16 Updated Therapeutic Protocols for Glioblastomas

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16.1 Introduction

16.1.1 Current Standard Treatment

16.1.1.1 Radiotherapy and Concomitant Temozolomide

Radiotherapy (RT) retains a major role in the standard treatment of glioblastoma (GBM), as it is useful for tumor control and improvement of both progression-free and overall survival (OS). Conventional standard RT is indicated after surgery (ideally within 2 months from resection) and should be associated with concurrent temozolomide (TMZ) [1]. The most employed RT schedule delivers 60 Gy in 2-Gy daily fractions over 6 weeks. TMZ is given orally (75 mg/m^2 daily) during all the radiation treatment period [2, 3]. Other dose schedules have been investigated but without clear benefit. In particular, there is no indication for doses >60 Gy [4].

The association with radiotherapy and concurrent TMZ is a milestone of GBM treatment, which was established as a standard after the publication of a phase III trial of the European Organization for Research and Treatment of Cancer (EORTC) and National Cancer Institute of Canada Clinical Trials Group (NCIC) in 2005. This trial compared the association of RT and TMZ to RT alone, and results clearly favored the combination modality [2]. In the long-term 5-year analysis, OS was 27.2% (95% CI 22.2–32.5) at 2 years, 16.0% (12.0–20.6) at 3 years, 12.1% (8.5–16.4) at 4 years, and 9.8% (6.4–14.0) at 5 years with RT and TMZ, versus 10.9% (7.6–14.8), 4.4% (2.4–7.2), 3.0% (1.4–5.7), and 1.9% (0.6–4.4) with RT alone (hazard ratio 0.6, 95% CI 0.5–0.7; $p < 0.001$). A benefit of combined therapy was observed in all prognostic subgroups, including patients aged 60–70 years. *MGMT* promoter methylation was the strongest predictor of outcome and benefit from TMZ chemotherapy, even if the advantage of the combined modality was significant is higher in methylated patients (OS 23.4 versus 15.3 months for combined RT/TMZ versus RT alone, $p = 0.004$) as compared to unmethylated patients (OS 12.6 versus 11.8 months for combined RT/TMZ versus RT alone, $p = 0.035$) [3].

RT employs ionizing radiations that induce double-strand DNA damage and tumor cell death. However, endothelial cells of the neovasculature of the tumors are also vulnerable to radiation-

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induced damage [5], and their disruption may lead to radiation-induced MRI changes, known as pseudoprogression and radionecrosis [6]. These conditions are common pitfall in GBM management and should be carefully considered after RT shortly.

The so-called pseudoprogression occurs most commonly within the first 3 months from RT. It is usually characterized by increased contrast enhancement peritumoural edema and necrosis [6] which mimic tumor regrowth However, patients with pseudoprogression typically do not experience neurological deterioration [7]. Furthermore, pseudoprogression prevails among patients with *MGMT*p methylated tumors, and in some studies, it has been associated to increased survival advantage. Even if timing of MRI changes and clinical correlates may help to distinguish pseudoprogression from radionecrosis and disease progression, advanced MRI (such as diffusion and perfusion studies) and positron emission tomography (PET) may be useful in the differential diagnosis [6, 8, 9].

16.1.1.2 Adjuvant Chemotherapy with Temozolomide

Standard first-line chemotherapy agent for GBM is represented by TMZ. TMZ is an alkylating agent, which causes DNA damage and interferes with tumor proliferation. One mechanism of TMZ action is the depletion of the repair enzyme O6-methylguanine-DNA methyltransferase (MGMT), which explains why TMZ is especially effective against tumors with methylation of the *MGMT* promoter, where the enzyme activity is silenced [10, 11]. There are different reasons supporting the use of TMZ as a standard chemotherapy treatment in GBM: penetration through the blood–brain barrier is good, oral formulation improves patient's compliance and toxicity profile is more favorable (no cumulative myelotoxicity) than other commonly used oral chemotherapies, such as lomustine and procarbazine. Moreover, TMZ it does not induce or inhibit significantly the liver cytochrome system, which translate in a low risk of interactions with other drugs, such as steroids or antiseizure medications (ASMs) [12].

Common adverse events include nausea and/or vomiting, fatigue, dose-dependent myelosuppression (especially thrombocytopenia and leukopenia). Other rarer adverse events are dose-independent pancytopenia, increase of liver enzymes, skin rash, photosensitivity. Very rare adverse events include hair loss, fragility of cutaneous appendages, and periodontitis. Second hematological tumors are uncommon as potential complication of long-term treatment with TMZ [13]. The standard schedule of adjuvant TMZ is 150–200 mg/m^2 on days 1–5 every 28 days [2]. Dose should be reduced in case of poor tolerability and/or hematologic toxicity. In particular, patients undergoing treatment with TMZ should be carefully monitored with monthly blood tests to rule out thrombocytopenia <100,000/mm^3 and neutropenia <1500/mm^3; similarly, occurrence of any drug-related adverse events must be promptly recognized with careful periodic clinical evaluations.

