involving ICB in order to address the immunosuppressive environment of GBM, including: multiple ICB exposure, radiation in conjunction with ICB, antigen priming with vaccination in combination with ICB, and local chemotherapy with ICB. This review will also discuss the role of neoadjuvant ICB and new targets for treatment, including myeloid cells in GBM. Through this discussion, we hope to both provide an up to date compendium of combination immunotherapy clinical trials, but also elicit discussions as to the future of immunotherapy including exciting prospects fueled by recent preclinical and clinical findings.

Multiple immune checkpoint blockade

The concept of "exhausted T cells" has garnered attention as one of several reasons for the lack of response to immune cells. Particularly in GBM, tumor infiltrating lymphocytes (TILs) upregulate expression of multiple inhibitory immune checkpoint molecules such as PD-1, CTLA-4, Indoleamine 2,3-dioxygenase (IDO1), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), and Lymphocyte-activation gene 3 (LAG-3) [15]. These findings support the rationale of combinatorial checkpoint blockade, since targeting only one of the checkpoint molecules could be insufficient if other checkpoint-based immunosuppressive pathways remain.

The success of combining multiple ICB is notable in metastatic melanoma. Ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) combination therapy was approved by the FDA in 2016 as a first line therapy for patients with metastatic or inoperable melanoma. Recently at a 4-year follow up, the combination of ipilimumab and nivolumab demonstrated promising results in terms of improved progression-free survival (PFS) when compared with mono-checkpoint therapy [16]. Similarly, preclinical GBM models have also demonstrated that the combination of CTLA-4 and PD-1 blockade improved long-term survival when compared with ICB monotherapy, with longstanding memory T cell responses when re-challenged with tumors [17]. To date, there are six clinical trials involving the use of both CTLA-4 and PD-1 blockade for patients with GBM (Table 1).

Several other checkpoint molecules have garnered interest as additional therapeutic targets in GBM. Indoleamine 2,3-dioxygenase 1 (IDO1) is a checkpoint molecule found on GBM and immune cells that works to convert tryptophan to its eventual downstream catabolite kynurenine, which has since been implicated in immunosuppression. The expression of IDO1 is positively correlated to immunosuppressive regulatory T cell (Treg) infiltration and is negatively correlated with patient prognosis [18]. Pre-clinical data has shown that triple checkpoint blockade involving antibodies against IDO1, CTLA-4, and PD-L1 decrease the presence of tumor infiltrating Tregs and confer a durable survival benefit [19]. There are two phase I clinical trials that are now examining the use of combination PD-1 and IDO1 dual blockade (NCT04047706 and NCT03707457).

Moreover, T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and Lymphocyte-activation gene 3 (LAG-3) are two other inhibitory checkpoint molecules that demonstrate upregulation in GBM, particularly after patients receive anti-PD-1 therapy [20]. TIM-3 and PD-1 blockade [21] as well as LAG-3 and PD-1 blockade [22] demonstrate significant survival benefit in preclinical models. There is currently a phase I clinical trial

Table 1 Clinical trials with multiple ICB

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT02017717 (CheckMate 143) [9, 59]	III	A study of the effectiveness and safety of nivolumab Compared to Bevacizumab and of Nivolumab With or Without Ipilimumab in Glioblastoma Patients (CheckMate 143)	Primary	626	2: First recurrence of GBM; Prior 1L treatment with at least RT and TMZ 1c: Methylated, unmethylated, or indeterminate MGMT 1d Unmethylated MGMT	1. nivolumab (Cohort 1c, 1d, 2) 2. nivolumab + Ipilimumab (Cohort 1, 1b) 3. Bevzcizumab (Cohort 2)	Cohort 1, 1b, 1c, and 1d: Safety and Tolerability (8 months) Cohort 2: OS of nivolumab vs Bevacizumab (36 months)	Active, not recruiting Primary Endpoint Not Met (nivolumab arm failed to meet OS endpoint)
NCT04145115	II	A Phase II Study of Checkpoint Blockade Immunotherapy in Patients with Somatically Hypermutated Recurrent Glioblastoma	Recurrent	~37	N/A	Single arm: nivolumab + Ipilimumab	Overall Response Rate (32 months)	Not Yet Recruiting
NCT03233152	I	Intra-tumoral ipilimumab plus intravenous nivolumab Following the Resection of Recurrent Glioblastoma (GlitIpNi)	Recurrent	6	N/A	Single arm: Ipilimumab (at the end of neurosurgical resection) + Neoadjuvant and Adjuvant nivolumab	PFS (up to 30 weeks) OS (1 year)	Recruiting
NCT04003649	I	IL13Ralpha2-targeted chimeric antigen receptor (CAR) T cells with or without nivolumab and ipilimumab in treating patients with recurrent or refractory glioblastoma	Recurrent or Refractory	60	N/A	1. Ipilimumab + nivolumab + IL13Ralpha2 CAR T cells 2. nivolumab + IL13Ralpha2 CAR T cells	1. Dose Limiting Toxicities (28–42 days) 2. Cytokine Release Syndrome (28–42 days) 3. Toxicities (up to 15 years) 4. Feasibility of participants to either (i) receive Ipi/Nivo followed by 4 weekly CAR T cell with alternating weeks of Nivo infusions OR (ii) 4 weekly CAR T cell with alternating weeks of Nivo infusions (14–28 days) 5. OS (9 months)	Recruiting

Table 1 (continued)

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT03422094	I	Neoantigen-based personalized vaccine combined with immune checkpoint blockade therapy in patients with newly diagnosed, unmethylated glioblastoma	Primary	30	N/A	1. NeoVax + nivolumab (Start at time of progression) 2. NeoVax + nivolumab (Start at cycle 2) 3. NeoVax + nivolumab (Start at cycle 1) 4. NeoVax + ipilimumab + nivolumab (Start at cycle 3, q2w) 5. NeoVax + Ipilimumab + nivoluab (Start at cycle 1, q2w)	1. Safety and tolerability of regimen as measured by dose-limiting toxicity (up to 90 days) 2. Ability to identify candidate tumor-specific neoantigens (~ 14 weeks) 3. Ability to manufacture neoantigen-based synthetic long peptide vaccine (~ 14 weeks) 4. Ability to administer vaccines to patients at 4 weeks post-completion of radiotherapy (~ 14 weeks)	Recruiting
NCT02311920 [60]	I	Ipilimumab and/or nivolumab in combination with temozolomide in treating patients with newly diagnosed glioblastoma or gliosarcoma	Primary	32	N/A	1. Temozolomide + ipilimumabidentify (within 5 weeks after completion of chemoradiation) 2. Temozolomide + nivolumab (within 5 weeks after completion of chemoradiation) 3. Temozolomide + nivolumab + Ipilimumab (within 5 weeks after completion of chemoradiation)	Immune-related dose-limiting toxicities (up to 8 weeks)	Active, Not Recruiting
NCT04047706	I	Nivolumab, bms-986205, and radiation therapy with or without temozolomide in treating patients with newly diagnosed glioblastoma	Primary	30	1. Patients with MGMT methylated promoter 2. Patients with MGMT unmethylated promoter	1. Radiation + temozolomide + BMS-986205 (anti-IDO1) + nivolumab (Cohort I) 2. Radiation + BMS-986205 (anti-IDO1) + nivolumab (Cohort II)	Incidence of adverse events (up to 30 days after last dose)	Recruiting
NCT03707457	I	Biomarker-driven therapy using immune activators with nivolumab in patients with first recurrence of glioblastoma	Recurrent	30	Tumor tissue will be tested for biomarkers to determine immunotherapy combination	1. Nivolumab + Anti-GITR 2. Nivolumab + IDO1 inhibitor 3. Nivolumab + Ipilimumab	Dose limiting toxicities evaluated according to NCI-CTCAE (v.5) (up to 9 weeks after initial dose)	Recruiting

