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REVIEW ARTICLE

Pharmacotherapy of Glioblastoma: Established Treatments and Emerging Concepts

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Abstract Glioblastoma is the most frequent malignant brain tumor and is characterized by poor prognosis, increased invasiveness, and high recurrence rates. Standard treatment for glioblastoma includes maximal safe surgical resection, radiation, and chemotherapy with temozolomide. Despite treatment advances, only 15–20% of glioblastoma patients survive to 5 years, and no therapies have demonstrated a durable survival benefit in recurrent disease. In the last 10 years, significant advances in knowledge of the biology and molecular pathology of the malignancy have opened the way to new treatment options. Clinical management of patients (pseudo-progressions, side effects of therapies, best supportive care, centralization in expertise care centers) has improved. In brain tumors, such as in other solid tumors, we have entered an era of immune-oncology. Immunotherapy seems to have an acceptable safety and tolerability profile in the recurrent setting and is under investigation in clinical trials in newly diagnosed glioblastoma patients. This review focuses on novel targeted therapies recently developed for the management of newly diagnosed and recurrent glioblastomas.

Key Points

The standard therapy for newly diagnosed glioblastoma is temozolomide concurrent with and adjuvant to radiotherapy.

Many new targeted therapies have been investigated but none have provided a greater benefit compared with standard therapy.

No drug has yet improved overall survival for recurrent disease.

1 Introduction

Glioblastoma is the most frequent malignant brain tumor with an incidence rate of about 3 per 100,000 people, accounting for about 50% of all gliomas. The prognosis is poor with a median overall survival (OS) of 12–18 months and <10% of patients are alive 5 years after diagnosis. Treatment of these tumors is complex and needs a multidisciplinary approach.

2 Treatment of Newly Diagnosed Glioblastoma

Macroscopic complete surgical resection is the way to improve the patient's prognosis, decreasing tumor burden and intracranial pressure and improving tissue oxygenation and sensitivity to adjuvant treatments. However, a radical approach is difficult considering the invasiveness of these tumors, which can arise in eloquent areas that control speech, motor function, and senses.

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Before the introduction of temozolomide, radiation therapy was the only strategy associated with a significant survival benefit. The best studied chemotherapy agent for newly diagnosed glioblastoma was carmustine [1, 2]. The combination procarbazine/lomustine/vincristine failed to show a survival benefit and resulted in more acute toxicity [3].

For patients affected by high-grade gliomas, the implantation of carmustine wafers (Gliadel) followed by adjuvant radiotherapy resulted in a survival benefit that was not statistically significant for glioblastoma patients [4]. Only retrospective studies compared the implantation of carmustine wafers with standard chemoradiation.

In 2005, the European Organisation for Research and Treatment of Cancer and the National Cancer Institute of Canada Clinical Trials Group (EORTC/NCIC) phase III trial radically changed the standard of care for glioblastoma by leading to the acceptance of radiotherapy with concurrent temozolomide as a standard first-line treatment for glioblastoma. In this study, temozolomide concurrent with and adjuvant to radiotherapy provided a median survival benefit of 2.5 months, and a 2-year survival rate improvement from 15 to 20% [5]. In the last 10 years, many new drugs have been evaluated for newly diagnosed glioblastoma.

The role of bevacizumab has been extensively evaluated in two randomized phase III trials and recently in a phase II study. The AVAglio (Avastin in Glioblastoma) phase III trial randomized patients to receive temozolomide radiotherapy followed by adjuvant temozolomide plus bevacizumab (10 mg/kg every 2 weeks) or placebo. Adding bevacizumab provided a progression-free survival (PFS) benefit (10.6 vs 6.2 months, $p < 0.001$), but it did not impact on OS. Toxicity (grade 3–4 adverse events) was higher for the bevacizumab group [6]. Moreover, the authors identified a gene signature, the 'proneural' group, that characterized patients who achieved a significant OS benefit with bevacizumab (17.1 vs 12.8 months, $p = 0.002$) [7]. Similarly, another randomized phase III trial by the Radiation Therapy Oncology Group, RTOG 0825, with a similar design, reported an improvement in PFS (10.7 vs 7.3 months, $p = 0.004$) but not in OS (16.1 vs 15.7 months) [8]. The phase II GLARIUS trial has explored bevacizumab plus irinotecan in newly diagnosed glioblastoma. Patients were randomly assigned 2:1 to bevacizumab (10 mg/kg every 2 weeks) during radiotherapy followed by maintenance bevacizumab (10 mg/kg every 2 weeks) plus irinotecan (125 mg/m^2 every 2 weeks) or to standard therapy with temozolomide. The combination bevacizumab + irinotecan resulted in a superior median PFS compared with temozolomide but there was no benefit in OS or quality of life (QoL) [9].