TMZ may also be administered with intensified schedules, which include metronomic and dose-dense TMZ [14]. The metronomic schedule consists in a lower dose of TMZ (50 mg/m^2), which is given daily without interruptions. Metronomic TMZ seems to have a direct toxic effect on tumor endothelial cells, which would result also in an antiangiogenic activity [15]. Moreover, continuous exposition to TMZ seems to increase methylation and inhibition of the *MGMT* promoter, thus enhancing antitumor activity especially in *MGMT*p methylated tumors. Dose-dense chemotherapy is given at the dose of 150 mg/m^2 days 1–7 and 15–21 of a 28-day cycle. With dose-dense TMZ, the interval between doses is reduced, whereas the dose density is boosted. Thus, tumor cells should be more likely to be damaged between cycles, with higher treatment efficacy [16]. In a phase II trial investigating the activity and tolerability of dose-dense versus metronomic TMZ, both regimens appeared to be feasible and quite well-tolerated. Dose-dense TMZ was associated with a median OS of 17.1 months and 1-year survival of 80%, while in case of metronomic TMZ OS was 15.1 months and 1-year survival 69%. Main toxicities were

leukopenia and elevation of liver enzymes (in the latter case, especially in the metronomic subgroup) [14]. However, a significant benefit from dose-dense regimens of TMZ was not confirmed by other studies, where higher doses were associated with greater toxicity and deterioration in function and quality of life [17, 18]. Moreover, in a phase II randomized, multicentre, open-label trial of continuing adjuvant TMZ beyond six cycles (GEINO 14–01), patients exposed to 12 months of adjuvant TMZ did not seem to benefit in terms 6-month PFS and OS as compared to those undergoing only 6 cycles (median PFS 9.5 months versus 7.7 months, $p = 0.95$; median OS 18.2 months versus 23.3 months, $p = 0.16$). In addition, 12 cycles of TMZ were associated to higher adverse effects (thrombocytopenia, lymphopenia, nausea, and vomiting) [19]. However, it is unclear whether an extended duration of TMZ treatment is useful in some certain subgroups of patients, such as incompletely resected or MGMT methylated tumors.

Ultimately, the development of resistance to TMZ acts as the limiting factor in GBM treatment, one of the major factors is the presence of tumor stem cells, which are intrinsically TMZ-resistant. Furthermore, many other molecular mechanisms are emerging, including the involvement of other DNA repair systems, aberrant signaling pathways, autophagy, epigenetic modifications, microRNAs, and extracellular vesicle production [20].

The addition of other chemotherapy agents to adjuvant TMZ has been the object of several investigations. In an open-label, phase III trial on newly diagnosed GBM patients with *MGMT*p methylation, the addition of lomustine (CCNU) to TMZ versus standard TMZ was associated with better OS (48.1 versus 31.4 months, respectively), and the occurrence of significant adverse events (grade 3 according to Common Terminology Criteria for Adverse Events—CTCAE) was similar in the two groups (51% versus 59% in TMZ and TMZ/CCNU groups, respectively). However, the limitation of this trial is the small sample size. The association of TMZ with antiangiogenic agents, such as bevacizumab, has also been explored. Bevacizumab is a humanized monoclonal antibody that inhibits vascular endothelial growth factor (VEGF). A combination of bevacizumab with has been investigated in two large, randomized phase III trials. However, despite prolonging PFS in both trials, the addition of bevacizumab did not translate in an OS advantage [21, 22].

16.1.2 New Perspectives

16.1.2.1 Targeted Therapy

Targeted therapies employ drugs that are specifically engineered to interact with molecular pathways involved in the proliferation and survival of cancer cells. Such an approach provides a more accurate and less toxic alternative to conventional chemotherapy. Additionally, the combination of TMZ with targeted agents not only may enhance the treatment effectiveness for brain tumors but also minimize chemotherapy side effects, ultimately leading to improved patient outcome and progress in cancer treatment.

Epidermal Growth Factor Receptor (EGFR)

The epidermal growth factor receptor (EGFR) gene has been extensively studied in GBM due to an overexpression in approximately 60% of tumors and amplification in over 40% [23]. Nearly 50% of GBM tumors with EGFR amplification have a deletion mutation known as EGFRvIII, which leads to disrupted gene regulation [24]. In recent years, various clinical trials have explored therapies targeting EGFR or EGFRvIII.

Rindopepimut, a vaccine targeting EGFRvIII conjugated with keyhole limpet hemocyanin, was evaluated in the Phase III ACT IV trial (NCT01480479). This trial assessed the efficacy of adding rindopepimut to standard chemoradiation but found no significant survival benefit for patients with newly diagnosed GBM [25].

The antibody-drug conjugate depatuxizumab mafodotin (Depatux-M), which targets cells with EGFR amplification, was studied in GBM patients [26]. Depatuxizumab mafodotin internalizes and releases the antimicrotubule agent

monomethyl auristatin F. However, when used with concurrent TMZ, it did not improve survival outcomes compared to TMZ alone in patients with recurrent *EGFR*-amplified GBM [27]. Additionally, in a study involving newly diagnosed GBM patients (NCT02573324), it did not significantly enhance progression-free survival compared to standard therapy [28].

