Chapter

Systemic Treatment in Glioblastoma

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Abstract

Glioblastoma is the most common primary brain tumor and the initial treatment with maximal safe resection is not curative. In order to improve the prognosis, surgery is completed with radiotherapy and temozolomide, an oral chemotherapy, but overall survival remains poor. Therefore, new efforts are needed to improve these results. In fact, different systemic treatments have been tested but, nevertheless, few advances have been reached despite the development of large clinical trials. This chapter will review the most important findings, achievements, and main studies in this pathology. Standard of care in newly diagnosed and recurrent glioblastoma will be reassessed with the results of clinical trials with targeted agents and immunotherapy. Ongoing studies are evaluating advanced treatments, with chimeric antigen receptor T-cells, biospecific T-cell antibodies, tumor vaccines, and oncolytic viruses, although results are pending, a wide review of these new-generation agents is important to better understand the advances in glioblastoma in the coming years.

Keywords: glioblastoma, chemotherapy, immunotherapy, clinical trials

1. Introduction

Glioblastoma is the most frequent primary brain tumor in adults.

The median age of diagnosis is 64 years and the average age-adjusted incidence rate is 3.2 per 100.000 population [1].

Initial treatment includes maximal safe resection, radiotherapy, and chemotherapy with temozolomide based on a phase III pivotal study published in 2005.

Despite all of this, the prognosis remains poor with a median overall survival of 14 months [2].

Therefore, new efforts are needed to improve these results.

Systemic treatments have been tested in different clinical trials. Nevertheless, few advances have been reached.

This chapter will review the most important achievements and main studies in this pathology.

To understand the difficulties to advance in glioblastomas, here we expose some characteristics of this tumor.

Glioblastoma is characterized by the presence of several mechanisms of resistance to different treatments.

One of them is the presence of the blood-brain barrier (BBB*).* The BBB is composed of a neurovascular structure, with specialized capillary endothelial cells adhered with tight junctions, a basal lamina, and a complex of astrocytic endfeet, pericytes, and intermittent end of neurons. Only small molecules can passively diffuse across this barrier. Other molecules need mechanisms such as pinocytosis or receptor or carried-mediated transcytosis.

Moreover, several drug-resistance proteins (such as P-glycoprotein and multidrug resistance-1) are expressed in the vessel wall to reinforce this barrier.

In glioblastoma, the BBB is heterogeneously disrupted with reduced tight junctions, altered pericytes, and astrocytic end-feet, leading to tumoral areas with different blood permeability to the different drugs [3].

Another mechanism of resistance is tumor heterogeneity, which is perhaps the most challenging obstacle to finding successful treatments for glioblastoma. At a cellular level, glioblastoma tumors are composed of various groups of cells and glioma stem cells (GSCs), each with a specific transcriptional signature.

Moreover, glioblastoma is also characterized by spatial heterogeneity due to the presence of diverse hypoxia gradients and heterogeneity of the tumoral microenvironment.

On the other hand, primary and recurrent glioblastoma can have subclonal genetic alterations, with the presence of regions with different drug sensitivity [3].

Other studied factors that have contributed to systemic treatment failure are related to mechanisms of chemoresistance such as the presence of unmethylated DNA repair enzyme O6Meg DNA methyltransferase (MGMT) [4].

Although the increased knowledge of molecular alteration in this disease, a lack of success has been reported in different approaches to targeted therapy probably related to the tumoral heterogeneity and signaling-pathway redundancy [5, 6] as well as the absence of a biomarker selection.

All of these considerations should be taken into account in the design of the clinical trials, given that several trials fail to demonstrate a clinical benefit for this disease.

As a result of these difficulties, today the standard of care is a maximal initial resection followed by concurrent radiation and temozolomide.

About 70% of GM will experience recurrence within one year of diagnosis with less than 5% of patients surviving after diagnosis.

In recurrent glioblastoma, there is no standard of treatment. The USA Food and Drug Administration (FDA) has approved bevacizumab (but not by EMA) and TTF.

2. Systemic treatment in newly diagnosed glioblastoma: positive trials

The EORTC/NCIC clinical trial demonstrated the clinical benefit of adding chemotherapy to the treatment of surgery and radiotherapy in patients with glioblastoma.

In this study, 573 patients were randomized to receive involved-field radiation therapy alone or radiation plus concurrent temozolomide followed up to six cycles of adjuvant temozolomide.

A statistically significant benefit was observed with the addition of temozolomide, with a median overall survival (OS) of 14.6 months vs. 12.1 months [2]. Since the publication of this study, the standard of care (SOC) in newly diagnosed glioblastoma is temozolomide 75 mg/m2 daily during RT followed by 6 adjuvant cycles of 150–200 mg/m2 on days 1-5/28.