Cilengitide, an integrin inhibitor, has been evaluated in two randomized studies: the first for O^6-methylguanine-DNA methyltransferase (MGMT) methylated patients (CENTRIC, phase III [Cilengitide, Temozolomide, and Radiation Therapy in Treating Patients with Newly Diagnosed Glioblastoma and Methylated Gene Promoter Status]) and the second for MGMT unmethylated patients [CORE, phase II [(Cilengitide, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma and Unmethylated Gene Promoter Status)]. Results from the CENTRIC study reported no improvement in PFS and OS for the cilengitide group [10, 11]. The CORE trial reported a limited efficacy benefit, with contradictory findings of more pronounced effects in the standard cilengitide arm compared with the intensive cilengitide arm [12]. The results observed in the CENTRIC and CORE trials suggest that the addition of cilengitide as administered in these studies does not improve outcomes when added to temozolomide and radiotherapy for newly diagnosed glioblastoma patients.

Temsirolimus was evaluated in the EORTC 26082 trial where MGMT unmethylated patients received standard treatment with temozolomide concomitant with radiotherapy or radiotherapy plus weekly temsirolimus 25 mg. The 1-year survival was 72.2% in the temozolomide arm and 69.6% in the temsirolimus arm ($p = 0.47$). Phosphorylation of mTOR Ser2448 in tumor tissue was detected in 37.6% of patients and was associated with benefit from temsirolimus ($p = 0.002$) [13].

Other studies addressed to target tumor-specific antigens, such as a truncated form of epidermal growth factor receptor (EGFR), called EGFRvIII. Rindopepimut, or CDX-110, is a 14-amino acid peptide that has been used to raise an immune response against EGFRvIII. To enhance its immunogenicity, the peptide is conjugated to the carrier protein keyhole limpet hemocyanin (KLH). In a phase III trial (ACT IV trial) rindopepimut plus temozolomide failed to improve overall survival compared with temozolomide alone for patients with newly diagnosed EGFRvIII-positive glioblastoma [20.4 vs 21.1 months, hazard ratio (HR) 0.99], respectively.

Moreover, temozolomide dose-dense regimens have been investigated in this setting. The RTOG 0525 phase III trial compared dose-dense adjuvant temozolomide (75–100 mg/m^2 × 21 days) or standard temozolomide (150–200 mg/m^2 × 5 days) every 4 weeks for 6–12 cycles. No survival benefit (PFS or OS) was observed for the dose-dense regimen. This trial did not show improved efficacy for intensified strategies of temozolomide for newly diagnosed glioblastoma [14].

3 Knowledge Improvement for Newly Diagnosed Glioblastoma: Who, How, Why?

In the last 10 years we have observed an improvement in knowledge of the biology of these tumors, as well as translational application of biological findings to patients. Moreover, another important step has been to recognize the role of the expertise of tertiary cancer centers in the treatment of these rare cancers.

3.1 Biology of Glioblastoma

The *MGMT* gene encodes a DNA-repair protein (O^6-methylguanine-DNA methyltransferase) which removes alkyl groups from the O^6 position of guanine, an important site of DNA alkylation. High levels of MGMT protein can create a resistant phenotype by counteracting effects of alkylating agents and may determine treatment failure. Epigenetic silencing of the *MGMT* gene through *MGMT* promoter methylation is associated with the loss of MGMT activity and decreased DNA-repair activity. *MGMT* promoter methylation is an important prognostic factor and a relevant predictive factor of response to temozolomide chemotherapy. Patients with unmethylated *MGMT* promoter appear to have little or no benefit from adding temozolomide to radiotherapy. Determination of *MGMT* methylation status by using methylation-specific PCR (polymerase chain reaction) is essential for a treatment decision, especially to identify pseudoprogressions and to decide whether to continue temozolomide despite an early appearance of increasing contrast enhancement at MRI [15].