The monoclonal antibody GC118, which has strong inhibitory effects against high-affinity EGFR ligands, was tested in patients with *EGFR*-amplified recurrent GBM in an open-label, single-arm phase II trial (NCT03618667). Unfortunately, the primary outcome of a 6-month progression-free survival rate was only 5.6% (95% CI 0.3–25.8%), with a median overall survival of 5.7 months (range 2–22.0 months) [29]. Another approach involved AMG 596, a bispecific T cell engager targeting EGFRvIII-positive tumor antigens. In its initial trial (NCT03296696), among the eight evaluable patients, one experienced a partial response and two had stable disease at initial follow-up [30].

Inhibitors of the EGFR tyrosine kinase have also been largely investigated. Erlotinib, a reversible *EGFR* tyrosine kinase inhibitor, failed to demonstrate improved OS when combined with small molecules bevacizumab and TMZ in newly diagnosed patients in two phase 2 studies [31, 32]. Similar outcomes were observed in a phase 2 study evaluating erlotinib in combination with sorafenib [33]. Gefitinib, a reversible and specific EGFR tyrosine kinase inhibitor, did not improve OS when concurrent with RT in newly diagnosed patients [34], or as adjuvant therapy after RT. Afatinib, an irreversible pan-inhibitor of the ErbB family (including *EGFR* and EGFRvIII), did not outperform TMZ in a phase 2 study (NCT00727506). However, it showed increased PFS in patients with tumors expressing EGFRvIII or *EGFR* amplification [35]. Dacomitinib, a pan-HER family inhibitor approved for *EGFR*-mutant NSCLC, provided minimal benefits in GBM when tested as monotherapy in tumors with *EGFR* amplification or the common *EGFR* mutation EGFRvIII [36].

ERAS-801, a newly developed EGFR inhibitor with better CNS penetration, is under evaluation in patients with recurrent GBM (NCT05222802). In preclinical models, ERAS-801 demonstrated a survival benefit in 93% of EGFR mutant and/or amplified cases, showing significant brain penetration and prolonged survival compared to erlotinib [37]. Another EGFR inhibitor, WSD0922-Fu, is being studied for its potential to prolong stable disease in patients with EGFRvIII-mutant high-grade astrocytoma (NCT04197934) [38].

BRAF

BRAF, part of the Raf kinase family, facilitates cellular proliferation via the MAP kinase/ERK signaling pathway. The *BRAF* V600E mutation is present in several tumor types, including a minority of GBM patients. This mutation is prevalent in epithelioid GBM, where it occurs in 50–93% of cases [39]. Vemurafenib, an early BRAF inhibitor, was tested in patients with *BRAF* V600-mutated glioma (NCT01524978). Among 11 patients with *BRAF*-mutant malignant diffuse glioma, one showed a partial response, and five had stable disease for 6 months [40].

Further investigations have explored the concurrent use of BRAF and MEK inhibitors for treating low-grade and high-grade glioma, showing promising results. A phase 2 clinical trial (NCT02684058) involving 110 pediatric patients with *BRAF* V600-mutated low-grade glioma randomly assigned participants to receive either dabrafenib plus trametinib or standard chemotherapy. At a median follow-up of 18.9 months, an overall response rate of 47% was observed in the combination therapy group, compared to 11% in the standard chemotherapy group (risk ratio, 4.31; 95% CI 1.7–11.2; $p < 0.001$). Additionally, significant improvements were seen in clinical benefit (risk ratio 1.88; 95% CI 1.3–2.7) and median progression-free survival (hazard ratio 0.31; 95% CI 0.17–0.55; $p < 0.001$) in the combination group [41]. Another study (NTC02034110) evaluated dabrafenib and trametinib in 45 adult patients with recurrent or refractory *BRAF* V600E-mutated high-grade glioma, reporting an objective response in 33% of patients, including three complete responses and 12 partial responses [42]. This combination has been recently

approved by registration authorities for any type of solid tumors with *BRAF* V600 mutation or fusion (Fig. 16.1). More recently, a study examined a new combination of BRAF and MEK inhibitors in patients with *BRAF*-mutant GBM (NCT03973918). In a small series, concurrent use of encorafenib (BRAF inhibitor) and binimetinib (MEK inhibitor) led to sustained responses in all GBM patients and in 50% of those with anaplastic pleomorphic xanthoastrocytoma, though the study closed early due to enrollment challenges [43]. Despite the rarity of *BRAF* V600E-mutated GBM these studies highlight the potential benefits of combination therapy using BRAF and MEK inhibitors in gliomas harboring the *BRAF* V600E mutation. An open issue is whether to search for BRAF alterations in all GBM patients or only in some subgroups (longer patients at recurrence?)