A retrospective analysis of 206 patients has been done to determine the MGMT methylation status. In 45% of the cases, MGMT was methylated and the benefit of the treatment with temozolomide was greater (median overall survival 21.7 months vs. 15.3 months).

In non-methylated patients, there was a survival benefit that was not statistically significant [4].

3. Systemic treatment in newly diagnosed glioblastoma: negative results

Since the publication of the previously mentioned study, by Roger Stupp [2], of what is now the standard of care (SOC), there have been few advances. Furthermore, despite a better understanding of the biology of the tumor, this has not translated into progress in first-line therapy or newly diagnosed GBM. However, this does not mean that efforts to search for new targets and/or therapeutic strategies for improving the prognosis of these patients have been null or void. It must be said that there has been a titanic effort and that the negative results of trials have helped us to steer the research path. Therefore, we are going to review the negative studies with the greatest impact.

3.1 Antiangiogenics

The rationale for the use of drugs that inhibit vascular endothelial growth factors, such as bevacizumab, was based on the concept that the tumor vasculature could be normalized. This would lead to a decrease in tumor interstitial pressure and, therefore, better access to cytotoxic drugs. Moreover, with increased oxygen supply, the efficacy of radiotherapy would also be improved [7]. On the other hand, it is known that GBM overexpresses vascular endothelial growth factor A (VEGF-A), a key regulator of tumor-associated angiogenesis, and these tumors are highly vascularized [8].

Given that bevacizumab has demonstrated activity in patients with recurrent GBM and there was evidence that indicates the combination of bevacizumab with SOC therapy was active for patients with newly diagnosed GBM, two studies were initiated for first-line patients.

In the **AVAglio trial** [9], 921 patients were randomized to receive bevacizumab (10 mg per kilogram of body weight every 2 weeks) or placebo, plus SOC: radiotherapy (2 Gy 5 days a week; maximum, 60 Gy) and temozolomide (75 mg per square meter of body-surface area per day) for 6 weeks. After a 28-day treatment break, maintenance bevacizumab (10 mg per kilogram intravenously every 2 weeks) or placebo, plus temozolomide (150 to 200 mg per square meter per day for 5 days), was continued for six 4-week cycles, followed by bevacizumab monotherapy (15 mg per kilogram intravenously every 3 weeks) or placebo until progression or unacceptable toxicity. Even though PFS was longer in the bevacizumab group (10.6 months vs. 6.2 months; stratified hazard ratio for progression or death, 0.64; 95% confidence interval [CI], 0.55 to 0.74; $P < 0.001$), the OS did not differ between groups (stratified hazard ratio for death, 0.88; 95% CI, 0.76 to 1.02; $P = 0.10$). Maintenance of quality of life and performance status were observed with bevacizumab even though the bevacizumab group had more adverse events (arterial thromboembolic events, hypertension, and complications of wound healing). No predictive influence of MGMT status or any other subgroup variable was observed concerning progression-free survival or overall survival.

The addition of bevacizumab to SOC was also investigated in the Radiation Therapy Oncology Group **(RTOG)-0825 study** [10]. It showed a similar trend toward

improvement in PFS (HR 0.79; 95% CI, 0.66 to 0.94; P = 0.007), with a 3.4-month extension of PFS; the difference was not significant according to the prespecified alpha level (P < 0.004) and there was also no statistically significant difference in OS (HR, 1.13; 95% CI, 0.93 to 1.37; P = 0.21).

Finally, the exhaustive review by Cochrane concluded that there is insufficient evidence to support the use of antiangiogenic therapy for people with newly diagnosed glioblastoma [11].

3.2 Integrin inhibition

Integrins are adhesion molecules involved in several tumorigenic processes such as survival, proliferation, migration, invasion, and angiogenesis [12]. Cilengitide is a selective inhibitor of αvβ3 and αvβ5 integrins that are expressed both in GBM tumor cells and in the vasculature. Moreover, several studies demonstrated a potentiation or synergy when cilengitide is combined with radiation therapy and chemotherapy. That was the rationale for exploring cilengitide in newly diagnosed GBM.

The first trial, **CENTRIC EORTC 26071-22072**, was carried out in methylated MGMT promoter tumors. Cilengitide, 200 mg intravenously twice weekly, was added to SOC, maintenance temozolomide was given for up to six cycles, and cilengitide was given for up to 18 months or until disease progression or unacceptable toxic effects. The primary endpoint was overall survival [13]. Unfortunately, the addition of this drug did not improve the outcome: OS was 26·3 months in both arms (HR,1·02;95%CI,0·81–1·29;p = 0·86) and PFS 10·6 months in the cilengitide arm and 7·9 months in the control arm (HR, 0·92; 95% CI, 0·75–1·12;p = 0·41).