Table 1 (continued)

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT02658981 [23]	I	anti-LAG-3 alone & in combination w/ nivolumab Treating Patients w/recurrent GBM (anti-CD137 arm Closed 10/16/18)	Recurrent	100	N/A	1. Anti-LAG-3 2. Anti-CD137 3. Anti-LAG3 + Anti-PD-1 4. Anti-CD137 + Anti-PD-1	1. MTD of anti-LAG-3 (4 weeks) 2. MTD of anti-CD137 (4 weeks) 3. MTD of Anti-LAG-3 + Anti-PD-1 (4 weeks) 4. MTD of Anti-CD137 + Anti-PD-1 (4 weeks)	Recruiting RP2D Monotherapy: 1. Anti-LAG-3: 800 mg 2. Anti-CD137: 8 mg Combination therapy: 3. Anti-LAG-3 (160 mg) + Anti-PD-1 (240 mg) 4. Anti-CD137 (3 mg) + Anti-PD-1 (240 mg)

Compendium of clinical trials with combination checkpoint inhibition, including antibodies against PD-1, CTLA-4, IDO1. Clinical trials studying costimulatory checkpoint agonists were also included, including antibodies for CD137 (4-1BB) and GITR

that involves combination ICB against PD-1 and LAG-3 (NCT02658981) [23].

While there has been much excitement with the advent of inhibitory checkpoint blockade, there are also costimulatory checkpoint molecules that have been explored for treatment with the use of checkpoint agonists in GBM. CD137 (4-1BB) is a costimulatory molecule that is implicated in the activation and infiltration of cytotoxic T lymphocytes (CTL) into tumor sites [24]. When combined with anti-CTLA-4 as well as focal radiation, mice treated with a 4-1BB agonist exhibited at least 50% long-term survival with increased infiltration of T cells in tumor [25]. The ongoing ABTC 1501 trial is examining anti-LAG-3 or anti-CD137 alone and in combination with anti-PD-1 in patients with recurrent GBM, with preliminary data supporting improved overall survival for patients receiving CD137 and anti-PD-1 combination therapy [23]. There are nine ongoing clinical trials examining a combination of ICB for GBM to date (Table 1).

ICB and radiation

As part of the Stupp protocol for GBM, radiotherapy has roles in directly inducing cell death as well as enhancing immunogenicity. In preclinical melanoma and breast carcinoma models, radiotherapy was found to increase the percentage of antigen-experienced T cells and effector memory T cells, with increased infiltration of these T cells in tumors. Antigen presenting cells also saw increased immunogenicity, with upregulation of tumor-associated antigen-major histocompatibility complexes as well as enhanced cross-presentation to T cells [26].

The immunological effects of combining ICB with radiation were first examined in a cohort of metastatic melanoma patients, in which ICB and radiation were found to work in an immunologically non-redundant manner. Radiation diversified the T cell receptor (TCR) repertoire while ICB promoted T cell effector responses [27]. Some of the first promising preclinical studies involving combination therapy for GBM involved radiation and ICB, in which antibodies against PD-1, CTLA-4, and IDO1 yielded significant survival benefit [28, 29]. When combining antibodies against TIM-3 and PD-1 with radiation therapy, preclinical GBM models demonstrate 100% long-term survival [21]. A Phase I trial examining the efficacy of anti-TIM-3 with anti-PD-1 and SRS in patients with recurrent GBM is ongoing (Table 2). Furthermore, similar to patients with metastatic melanoma, anti-PD1 treated GBM patients that demonstrate a more diversified T cell repertoire are associated with improved overall survival when compared with standard treatment [8]. There have been twelve clinical trials involving ICB and radiation in GBM (Table 2).

ICB and antigen priming with vaccinations

The immune resistance exhibited by GBM can also be contributed in part to poor antigen priming [5]. GBM's relatively low mutational burden compounds the challenges of ICB as a therapeutic agent due to less available cognate antigens. Antigen priming can improve the efficacy of antigen presentation in ICB rescued T cells, thereby synergistically enhancing both antigen recognition and effector function. The strategy of using cancer vaccination with personalized neoantigens and/or tumor associated antigens has seen success in melanoma as well as prostate cancer, a cancer type traditionally deemed immunologically cold [30]. Of note, the combination of adjuvant vaccination and anti-PD-1 has found success in stage IIIC and IV high-risk melanoma, promoting relapse-free survival [31]. In GBM, transfer of autologous dendritic cells (DC) pulsed with tumor neoantigens demonstrate modulation of the TME by increasing T cell infiltration in preclinical models [32]. Clinically, a phase III trial investigating DC vaccination following standard of care in newly diagnosed GBM has found the treatment to be compatible with standard of care with comparable adverse events [33]. While analysis of the trial is ongoing, preliminary data suggests that DC vaccination might prolong survival [33]. A phase Ib trial involving personalized neoantigen vaccination has found increased T cell infiltration and enriched neoantigen-specific CD4+ and CD8+T cells with memory phenotypes [34].

Both preclinical and clinical data support that DC vaccination results in upregulation of PD-1 expression on T cells [35]. Moreover, when DC vaccination is combined with anti-PD-1, an increase in expression of memory markers in addition to integrin homing markers are observed in TILs [35]. There are currently seven GBM clinical trials involving ICB in combination with vaccinations for patients with both recurrent and newly diagnosed GBM (Table 3).