PI3K/AKT/mTOR Pathway

The loss of the tumor suppressor gene phosphatase and tensin homolog (*PTEN*) on chromosome ten occurs in up to 60% of GBM patients [44]. This loss leads to activating mutations in the PIK3CA and PI3K/mTOR pathways, promoting uncontrolled cell proliferation [23]. Consequently, mTOR-targeted compounds, like temsirolimus (CCI-779) and everolimus, have been explored in GBM treatment. Both everolimus and temsirolimus have been evaluated in newly diagnosed GBM setting. When combined with radiotherapy (RT) and temozolomide (TMZ), everolimus did not significantly improve survival compared to

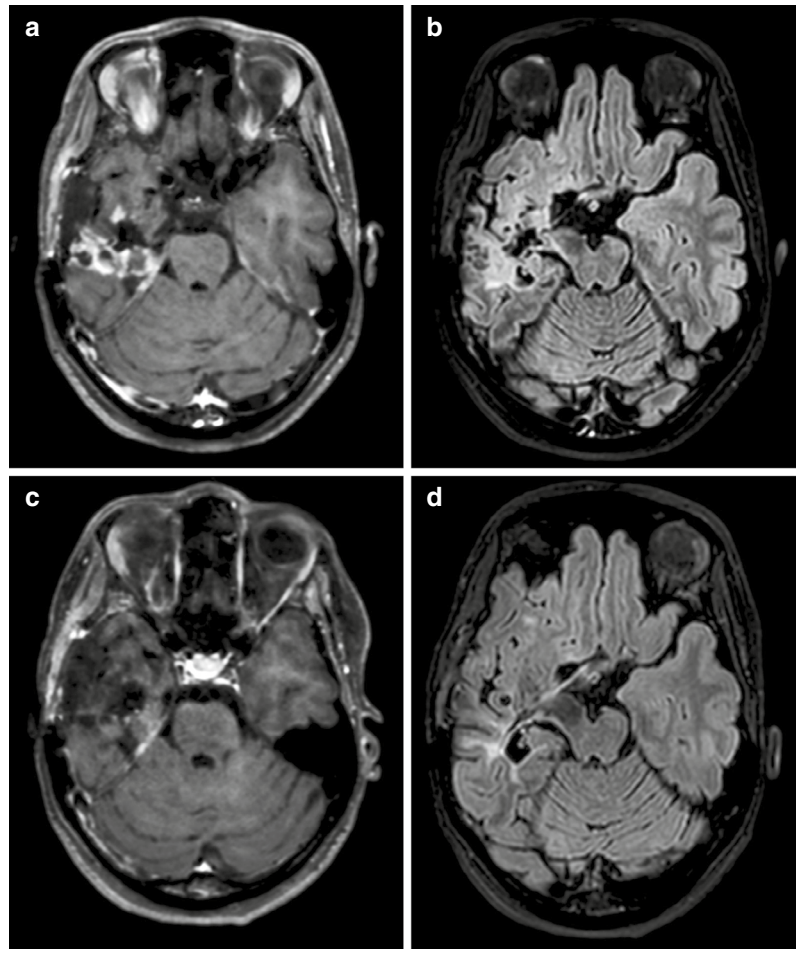

Fig. 16.1 In this figure, we present the case of a 20-year-old woman diagnose with an epithelioid GBM, BRAF V600E mutant, who was treated with dabrafenib 300 mg daily and trametinib 2 mg daily at recurrence after first-line radiotherapy and temozolomide. (**a**) Axial post-contrast T1 sequence at recurrence (before receiving dabrafenib and trametinib); (**b**) axial FLAIR sequence at recurrence (before receiving dabrafenib and trametinib); (**c**) axial post-contrast T1 sequence after two cycles of dabrafenib and trametinib; (**d**) axial FLAIR sequence after two cycles of dabrafenib and trametinib. Overall, the patient presented a near complete response (CR) of the contrast-enhanced target lesion according to RANO criteria

standard therapy [45], and the same was true for temsirolimus [46]. Additionally, mTOR inhibitors have been poorly tolerated in these clinical trials. Paxalisib, a brain-penetrant small molecule inhibitor of PI3K and mTOR, initially showed promise by prolonging progression-free survival and overall survival at maximum tolerated dose [47], but subsequent studies did not meet the criteria to advance to a major GBM trial.

Cyclin-Dependent Kinase (CDK)

The retinoblastoma (Rb) cell cycle control pathway is commonly altered in GBM due to homozygous deletions of CDKN2A/B, amplifications of CDK4 or CDK6, or alterations in the RB1 gene. Abemaciclib, a potent CDK4/6 inhibitor with good brain penetration, was investigated in newly diagnosed GBM patients in the INSIGHT trial. Patients were randomized to receive either standard radiotherapy and TMZ or radiochemotherapy followed by adjuvant abemaciclib. While abemaciclib was well-tolerated and associated with longer progression-free survival, it did not demonstrate an improvement in overall survival compared to standard radiochemotherapy [48].