Later, data were published on unmethylated tumors [13]. It offered the opportunity to use dose intensification as a means of overcoming resistance. Patients were randomized to standard cilengitide (2000 mg twice weekly until progression) or intensive cilengitide (2000 mg daily for 5 days during radiotherapy followed by twice weekly until progression) with radiotherapy and temozolomide or a control arm with SOC. Median PFS was 5.6 months and 5.9 months in the standard and intensive cilengitide arms, respectively, versus 4.1 months in the control arm. The median OS was 13.4 months (range, 0–30 mo) in the control arm, 16.3 months (range, 0–29) in the standard cilengitide arm, and 14.5 months (range, 0–29) in the intensive cilengitide arm, which is statistically non-significant. No benefit was observed despite dose escalation and most striking was the improvement in OS in patients who were expected to have a worse prognosis as they were unmethylated. The study was underpowered to consider the 3-month improvement in OS was enough. In addition, we do not have a biomarker to select responders.

Integrins are an important target in GBM, but a better understanding of the interaction between the tumor and the extracellular matrix is needed [14].

3.3 PARP inhibitors

Half of the patients with GBM have methylated MGMT and there is a rationale for combining PARP inhibitors with temozolomide, based on the importance of PARP in mediating basic tissue repair as well as homologous recombination.

The combination of veliparib and SOC did not provide benefits [15]. In ASCO 2022, the **Alliance A071102 trial** was presented [16]. Total of 447 patients with MGMT promoter hypermethylated GBM after radiotherapy and temozolomide were randomized to receive adjuvant temozolomide, given on days 1 to 5 every 28 days, combined with either placebo (n = 224) or veliparib (n = 223), given on days 1 to 7

every 28 days. Treatment was continued for up to six cycles. For phase II, PFS was the primary endpoint. The results were disappointing as the PFS was similar in both groups: 13.2 months with veliparib versus 12.1 months with placebo (HR 1.05, 95% confidence interval 0.86–1.29, p = .31). Median OS was 28.1 months with veliparib and 24.8 months with placebo (hazard ratio 0.89, 95% confidence interval 0.71–1.11, P = .15). The study is negative despite the different hypotheses put forward by the authors about improved survival at intermediate time points.

Effective biomarkers are needed to identify patients who are most likely to benefit from the addition of veliparib.

3.4 ANTI EGFR therapies

Epidermal growth factor receptor (EGFR) gene amplification on chromosome 7 (EGFR-amp) is expressed in 50% of GBMs. The EGFR variant 3 mutation (EGFRvIII), a tumor-specific deletion of exons (2–7), is active and is observed in approximately 50% of GBMs with EGFR (~25% overall) [17]. Nevertheless, EGFR-targeted treatments in GBM have been disappointing.

3.5 Antibody-drug conjugate

Depatuxizumab mafodotin (depatux-m) is an antibody-drug conjugate composed of a monoclonal antibody that binds to activated EGFR and is bound to a microtubule inhibitor toxin. It was tested in a phase III trial, adults with centrally confirmed, EGFR-amp newly diagnosed GBM [18]. Patients were randomized to receive SOC plus depatux-m at 2.0 mg/kg during RT, then 1.25 mg/kg on days 1 and 15/28, and continue until disease progression versus SOC. The trial was a phase III with OS as the primary endpoint. There was no improvement with the addition of the antibody; OS for depatux-m over placebo (median 18.9 vs. 18.7 months, HR 1.02,95% CI 0.82–1.26, 1-sided p = 0.63). PFS was longer for depatux-m than placebo (median 8.0 vs. 6.3 months; HR 0.84, 95% confidence interval [CI] 0.70–1.01, p = 0.029), particularly among those with EGFRvIII-mutant (median 8.3 vs. 5.9 months, HR 0.72, 95% CI 0.56–0.93, 1-sided p = 0.002) or MGMT unmethylated (HR 0.77, 95% CI 0.61–0.97; 1-side p = 0.012) tumors but without an OS improvement. One of the most peculiar toxicities of this drug is the corneal epitheliopathy that occurred in 94% of depatux-m-treated patients (61% grade 3–4), causing 12% to discontinue.

4. Pharmacologic treatment of recurrent glioblastoma

In the recurrence set, the prognosis of these patients is poor, with an estimated survival of about 6 months [19].

Compared to newly diagnosed glioblastoma, the management of the recurrent disease is not curative and less standardized without randomized trials. Different approaches should be considered including systemic agents (chemotherapy and target therapy) or locoregional treatments (radiation therapy and surgery) [20].

There is limited evidence for the systemic therapy of recurrent GB (rGB).

Several prognostic factors should be taken into account to select the patients that can benefit from systemic treatment after recurrence. Some of these factors are tumor size, location, performance status, and administration of steroids.