ICB and local delivery

The blood brain barrier (BBB) offers a unique challenge for immune-mediated anti-tumor activity. This separation of compartments leads to immune phenotypic differences between cells in peripheral blood and the central nervous system (CNS) [15]. Difficulties penetrating the blood brain barrier extend beyond immune cells and have historically involved novel delivery strategies for other treatments, including chemotherapy. One of these strategies involve local delivery of chemotherapeutic agents such as carmustine (BCNU) or temozolomide (TMZ) through the form of biodegradable wafers or thermo-responsive biodegradable pastes [36, 37]. Similarly, there is now increased interest in delivering ICB intracranially to the tumor site, with a phase I clinical trial currently recruiting patients to examine the use of intra-tumoral ipilimumab at the time of surgical resection with systemic nivolumab for recurrent GBM (Table 4).

Beyond bypassing the issue of penetrating the BBB, local chemotherapy has an immunological advantage over its systemic counterpart in that the latter has disadvantages in globally suppressing immune function from cytotoxic lymphodepletion [38, 39]. In a preclinical study that compared the use of systemic chemotherapy against local chemotherapy with or without anti-PD-1, the combination of local chemotherapy with ICB resulted in the most optimal long-term survival benefit. Furthermore, systemic chemotherapy with anti-PD-1 therapy was shown to result in severe lymphodepletion as well as decreased infiltration of T cells to the tumor site [40]. As such, the benefits of exploring the combination of local chemotherapy and ICB remain of interest.

Neoadjuvant therapy

Despite initial responses to ICB in metastatic melanoma and non-small cell lung cancer, tumors can develop acquired resistance to anti-tumor responses and result in recurrence [20]. In a chronic viral antigen model, T cells that were continuously exposed to foreign antigens succumbed to terminal exhaustion, with their transcription profiles differentiating them from effector T cells [15, 41]. The temporal association of ICB delivery to immunologic tolerance opens up potential investigation of ICB as a neoadjuvant therapy. By first providing ICB in the setting of maximal tumor density, antigen presenting cells and T cells would have increased opportunities for developing tumor specific immunity. Subsequent resection of the tumor would then physically decrease the number of tumor antigens after having already activated the immune response and thereby mitigate immune cell tolerance due to chronic antigen exposure [42]. This might be particularly relevant to GBM given the intrinsic immunosuppressive TME.

Data supporting the use of neoadjuvant ICB has been demonstrated in several models. In a preclinical breast cancer model, neoadjuvant therapy with ICB conferred a survival benefit over adjuvant therapy only counterparts [43]. Results from neoadjuvant ICB in melanoma and non-small cell lung cancer have also seen promising responses; neoadjuvant anti-PD-1 in these cancer tissues demonstrated an increase in both T cell clonality and diversity upon treatment, thereby indicating viable T cell activation from antigen presenting cells [44, 45]. This strategy of pre-treating patients with anti-PD-1 may also be beneficial for GBM given the immunosuppressive effects of current standard of care involving chemoradiation.

Table 2 Clinical trials involving ICB with radiation

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT02617589 (CheckMate 498) [61]	III	An investigational immuno-therapy study of nivolumab compared to temozolomide, each given with radiation therapy, for newly-diagnosed patients with glioblastoma (GBM, a malignant brain cancer) (CheckMate 498)	Primary	550	N/A	1. Nivolumab + radiotherapy 2. Temozolomide + radiotherapy	Overall survival (3 years)	Recruiting; primary endpoint not met–overall survival
NCT02667587 (CheckMate 548) [62]	III	An investigational immuno-therapy study of temozolomide plus radiation therapy with nivolumab or placebo, for newly diagnosed patients with glioblastoma (GBM, a malignant brain cancer) (CheckMate548)	Primary	693	N/A	1. Nivolumab + temozolomide + Radiotherapy 2. Nivolumab placebo + temozolomide + Radiotherapy	1. Overall survival (24 months) 2. Progression free survival (35 months)	PFS not met; continual evaluation of OS
NCT03743662	II	Nivolumab with radiation therapy and bevacizumab for recurrent MGMT methylated glioblastoma	Recurrent	94	1. Patients with recurrent GBM not undergoing surgical debulking as part of their treatment plan 2. Patients with recurrent GBM who are undergoing surgery as part of their treatment	1. Nivolumab followed by re-radiation + bevacizumab (if deemed beneficial) 2. Nivolumab followed by re-resection, then re-radiation + bevacizumab (if deemed beneficial)	Overall survival (2 years)	Recruiting; primary endpoint not met
NCT03661723	II	Pembrolizumab and Reirradiation in bevacizumab naïve and bevacizumab resistant recurrent glioblastoma	Recurrent	60	1. Bevacizumab naïve 2. Bevacizumab recurrent	1. Pembrolizumab + re-Irradiation (lead-in) 2. Pembrolizumab + bevacizumab + re-irradiation (lead-in) 3. Pembrolizumab + re-irradiation 4. Pembrolizumab + bevacizumab + re-irradiation	Objective response rate (2 years) Overall survival (12 months)	Recruiting

Table 2 (continued)

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT03367715	II	Nivolumab, ipilimumab, and short-course radiotherapy in adults with newly diagnosed, MGMT unmethylated glioblastoma	Primary	24	N/A	Single arm: Nivolumab + Ipilimumab + short-course radiation	Overall survival (1 year)	Not yet recruiting
NCT03018288	II	Radiation therapy plus temozolomide and pembrolizumab with and without HSPPC-96 in newly diagnosed glioblastoma (GBM)	Primary	108	N/A	1. Radiotherapy + temozolomide + pembrolizumab 2. Radiotherapy + temozolomide + HSPPC-96 vaccine 3. Radiotherapy + temozolomide + placebo	Overall survival (1 year)	Recruiting
NCT03174197	II/I	Atezolizumab in combination with temozolomide and radiation therapy in treating patients with newly diagnosed glioblastoma	Primary	60	One cohort, Phase I followed by Phase II	1. Phase II: concurrent Atezolizumab + temozolomide + radiotherapy 2. Phase I: Adjuvant atezolizumab + temozolomide	Phase II: overall survival (3 years) Phase I: Dose-limiting toxicities (10 weeks) Phase I + II: incidence of adverse events (3 years)	Recruiting
NCT02052648 [63, 64]	II/I	Study of the IDO pathway inhibitor, indoximod, and temozolomide for pediatric patients with progressive primary malignant brain tumors	Primary	160	1. Bevacizumab-naïve patients 2. Patients receiving of have received and failed Bevacizumab 3. Patients who will receive stereotactic radiosurgery	Phase Ib Single arm: indoximod (dose escalation) + temozolomide Phase II Single arm: Indoximod + temozolomide (dosed at 150–200 mg/m2) cohort 1, 2,3	Phase I: Determine Phase 2 dosing Phase II: Efficacy (18 month)	Recruitment completed; indoximod MTD: 1200 mg BID
NCT04047706	I	Nivolumab, BMS-986205, and radiation therapy with or without temozolomide in treating patients with newly diagnosed glioblastoma	Primary	30	1. Patients with MGMT methylated promoter 2. Patients with MGMT unmethylated promoter	1. Radiation + temozolomide + BMS-986205 (anti-IDO1) + nivolumab (Cohort I) 2. Radiation + BMS-986205 (anti-IDO1) + nivolumab (cohort II)	Incidence of adverse events (up to 30 days after last dose)	Recruiting