In 2008, a multigroup genome sequencing study found recurrent somatic mutations in the metabolic gene isocitrate dehydrogenase (IDH) in about 12% of glioblastomas. IDH is a metabolic enzyme of the tricarboxylic acid cycle that converts isocitrate into α-ketoglutarate. Mutations in *IDH*1 and *IDH*2 result in a change of function that generates D-2-hydroxyglutarate rather than α-ketoglutarate [16]. These mutations are common in younger patients affected by secondary glioblastomas. *IDH* mutations are associated with increased DNA methylation, called G-CIMP (Glioma CpG Island Methylation Phenotype). *IDH* mutation and G-CIMP phenotype are important prognostic factors improving prognosis compared with IDH wild-type gliomas. They typically characterize secondary glioblastomas and may be predictive of response to radiation therapy and/or chemotherapy [17]. Many IDH-targeting agents are under investigation for secondary glioblastomas [16].

The 2016 World Health Organization (WHO) classification of central nervous system (CNS) tumors integrates their pathological, genotypic, and molecular features. In this classification, glioblastomas are divided into IDH wild-type glioblastomas (about 90%), which are the so-called primary or de novo glioblastomas and are frequent in patients older than 55 years, IDH-mutated glioblastomas (10%), called secondary glioblastomas, and glioblastoma NOS, a diagnosis reserved for those tumors for which full IDH evaluation cannot be performed [18].

3.2 From Bench to Bedside

Temozolomide concomitant with radiotherapy can lead to a strengthening of radiation effects on brain tissue and to an increase of necrosis in neoplastic areas. Radiotherapy injury depends on increased capillary permeability leading to fluid transudation and brain edema. The disruption of the blood–brain barrier can lead to the observation of a radiological increase of contrast enhancement at MRI evaluation after 1 month from the end of radiotherapy due to the passage of the contrast medium. This results in neuroimaging images of pseudo-progression, almost indistinguishable from true disease progression. The incidence of pseudo-progression is about 20–30%. This phenomenon is probably linked to *MGMT* methylation status: the higher rates of pseudo-progression among *MGMT* methylated patients could be explained by the efficacy of concurrent chemo-radiation on residual tumor [19] and can correlate with survival of glioblastoma patients. Recently, a prospectively designed algorithm was used to assess pseudo-progressions during the AVAglio phase III trial [20]. In this algorithm, new lesions or clinical worsening in the disease assessment after the treatment break were considered sufficient to define disease progression. This pre-defined definition might be, at least in part, responsible for the lower incidence of pseudo-progressions (about 10%) in the standard arm.

MGMT methylation status also influences the pattern of, and time to, recurrence. After concomitant and adjuvant therapy, recurrence may occur inside or at the margin of the radiotherapy field more commonly in patients with *MGMT* unmethylated status than in methylated patients. Furthermore, recurrences outside the radiotherapy field occur after a longer time than those inside [21].

An interesting interaction between clinical characteristics and molecular features is represented by gender with MGMT and IDH. High expression of Wnt receptor Frizzled-7 (FZD7) is associated with a poor prognosis in male patients. *IDH*1 mutations are a predictor of longer survival in male patients but not in females. *MGMT* methylation status can predict longer survival only in female patients. Combination of FZD7 and *MGMT* methylation seems to delete sex differences. Combination of FZD7 and *IDH*1

mutations is a predictor of survival in male patients [22]. Moreover, for reasons not fully explained, gender is a useful clinical factor to predict toxicity of chemotherapy with temozolomide. The risk of developing severe (grade 3–4) myelotoxicity is greater for females (range 0–100%) than for males (range 0–33%) [23, 24].

3.3 Treatment of Elderly Glioblastoma Patients

The optimal therapeutic approach of elderly patients with glioblastoma is of great interest for clinicians considering that more than half of newly diagnosed patients are older than 65 years and older patients frequently cannot tolerate the combined therapy. Age is reported in many studies as the most important prognostic factor.