Vascular Endothelial Growth Factor (VEGF)

VEGF is a critical mediator of angiogenesis in GBM. Bevacizumab, a VEGF inhibitor, has been extensively studied in GBM. It has been already reported in a previous section that the association of bevacizumab with chemoradiation as first-line therapy was ineffective. Also, in one study of recurrent GBM, bevacizumab achieved a median progression-free survival of 16 weeks and a radiographic response in 71% of patients [49]. Other VEGF inhibitors have also been tested in GBM. Among those, regorafenib is probably the one that acquired the greater interest in recent times. Regorafenib is an oral inhibitor of several kinases involved in tumor angiogenesis (VEGFR1–3 and TIE2), oncogenesis (KIT, RET, RAF1, and BRAF), and in the interaction between tumor and microenvironment (platelet-derived growth factor receptor [PDGFR] and fibroblast growth factor receptor [FGFR]), and tumor immunity (colony-stimulating factor 1 receptor [CSF1R]) [50–53]. In the randomized, open-label, phase 2 REGOMA trial, regorafenib improved OS (median OS: 7.4 vs 5.6 months, $p = 0.0009$) and PFS (6-month PFS, 16.9% vs 8.3%, $p = 0.022$) of recurrent GBM patients as compared with lomustine [54]. Other subsequent real-life studies showed similar impact on survival, but a higher rate of adverse than in REGOMA [55–60]: interestingly, dose adjustment and an escalation schedule has been proposed to improve tolerability with similar efficacy [61].

Despite these initial promising results, the regorafenib arm of the GBM Adaptive Global Initiative Learning Environment (AGILE) trial was closed because regorafenib failed to demonstrate an improvement in OS in both recurrent and newly diagnosed tumors.

16.1.2.2 Targeting DNA Repair Pathways

Radiation therapy is highly effective for GBM due to the ability to damage tumor DNA. However, tumor cells acquire radioresistance through enhanced DNA damage response mechanisms [62].

There are several mechanisms of DNA damage repair (DDR) in tumor cells and compounds to inhibit. Among these, poly (ADP-ribose) polymerase (PARP) inhibitors, which prevent single-strand DNA repair, are the most studied in GBM [63]. PARP inhibitors are being evaluated in combination with radiotherapy, chemotherapy, or antiangiogenic agents [64, 65].

Unfortunately, DNA damage repair (DDR) inhibitors have generally yielded disappointing results thus far. The myelosuppressive effects of PARP inhibitors also raise concerns about their safety when used in combination with TMZ.

16.1.3 Immunotherapies

16.1.3.1 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) have transformed the treatment landscape for certain solid tumors, such as metastatic melanoma [66], non-small cell lung cancer [67], and renal cell carcinoma [68], generating interest in their potential

role in treating GBM [69]. The primary mechanism of ICIs is to reduce tumor-induced immunosuppression, thereby enhancing the immune system's ability to recognize and attack the tumor [70].

The two main checkpoint regulators, currently targeted in immunotherapy, are cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) [71]. T cell receptor activation leads to the expression of CTLA-4, which promotes self-tolerance and prevents autoimmunity [72]. PD-1, an inhibitory receptor on T cells, binds to its ligand PD-L1, which is expressed on tumor cells, thus inhibiting T cell activation [72]. Blocking these pathways with anti-CTLA-4 agents (such as ipilimumab, tremelimumab) or anti-PD-1/PD-L1 agents (such as pembrolizumab, nivolumab, atezolizumab) may trigger a more robust immune response against the tumor.

However, ICIs have not been proven effective in newly diagnosed GBM [69]. The phase III study, CheckMate 498 (NCT02617589), compared the efficacy of nivolumab plus RT with TMZ plus RT in 560 patients with newly diagnosed *MGMT*p unmethylated GBM [73]. The median overall survival was longer in the TMZ plus RT group (14.9 months) compared to the nivolumab plus RT group (13.4 months) (p = 0.0037), reaffirming TMZ plus RT as the standard of care [73]. The CheckMate 548 trial (NCT02667587), another phase III study, involved 716 patients with newly diagnosed *MGMT*p methylated GBM [74]. All patients received standard care treatment with RT plus TMZ, with either nivolumab or a placebo. Median progression-free survival was similar between the two groups (10.6 months for nivolumab versus 10.3 months for placebo), and median overall survival was slightly longer in the placebo group (32.1 months) compared to the nivolumab group (28.9 months).

Several hypotheses could explain the limited efficacy of ICIs in GBM in comparison with other cancers. GBM is now considered "immunologically cold," characterized by a low number of infiltrating lymphocytes leading to a loss of effector function [70, 75]. Additionally, GBMs exhibit a low mutational burden resulting in fewer neoantigen development for immune attack [69, 70]. Furthermore, GBMs generally express lower levels of PD-L1 compared to other tumors responsive to ICIs, with PD-L1 expression observed in 61–88% of GBM cases [76].

GBM promotes immune quiescence through the secretion of immunosuppressive paracrine factors such as transforming growth factor-ß (TGF-ß), interleukin-10 (IL-10), and prostaglandin E2 [77]. It also induces an inhibitory phenotype in tumor-associated macrophages and microglia [78]. Moreover, GBM may cause systemic immune dysfunction, evidenced by T cell lymphopenia and sequestration in the bone marrow in treatment-naïve patients with glioblastoma and other intracranial tumors [79]. The use of corticosteroids and TMZ in GBM treatment further enhances immunosuppression.