Table 2 (continued)

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT03426891 [65]	I	Pembrolizumab and vorinostat combined with temozolomide for newly diagnosed glioblastoma	Primary	32	Part 1: Dose escalation of Vorinostat Part 2: Dose Expansion (All participants receiving same dose of Vorinostat, MTD determined by part 1	Single arm: Pembrolizumab + vorinostat + temozolomide + Radiotherapy	MTD (12 weeks)	Recruiting Completed enrollment to dose level 1 No dose liming adverse event observed Most common adverse event: thrombocytopenia and fatigue
NCT02287428 [17]	I	Personalized neoantigen cancer vaccine w RT plus pembrolizumab for patients With MGMT unmethylated, newly diagnosed GBM	Primary	46	1. Radiotherapy, then NeoVax 1a,b,c. Radiotherapy, then NeoVax + Pembro (at different treatment time point)	1. Standard radiotherapy, then NeoVax 2. Concurrent radiotherapy + temozolomide + pembrolizumab, then NeoVax + pembrolizumab 3. Concurrent radiotherapy + temozolomide, then NeoVax + pembrolizumab 4. Concurrent Radiotherapy + temozolomide + 1 dose of pembrolizumab, then NeoVax + pembrolizumab	1. Safety and tolerability (2 years) 2. Cohort 1: # patients with at least 10 actionable peptides (2 years) 3. Cohort 1: # patients able to initiate post radiotherapy vaccine therapy within 12 weeks from date of surgery (2 years)	Active, not recruiting "Individualized, multi-neo-epitope vaccines are feasible, safe and capable of generating systemic and intra-tumoral immune responses in GBM patients that appear to be abrogated by dex"
NCT03197506	II	Pembrolizumab and standard therapy in treating patients with glioblastoma	Recurrent	90	N/A	Single arm: Neoadjuvant pembrolizumab + adjuvant pembrolizumab + temozolomide + radiotherapy	1. Dose limiting toxicities (5 years) 2. Overall Survival (18 months) 3. Progression-free Survival (5 years) 4. Time to progression (5 years) 5. Time to treatment failure (5 years)	Recruiting

Compendium of clinical trials that included radiation as part of therapy with checkpoint inhibition. Radiation is thought to have both direct tumoricidal and immunogenic effects

Table 3 Clinical trials involving combination therapy with ICB and immune vaccination

Clinical Trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT03018288	II	Radiation therapy plus temozolomide and pembrolizumab with and without HSPPC-96 in newly diagnosed glioblastoma (GBM)	Primary	108	N/A	1. Radiotherapy + temozolomide + pembrolizumab 2. Radiotherapy + Temozolomide + HSPPC-96 vaccine 3. Radiotherapy + temozolomide + Placebo	Overall survival (1 year)	Recruiting
NCT03750071 Wick W, 2019	II/I	VXM01 Plus avelumab combination study in progressive glioblastoma	Progressive glioblastoma	30	1. Patients with progressive glioblastoma that is non-resectable 2. Patients with progressive glioblastoma that is resectable	Single arm: VXM01 + avelumab	Treatment-emerging adverse events (up to 60 weeks)	Recruiting
NCT03665545	II/I	Pembrolizumab in association with the IMA950/poly-ICLC for relapsing glioblastoma (IMA950-106)	Recurrent	24	N/A	1. Poly-ICLC + IMA950 2. Poly-ICLC + IMA950 + pembrolizumab	Incidence of Treatment-Emergent Adverse Events (30 days after cessation of treatment)	Recruiting
NCT03422094	I	Neoantigen-based personalized vaccine combined with immune checkpoint blockade therapy in patients with newly diagnosed, unmethylated glioblastoma	Primary	30	N/A	1. NeoVax + nivolumab (start at time of progression) 2. NeoVax + nivolumab (start at cycle 2) 3. NeoVax + nivolumab (start at cycle 1) 4. NeoVax + ipilimumab + nivolumab (start at cycle 3, q2w) 5. NeoVax + ipilimumab + nivolumab (start at cycle 1, q2w)	1. Safety and tolerability of regimen as measured by dose-limiting toxicity (up to 90 days) 2. Ability to identify candidate tumor-specific neoantigens (~ 14 weeks) 3. Ability to manufacture neoantigen-based synthetic long peptide vaccine (~ 14 weeks) 4. Ability to administer vaccines to patients at 4 weeks post-completion of radiotherapy (~ 14 weeks)	Recruiting

Table 3 (continued)

Clinical Trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT02287428 Reardon DA, 2018	I	Personalized neoantigen cancer vaccine w RT Plus pembrolizumab for patients With MGMT unmethylated, newly diagnosed GBM	Primary	46	1. Radiotherapy, then NeoVax 1a,b,c. Radiotherapy, then NeoVax + Pembro (at different treatment time point)	1. Standard radiotherapy, then NeoVax 2. Concurrent radiotherapy + temozolomide + pembrolizumab, then NeoVax + pembrolizumab 3. Concurrent radiotherapy + temozolomide, then NeoVax + pembrolizumab 4. Concurrent radiotherapy + Temozolomide + 1 dose of pembrolizumab, then NeoVax + pembrolizumab	1. Safety and tolerability (2 years) 2. Cohort 1: # patients with at least 10 actionable peptides (2 years) 3. Cohort 1: # patients able to initiate post radiotherapy vaccine therapy within 12 weeks from date of surgery (2 years)	Active, not recruiting "Individualized, multi-neo-epitope vaccines are feasible, safe and capable of generating systemic and intra-tumoral immune responses in GBM patients that appear to be abrogated by dex"
NCT04201873	I	Pembrolizumab and a Vaccine (ATL-DC) for the treatment of surgically accessible recurrent glioblastoma	Recurrent	40	N/A	1.Neoadjuvant pembrolizumab, then Adjuvant pembrolizumab + ATL-DC w/ Poly ICLC 2. Neoadjuvant placebo, then adjuvant placebo + ATL-DC w/ poly ICLC	1. Cell cycle-related signature (6 years) 2. Expansion of TCR clones (6 years) 3. Incidence of adverse events (up to 30 days post treatment)	Not yet recruiting
NCT02529072 Peters KB, 2019	I	Nivolumab with DC vaccines for recurrent brain tumors (AVERT)	Recurrent	7	N/A	1. Neoadjuvant nivolumab, then adjuvant nivolumab + DC vaccines 2. Neoadjuvant nivolumab + DC vaccines, then adjuvant nivolumab	% patients who experience unacceptable toxicity due to combination treatment (12 months)	Active, not recruiting Safety of combination nivolumab and DC vaccination therapy is similar to nivolumab monotherapy

