ANTICANCER RESEARCH *37*: 21-34 (2017)
doi:10.21873/anticanres.11285

Review

Advances in Experimental Targeted Therapy and Immunotherapy for Patients with Glioblastoma Multiforme

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Abstract. *Glioblastoma multiforme (GBM) represents the most malignant primary brain tumor in adults with generally dismal prognosis, early clinical deterioration and high mortality. GBM is extremely invasive, characterized by intense and aberrant vascularization and high resistance to multimodal treatment. Standard therapy (surgery, radiotherapy and chemotherapy with temozolomide) has very limited effectiveness, with median overall survival of patients no longer than 15 months. Progress in genetics and epigenetics of GBM over the past decade has revealed various aberrations in cellular signaling pathways, the tumor microenvironment, and pathological angiogenesis. A number of targeted anticancer drugs, such as small-molecule kinase inhibitors and monoclonal antibodies, have been evaluated in clinical trials with newly-diagnosed, as well as recurrent GBM. Unfortunately, to date, only a single anti-angiogenic agent, bevacizumab, has been approved for the treatment of recurrent GBM in the USA and Canada. The novel possibilities of cancer immunotherapy, especially immune checkpoint inhibitors, are being evaluated in clinical trials of patients with GBM. The most recent clinical experiences with targeted therapy as well as immunotherapy of GBM are given in this review. The relative lack of success of some of these approaches recently revealed in well-designed randomized clinical trials is also discussed.*

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Key Words: Glioblastoma multiforme, GBM, targeted therapy, immunotherapy, immune checkpoint inhibitors, PD1 inhibition, CTLA4 inhibition, clinical trials, personalized medicine, review.

Glioblastoma multiforme (GBM) belongs to the largest group of primary central nervous system (CNS) tumors, so-called gliomas, which are formed from supporting glial cells in the brain parenchyma (1, 2). GBM represents the most common and most malignant tumor in this class, with an incidence of 3-4/100,000/year (3, 4). GBM is an extremely invasive and difficult to treat tumor, characterized by intense and aberrant vascularization and high resistance to radiotherapy (RT) and chemotherapy (CHT). The current standard of care for patients with newly-diagnosed GBM comprises of neurosurgery and subsequent concomitant chemoradiotherapy by fractionated external-beam RT and systemic temozolomide followed by systemic temozolomide in the adjuvant setting (5). There are only very limited possibilities for the treatment of subsequent recurrences, generally with minimal clinical efficacy (6). Despite intensive multimodal treatment strategies, the median survival of patients with GBM is still 12.1-14.6 months and only 3-5% of patients survive longer than 3 years (7).

Enormous progress has been made in the genetics and epigenetics of GBM during the past decade. The Cancer

Genome Atlas Research Network consortium carried out multiplatform analysis (DNA mutations, mRNA, microRNA expressions) of 500 tumor tissue samples from patients with untreated primary GBM (8). This initiative, together with the other large multiplatform studies, revealed several abnormalities in a diversity of mutated genes and cellular signaling pathways involved in high-grade glioma development and progression (8-12). The most important genetic and epigenetic aberrations were found in following cellular signaling pathways: i) Kirsten rat sarcoma viral oncogene homolog (KRAS) and phosphoinositide 3-kinase (PI3K) oncogenic pathways (88% of GBM), ii) the p53 pathway (87% of GBM), iii) cell-cycle regulatory pathway (78% of GBM), and iv) the newly-discovered alterations in metabolic pathways including isocitrate dehydrogenases 1 and 2 (*IDH1/2*) mutations (10% of GBM). The mutations in *IDH1/2* serve also as an independent and important GBM prognostic factor (13-15). Their routine assessment is now highly recommended in the clinical management of patients with glioma (including GBM) according to the recently updated World Health Organization (WHO) 2016 classification of CNS tumors (16-18).