It is recommended to enroll these patients in a clinical trial whenever possible.

Outside a clinical trial, a second-line treatment could be considered.

The most commonly used agents are nitrosoureas, bevacizumab, and temozolomide, but none is approved by EMA because most of the time the evidence was derived from small to no randomized studies [21]. Bevacizumab has been approved by the FDA for recurrent high-grade glioma.

Treatments as promising as immunotherapy and drugs against EGFR are not superior to the treatments cited. Several novel treatments are undergoing evaluation in clinical trials.

4.1 Nitrosoureas

Nitrosoureas (lomustine, carmustine, fotemustine) have shown activity in phase II trials in rGB.

Lomustine (CCNU) is an oral nitrosourea. It has shown a modest improvement in overall survival (median OS 7.1–9.8 months).

Lomustine has never been shown to be superior to other drugs in randomized studies but represents the control treatment arm in randomized clinical trials. Lots of new drugs, especially multitarget tyrosine kinase inhibitors, have been tried alone or in association with lomustine or against lomustine without any benefit in overall survival [22].

Fotemustine is a new intravenous nitrosourea with a better toxicity profile. It has proven activity in glioblastoma in several phase II studies and is mainly used in Europe [23].

4.2 Antiangiogenic agents

Bevacizumab (BV) is a VEGF-A (vascular endothelial growth factor A) targeting monoclonal antibody. It was a very promising agent in rGB. In phase II, studies showed a PFS-6 rate ranging from 18–42% and median OS from 6.5 to 9.2 months. However, in randomized clinical trials, it has not been proven to have better results than lomustine.

In the phase II randomized trial BELOP (5) the combination of BV and lomustine showed an OS benefit over lomustine. Nevertheless, the phase III EORTC 26102 study randomized more than 400 patients with rGB to BV plus lomustine versus lomustine. The results showed a significant difference in PFS, but without any impact on OS, which was the main endpoint [24]. It is not yet known, which subgroup of patients could benefit from BV and its real impact on OS. Combinations of BV with other agents do not appear to be superior to monotherapy.

Regorafenib, an oral multi-kinase inhibitor, has been investigated versus lomustine in the randomized phase II trial REGOMA. The primary endpoint was overall survival in the intention-to-treat population, which was higher in the experimental arm. However,the planned statistical design did not have enough power to estimate survival advantage. Therefore, the authors concluded that phase III is needed to confirm this benefit [20].

4.3 Temozolomide

Temozolomide rechallenge can be considered an option in patients who have tumor recurrence beyond four to six months from the end of the first-line treatment with temozolomide and have a methylated MGMT promotor.

Another strategy consists of the administration of temozolomide in an extended regimen. Extended schedules had been developed to overcome TMZ resistance in phase II studies [25].

There are small studies that yield modest results in rGB (PFS-6 rates 17–50%) [21].

4.4 AntiEGFR therapy

About 50% of all GB patients present an amplification of the epidermal growth factor receptor (EGFR) gene. Agents targeting this receptor failed to show a significant survival impact on patients with rGB.

The most promising agent has been depatuxizumab mafodotin, an antibody-drug conjugate, that consists of an antibody directed against EGFR and EGFRvIII, conjugated to a toxin (monomethyl auristatin F). The INTELLANCE-2 /EORTC 1410 phase II randomized study [26] investigated depatux-M in combination with temozolomide or as a single agent in recurrent EGFR amplified GB. Patients who received depatux-M and temozolomide had a trend toward improved survival but did not reach statistical significance.

4.5 Future promising agents

It is necessary to improve the design of clinical trials in GB.

Personalized treatments based on the tumor's molecular characteristics have had promising results.

There are small studies with inhibitors of NTRK (neurotrophic tropomyosin receptor kinase), BRAF (B-Raf proto-oncogene), FGFR (fibroblast growth factor receptor), PDGFR (platelet-delivered growth factor receptor), IDH (isocitrate dehydrogenase), and histones, mainly, that are showing interesting preliminary results.

Other types of immunotherapy, such as chimeric antigen receptor T-cells (CAR-Ts), chimeric antigen receptor macrophages (CAR-Ms), oncolytic viruses, and vaccines, are under evaluation [27].

5. Recurrence glioblastoma: radiotherapy and surgery

Despite systemic treatment, other options could be considered for recurrence.

As previously referred, in this context, treatment decisions must be individualized.

One of the most important prognostic factors for benefit from local treatment is the previous performance status. Other useful factors include young age, the extent of the disease, the histologic grade, the relapse-free interval, the recurrence pattern (i.e., local versus diffuse), and the extent of the second surgical resection [28].

A negative factor is ependymal involvement, which is independent of performance status, tumor size, and extent of resection [29].