Tumors with a higher mutational load, which correlates with increased immunogenicity, might be more responsive to ICIs. High tumor mutational burden can result from mutations in mismatch repair genes like MSH2, MLH1, MSH6, and PMS2, suggesting a greater likelihood of response to ICIs. Another factor contributing to high mutational burden is the post-treatment effect of radiation and TMZ [80]. Approximately, 10% of GBM patients develop hypermutated tumors after chemoradiation, indicating that ICIs may be beneficial for recurrent GBM with a high mutation load [80], but this has not been confirmed in the clinical setting. Future research should explore the potential of combining ICIs with other immunotherapies, such as CAR-T cells, oncolytic viruses, vaccines, or integrating them with other treatment modalities to achieve better tumor control [69, 70, 72].

16.1.3.2 Vaccine-Based Therapies

Vaccine-based therapies represent another approach to leveraging the immune system against GBM. These therapies work by exposing patient's antigen-presenting cells to tumor-associated antigens, thereby stimulating an immune response targeting the tumor [70, 72]. Unlike traditional chemotherapy, these vaccines are disease-targeted. The primary mechanisms of

vaccine therapy for GBM include peptide vaccines, dendritic cell vaccines, and individualized vaccines.

16.1.3.3 Peptide Vaccines

Peptide vaccines, among the most extensively studied for GBM treatment, consist of amino acids forming either single or multiple peptides [81]. A significant challenge in their development is selecting a target antigen consistently expressed on GBM cells. This was evident in a phase III, randomized clinical trial involving newly diagnosed GBM patients with confirmed EGFRvIII expression, all of whom had undergone standard radiation therapy and concurrent TMZ [25]. In this trial, 371 patients received monthly doses of rindopepimut, an EGFRvIII-targeting peptide vaccine, while 374 received a control treatment. The trial was prematurely halted due to futility, as there was no significant difference in survival between the two groups. Notably, both groups experienced a loss of EGFRvIII expression in 57–59% of patients' tumors [82], suggesting that tumor evolution occurred independently of rindopepimut therapy. This phenomenon, known as "antigen escape," occurs when a targetable mutation loses relevance as the tumor progresses.

Other experimental vaccines have targeted various peptides in GBM. For example, survivin, an intracellular anti-apoptotic protein that regulates cell division, has high expression in GBM cells, being a potential vaccine target [83]. Another peptide vaccine, DSP-7888, targets the Wilm's tumor 1 peptide (WT1), a cell growth transcription factor present in GBM and other solid tumors [84].

To address the heterogeneity and evolving nature of GBM, multipeptide vaccines like IMA950 have been developed. IMA950 includes nine MHC I and II peptides from the GBM surface. In a phase I/II study, newly diagnosed GBM patients treated with IMA950, combined with poly-ICLC, exhibited 63.2% single-peptide CD8+ T cell responses and 36.8% multipeptide CD8+ T cell responses [85]. Furthermore, 84.6% of patients showed tumor-specific CD4+ T cell responses, indicating the immunogenicity of IMA950 against GBM.

The promising initial results for SurVaxM, DSP-7888, and IMA950 are being further explored in combination with other immunotherapies, including pembrolizumab (NCT04013672, NCT03665545) and bevacizumab (NCT03149003).

16.1.3.4 Dendritic Cell Vaccines

Dendritic cells, key antigen-presenting cells, are crucial for establishing both innate and adaptive immunity. In GBM-targeting vaccines, dendritic cells are used to enhance the immune response. The process involves isolating dendritic cells from the patient blood, exposing them to tumor antigens ex vivo, maturing them with cytokines, and then reintroducing them into the patient [86]. After administration, these dendritic cells migrate to lymphoid organs to present antigens to T cells, thereby initiating an immune response [87].

To date, dendritic cell vaccinations have not consistently shown a definitive survival benefit in GBM patients, though there is some encouraging evidence. For instance, a phase III clinical trial (NCT00045968) involving 331 newly diagnosed glioblastoma patients who had undergone surgery and chemoradiotherapy compared TMZ plus DCVax-L to TMZ plus placebo [88]. The primary endpoint was progression-free survival, which was 6.2 months for DCVax-L recipients versus 7.6 months for the placebo group ($p = 0.47$), not meeting the primary endpoint [89]. However, patients could cross over to the vaccine at progression. The median overall survival for newly diagnosed glioblastoma patients receiving DCVax-L was 19.3 months from randomization, compared to 16.5 months in the control group ($p = 0.002$). Additionally, survival was higher at 48 months (15.7% vs. 9.9%) and at 60 months (13% vs. 5.7%) in the DCVax-L group [90]. Patients with *MGMT*p methylation receiving DCVax-L also had better survival (median overall survival of 30.2 months from randomization) compared to controls (21.3 months) ($p = 0.03$) [90].