Radiotherapy is the backbone of care in elderly patients. In 2005, a randomized trial showed that radiotherapy combined with supportive care determines a survival benefit without adverse event increase or QoL worsening in patients older than 70 years with a performance status $\geq$70% [25]. Moreover, no survival differences between standard radiotherapy (60 Gy in 30 fractions over 6 weeks) and short-course radiotherapy (40 Gy in 15 fractions over 3 weeks) were found in patients older than 60 years [26]. In 2012, the NOA-08 phase III trial randomized patients older than 65 years with anaplastic astrocytoma or glioblastoma to dose-dense temozolomide alone versus radiotherapy alone. Median OS was 8.6 months in the temozolomide group and 9.6 months in the radiotherapy group. Median event-free survival (EFS) did not differ for the two groups. *MGMT* promoter methylation analysis was performed retrospectively in about 56% of patients, suggesting a potential benefit in terms of EFS in patients treated with temozolomide (8.4 vs 4.6 months). EFS was longer for the radiotherapy group in patients without *MGMT* promoter methylation (4.6 vs 3.3 months) [27]. The NORDIC phase III trial stratified patients aged $\geq$60 years to receive standard adjuvant radiotherapy (60 Gy in 30 fractions over 6 weeks), hypofractionated radiotherapy (34 Gy administered in 3–4 Gy over 2 weeks) or temozolomide (200 mg/m^2 days 1–5 every 28 days for six cycles). Standard radiotherapy resulted in poor outcomes above all in patients aged $\geq$70 years. Hypofractionated radiotherapy or temozolomide alone can be considered as alternative treatments [28]. In the CCTG CE.6, EORTC 26062-22061, TROG 08.02 randomized phase III trial, newly diagnosed glioblastoma patients older than 65 years were randomized to receive hypofractionated radiotherapy (40 Gy in 15 fractions) alone and hypofractionated radiotherapy with concomitant temozolomide followed by 12 cycles of adjuvant temozolomide. Primary endpoint was OS. The study has reported a significant improvement in OS (9.3 vs 7.6 months) and PFS (5.3 vs 3.9 months). Survival benefit was particularly evident in patients with *MGMT* promoter methylation (13.5 vs 7.7 months in the radio-chemotherapy group and 10 vs 7.9 months in the radiotherapy group). Toxicity analyses showed similar hematologic adverse events (above all grade 3–4 leucopenia and thrombocytopenia) between older and younger patients since elderly patients have reduced bone marrow progenitors. QoL evaluation showed no differences in patients' functional domains but was worse in the radio-chemotherapy group for nausea, vomiting, and constipation [29].

3.4 Expertise in Treating Rare Tumors

As previously mentioned, the expertise of the care center should be considered as a prognostic factor. In a prospective study of the Project of Emilia-Romagna on Neuro-Oncology (PERNO), OS was related to Karnofsky Performance Status, *MGMT* methylation status, and treatment received in a high- versus low-volume center [30]. Glioblastomas are rare tumors and, according to the European Network for Rare Adult Solid Cancer (EURACAN), rare tumors should be treated in centers with experience in networks, establishing guidelines, clinical trials, and the development of research projects.

Best supportive care must be considered early in the decision-making process for patients with glioblastoma. Patients with seizures need a prophylactic antiepileptic therapy [31]. In the case of anamnesis negative for seizures, the use of an antiepileptic therapy is not recommended, even if it is often used in clinical practice. Among antiepileptic treatments, non-enzyme-inducing drugs could be chosen [32], even though no significant interactions have been proven with temozolomide or nitrosoureas. Prolonged use of antiepileptic drugs seems to be associated with early cognitive impairment [31, 33].

Patients with brain tumors have an absolute risk of >20% to develop thromboembolic events and it has been demonstrated that the use of low molecular weight heparin decreases the incidence of these events without increasing the risk of brain hemorrhage [34]. Steroid-related osteoporosis should be prevented by using calcium replacement therapy and cholecalciferol [35].