The GBM microenvironment and its involvement in cancer development and progression was also extensively studied, especially tumor angiogenesis and aberrations in anticancer immune responses (19, 20). Together these findings allow the possibility of developing innovative and better personalized treatment strategies for clinical patient management in the future. The most recent advances in targeted therapy as well as immunotherapy for GBM are given in this review. The relative lack of success of some of these approaches, as recently revealed from well-designed randomized clinical trials, is also discussed.

Perspectives on Novel Therapies for GBM

The standard therapeutic protocols for the treatment of GBM have only limited benefits and provide a median survival of patients of no longer than 15 months (7). The novel so-called targeted therapies can affect and inhibit various tumor-specific features, such as altered cellular signaling pathways, aberrant vascularization, or the tumor microenvironment and impaired anticancer immune response (21, 22). Genetic and epigenetic studies in GBM have revealed potential new targets in cancer cells and the surrounding tumor microenvironment that can be therapeutically influenced by the small molecules and monoclonal antibodies (4, 23). New approaches of GBM immunotherapy could lead to a fundamental breakthrough into the treatment of high-grade gliomas (20).

However, the vast molecular heterogeneity associated with aberrant GBM signaling pathways contributes to the relative lack of success of these therapeutic strategies. A recent study identified a distinct mutation profile of recurrent glioma that varied from the initial mutation analyses in the same patient (24). The exomes of 23 initially low-grade gliomas and recurrent tumors resected from the same patients were sequenced and the mutation profiles were mutually compared. In 10 (43%) of these cases, 50% or fewer of the mutations present in the initial tumor were found at recurrence. Moreover, the mutational profile of GBM is also affected by CHT as recurrent tumors exhibit temozolomide-induced damage to the DNA mismatch repair system resulting in a hypermutated phenotype (25). Another study revealed the possibility of transition proneural gene expression pattern in GBM to a mesenchymal pattern at recurrence that also negatively influenced the effectiveness of new drugs applied at the beginning of the treatment in newly-diagnosed disease (10).

These and other mechanisms, such as the lack of tumor dependence on the proposed target, failure of drug penetration into the CNS, or clonal evolution and antigen escape of tumor after effective therapeutic intervention, could be reasons for the relative lack of success of targeted approaches in the treatment of gliomas. Only a small clinical benefit has been demonstrated with these agents so far, which is discussed in more detail in the following text.

Overcoming these barriers will probably require the use of individualized molecular profiling of each GBM tumor at initial diagnosis and also at recurrence, and application of personalized medicine principles for combinations of targeted therapies with other types of treatment for high-grade gliomas, including GBM.

Inhibitors of Growth Factors and Their Receptors, Inhibitors of Intracellular Signaling Pathways

These are relatively new molecules are able to specifically inhibit various aberrantly activated cell signaling pathways which lead to the formation and progression of cancer (4, 22, 26). Such effects can be achieved by inhibiting specific growth factors and their receptors, including the epidermal growth factor family (EGF) and their receptors (EGFR), platelet-derived growth factors (PDGF) and their receptors (PDGFR), insulin-like growth factors (IGF), fibroblast growth factor (FGF) and their receptors and others that are overexpressed or mutated in a high proportion of GBMs (9, 27).

Molecular aberrations in EGFR signalling, comprising mutations and gene overexpression, are described in approximately 50% of GBMs (8). Therefore aberrantly activated EGFR could be a possible therapeutic target, similar to the situation common in other tumor types (28-30). One of the approved drugs directed against EGFR is the small-molecule kinase inhibitor gefitinib. In an early phase II clinical trial of recurrent GBM treated with gefitinib, the progression-free survival (PFS) at 6 months was 13% and the median overall survival (OS) was 10 months (31). There were more recent studies with gefitinib as monotherapy or in combinations for GBM treatment with results of only very