Patients with a localized recurrence are better candidates for reoperation or reirradiation interventions than those with primary refractory disease or diffuse or multifocal relapse.

It is important to point out that these patients should be referred to a multidisciplinary brain tumor center with a multidisciplinary team to revise images, evolution, and options of treatment [28, 30, 31].

In conclusion, the best candidates for reoperation are patients with large but well-circumscribed, symptomatic tumors that are amenable to complete or near-complete resection, particularly if the tumor has recurred after an extended interval.

The benefit of reirradiation of glioblastoma is uncertain and can be considered in selected patients. Occasionally used in patients with a localized or out-of-field glioblastoma recurrence. Instead, patients with a poor performance status have poor prognostic, and the risks of receiving subsequent treatment outweigh the benefits [32, 33].

5.1 Reoperation

Approximately, only 20 to 30 percent of patients with recurrent glioblastoma are candidates for a re-operation [19, 34, 35].

The technics used are the same as for primary resection and included 5-aminolevulinic acid (5-ALA) guided resection that showed benefit in recurrent glioblastoma [36–38].

There is no evidence to suggest that these results are better than and can be expected with radiation and/or chemotherapy alone.

Two meta-analyses have analyzed surgery as a treatment approach in recurrent glioblastoma.

The first study assessed eight observational studies for a total of 1906 patients with glioblastoma who underwent primary surgery and 709 patients with secondary surgery. The pooled hazard ratio (HR) showed a longer OS for patients receiving surgery at the time of recurrence (HR: 0.722; p: 0.001).

The second meta-analysis selected nine studies for a total of 1507 patients with glioblastoma and 1335 patients treated with re-intervention.

Among these studies, OS after repeat surgery ranged from 8 to 13 months. Maximal safe resection appears to confer a significant OS benefit (HR 0.59, p: 0.1). Radiographic confirmed gross total resection was the most prognostic variable related to the extent of surgery and was associated with longer OS (HR 0.52, p: 0.01) [39].

Another interesting option is carmustine polymer wafers. A review revealed three trials in which patients with glioblastoma who received carmustine wafers had statistically significant longer overall survival. Overall results of these trials seem to suggest that carmustine wafer implantation demonstrates promise as an effective and tolerable treatment strategy for GBM [40]. Daily practice is not commonly used due to potential surgical complications.

In conclusion, surgery should be included in the treatment algorithm for recurrent glioblastoma. It should be proposed when it is technically safe and associated with a feasible total resection, especially in patients with good performance status. Optimal management after surgery is still unknown, and prospective studies are ongoing to study different strategies, such as RESURGE trial [41].

5.2 Reirradiation

Salvage reirradiation has been utilized in the treatment of recurrent diseases for years. The role of reirradiation in patients with recurrent glioblastoma is uncertain, and there is little prospective data. For this reason, participation in clinical trials is encouraged.

As most recurrences occur within the high-dose radiation field (90–95%), reir-radiation is generally poorly considered as a treatment option due to the high risk of toxicity.

The adequate selection of patients suitable for reirradiation is a key issue. Age, performance status, target volume, time to progression, type of progression, and site of recurrence are essential elements to consider. Different techniques can be used: conventionally fractionated radiotherapy (RT), hypofractionated stereotactic radiosurgery (HFSRT), and stereotactic radiosurgery (SRS) [42].

Based on mostly retrospective series, selected patients with small recurrent tumors and a good performance status may benefit from repeat radiation using modern high-precision techniques to deliver total doses of 30 to 35 Gy in 5 to 15 fractions [43].

Reirradiation with conventional involved field radiation at therapeutic doses (54 to 60 Gy) is not recommended in patients with relapsed disease due to treatment-related toxicity. The most common form used is fractionated radiosurgery or hypofractionated radiotherapy (e.g., 30 to 35 Gy in 5 to 15 fractions). Selection is based on the preference of the treating radiation oncologist and local availability since there are no clear differences in efficacy [44].

Reirradiation can be given with both concurrent or sequential administration of systemic therapy (TMZ, bevacizumab, and immunotherapy). The available data in patients with recurrent glioblastoma generally suggest that reirradiation modestly improves progression-free survival compared with systemic therapy alone, but overall survival is similar [45].

A few prospective data are available in a phase II trial 182 patients with recurrent glioblastoma were randomized to receive bevacizumab alone or in combination with radiation treatment (35 Gy in 10 fractions). The combination of radiation therapy and bevacizumab prolonged the PFS of these patients without significant improvement in OS [45].

The risk of radionecrosis should also be considered [46].

The benefit of the addition of bevacizumab to radiotherapy treatment was published in a recent systematic review. Data from a total of 1399 patients, were analyzed (954 patients receiving RT alone and 445 patients receiving RT and bevacizumab). Multivariate analysis showed that bevacizumab was associated with significantly improved. Patients receiving BVZ also had significantly lower rates of radionecrosis (2.2% vs. 6.5%) [47].