A phase II randomized trial involving newly diagnosed GBM patients tested a dendritic cell vaccine (ICT-107) [91]. The dendritic cells were exposed to six stem-cell-associated peptides in

GBM. Even if no significant difference in median OS between ICT-107 recipients and controls was seen (17 versus 15 months), patients with HLA-A2 receiving ICT-107 had a stronger immune response and longer median overall survival (33.7 months compared to 23.9 months in the control group). This indicates that patient factors, like HLA status, are crucial for the response to dendritic cell vaccine therapy and potentially other immunotherapies for GBM.

Another dendritic cell vaccine, Audencel, was tested in a randomized phase II trial involving GBM patients who had undergone at least 70% surgical resection. The study found no difference in median OS between those receiving standard care and those receiving standard care plus Audencel [92]. In contrast, a study from China Medical University on 34 newly diagnosed GBM patients showed that an adjuvant dendritic cell vaccine, administered post-operatively and continued for 6 months, resulted in a median OS of 31.9 months, compared to 15 months for the control group receiving standard care [93].

16.1.3.5 Individualized Vaccines

Individualized vaccines leverage data from patients' tumors to tailor treatments. A phase I trial involving glioblastoma patients treated with APVAC1, a personalized vaccine derived from patient transcriptomes and tumor peptides, showed CD8+ T cell responses [94]. APVAC2, targeting neoepitopes, also elicited CD4+ T cell responses against the neoepitopes [94]. Another phase I/Ib trial demonstrated that personalized antigen vaccines could induce neoantigen specific responses in both CD4+ and CD8+ T cells in patients not treated with dexamethasone [95].

16.1.3.6 Viral Oncolytics

Genetically engineered viruses are appealing as antineoplastic agents because they can target tumor cells, replicating within them and causing cell death—a process known as "oncolysis." Additionally, these viruses can deliver beneficial genetic material to the infected tumor cells, thus inducing cytotoxicity and stimulating a more robust and sustained local immune response against the antigens released by the lysing tumor cells [96]. Although viral oncolytics have not yet demonstrated significant efficacy either as standalone treatments or in combination with other therapies for GBM, there is considerable potential to explore various viral vector types, delivery methods, patient subsets, and combinatorial strategies with other immunotherapies.

Adenoviruses are a class of viral oncolytics utilized in the treatment of GBM. One notable adenoviral vector is aglatimagene besadenovec (AdV-tk), which incorporates herpes simplex virus (HSV) thymidine kinase. When AdV-tk is injected into the tumor environment, it preferentially infects tumor cells. Subsequent administration of anti-HSV drugs like valacyclovir results in targeted tumor cell death [97]. In a phase IIa study (NCT00589875), patients with GBM who received AdV-tk followed by valacyclovir had a median post-therapy survival of 16.7 months, compared to 13.7 months in the control group [98]. Another phase II study (NCT00870181) explored intra-arterial infusion of AdV-TK followed by ganciclovir and mannitol to disrupt the blood-brain barrier in patients with recurrent GBM. This approach resulted in significantly longer median progression-free survival (34.9 weeks vs. 7.4 weeks; $p < 0.001$) and OS (45.7 weeks vs. 8.6 weeks; $p < 0.001$) compared to controls [99]. Another adenovirus-based therapy, Ad-RTs-IL-12, contains an interleukin-12 (IL-12) transgene. IL-12 has antineoplastic properties but is highly toxic when administered systemically. The Ad-RTs-IL-12 vector allows for ligand-inducible expression of IL-12 through velemidex, limiting IL-12 effects to the tumor microenvironment [72, 100]. A phase I study (NCT04006119) confirmed the safety of Ad-RTs-IL-12, velemidex, and nivolumab [100].

16.1.4 CAR-T Cell Therapy

CAR-T cell therapy is an innovative approach for treating GBM by targeting tumor-specific antigens. This therapy consists of extracting T cells from a patient's blood, genetically modifying to express chimeric antigen receptors (CARs), that recognize specific antigens on tumor cells, and

then reinfusing these engineered cells into the patient [96, 101]. CARs are synthetic proteins that combine an antigen-recognition domain with T cell activating domains, enabling targeted immune responses against tumor cells. While CAR-T therapy has shown success in hematologic cancers and is FDA-approved for some lymphomas and leukemias, its application in GBM is still under investigation [102].

A Phase I/II trial (NCT01454596) with anti-EGFRvIII CAR-T cells in recurrent EGFRvIII-positive GBM patients noted a patient death, possibly due to T cell-induced increased pulmonary vascular permeability, and no significant clinical improvement [103]. Another Phase I study (NCT02209376) reported a median OS of around 8 months, with CAR-T-EGFRvIII cells detectable for up to 30 days post-infusion [104].

IL13Rα2, overexpressed in multiple malignancies and 75% of GBM cases, represents another target. A pilot study of intracranially delivered IL13Rα2-targeted CAR-T cells showed temporary brain inflammation but no survival benefit, despite transient responses [105].

HER2, overexpressed in many cancers including up to 80% of GBMs, was targeted in a Phase I study (NCT01109095) where patients showed a median overall survival of 11.1 months from first T cell infusion [106]. However, presence of HER2 on normal tissues poses toxicity risks [107]. Other targets, including B7-H3, CD147 (EMMPRIN), disialoganglioside GD2, and chlorotoxin (CLTX), are also under investigation.

Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity (ICANS) are common complications of CAR-T therapy, with the latter potentially causing fatal cerebral oedema [108]. Due to the immunosuppressive microenvironment and limited leukocyte migration through the blood–brain barrier, direct administration of CAR-T cells into the tumor has been proposed, though the risk of ICANS remains uncertain [109].

CAR-Natural Killer (NK) cells are also being studied for their ability to recognize multiple tumor antigens and potentially avoid CRS [110]. HER2-specific CAR-NK cells have demonstrated anti-GBM activity, leading to a Phase I trial (NCT03383978) [111]. However, NK cells face barriers such as lower numbers in peripheral blood and challenges in crossing the blood–brain barrier [112]. They must be irradiated before infusion to prevent secondary malignancy, which limits their lifespan for antitumor activity [110]. In summary, while CAR-T therapy is a promising treatment for GBM, questions remain about the optimal antigen targets, delivery methods, and therapy duration before it can become an effective treatment for glioblastoma.

16.1.5 Other Approaches in Glioblastoma Treatment

16.1.5.1 Tumor Treating Fields (TTFs)

Tumor treating fields (TTFs) represent an additional treatment for newly diagnosed GBM, in combination with TMZ. TTFs consist of four transducer arrays applied for at least 18 h daily to the shaved scalp and connected to a portable device [113]. In a phase III trial (EF-11) comparing TTFs with physician's-choice chemotherapy in the recurrent setting, TTFs demonstrated efficacy, with an objective response rate of 14% compared with 9.6% of chemotherapy. However, no improvement in overall survival was demonstrated [114]. Conversely, in a phase III trial on 695 newly diagnosed GBM (EF-14), a benefit in progression-free survival and overall survival was seen in the combination TMZ/TTFs group versus TMZ alone (6.7 versus 4.0 months, $p < 0.001$, and 20.9 versus 16.0 months, $p < 0.001$, respectively) [115]. With regard to tolerability, treatment with TTFs was not associated with poorer quality of life, as reported in a dedicated sub-analysis of the EF-14 trial [116].

16.1.5.2 Focused Ultrasound Therapy

Focused ultrasound (FUS) therapy uses sound waves to treat various medical conditions, including GBM. It can operate at high or low intensity, each with distinct mechanisms and applications:

High-Intensity Focused Ultrasound (HIFU): Utilizes thermal effects to cause protein denaturation and DNA fragmentation, leading to coagulative necrosis of tumor cells [117, 118].

Low-Intensity Focused Ultrasound (LIFU): Relies on acoustic cavitation to temporarily open the blood-brain barrier (BBB), enhancing drug delivery to brain tumors. LIFU is being explored in multiple clinical trials for its potential to improve the outcome in GBM [119]. Sonodynamic therapy, which combines LIFU with sonosensitising agents like 5-ALA, is also under investigation for its ability to generate reactive oxygen species and kill tumor cells [120].

16.1.5.3 Magnetic Nanoparticles

Magnetic nanoparticles are being developed for their potential to cross the BBB and deliver therapeutic agents directly to GBM cells:

Biomimetic Therapeutic Nanoparticles: These nanoparticles, mimicking glioma cells, can convert lactate to pyruvic acid and hydrogen peroxide, inducing cell cycle arrest and tumor cell death. They show promise in preclinical models for their stability and targeting efficiency [121].

Gold Nanoparticles: Designed to target the tight junction protein JAM-A, these nanoparticles can temporarily disrupt the BBB, allowing enhanced delivery of chemotherapy drugs like paclitaxel to brain tumors when activated by a laser [122].

16.1.5.4 Carbon Ion Radiotherapy (CIRT)

CIRT involves using carbon ions to deliver high-energy radiation, causing severe double-strand breaks in DNA. This method is particularly attractive against radioresistant tumors like GBM and is being explored in several international clinical trials [123]. CIRT offers the advantage of delivering higher radiation doses precisely to the tumor while sparing surrounding healthy tissue.

16.1.5.5 Surgically Targeted Radiation Therapy

Surgically targeted radiation therapy aims to deliver radiation at the time of surgery, potentially improving local tumor control:

Intraoperative Radiotherapy (IORT): Includes techniques like intraoperative electron radiotherapy (IOERT) and low-energy X-ray IORT (LEX-IORT), which deliver radiation during surgery but have not yet shown significant improvements in overall survival [124, 125].

Interstitial Brachytherapy (IBT): Uses radiation-emitting seeds implanted post-surgery to continue to deliver to the tumor site. GammaTile, a newer form of IBT, uses cesium-131 seeds in a collagen matrix to provide localized radiation therapy, showing promise in extending recurrence-free survival [126].

16.1.6 Convection Enhanced Delivery (CED)

CED is a method of delivering therapeutics directly to brain tumors by creating pressure gradients that force drugs through the interstitial spaces of the brain bypassing the BBB. CED is being explored for delivering various treatments, including conjugated toxins, liposomal carriers, and viral therapies [127].

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