limited efficacy compared to standard treatment (32-34). Another EGFR inhibitor also examined in the GBM setting is erlotinib. A number of phase II trials of erlotinib as a single agent showed minimal benefit for GBM treatment and modest survival benefit in combination with other agents (35-37). Another potentially promising EGFR inhibitor is lapatinib. This agent was tested in multiple clinical trials in patients with GBM but again with very limited antitumor effect (38-40). The newer irreversible EGFR inhibitor afatinib was recently evaluated as a monotherapy and in combination with temozolomide in phase I/II study of patients with recurrent GBM (41). Afatinib had a manageable safety profile but only limited activity. Cetuximab is a chimeric monoclonal antibody with activity against EGFR. Cetuximab was tested in the small group of patients with GBM but also with poor results (42). Some improvement was observed in a phase II study evaluating the combination of cetuximab, irinotecan, and bevacizumab. However, the efficacy data were not superior compared to results with bevacizumab and irinotecan alone (43). The observed effects of EGFR inhibitors in the treatment of patients with GBM are generally weak. Better results could possibly be achieved by stratification of patients eligible for treatment by presence of overexpression or specific mutations of *EGFR* in their tumor tissue (44-46).

PDGFR is another cell surface receptor frequently overexpressed and activated in GBM, especially in its proneural subtype (8, 47). The aberrant activation of PDGFR assists in the transition from lower-grade glioma to GBM and PDGF ligand stimulates tumor growth and angiogenesis (48, 49). Imatinib is a kinase inhibitor of PDGRF, mast/stem cell growth factor receptor (c-KIT), and oncogene fusion protein BCR–ABL that was also extensively examined in patients with GBM. PFS of 16% at 6 months was observed in one phase II trial of patients with recurrent disease (50). Further multicenter phase II studies confirmed that imatinib as a monotherapy or in combination therapy failed to improve PFS or OS in patients with GBM (51, 52). Multikinase inhibitors influencing tumor angiogenesis, namely sunitinib, sorafenib, and vandetanib, also have inhibitory effect on PDGFR. These substances were also evaluated in the treatment of GBM (53, 54). However, more recent multicentric randomized phase II clinical trial of RT and temozolomide with and without vandetanib in patients with newly diagnosed GBM showed no significant OS benefit of combination compared with the parallel control arm, which led to an early termination of the study (55). Newer multikinase inhibitors affecting PDGFR such as dasatinib or nintedanib also failed to improve OS in patients with recurrent GBM (56-58). Based on the results from these and other clinical trials with various targeted drugs inhibiting overexpressed PDGFR, this approach unfortunately does not seem to be an effective therapeutic strategy for patients with GBM at the moment.

Intracellular signaling pathway components mediate the response of cells to such growth factors and their interactions with cell surface receptors. Inhibition of such aberrant signaling components can be a promising targeted therapeutic strategy in GBM (4, 22). Protein kinase C (PKC) is an important driver of the signal propagation from several growth factors, such as EGF and PDGF, stimulating glioma cell proliferation. Examples of drugs that inhibit PKC and were evaluated in patients with GBM are tamoxifen (59, 60) and enzastaurin (61, 62). Again, minimal or no benefit was observed. Mammalian target of rapamycin (mTOR) is a crucial intracellular protein kinase involved in cell growth signaling. It transduces the signals from PI3K as well as the KRAS pathways (22). Mutations in the tumor suppressor phosphatase and tensin homolog (*PTEN*), which normally inhibits PI3K signaling, often increases mTOR activity in GBM (27, 63). Therefore selective mTOR inhibition was extensively examined in GBM settings. The small-molecule mTOR inhibitor sirolimus was not effective as a single agent and had limited efficacy in a phase II trial in combination with erlotinib (37, 64). Another mTOR inhibitor, everolimus, showed no clear clinical benefit in combination with gefitinib for recurrent GBM (33). A recent phase II study evaluating everolimus, temozolomide, and RT in patients with newly diagnosed glioblastoma showed no appreciable survival benefit of the combination compared to historical controls treated with conventional therapy (65). The newer selective PI3K inhibitor PX-866 had a low overall response rate, with median PFS at 6 months of only 17% in a phase II study of patients with recurrent GBM (66). However, 21% of participants had durable stable disease even if no association between stable disease and molecular biomarkers was seen. There are many other targeted therapeutics affecting various aberrantly activated intracellular signaling pathways of cancer cells that are being examined in the GBM setting, such as inhibitors of poly (ADP-ribose) polymerase (PARP), signal transducer and activator of transcription 3 (STAT3) and others (4, 23, 67-69).