Other initial trials (phase I) studied the combination of RT, bevacizumab, and immunotherapy with promising results, but further controlled studies are needed to confirm these effects [48, 49].

Interstitial brachytherapy has been used in patients with recurrent high-grade gliomas, with several observational studies suggesting a survival benefit. However, brachytherapy is associated with a high incidence of radiation necrosis [50, 51].

An alternate form of brachytherapy uses an inflatable balloon catheter containing a liquid I-125 radioisotope (GliaSite) inserted at the time of surgical resection, which allows delivery of a quantifiable high dose of radiation to the tissue. No randomized clinical trials have been reported comparing this form of brachytherapy with other approaches. The role of brachytherapy is diminishing as experience with SRS and fractionated localized limited field radiation evolves [52, 53].

6. Immnunotherapy

Historically, the central nervous system (CNS) was considered to be immunologically isolated. However, today we know that the immunity system of the CNS is different but not incapable. There are functional lymphatic vessels and there are

antigen-presenting cells: microglia, macrophages, astrocytes, and classical APCs such as dendritic cells [54].

Glioblastoma is a cold tumor, with a low mutational burden; furthermore, as detailed below, it has demonstrated a poor response to immune stimulation therapies, such as immune checkpoint blockade. Even when T-cell responses are induced in CNS tumors by means such as vaccination, as discussed above, the number of antigen-specific TILs can remain relatively low, and the cells that are present often show a depleted phenotype. The reduced number and limited activity of T-cells in CNS tumors are largely due to the unique immunosuppressive immune environment of the brain [55].

The final step of the immune response in glioblastoma is the destruction by the active T-lymphocyte of the GBM cells after binding to their tumor antigen on MHC-I *via* the T-cell receptor (TCR). These T-cells are activated after recognizing the GBM cells, secreting inflammatory cytokines, and inducing GBM cell death.

Glioblastomas are characterized as tumor with a low median TMB and a lack of infiltrating lymphocytes [56]. Current approaches focus on: (1) Increasing glioma immunogenicity and activating the adaptative immune response by using tumor vaccines and oncolytic viruses, (2) Revert T-cell energy and promote a more inflamed tumor microenvironment by using immune cytokines, chemokines, and cytokine modulators, and (3) Overcome the lack of resident tumor infiltrating lymphocytes by directly engaging T-cells through direct activators such as CAR T-cells, TCBs, and bispecific T-cell engager antibodies.

6.1 Checkpoint inhibitor

GBM overexpresses PDL1, leading to PD-L1 binding to PD-1 and thus inhibiting the immune response [55].

Treatment with immune checkpoint blockade has shown improved survival in murine glioma models. However, data from phase III studies with the anti-PD-L1 nivolumab did not meet their primary endpoint of OS in the final analysis.

For newly diagnosed patients with MGMT-methylated or indeterminate GBM, the SOC therapy was compared with the same scheme plus nivolumab [57]. The trial included 716 patients who were required to have a centrally assessed methylated MGMT promoter, a Karnofsky performance status (KPS) of ≥70, and ≤ 3 mg dexamethasone at baseline. This study had two primary endpoints: PFS and OS. Regrettably, there were no significant differences observed for the 2 primary endpoints of the study. The median PFS for patients on the nivolumab arm was 10.6 months, compared with 10.3 months for the control arm. For patients not on corticosteroids, the median OS was 31.3 months for the nivolumab arm and 33.3 months for the control arm.

Nivolumab was also investigated for MGMT unmethylated GBM [58]. The trial compared nivolumab concurrent with RT followed by nivolumab until disease progression or unacceptable toxicity versus SOC. The addition of nivolumab did not improve efficacy. A total of 560 patients were randomized; median OS was 13.4 months (95% CI, 12.6–14.3) with NIVO+RT and 14.9 months (95% CI, 13.3–16.1) with TMZ + RT (HR, 1.31; 95% CI, 1.09–1.58; P = 0.0037). Median PFS was 6.0 months (95% CI, 5.7–6.2) with NIVO+RT and 6.2 months (95% CI, 5.9–6.7) with TMZ + RT (HR, 1.38; 95% CI, 1.15–1.65). A subgroup analysis based on established prognostic factors, including age, KPS, and degree of surgery, showed no significant benefit for the addition of nivolumab in any patient subgroup. One interesting feature was the baseline PD-L1 expression in tumor tissue: <1% in >55% of RT-TMZ and > 62% of RT-nivolumab patients. Although debate still rages regarding the role

and predictive value of this biomarker as well as optimal threshold, such a high level of lack-of expression of a key mechanistic molecule is worrisome.