Compendium of clinical trials that feature antigen priming with immune cell vaccination, including activation of toll-like receptor 3 (TLR3) on dendritic cells with Poly-ICLC

Table 4 Clinical trials involving local therapy as well as neoadjuvant ICB

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT03233152	I	Intra-tumoral Ipilimumab Plus Intravenous nivolumab Following the Resection of Recurrent Glioblastoma (GlitIpNi)	Recurrent	6	N/A	Single Arm: Ipilimumab (at the end of neurosurgical resection) + Neoadjuvant and Adjuvant nivolumab	PFS (up to 30 weeks) OS (1 year)	Recruiting
NCT04201873	I	pembrolizumab and a Vaccine (ATL-DC) for the Treatment of Surgically Accessible Recurrent Glioblastoma	Recurrent	40	N/A	1. Neoadjuvant pembrolizumab, then Adjuvant pembrolizumab + ATL-DC w/ Poly ICLC 2. Neoadjuvant placebo, then adjuvant placebo + ATL-DC w/ poly ICLC	1. Cell cycle-related signature (6 years) 2. Expansion of TCR clones (6 years) 3. Incidence of adverse events (up to 30 days post treatment)	Not yet recruiting
NCT02529072 Peters KB, 2019	I	nivolumab With DC Vaccines for Recurrent Brain Tumors (AVERT)	Recurrent	7	N/A	1. Neoadjuvant nivolumab, then adjuvant nivolumab + DC vaccines 2. Neoadjuvant nivolumab + DC vaccines, then adjuvant nivolumab	% patients who experience unacceptable toxicity due to combination treatment (12 months)	Active, not recruiting Safety of combination nivolumab and DC vaccination therapy is similar to nivolumab monotherapy
NCT02550249 Schalper KA, 2019	II	Neoadjuvant nivolumab in Glioblastoma (Neo-nivo)	Primary and Recurrent	29	N/A	Single arm: Neoadjuvant nivolumab	Changes in % and PD-L1 expression by tumor cells and lymphocytes (4 weeks)	Completed Neoadjuvant nivolumab enhanced chemokine expression, immune cell infiltration, increased T cell diversity, and had a local immunomodulatory effect
NCT03197506	II	pembrolizumab and Standard Therapy in Treating Patients With Glioblastoma	Recurrent	90	N/A	Single arm: Neoadjuvant pembrolizumab + Adjuvant pembrolizumab + Temozolomide + Radiotherapy	1. Dose limiting toxicities (5 years) 2. Overall Survival (18 months) 3. Progression-free Survival (5 years) 4. Time to progression (5 years) 5. Time to treatment failure (5 years)	Recruiting
NCT03233152	I	Intra-tumoral Ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma (GlitIpNi)	Recurrent	6	N/A	Single arm: Ipilimumab (at the end of neurosurgical resection) + Neoadjuvant and Adjuvant nivolumab	PFS (up to 30 weeks) OS (1 year)	Recruiting

Table 4 (continued)

Clinical trial identifier	Phase	Name of trial	Primary/recurrent	N	Cohort(s)	Treatment arms	Primary endpoint	Status/conclusion
NCT03233152	I	Intra-tumoral ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma (GlitIpNi)	Recurrent	6	N/A	Single arm: Ipilimumab (at the end of neurosurgical resection) + Neoadjuvant and adjuvant nivolumab	PFS (up to 30 weeks) OS (1 year)	Recruiting
NCT04201873	I	Pembrolizumab and a vaccine (ATL-DC) for the treatment of surgically accessible recurrent glioblastoma	Recurrent	40	N/A	1.Neoadjuvant pembrolizumab, then Adjuvant pembrolizumab + ATL-DC w/ poly ICLC 2. Neoadjuvant placebo, then adjuvant placebo + ATL-DC w/ poly ICLC	1. Cell cycle-related signature (6 years) 2. Expansion of TCR clones (6 years) 3. Incidence of adverse events (up to 30 days post treatment)	Not yet recruiting
NCT02529072 Peters KB, 2019	I	Nivolumab With DC vaccines for recurrent brain tumors (AVERT)	Recurrent	7	N/A	1. Neoadjuvant nivolumab, then adjuvant nivolumab + DC vaccines 2. Neoadjuvant nivolumab + DC vaccines, then adjuvant nivolumab	% patients who experience unacceptable toxicity due to combination treatment (12 months)	Active, not recruiting Safety of combination nivolumab and DC vaccination therapy is similar to nivolumab monotherapy
NCT02550249 Schalper KA, 2019	II	Neoadjuvant nivolumab in glioblastoma (Neo-nivo)	Primary and recurrent	29	N/A	Single arm: Neoadjuvant nivolumab	Changes in % and PD-L1 expression by tumor cells and lymphocytes (4 weeks)	Completed Neoadjuvant nivolumab enhanced chemokine expression, immune cell infiltration, increased T cell diversity, and had a local immunomodulatory effect
NCT03197506	II	pembrolizumab and Standard Therapy in Treating Patients With Glioblastoma	Recurrent	90	N/A	Single arm: Neoadjuvant pembrolizumab + Adjuvant pembrolizumab + Temozolomide + Radiotherapy	1. Dose limiting toxicities (5 years) 2. Overall survival (18 months) 3. Progression-free survival (5 years) 4. Time to progression (5 years) 5. Time to treatment failure (5 years)	Recruiting

Compendium of clinical trials involving direct treatment with anti-PD-1 during resection as well as several neoadjuvant trials with anti-PD-1 and anti-CTLA-4

There are currently four clinical trials of GBM involving neoadjuvant treatment with ICB and/or vaccines (Table 4). Recently, results from several trials show the ability of neoadjuvant ICB therapy to affect the immune landscape of GBM. Efforts led by Schalper et al. demonstrated that neoadjuvant nivolumab when compared with standard treatment had opposing effects on the number of TCR clonotypes [8]. Similar to melanoma models, neoadjuvant nivolumab for GBM diversified the TCR repertoire when comparing tumor samples from pre- and post-surgical resection [44]. They also reported an increase in detection of chemokine mRNA transcripts in the nivolumab group, potentially indicating recruitment of immune cells [44].