However, despite enormous advances in the research of targeted oncological therapy during the past two decades, none of these therapeutics have had proven significant PFS or OS benefit in well-designed phase III clinical trial for patients with newly diagnosed or recurrent GBM as a monotherapy or in combination with standard treatment regimes, which remains truly disappointing.

Inhibition of Angiogenesis in GBM

Cancer research has increasingly highlighted the fundamental role of the tumor microenvironment together with pathological angiogenesis and tumor neovascularization for the development and progression of malignant diseases (21, 70). The processes of pathological angiogenesis and possible

mechanisms for their therapeutic inhibition have been extensively studied in GBM (19, 71, 72). The major role in tumor angiogenesis is played by vascular growth factors, especially vascular endothelial growth factor (VEGF) and its variant VEGF-A, primarily through its interactions with the VEGFR1 and VEGFR2 receptors found on endothelial as well as cancer cells. Excessive microvascular proliferation and VEGF overexpression were identified in tumor tissues from patients with GBM. Higher intra-tumoral as well as plasma VEGF concentrations were associated with rapid disease progression and presence of early recurrence of GBM (72-77). Therefore, the evaluation of antiangiogenic and anti-VEGF agents in GBM is highly needed.

The most widely used inhibitor of angiogenesis in advanced cancer treatment at the moment is bevacizumab, a humanized IgG1 monoclonal antibody against VEGF-A. Bevacizumab was extensively examined in clinical trials for treatment of recurrent as well as newly-diagnosed GBM, as a single agent and in various combinations with CHT and other targeted therapeutics (71, 78-83). Bevacizumab gained accelerated approval by the US Food and Drug Administration (FDA) for the treatment of recurrent GBM in 2009 based on a high radiographic response rates and prolonged PFS (84). The multicenter phase II BELOB clinical trial undertaken in 14 hospitals in the Netherlands suggested the possible OS benefit for patients with recurrent GBM treated with the combination of bevacizumab plus lomustine *versus* bevacizumab or lomustine alone (85). However, recently published results from the well-designed phase III European Organization for Research and Treatment of Cancer (EORTC) 26101 clinical trial failed to confirm the OS benefit of bevacizumab plus lomustine by comparison with lomustine alone [9.1 *vs*. 8.6 months, hazard ratio (HR)=0.95, 95% confidence interval (CI)=0.74-1.21; p=0.65] in patients with first progression of GBM (86). On the other hand, PFS was longer in the combination arm by comparison with lomustine alone arm (4.2 *vs*. 1.5 months, HR=0.49, 95% CI=0.39-0.61; p<0.0001). Combinations of bevacizumab with standard treatment for newly-diagnosed GBM were also examined with encouraging results in initial phase II studies (87-89). Based on the previous results, two large phase III clinical trials were designed, AVAglio (NCT00943826) and RTOG-0825 (NCT00884741), evaluating bevacizumab-containing regimes for patients with newly-diagnosed GBM. Unfortunately, neither trial demonstrated a benefit in OS for the combination of bevacizumab with standard RT plus temozolomide treatment compared to standard regimen alone (90, 91). Both studies demonstrated PFS survival benefit of combination, but it reached the pre-specified statistical significance only in the AVAglio trial (10.6 *vs*. 6.2 months, p<0.001). Moreover, the baseline health-related quality of life and performance status were maintained longer and glucocorticoid use was lower in the bevacizumab arm in the AVAglio trial but with more grade 3 and 4 adverse events (66.8% *vs*. 51.3%). The retrospective analysis of molecular biomarkers in the AVAglio trial showed that patients with both isocitrate dehydrogenase 1 (*IDH1*) wild-type tumors and proneural pattern of gene expression may have derived 4.3 months of OS benefit with the addition of bevacizumab to a standard regimen (92). Because of the *post-hoc* nature of this analysis, the predictive effect in relation to bevacizumab treatment must be interpreted with caution. A recent meta-analysis examined clinical trials that compared bevacizumab plus RT/temozolomide with RT/temozolomide alone in patients with newly diagnosed GBM (79). The meta-analysis included 1,738 patients from three well-designed clinical trials. The result failed to demonstrate OS benefit (HR=1.04, 95% CI=0.84-1.29; p=0.71) but identified increased PFS (HR=0.74, 95% CI=0.62-0.88; p=0.0009) for combined treatment with bevacizumab. Moreover, there was no increase in the 6-month survival (p=0.13) and the rate of serious adverse events was higher in the bevacizumab-treated compared to the placebo group. Based on the results from the AVAglio and RTOG-0825 trials, bevacizumab was not approved for the treatment of patients with newly diagnosed GBM and remains a treatment alternative only in the recurrent setting in the USA and in Canada.