4 Recurrent Disease

Recurrence of glioblastoma is an almost inevitable event. To date, the standard systemic treatment at recurrence differs between Europe and the US. In Europe, it is represented by nitrosoureas: lomustine, fotemustine [36], or combination PCV (procarbazine, lomustine, vincristine) [37]; in the US (as well as in Japan), bevacizumab has been

approved in this setting. Other approaches, (i.e. the use of alternative temozolomide schedules) were investigated in different trials with contradictory and unsatisfactory results [38–43].

However, the most debated topic remains the role of bevacizumab. Phase II studies in the US led to approval in 2009 by the US Food and Drug Administration (FDA) for the treatment of recurrent glioblastoma. In Europe, the BELOB phase II study suggested a potential overall survival benefit for bevacizumab plus lomustine in patients with progressive glioblastoma [44]. Results from the AVAREG phase II trial suggested a superimposable survival rate between bevacizumab and fotemustine [45], with an increased early tumor shrinkage (ETS) for patients treated with bevacizumab, and a consequent improvement in survival for patients who achieve ETS [46]. From these studies, the EORTC 26101 study was designed. Patients with recurrent glioblastoma were randomized 2:1 to receive lomustine 90 mg/m^{2}every 6 weeks plus bevacizumab 10 mg/kg every 2 weeks or lomustine 110 mg/m^2 alone. The primary endpoint was OS. Disease assessment according to RANO (Response Assessment in Neuro-Oncology) criteria was centrally reviewed. OS was not superior in the combination therapy arm (HR 0.95, 95% CI 0.74–1.21, $p = 0.650$), whereas locally assessed PFS was longer with the addition of bevacizumab to lomustine (HR 0.49, 95% CI 0.39–0.61) [47]. Due to these findings, bevacizumab is being used for the treatment of recurrent glioblastomas in the US but not in Europe, although in many countries it is used for off-label use, as a single agent or combined with irinotecan [48]. Anyway, in clinical practice bevacizumab is used because of improvement in QoL, delay of progression, and reduced use, or in some cases definitive suspension, of corticosteroids. Therefore, it is difficult to achieve a consensus on eliminating bevacizumab for treatment of relapsed glioblastoma.

5 Welcome to the Future

5.1 New Perspectives for *MGMT* Methylated Newly Diagnosed Glioblastoma

The prognosis of patients with newly diagnosed glioblastoma is still poor with standard therapy with temozolomide and radiotherapy. Many attempts have been made to increase survival through combination chemotherapy with lomustine (CCNU) and temozolomide that has shown a median OS of 23 months in a non-randomized bicentric phase II single-arm trial [49, 50]. In this trial, the combination chemotherapy with CCNU/temozolomide proved promising, in terms of OS, for patients with a methylated *MGMT* promoter compared with patients with unmethylated *MGMT*. Thus, these data led to a phase III trial (CeTeG/NOA-09 trial), still ongoing, that investigates the efficacy of the combination of CCNU with temozolomide versus standard therapy [49–51].

5.2 Tumor-Treating Fields

Tumor-treating fields (TTFs) are low-intensity, intermediate-frequency, alternating electric fields that show antiproliferative features in vitro and in vivo. They have been studied for different cancers and preclinical and clinical data have demonstrated their potential efficacy for treatment of glioblastoma leading to clinical trials in recurrent and newly diagnosed settings [52, 53]. Based on the results of these studies, the FDA approved its use in 2011 as salvage therapy for recurrent glioblastoma and in 2015 in association with temozolomide for newly diagnosed glioblastoma. The available evidence in the literature supports the use of TTFs as an antimitotic, minimally toxic therapy for glioblastoma multiforme, both in recurrent and, combined with temozolomide, in adjuvant settings. Further studies are needed to document the real impact on patients' QoL [54].

5.3 Checkpoint Inhibition

In recent years, immune checkpoint inhibition has changed clinical practice in tumors, such as melanoma, lung, renal, bladder, head and neck cancers and Hodgkin lymphoma, and more approvals are to be expected in the coming years.