While another trial did not observe an increase in TCR diversity in relation to neoadjuvant therapy, they did however find a fraction of peripheral and tumor infiltrating T cell clonotypes that expanded post-surgical resection [46]. This could potentially be indicative of increased clonality as observed in the NSCLC neoadjuvant trial [45]. Other significant findings included patients with neoadjuvant treatment showing a significant decrease in monocytes in the periphery after surgery compared to adjuvant treated groups [46]. However, CTLA-4 was upregulated after just 1 dose of pembrolizumab in the neoadjuvant group, suggesting that there may be increased susceptibility to adaptive resistance in the form of upregulated expression of alternative checkpoint molecules [46]. While current neoadjuvant GBM trials have yet to show survival benefit when compared with the current standard of care in recurrent GBM, the T cell repertoire changes found in neoadjuvant GBM trials indicate the importance of exploring the chronic antigen environment as well as the timing of ICB for GBM further.

Targeting the myeloid compartment

Beyond the T lymphocyte population, the relatively higher number of myeloid cells present in GBM have garnered interest in targeting these cells for immune reactivation. There is an increasing body of literature that demonstrate the importance of immunosuppressive and pro-tumor characteristics in microglia, macrophages, and dendritic cells in the tumor microenvironment for sustaining tumorigenesis [47, 48]. Several therapeutic strategies that have since evolved include reactivation of the intrinsic anti-tumor properties of dendritic cells, microglia, and macrophages, including agonists for toll-like receptors (TLR) such as polyinosinic-polycytidylic acid [poly(I:C)] for TLR3 [49] as well as oligodeoxynucleotides containing CpG motifs (CpG-ODN) for TLR9 [50] on dendritic cells. However, it should be noted that a phase II trial investigating intracerebrally administered CpG-ODN as an anti-tumor therapeutic for GBM did not result in significant improvement in PFS [51]. Other therapies have focused on inhibiting the pro-tumor functions of microglia and macrophages, including antibodies against colony stimulating factor-1 receptor (anti-CSF-1R) and C–C Motif Chemokine Receptor 2 (CCR2), with resultant tumor growth inhibition and enhanced survival in preclinical models [52–54].

Additionally, recombinant adenovirus carrying Interleukin 12 (rAAV2/IL-12) has shown promise for targeting myeloid cells as demonstrated by increased expression of CD68 and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in microglia, with resultant infiltration of activated microglial cells within the tumor mass and increased median overall survival in murine models [55]. As myeloid cells continue to be explored as a source of immunosuppression in GBM, it will be increasingly critical to comprehensively reactivate both T lymphocyte and myeloid cells. Future directions exploring combinatorial approaches for both of these compartments are of ongoing interest.

Discussion

Immune checkpoint blockade has been a cornerstone of cancer immunotherapy this past decade, with durable response rates to inhibition of PD-1 and CTLA-4 driving forward several ICB combination therapies. However, despite translating these treatments to GBM, significantly improved prognostic outcomes over standard of care have remained elusive.

While GBM has long since established itself as having a dynamic immune milieu, the immunosuppressive tumor microenvironment, the heterogeneity of the disease between patients, as well as challenges from the BBB present a unique challenge for achieving durable immunotherapeutic response. As such, there is need for caution in simply transposing treatment of other cancers to GBM. While there is still much to be gained in understanding basic immune mechanisms from immunotherapy in other cancers, GBM will require further studies that explore how it is different, including the relatively increased representation of myeloid cells compared to T lymphocytes in the tumor site [15]. As discussed in this review, the need for reinvigorating both T lymphocytes as well as the myeloid cells that activate the T cells should be further explored.

Furthermore, the role and timing of ICB in cancer immunotherapy remains important as new mechanisms and therapies are elicited from ongoing preclinical and clinical investigations. Already, several novel ICB combinations tailored to GBM are being explored, with efforts focusing on using chemokines such as C-X-C chemokine receptor type 4 (CXCR4) or cytokines such as IL-12, as well as tumor suppressor genes such as tumor protein p53 in conjunction with ICB [56–58]. Furthermore, as we embrace GBM as an immunological unique disease, interdisciplinary approaches

studying chronic CNS inflammatory conditions such as multiple sclerosis and chronic viral infections will be integral to understanding immune mechanisms within the brain. Through a combination study approach, it is our hope that GBM joins the success of recent years in immunotherapy for solid tumors.

Author contributions HYC (Literature review, data compilation, figures generation, writing, revisions); JC (Literature review, writing, revisions), CJ (Concept outline, literature review, revisions), ML (Concept outline, literature review, revisions).

Funding No funding.

Compliance with ethical standards

Conflict of interest Michael Lim (Funding from Arbor Pharmaceuticals, Accuray, BMS, Novartis; Consultant: BMS, Merck, SQZ Biotechnologies, Tocagen, VBI; Patents: Combining Focused Radiation and Immunotherapy, Combining Local Chemotherapy and Immunotherapy).