There is a substantial number of studies evaluating other inhibitors of angiogenesis in the treatment of newly-diagnosed as well as recurrent GBM. The VEGFR tyrosine kinase inhibitor cediranib showed activity in an early phase II clinical trial as a monotherapy in patients with recurrent GBM (93). Despite the promising results, cediranib demonstrated no PFS benefit as a monotherapy (HR=1.05, 95% CI=0.74-1.50, p=0.9) or in combination with lomustine (HR=0.76, 95% CI=0.53-1.08; p=0.16) *versus* lomustine alone in patients with recurrent GBM in a phase III clinical trial (94). Cilengitide is an inhibitor of αvβ3 and αvβ5 integrin receptors that also blocks pathological tumor angiogenesis. Cilengitide was evaluated with promising results in phase II study as a monotherapy in patients with recurrent GBM (95). However, the phase III clinical trial evaluating cilengitide combined with standard treatment compared to standard regime alone failed to show significant OS benefit of the combination in patients with newly diagnosed GBM with O-6-methylguanine DNA methyltransferase (*MGMT*) methylation (26.3 *vs*. 26.3 months; HR=1.02, 95% CI=0.81-1.29, p=0.86) (96). Another angiogenesis inhibitor aflibercept, a recombinant produced fusion protein that scavenges both VEGF and PDGF, was studied in the recurrent setting, but demonstrated minimal evidence of single-agent activity in patients with GBM with PFS at 6 months of only 7.7% (97). Unfortunately, apart from bevacizumab, no other inhibitor of angiogenesis has been approved for the treatment of patients with newly-diagnosed or recurrent GBM.

Immunotherapy of GBM

Immunotherapy represents a very promising area of multimodal treatment for many types of cancer (98-101). There has also been great progress in immunotherapy research in GBM over the past few years. There are many different approaches currently being evaluated in GBM clinical trials, including passive immunotherapy with antibodies, utilization of autologously stimulated lymphocytes and cytokines, oncolytic virotherapy, and active immunotherapy with vaccine strategies based on tumor cells, peptides, or dendritic cells (DCs) (20, 102, 103).