The first checkpoint inhibitor was ipilimumab, a monoclonal antibody against CTLA-4. Given the significant improvement in OS, this agent is now approved as frontline therapy for patients with metastatic melanoma [55, 56]. Another checkpoint pathway involves PD-1, a cell surface receptor on T cells. In 2014, two checkpoint inhibitors against PD-1, pembrolizumab and nivolumab, have had FDA approval for metastatic melanoma. Nivolumab is also approved for metastatic non-small-cell lung cancer (NSCLC), renal cell carcinoma, Hodgkin lymphoma, head and neck cancer, and urothelial carcinoma, whereas pembrolizumab is approved for head and neck cancer and in NSCLC as first-line therapy only for tumors that express PD-L1 [Tumor Proportion Score (TPS) $\geq$50%] and for recurrence with TPS >1% [57]. Atezolizumab is an anti-PD-L1 agent that is approved for NSCLC, but it has also shown promising activity in other cancers.

The role of PD-L1 expression as a potential biomarker has been investigated in many cancer types. It has been reported that PD-L1 expression in glioblastoma is 72% for recurrent and 88% for newly diagnosed disease [58]. For a long time, the CNS has been regarded as an immunologically privileged site, where the immune system is not

active. As proof, lymphatic vessels and native T cells were believed to be absent in the CNS [59, 60]. However, a recent study has shown the presence of lymphatic vessels in the dura [61]. An important factor in the development of immune checkpoint modulators in glioblastoma is advanced knowledge of the blood–brain barrier (BBB). Glioblastomas are often characterized by a disruption of the BBB. Open endothelial tight junctions allow peripherally activated macrophages and lymphocytes to cross the BBB [62], and glioblastoma cells have been found circulating in peripheral blood [63] in the patients affected.

PDL-1 ligand is expressed on the tumor cells, so PDL-1 inhibitors have to cross the BBB to reach tumor cells [64]; however, possibly the main mechanism of action of these antibodies is not direct but rather through the modulation of immune mechanisms and the production of tumor-reactive immune cells that really cross the BBB. It is unknown if anti-PDL-1 agents reach therapeutic concentrations in glioblastoma cells and if they can have site-specific toxic effects on the brain. In fact, checkpoint inhibitors may have local potential inflammatory and autoimmune side effects [65–68]. Subsequent associated brain edema could cause neurological symptoms miming radiological features of tumor progression and altering MRI evaluation. Furthermore, neuroimaging may often reveal temporary worsening and the appearance of new lesions because effective immune responses may require time to evolve and early imaging can show progressive disease.

Disease assessment with immunotherapies is investigated by the immunotherapy Response Assessment for Neuro-Oncology (iRANO) international working group [69]. Dynamic *O*-(2-[18F]fluoroethyl)-L-tyrosine PET imaging has been demonstrated to be useful to detect checkpoint-inhibitor-related pseudoprogression in patients with melanoma brain metastases treated with ipilimumab or nivolumab [70], but no data are available for high-grade gliomas.

The most frequent side effects of checkpoint inhibitors are adverse immune-related events (AEs) due to hyperactivation of the immune system and autoimmunity. They consist mainly of dermatologic, gastrointestinal, endocrine, hepatic, and pulmonary toxicities. If diagnosed early and appropriately treated, they are frequently mild and usually reversible, but they can become severe and life-threatening if not recognized early.

Clinical trials with checkpoint inhibitors for the treatment of glioblastoma are reported in Table 1. In the multicohort Checkmate-143 study (NCT02017717) [71], the role of nivolumab and ipilimumab was explored in recurrent glioblastoma. This trial was in fact composed of the safety cohorts [nivolumab monotherapy at 3 mg/kg every 2 weeks (NIVO) or nivolumab at 1 mg/kg combined with ipilimumab at 3 mg/kg every 3 weeks followed by nivolumab at 3 mg/kg every 2 weeks (NIVO 1–IPI 3) or nivolumab at 3 mg/kg combined with ipilimumab at 1 mg/kg every 3 weeks for four doses followed by nivolumab at 3 mg/kg every 2 weeks (NIVO 3–IPI 1)] and the phase III trial (nivolumab monotherapy at 3 mg/kg every 2 weeks vs bevacizumab 10 mg/kg every 2 weeks). Preliminary results from the safety part of the Checkmate-143 study showed that among treated patients in the safety cohorts, OS at 6 months was 75%, including 7/10 NIVO patients (70%) and 8/10 NIVO 1–IPI 3 patients (80%). These results were presented at the American Society of Clinical Oncology (ASCO) 2016 Meeting, including the results of the safety cohort that evaluated NIVO 3–IPI 1. Overall, three patients on NIVO 1–IPI 3, and one patient on NIVO 3–IPI 1, and no patients receiving NIVO alone discontinued due to treatment-related AEs. In the NIVO 1–IPI 3 cohort ($n = 10$ patients), nine patients (90%) experienced grade 3–4 treatment-related AEs, and seven patients (70%) had grade 3–4 treatment-related serious AEs. In the NIVO 3–IPI 1 cohort ($n = 20$ patients), the toxicity profile seems more acceptable: five patients (25%) experienced grade 3–4 treatment-related AEs, and two patients (10%) had grade 3–4 treatment-related serious AEs. Interestingly, we have almost no data regarding neurologic toxicities using checkpoint modulators in brain tumors.