References

1. Burki TK (2015) pembrolizumab for patients with advanced melanoma. Lancet Oncol 16(6):e264. https://doi.org/10.1016/S1470-2045(15)70193-2
2. Larkin J, Chiarion-Sileni V, Gonzalez R et al (2019) Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 381(16):1535–1546. https://doi.org/10.1056/NEJMoa1910836
3. Garon EB, Rizvi NA, Hui R et al (2015) pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 372(21):2018–2028. https://doi.org/10.1056/NEJMoa1501824
4. Le DT, Durham JN, Smith KN et al (2017) Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357(6349):409–413. https://doi.org/10.1126/science.aan6733
5. Jackson CM, Choi J, Lim M (2019) Mechanisms of immunotherapy resistance: lessons from glioblastoma. Nat Immunol 20(9):1100–1109. https://doi.org/10.1038/s41590-019-0433-y
6. Ostrom QT, Gittleman H, Liao P et al (2017) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2010–2014. Neuro Oncol 19(5):v1–v88. https://doi.org/10.1093/neuonc/nox158
7. Stupp R, Mason WP, Van Den Bent MJ et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996. https://doi.org/10.1056/NEJMoa043330
8. Schalper KA, Rodriguez-Ruiz ME, Diez-Valle R et al (2019) Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma. Nat Med 25(3):470–476. https://doi.org/10.1038/s41591-018-0339-5
9. Omuro A, Vlahovic G, Lim M et al (2018) nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. Neuro Oncol 20(5):674–686. https://doi.org/10.1093/neuonc/nox208
10. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12(4):252–264. https://doi.org/10.1038/nrc3239
11. Li B, Severson E, Pignon JC et al (2016) Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol. https://doi.org/10.1186/s13059-016-1028-7
12. Ribas A (2015) Adaptive immune resistance: how cancer protects from immune attack. Cancer Discov 5(9):915–919. https://doi.org/10.1158/2159-8290.CD-15-0563
13. Bouffet E, Larouche V, Campbell BB et al (2016) Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. J Clin Oncol 34(19):2206–2211. https://doi.org/10.1200/JCO.2016.66.6552
14. Johanns TM, Miller CA, Dorward IG et al (2016) Immunogenomics of hypermutated glioblastoma: a patient with germline POLE deficiency treated with checkpoint blockade immunotherapy. Cancer Discov 6(11):1230–1236. https://doi.org/10.1158/2159-8290.CD-16-0575
15. Woroniecka K, Chongsathidkiet P, Rhodin K et al (2018) T-cell exhaustion signatures vary with tumor type and are severe in glioblastoma. Clin Cancer Res 24(17):4175–4186. https://doi.org/10.1158/1078-0432.CCR-17-1846
16. Hodi FS, Chiarion-Sileni V, Gonzalez R et al (2018) nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol 19(11):1480–1492. https://doi.org/10.1016/S1470-2045(18)30700-9
17. Reardon DA, Neuberg DS, Keskin DB et al (2018) Effect of dexamethasone in glioblastoma (GBM) patients on systemic and intratumoral T-cell responses induced by personalized neoantigen-targeting vaccine. J Clin Oncol 36(15_suppl):2020. https://doi.org/10.1200/jco.2018.36.15_suppl.2020
18. Zhai L, Ladomersky E, Lauing KL et al (2017) Infiltrating T cells increase IDO1 expression in glioblastoma and contribute to decreased patient survival. Clin Cancer Res 23(21):6650–6660. https://doi.org/10.1158/1078-0432.CCR-17-0120
19. Wainwright DA, Chang AL, Dey M et al (2014) Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. Clin Cancer Res 20(20):5290–5301. https://doi.org/10.1158/1078-0432.CCR-14-0514
20. Koyama S, Akbay EA, Li YY et al (2016) Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun. https://doi.org/10.1038/ncomms10501
21. Kim JE, Patel MA, Mangraviti A et al (2017) Combination therapy with anti-PD-1, anti-TIM-3, and focal radiation results in regression of murine gliomas. Clin Cancer Res 23(1):124–136. https://doi.org/10.1158/1078-0432.CCR-15-1535
22. Harris-Bookman S, Mathios D, Martin AM et al (2018) Expression of LAG-3 and efficacy of combination treatment with anti-LAG-3 and anti-PD-1 monoclonal antibodies in glioblastoma. Int J Cancer 143(12):3201–3208. https://doi.org/10.1002/ijc.31661
23. Lim M, Ye X, Piotrowski AF et al (2019) Updated phase I trial of anti-LAG-3 or anti-CD137 alone and in combination with anti-PD-1 in patients with recurrent GBM. J Clin Oncol. 37(15_suppl):2017. https://doi.org/10.1200/jco.2019.37.15_suppl.2017
24. Vinay DS, Kwon BS (2014) 4–1BB (CD137), an inducible costimulatory receptor, as a specific target for cancer therapy. BMB Rep 47(3):122–129. https://doi.org/10.5483/bmbrep.2014.47.3.283
25. Belcaid Z, Phallen JA, Zeng J et al (2014) Focal radiation therapy combined with 4–1BB activation and CTLA-4 blockade yields long-term survival and a protective antigen-specific memory response in a murine glioma model. PLoS ONE 9(7):1–9. https://doi.org/10.1371/journal.pone.0101764