More than 40% of GBMs carry the unique deletion mutant variant of *EGFR* called *EGFRvIII* that is characterized by a deletion of 267 amino acids in the receptor extracellular domain (104, 105). This mutation causes constitutive ligand-independent receptor activation and signal propagation leading to cancer cell proliferation. The enhanced proliferation of EGFRvIII-positive cancer cells together with the lack of EGFRvIII expression in normal non-cancerous cells makes it an ideal candidate for targeted therapy and the use of personalized medicine in GBM treatment. Rindopepimut is a peptide-based vaccine (containing 13 EGFRvIII-specific amino acid sequences) targeted against EGFRvIII surface antigens. The phase I/II multicenter study evaluating rindopepimut in patients with newly diagnosed GBM showed very promising results, with a median PFS of 15.2 months and an OS of 23.6 months (106). Subsequent phase II clinical trial (ACT III) examined rindopepimut in combination with standard RT and temozolomide in 65 patients with newly diagnosed GBM overexpressing EGFRvIII (107). The median OS was 21.8 months. Patients with unmethylated *MGMT* promoter had an OS of 20.9 months, whereas those with methylated *MGMT* had longer OS of 40 months. Based on the very promising results from these early clinical trials, the double-blinded randomized multicenter phase III ACT IV clinical trial (NCT01480479) of rindopepimut in patients with newly diagnosed GBM was designed and started enrollment of patients (108). Unfortunately, rindopepimut combined with temozolomide failed to improve OS by comparison with the standard treatment (20.1 *vs*. 20 months; HR=1.01, 95% CI=0.79-1.30, p=0.93). The study was discontinued in March 2016 after the second interim analysis, which was a true disappointment. At the same time, this example shows the importance of verification of promising preliminary results from early drug development in well-designed randomized placebo controlled phase III clinical trials. Rindopepimut is still being evaluated in the phase II ReACT clinical trial in combination with bevacizumab in patients with recurrent EGFRvIII-positive GBM (NCT01498328).

There are other peptide vaccines targeting tumor antigens, such as the HLA-restricted Wilms tumor 1 (WT1) 9-mer peptide vaccine, that was examined in patients with recurrent GBM in a phase II clinical trial (109). The median PFS was 20 weeks and the PFS at 6 months was 33.3%. A more recent phase II clinical trial with an autologous heat-shock protein-peptide vaccine HSPPC-96 (vitespen) showed promising results in patients with recurrent GBM, with a median OS of 42.6 weeks (110).

DC vaccines use autologous tumor lysates or common tumor antigens to induce an immune response against cancer. These strategies were evaluated in early-phase clinical trials in newly-diagnosed GBM (111-114). DC vaccine loaded with autologous tumor lysate was examined in a phase I clinical trial with 56 patients with relapsed GBM, resulting in a median PFS of 3 months and median OS of 9.6 months, with 2-year OS of 14.8% (115). The same group investigated the integration of DC vaccine into the standard treatment of patients with newly-diagnosed GBM and achieved an unexpected median OS of 24 months (116). Another large double-blinded randomized phase II trial of DC vaccine (DCVax-L) in patients with newly diagnosed GBM also showed encouraging results, with a median OS of 3 years, with 4-year survival reaching 33%, and 27% of patients exceeding 6-year survival from initial surgery (117, 118). However, the clarification of these promising results with DC vaccine strategies are essential in well-designed randomized phase III clinical trials, such as the DCVax-L phase III study (NCT00045968) which is now ongoing and whose final results are eagerly awaited.

The Role of Immune Checkpoint Inhibitors in Glioblastoma Immunotherapy

There has been dramatic success in the treatment of various advanced solid tumor types such as melanoma, renal cancer, lung cancer, head and neck cancer with the novel class of immunomodulatory anticancer agents called immune checkpoint inhibitors (98-101, 119-121). These therapeutics are able to block inhibitory molecules and their receptors on effector immune cells, which leads to a robust T-cell response against the tumor. At the moment there are FDA-approved monoclonal antibodies directed against distinct inhibitory molecules such as ipilimumab targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), nivolumab and pembrolizumab targeting programmed cell death 1 (PD-1) and atezolizumab targeting programmed cell death ligand 1 (PD-L1), and many others are in development. The great success of immune checkpoint inhibitors in a therapy of a number of advanced solid tumor type1 leading to the evaluation of these compounds in CNS gliomas, including GBM (122, 123).