Limitations of immune-based therapy may be related to tumor-associated factors, such as poor immunogenicity and tumor-induced immune tolerance, but it is important that treatment (SOC) induced immune regulatory effects may also play major roles, both adversely and beneficially [54]. In recurrent glioblastoma:

The CheckMate 143 trial included Cohort 1 in which patients with refractory glioblastoma were randomized to nivolumab monotherapy at 3 mg/kg every 2 weeks (10 pts), or nivolumab 1 mg/kg + ipilimumab 3 mg/kg every 3 weeks (10pts) and a non-randomized cohort 1b of 20 pts. that received nivolumab 3 mg/kg + ipilimumab 1 mg/kg Q3W for 4 doses, followed by nivolumab 3 mg/kg Q2W. A total of 3 pts. achieved a partial response (1 pt. with monotherapy and 2 with the combination). Based on the similar OS, RR and the lower degree of G3/4 toxicity, monotherapy with nivolumab was chosen as the treatment strategy for further development.

In the phase III trial, 369 patients were randomized to receive either nivolumab 3 mg/kg Q2W or bevacizumab 10 mg/kg Q2W. This study was negative for its principal objective of overall survival (OS of 9.8 months vs. 10 months and hazard ratio 1.04). OR and PFS favored bevacizumab, but ORR in the nivolumab was 7.8% and the duration of response was better than the one achieved with bevacizumab (11.1 versus 5.3 months) [59].

Other similar trials with checkpoint inhibitor monotherapy worth mentioning are the KEYNOTE-028 trial and the NCT01375842 trial of Atezolizumab. The KEYNOTE-028 [60] included 26 pts with bevacizumab-naïve recurrent glioblastoma have received pembrolizumab 10 mg/kg Q2W for a maximum of 24 months. 2 patients achieved partial responses (ORR of 7.6%) that lasted 8.3 and 22.8 months respectively and the 6 months PFS was 37.7%. On the NCT01375842 [61], 1 of 16 patients with recurrent glioblastoma showed a partial response (ORR of 6.25%).

The fact that in the Checkmate 143 trial nivolumab achieved similar outcomes to Bevacizumab (which is considered an active treatment in the recurrent setting), and the preclinical found that VEGF can mediate immunosuppression on the tumor stroma, the combination of immunotherapy with antiangiogenics could be worth investigating in the recurrent GBM setting. Three different clinical trials that have combined pembrolizumab with bevacizumab [62], avelumab with axitinib [63] or durvalumab with bevacizumab [64], have failed to show better results to those reported with antiangiogenic monotherapy.

Only a selected number of patients with recurrent glioblastoma will be considered candidates for a secondary resection, and as a consequence, the studies in the neoadjuvant setting have included a small number of patients. One small study with nivolumab [65] showed an increase in infiltrating T cells and an increase in the IFN response, while a similar one with pembrolizumab [66] failed to show any changes in the number of CD8 positive cells.

Intriguingly, the duration of response in some patients was high, but the expression of PD1 or the presence of hypermutation has not been associated with response to immunotherapy in gliomas.

6.2 Oncolytic viruses and tumor vaccines

Apart from the direct tumor cell killing that occurs after tumor cells are infected, oncolytic viruses have the potential of increasing immunogenicity in glioblastoma by delivering PAMPs (pathogenic associated molecular patterns) and facilitating the release of tumor-antigens by dying virus-infect cells.

HSV is the most studied virus in immunotherapy [67]. It can function as both an oncolytic agent and a transgene vector, which can be armed with immunomodulatory or angiogenic modulatory genes (i.e., GM-CSF in TVEC and IL2 in G47delta).

Results with G47delta injected intratumorally in patients with recurrent glioblastoma have shown issues with immediate enlargement of the contrast-enhanced area of the target lesion on MRI caused by the treatment, that is produced tumor destruction and lymphocyte infiltration, but the results in terms of OS (1-year OS of 38.5%) and especially the presence of a subset of patients with longer OS seem promising [68].

To create the DNX-2401 adenovirus, a 24-base pair deletion in the E1A gene that renders the virus unable to infect non-tumoral cells was introduced alongside an RGD-motif that enables the virus to infect integrin-rich cells, that are enriched in the tumor cells. Preliminary results of a phase I study show that 20% of patients with recurrent glioblastoma treated with intratumoral injection achieve OS 3y [69].

Another pilot study in patients with pediatric DIPG (a terrible disease where the historic series show a median OS of around 12 months) showed a reduction in tumor size in 9/12 patients, a 25% OR, and a median OS of 17.8 months [70].