26. Sharabi AB, Nirschl CJ, Kochel CM et al (2015) Stereotactic radiation therapy augments antigen-specific PD-1-mediated antitumor immune responses via cross-presentation of tumor antigen. Cancer Immunol Res 3(4):345–355. https://doi.org/10.1158/2326-6066.CIR-14-0196
27. Twyman-Saint Victor C, Rech AJ, Maity A et al (2015) Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 520(7547):373–377. https://doi.org/10.1038/nature14292
28. Zeng J, See AP, Phallen J et al (2013) Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 86(2):343–349. https://doi.org/10.1016/j.ijrobp.2012.12.025
29. Ladomersky E, Zhai L, Lenzen A et al (2018) IDO1 inhibition synergizes with radiation and PD-1 blockade to durably increase survival against advanced glioblastoma. Clin Cancer Res 24(11):2559–2573. https://doi.org/10.1158/1078-0432.CCR-17-3573
30. Comiskey MC, Dallos MC, Drake CG (2018) Immunotherapy in prostate cancer: teaching an old dog new tricks. Curr Oncol Rep 20(9):1–10. https://doi.org/10.1007/s11912-018-0712-z
31. Gibney GT, Kudchadkar RR, DeConti RC et al (2015) Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. Clin Cancer Res 21(4):712–720. https://doi.org/10.1158/1078-0432.CCR-14-2468
32. Liau LM, Prins RM, Kiertscher SM et al (2005) Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res 11(15):5515–5525. https://doi.org/10.1158/1078-0432.CCR-05-0464
33. Liau LM, Ashkan K, Tran DD et al (2018) First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. J Transl Med 16(1):1. https://doi.org/10.1186/s12967-018-1507-6
34. Keskin DB, Anandappa AJ, Sun J et al (2019) Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature 565(7738):234–239. https://doi.org/10.1038/s41586-018-0792-9
35. Antonios JP, Soto H, Everson RG et al (2019) PD-1 blockade enhances the vaccination-induced immune response in glioma. JCI Insight. https://doi.org/10.1172/jci.insight.87059
36. Brem H, Piantadosi S, Burger PC et al (1995) Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. Lancet 345(8956):1008–1012. https://doi.org/10.1016/S0140-6736(95)90755-6
37. Smith SJ, Tyler BM, Gould T et al (2019) Overall survival in malignant glioma is significantly prolonged by neurosurgical delivery of etoposide and temozolomide from a thermo-responsive biodegradable paste. Clin Cancer Res 25(16):5094–5106. https://doi.org/10.1158/1078-0432.CCR-18-3850
38. van der Most RG, Robinson BWS, Lake RA (2005) Combining immunotherapy with chemotherapy to treat cancer. Discov Med 5(27):265–270
39. Jackson C, Ruzevick J, Brem H, Lim M (2013) Vaccine strategies for glioblastoma: progress and future directions. Immunotherapy 5(2):155–167. https://doi.org/10.2217/imt.12.155
40. Mathios D, Kim JE, Mangraviti A et al (2020) Anti-PD-1 antitumor immunity is enhanced by local and abrogated by systemic chemotherapy in GBM. https://stm.sciencemag.org/. Accessed 12 Jan 2020
41. Sen DR, Kaminski J, Barnitz RA et al (2016) The epigenetic landscape of T cell exhaustion. Science 354(6316):1165–1169. https://doi.org/10.1126/science.aae0491
42. Melero I, Berraondo P, Rodríguez-Ruiz ME, Pérez-Gracia JL (2016) Making the most of cancer surgery with neoadjuvant immunotherapy. Cancer Discov. https://doi.org/10.1158/2159-8290.CD-16-1109
43. Liu J, Blake SJ, Yong MCR et al (2016) Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discov 6(12):1382–1399. https://doi.org/10.1158/2159-8290.CD-16-0577
44. Blank CU, Rozeman EA, Fanchi LF et al (2018) Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. Nat Med 24(11):1655–1661. https://doi.org/10.1038/s41591-018-0198-0
45. Forde PM, Chaft JE, Smith KN et al (2018) Neoadjuvant PD-1 blockade in resectable lung cancer. N Engl J Med. https://doi.org/10.1056/NEJMoa1716078
46. Cloughesy TF, Mochizuki AY, Orpilla JR et al (2019) Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med 25(3):477–486. https://doi.org/10.1038/s41591-018-0337-7
47. Choi J, Mai N, Jackson C, Belcaid Z, Lim M (2018) It takes two: potential therapies and insights involving microglia and macrophages in glioblastoma. Neuroimmunol Neuroinflamm 5(10):42. https://doi.org/10.20517/2347-8659.2018.47
48. Srivastava S, Jackson C, Kim T, Choi J, Lim M (2019) A characterization of dendritic cells and their role in immunotherapy in glioblastoma: from preclinical studies to clinical trials. Cancers (Basel). https://doi.org/10.3390/cancers11040537
49. Garzon-Muvdi T, Theodros D, Luksik AS et al (2018) Dendritic cell activation enhances anti-PD-1 mediated immunotherapy against glioblastoma. Oncotarget 9(29):20681–20697. https://doi.org/10.18632/oncotarget.25061
50. Kees T, Lohr J, Noack J et al (2012) Microglia isolated from patients with glioma gain antitumor activities on poly (I:C) stimulation. Neuro Oncol 14(1):64–78. https://doi.org/10.1093/neuonc/nor182
51. Carpentier A, Metellus P, Ursu R et al (2010) Intracerebral administration of CpG oligonucleotide for patients with recurrent glioblastoma: a phase II study. Neuro Oncol 12(4):401–408. https://doi.org/10.1093/neuonc/nop047
52. Pyonteck SM, Akkari L, Schuhmacher AJ et al (2013) CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med 19(10):1264–1272. https://doi.org/10.1038/nm.3337
53. Yan D, Kowal J, Akkari L et al (2017) Inhibition of colony stimulating factor-1 receptor abrogates microenvironment-mediated therapeutic resistance in gliomas. Oncogene 36(43):6049–6058. https://doi.org/10.1038/onc.2017.261
54. Zhu X, Fujita M, Snyder LA, Okada H (2011) Systemic delivery of neutralizing antibody targeting CCL2 for glioma therapy. J Neurooncol 104(1):83–92. https://doi.org/10.1007/s11060-010-0473-5
55. Chiu TL, Wang MJ, Su CC (2012) The treatment of glioblastoma multiforme through activation of microglia and TRAIL induced by rAAV2-mediated IL-12 in a syngeneic rat model. J Biomed Sci. https://doi.org/10.1186/1423-0127-19-45
56. Chiocca EA, Yu JS, Lukas RV et al (2019) Regulatable interleukin-12 gene therapy in patients with recurrent high-grade glioma: results of a phase 1 trial. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aaw5680
57. Wu A, Maxwell R, Xia Y et al (2019) Combination anti-CXCR4 and anti-PD-1 immunotherapy provides survival benefit in glioblastoma through immune cell modulation of tumor microenvironment. J Neurooncol 143(2):241–249. https://doi.org/10.1007/s11060-019-03172-5

58. Kim SS, Harford JB, Moghe M, Slaughter T, Doherty C, Chang EH (2019) A tumor-targeting nanomedicine carrying the p53 gene crosses the blood–brain barrier and enhances anti-PD-1 immunotherapy in mouse models of glioblastoma. Int J Cancer 145(9):2535–2546. https://doi.org/10.1002/ijc.32531
59. Filley AC, Henriquez M, Dey M (2017) Recurrent glioma clinical trial, CheckMate-143: the game is not over yet. Oncotarget 8(53):91779–91794. https://doi.org/10.18632/oncotarget.21586
60. Ranjan S, Quezado M, Garren N et al (2018) Clinical decision making in the era of immunotherapy for high grade-glioma: report of four cases. BMC Cancer 18(1):239. https://doi.org/10.1186/s12885-018-4131-1
61. Bristol-Myers Squibb Announces Phase 3 CheckMate-498 study did not meet primary endpoint of overall survival with opdivo (nivolumab) plus radiation in patients with newly diagnosed MGMT-Unmethylated glioblastoma multiforme | BMS newsroom. https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-announces-phase-3-checkmate-498-study-did. Accessed 7 Jan 2020
62. Bristol-Myers Squibb Provides Update on Phase 3 Opdivo (nivolumab) CheckMate-548 trial in patients with newly diagnosed MGMT-methylated glioblastoma multiforme | BMS newsroom. https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-provides-update-phase-3-opdivo-nivolumab. Accessed 7 Jan 2020
63. Zakharia Y, Johnson TS, Colman H, et al. A phase I/II study of the combination of indoximod and temozolomide for adult patients with temozolomide-refractory primary malignant brain tumors. *J Clin Oncol*. 2014;32(15_suppl):TPS2107-TPS2107. doi:10.1200/jco.2014.32.15_suppl.tps2107
64. Colman H, Mott F, Spira AI et al (2015) A phase 1b/2 study of the combination of the IDO pathway inhibitor indoximod and temozolomide for adult patients with temozolomide-refractory primary malignant brain tumors: safety analysis and preliminary efficacy of the phase 1b component. J Clin Oncol 33(15_suppl):2070–2070. https://doi.org/10.1200/jco.2015.33.15_suppl.2070
65. Sahebjam S, Forsyth P, Tran N et al (2018) ATIM-08 A phase I trial of pembrolizumab and vorinostat combined with temozolomide and radiation therapy for newly diagnosed glioblastoma (NCT03426891). Neuro Oncol 20(suppl_6):vi2. https://doi.org/10.1093/neuonc/noy148.005