The phase III trial of nivolumab compared with bevacizumab in patients with recurrent glioblastoma started enrollment in 2014. Recently, this trial has been announced as negative because it did not meet its primary endpoint of improved OS over bevacizumab monotherapy [72].

In phase I cohorts 1c and 1d of CheckMate-143, the safety and tolerability of nivolumab with radiotherapy ± temozolomide was also evaluated in patients with newly diagnosed glioblastoma. These results were presented at the Society of Neuro-Oncology (SNO) 2016 Meeting [73]. Most frequent treatment-related AEs included fatigue (23–26%), headache (8–23%), and increased AST (0–23%). Treatment-related serious AEs reported in two or more patients were pneumonia (0–6%), pyrexia (0–6%), and tumor flare (3–8%), characterizing either disease progression or pseudoprogression. AEs leading to discontinuation were increased aminotransferases ($n = 2$). No toxic deaths have been reported. These preliminary results suggest that the combination of nivolumab with radiotherapy ± temozolomide is feasible and well tolerated. This analysis from the CheckMate-143 study supports further clinical evaluation of nivolumab plus radiotherapy with or without temozolomide in patients with newly diagnosed glioblastoma.

Two other studies are also investigating nivolumab in combination with radiotherapy: CheckMate-498 for patients with unmethylated *MGMT* and CheckMate-548 for patients with methylated or indeterminate *MGMT*.

Table 1 Clinical trials with checkpoint inhibitors for newly diagnosed and recurrent glioblastoma

Trial	Name	NTC number	*N* (enrolled patients) and main eligibility criteria	Treatment	Main efficacy data
CheckMate-143 (closed)	A Randomized Phase III Open Label Study of Nivolumab Versus Bevacizumab and Multiple Phase I Safety Cohorts of Nivolumab or Nivolumab in Combination With Ipilimumab Across Different Lines of Glioblastoma	NCT02017717	440 Recurrent glioblastoma	ARM N: nivolumab 3 mg/kg Q2 W ARM B: bevacizumab 10 mg/kg Q2 W	Nivolumab does not improve OS compared with bevacizumab monotherapy
CheckMate-498 (enrollment closed)	A Randomized Phase III Open Label Study of Nivolumab vs Temozolomide Each in Combination With Radiation Therapy in Newly Diagnosed Adult Subjects With Unmethylated MGMT (Tumor O-6-methylguanine DNA Methyltransferase) Glioblastoma	NCT02617589	550 Newly diagnosed *MGMT* unmethylated glioblastoma	Experimental arm: RT + nivolumab 240 mg Q2 W, switch to 480 mg Q4 W after 16 wk Comparator arm: RT + TMZ then TMZ for 6 cycles	NA
CheckMate-548 (ongoing)	A Randomized Phase II Single Blind Study of Temozolomide Plus Radiation Therapy Combined With Nivolumab or Placebo in Newly Diagnosed Adult Subjects With MGMT-Methylated (Tumor O^6-methylguanine DNA Methyltransferase) Glioblastoma	NCT02667587	320 Newly diagnosed *MGMT* methylated glioblastoma	Experimental arm: RT + TMZ + nivolumab 240 mg Q2 W with switch to 480 mg Q4 W after 16 wk + TMZ for 6 cycles Comparator arm: RT + TMZ then TMZ for 6 cycles	NA