There is comprehensive pre-clinical research supporting a role for immune checkpoint inhibitors in the treatment of GBM. In a preclinical study with a murine glioma model, treatment with CTLA-4 blockade effectively reversed

glioma-induced changes to the $CD4^+$ T-cell compartment and enhanced the antitumor immunity (124). Another study with a mouse model of GBM showed the synergistic effect of combined treatment of systemic CTLA-4 blockade together with intratumoral interleukin 12 (IL12) application leading to tumor eradication even at advanced disease stages (125). The effectiveness of combined CTLA-4 with PD-L1 and indoleamine-2,3-dioxygenase 1 blockade was studied in mouse model of GBM. It was shown that 100% of mice (n=5) survived on the triple combination therapy for a long time (126). A more recent study with a murine model of GBM showed that anti-CTLA-4 plus anti-PD-1 therapy was able to cure 75% of the animals (12 out of 16), even with advanced and late-stage tumors (127).

Support for the rationale of using immune checkpoint inhibitors in the GBM setting has also come from substantial clinical findings. Firstly, these drugs effectively overcome the blood–brain barrier and are active in the CNS. The CTLA-4 inhibitor ipilimumab showed activity in patients with brain metastases of melanoma without significant CNS toxicity (128, 129). The PD-1 inhibitor pembrolizumab was active in the treatment of brain metastases in patients with melanoma or non-small-cell lung cancer with an acceptable safety profile (130, 131). Furthermore, PD-L1 expression level in the tumor tissue was positively associated with the likelihood of clinical benefit with PD-1 inhibitor in non-small-cell lung cancer as well as other tumor types (132, 133). A recent study showed robust and diffuse expression of PD-L1 assessed by immunohistochemistry in newly-diagnosed as well as recurrent GBM (88% *vs*. 72.2%, respectively), which is a relatively high percentage compared to other cancer types such as melanoma (134). Higher expression of PD-L1 in tumor tissue correlated with worse outcome in another study with 94 patients with GBM (135). Because of the promising pre-clinical experiments, proven activity in the CNS and the presence of targets in GBM tumor tissue, clinical trials with specific immune checkpoint inhibitors are warranted in patients with GBM in newly diagnosed as well as recurrent settings.

Clinical trials evaluating immune checkpoint inhibitors, including ipilimumab, nivolumab, pembrolizumab and others, in patients with GBM are currently being conducted (Table I). The phase III CheckMate 143 study (NCT02017717) is evaluating nivolumab alone and nivolumab plus ipilimumab *versus* bevacizumab as an active comparator in patients with recurrent GBM. The latest results included the observation of high activity of the nivolumab arm, with 12-month OS of 40% (136). Nivolumab alone was also the best tolerated arm with no new safety signals. Another currently running phase II clinical trial (NCT02337491) is validating the combination of pembrolizumab and bevacizumab in patients with recurrent GBM. Very preliminary results from six patients treated with the combination showed a median OS of 6.8 months and two patients remained alive long-term (327 and 328 days) (137). Pembrolizumab is also being examined in combination with bevacizumab and hypofractionated stereotactic RT in a phase I/II study (NCT02313272) in patients with recurrent GBM. Preliminary results from six patients who were treated with the combination showed durable disease control in all three patients evaluable for response (138). The phase II CheckMate 548 (NCT02667587) study is examining the combination of standard RT plus temozolomide treatment with nivolumab or placebo in patients with newly-diagnosed GBM. Moreover, the phase III CheckMate 498 (NCT02617589) clinical trial is evaluating the head to head comparison of nivolumab to temozolomide both in combination with standard RT in patients with newly diagnosed GBM with unmethylated *MGMT* promoter in tumor tissue. The preliminary results from both these studies are eagerly awaited. Pembrolizumab is also being examined in the newly diagnosed setting in combination with standard RT plus temozolomide regime in a phase I/II (NCT02530502) clinical trial. The results have not been published yet. Newer checkpoint inhibitors are also being evaluated for the treatment of GBM patients, such as the phase II study of PD-L1 inhibitor durvalumab in newly diagnosed as well as recurrent settings (NCT02336165). On the other hand, there are also conflicting results. A recently published retrospective analysis of 22 patients with heavily pretreated refractory progressive primary brain tumors (including 10 with GBM) treated with pembrolizumab as a salvage agent in four major Israeli brain tumor centers showed only limited OS of no more than 3.2 months (139). The authors do not recommend further use of pembrolizumab for the recurrent primary brain tumors until convincing positive prospective clinical trial data is published.