Poliovirus PSRIVO is introduced in the tumor area through convention-enhanced delivery and recognizes the poliovirus receptor CD155, which is widely expressed in neoplastic cells in comparison with normal tissues. In a dose-finding study that included 61 patients with glioblastoma, also benefited a subset of patients (21%) that achieved long-term control at 24 and 36 months [71].

Compared with other tumors, the low TMB burden leads to a lower potential of Tumor-specific antigens (TSAs), mutant proteins expressed exclusively in tumor cells. Most studies using peptidic vaccines have focused either on personalized vaccines, with only two small pilot projects being published [72, 73], or vaccination against Tumor-associated antigens(TAAs), proteins present in normal tissues but overexpressed in tumors. A vaccine, called rindopepimut, designed to target the EGFRvIII, which is present in 30% of GBM cases was recently tested in a randomized phase III, after showing immunogenicity, safety, and activity in earlier clinical trials [74]. A phase III study, that randomized 745 patients that had completed their initial chemoradiation without progression showed negative results for OS (20.0 vs 20.1 m) in both patients with and without residual disease [75].

In comparison with peptidic vaccines, DC vaccines have the potential of being generated directly from coculture with tumor lysates, allowing the co-targeting of both TSAs and TAAs. Preclinical studies on glioblastoma have demonstrated that DC vaccines can reduce tumor growth, prolong survival and induce tumor-specific IFN-γ, and cytotoxic T-lymphocytes responses associated with T-cell infiltration of tumors.

Several small clinical trials have shown that this approach is safe and feasible [76–78], and the preliminary results of the largest clinical trial to date, testing DC-Vax, vs placebo with crossover at progression in 331 patients seem promising. But the final unblinded results have yet to be published [79]. Another potential source of dendritic vaccines is the ones generated by exposing cells to pp65, which is a major structural protein of CMV a virus that is frequently present in glioblastoma cells. Although studies using pp65 vaccines are small the median PFS of 25.3 m and OS of 41.1 m are intriguing [80].

6.3 Immunocytokines, chemokines, and other cytokine modulators

Immunocytokines are molecules that target immunostimulating cytokines such as TNF or IL-2 to the tumor microenvironment using signals that direct them to the tumor cells, immune infiltrating cells, or components of the tumor stroma. One

potential therapeutic agent in this class is L19TNF, a multimer of TNF fused to the antibody L19 that binds a tumor-specific epitope of the extracellular matrix protein fibronectin. Preliminary studies have shown the safety, feasibility, and intriguing preliminary clinical results in both combinations with lomustine in refractory GBM, and combination with chemoradiation in front-line patients.

TGF-B upregulation in glioblastoma has been linked to increases in the migratory potential, promoting EMT, inducing a CSC-like drug-resistant phenotype, and an immunosuppressive microenvironment [81]. Despite some patients treated with TGFB inhibitors in clinical trials showing prolonged responses [82, 83], the overall results with oral TGFBR have been disappointing [84] most likely due to insufficient target inhibition due to concerns over cardiotoxicity.

CSF-1R inhibitors are cytokine modulators that try to repress tumor-associated myeloid cells that form a substantial proportion of the immunosuppressive glioblastoma microenvironment, by downregulating CSF1R, an important receptor for macrophage differentiation and survival. However clinical trials both in first-line patients combined with chemoradiation and in patients with recurrent disease in both monotherapy and combination with checkpoint inhibitors show limited clinical efficacy.

6.4 CAR T-cells, TCBs, and bispecific T-cell engager antibodies

CAR-T cell treatment share with vaccines the necessity of identifying targets that are primarily present in tumor cells with low expression in normal tissues (TAAs/TSAs).

Accordingly, EGFRvIII has also been chosen as a target for CAR T-cell treatment. One potential issue is that although most patients treated with CAR T-cells developed noticeable peripheral levels of EGFRvIII-directed CAR T-cells when their tumors were resected half of the patients had lost their baseline expression of EGFRvIII [85].

Subsequent small trials with second and third-generation trials including expression of costimulatory proteins show only minimal signs of activity in a few patients [86].

IL13Rα2 CAR-T has also been tested in small clinical trials, with some patients achieving clinical benefit, including 1 patient presenting a complete response. Finally.

HER2 CAR-T cells were deemed to be safe in the phase I clinical trial that included 17 patients, including 1 patient with partial response and 3 with disease stabilization for more than 4 months [87].

Another potential way to overcome the lack of antigen presentation and infiltrating T-Cell is by the use of bispecific antibodies that target at the same time a target present in immune cells, that many times is CD3 and a TSA or TAA. Several modifications to the bispecific antibody structure can be made to modify its protein and characteristics. For example, AMG 596 is composed of two single-chain variable fragments one binding to CD3, and the other to EGFRvIII, while RO7428731 contains both variable regions against EGFRvIII and CD3 and an IgG structure [88].

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