MGMT O^6-methylguanine-DNA methyltransferase, *NA* not available, *NTC* ClinicalTrials.gov identifier, *OS* overall survival, *QxW* every x weeks, *RT* radiation therapy, *TMZ* temozolomide

CheckMate-498 randomizes patients to receive radiotherapy plus temozolomide followed by six cycles of maintenance temozolomide or radiotherapy plus nivolumab 240 mg every 2 weeks followed by 16 weeks of a maintenance dose of 480 mg every 4 weeks. CheckMate-548 randomizes patients to receive temozolomide concurrent with and adjuvant to radiotherapy or the same regimen with nivolumab 240 mg every 2 weeks for 16 weeks then 480 mg every 4 weeks. The primary endpoint is OS.

The potential for immune checkpoint inhibitors to benefit patients with glioblastoma is of great interest, due to the poor prognosis and few effective treatment options. Despite the limited number of current studies, the safety and tolerability of checkpoint inhibitors seem to be acceptable and similar to other cancer types. The brain-specific symptoms (i.e. brain edema, seizures) should be carefully investigated to discriminate between disease progression sequelae and potential treatment toxicities. To date, despite interesting pre-clinical data and scientific rationale, results from clinical trials investigating immunotherapies for glioblastoma have been disappointing.

5.4 Vaccination and New Concepts of Combining Vaccines and Other Immunotherapies

Several strategies to revert glioblastoma-associated response and promote tumor-directed immune response have been investigated in large clinical trials of immunotherapy. Vaccination is the most explored immunotherapy sector. Some vaccine-based approaches have been investigated in phase III clinical trials, including vaccines targeting EGFRvIII (such as Rindopepimut, described previously) and the use of either immunogenic peptides or tumor lysates to stimulate autologous dendritic cell response. Other vaccines under investigation are in early phases of clinical development: multipeptide vaccines such as IMA-950, cytomegalovirus-derived peptides or tumor-derived peptides such as heat shock protein-96 peptide complexes and the Arg132His mutant form of isocitrate dehydrogenase. Recently, preclinical data suggest that the addition of immune checkpoint inhibitors could increase activity of vaccination to overcome glioblastoma-associated immunosuppressive properties [74].

5.5 Isocitrate Dehydrogenase (IDH)-Inhibiting Agents

Mutations in the genes for IDH, a metabolic enzyme of the tricarboxylic acid cycle, are one of the most important biomarkers in gliomas. Primary glioblastomas generally lack *IDH* mutations but secondary glioblastomas are most frequently *IDH* mutated and originate from lower-grade gliomas. *IDH* mutations precede the main oncogenic alterations thought to drive malignant transformation [75]. These mutations are potentially targetable with drug inhibitors, and phase I trials with inhibitors of IDH1 or IDH2 mutant proteins are ongoing. The main consequence of *IDH* mutation is the accumulation in the cell of D-2-hydroxyglutarate (2HG) that inhibits many dioxygenases, such as hypoxia-inducible factor, histone demethylases, and 5-methylcytosine hydroxylases resulting in global DNA hypermethylation [76]. The depletion of coenzyme NAD+ is the most important stimulator of growth and progression of cancer cells. In mice, the use of inhibitors that deplete NAD+ in *IDH* mutated gliomas stops their progression and increases survival [76]. Thus, NADPT inhibitors could have a promising role in the near future [76]. Preclinical data also documented the efficacy of peptide vaccines directed against the IDH1 R132H mutation, and clinical trials are ongoing to determine their activity in patients [16, 77, 78].

6 Conclusion

The treatment of glioblastoma remains a challenge. Despite new compounds and therapeutic approaches, no drug evaluated in a phase III trial has showed a survival improvement in newly diagnosed or recurrent glioblastoma in the last 10 years. However, over the years our knowledge of molecular characterization has improved and the clinical management of patients (pseudo-progressions, best supportive care, centralization in tertiary healthcare centers) has led to an increase in overall survival. Now the treatment of brain tumors has entered the era of immune-oncology. Large phase III trials have been conducted and are ongoing in both the newly diagnosed and recurrent settings.

Compliance with Ethical Standards

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Conflict of interest The authors declare no conflict of interests.

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