Immune checkpoint inhibitors represent a significant breakthrough in the treatment of various advanced tumor types (such as melanoma, renal cancer, lung cancer, head and neck cancer, and urinary bladder cancer) in recent years that has dramatically changed the prognosis of patients with cancer (98-101, 119-121). In some cases, therapy with these drugs means long-term disease control or hopefully even cure. Although the preliminary results for these drugs are promising and generally also encouraging in patients with GBM, it is necessary to wait for the mature results from multiple phase III clinical trials that are expected to finish in 2018 and beyond.

Summary

GBM remains a devastating disease for patients and their families, with very poor prognosis and early clinical deterioration, despite aggressive multimodal treatment with surgery, RT and CHT. On the other hand, there have been many novel discoveries in basic and translational research in recent years. The genetic and epigenetic basis of GBM

Table I. *The major ongoing clinical trials incorporating immune checkpoint inhibitors into glioblastoma multiforme (GBM) treatment.*

Immune checkpoint inhibitor	Phase of the trial	GBM setting	Estimated completion date	Other intervention	Clinical trial identifier
Nivolumab	III (CheckMate 498)	Newly diagnosed (*MGMT* unmethylated)	October 2019	Temozolomide, Radiotherapy	NCT02617589
Nivolumab	II (CheckMate 548)	Newly diagnosed (*MGMT* methylated)	November 2020	Temozolomide Radiotherapy	NCT02667587
Nivolumab	III (CheckMate 143)	Recurrent	January 2018	Ipilimumab, Bevacizumab	NCT02017717
Nivolumab	I	Recurrent	August 2019	Dendritic cell vaccine	NCT02529072
Pembrolizumab	I/II	Newly diagnosed	March 2018	Temozolomide Radiotherapy	NCT02530502
Pembrolizumab	II	Recurrent	2016	Bevacizumab	NCT02337491
Pembrolizumab	I/II	Recurrent	December 2017	Bevacizumab Hypofractionated stereotactic irradiation	NCT02313272
Durvalumab	II	Newly diagnosed and recurrent	July 2017	Bevacizumab Radiotherapy	NCT02336165
Durvalumab	II	Recurrent	December 2018	Tremelimumab	NCT02794883
BMS-986016 (anti-LAG3 MAb)	I	Recurrent	December 2019	Nivolumab Urelumab	NCT02658981

MGMT, *O*-6-Methylguanine DNA methyltransferase; *LAG3*, lymphocyte-activation gene 3; *MAb*, monoclonal antibody.

formation and progression has been explored well, together with the mutational changes occurring during the standard-treatment course. Possible molecular and genetic targets in tumor cells that can be affected and inhibit by novel therapeutics are known. A number of targeted anticancer drugs are being evaluated as single agents or in combinations for newly diagnosed as well as recurrent GBM. However, despite excessive research in this area, to date only a single antiangiogenic monoclonal antibody, bevacizumab, has been approved for the treatment of recurrent GBM in the USA and Canada.

There has been significant progress in the research of immunotherapy strategies for GBM. The very successful anticancer therapeutics, immune checkpoints inhibitors, are now also being extensively evaluated in clinical trials with GBM and the results are eagerly awaited. These and other immunotherapeutic strategies together with a better understanding of the role of targeted anticancer drugs in this context hopefully will shed new light onto the future treatment of this serious and almost exclusively fatal disease.

Conflicts of Interest

The Authors declare that they have no conflict of interests regarding the publication of this article.

Acknowledgements

This work was supported by MH CZ - DRO (Faculty Hospital Plzen - FNPl, 00669806) and by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

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Received November 15, 2016
Revised December 3, 2016
Accepted December 6, 2016