# Insights into pharmacotherapy of malignant glioma in adults

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Malignant gliomas are the most common primary brain tumors in adults. In the past 10 years significant advances in the treatment of this entity have been made, mainly owing to a better understanding of molecular pathways and biological behavior of the oncogenetic process. This review treats the proven effective and promising approaches with chemotherapy. The standard care for glioblastoma is surgery and concomitant radio- and chemotherapy with temozolomide (TMZ), followed by adjuvant treatment with TMZ. It has been demonstrated to be the most effective treatment protocol. This standardized care allows the application and study of new types of treatment mainly in recurrences and nonresponding patients. Many different approaches have been investigated: the combination of cytotoxic and cytostatic agents as well as molecular targeted therapies have given some encouraging results. Further intensified regimens with TMZ and the local postsurgical application of slow-release polymers loaded with carmustine remain to be defined. The characterization of molecular markers thus becomes particularly important for the stratification of patients raising the possibility to individualize treatment.

**Keywords:** bevacizumab, chemotherapy, erlotinib, glioblastoma, grade 3 gliomas, temozolomide

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## 1. Introduction

Malignant or high-grade gliomas comprise glioblastoma multiforme (GBM; grade 4), anaplastic astrocytomas, anaplastic oligodendrogliomas and oligoastrocytomas (grade 3), according to WHO classification (2007) [1]. These are the most frequent malignant primary brain tumors in adults, and despite important advances in surgical management, imaging, radiation therapy and experimental and clinical oncology, the survival rate for patients with malignant gliomas has improved only slightly in the last decade. There are several established reasons for the severe prognosis associated with high-grade gliomas. First of all, the glial origin of these tumors and the natural tendency to infiltrate normal brain tissue can explain the impossibility for the surgeon to obtain an effective total removal of all neoplastic cells. Second, the blood–brain barrier (BBB) represents an important obstacle to penetration of chemotherapeutic agents: as demonstrated by contrast enhanced TC and MRI, BBB disruption is variable, minimal or absent in the region of the brain adjacent to the tumor, where there is high infiltration of neoplastic cells; moreover, the frequent use of steroids in these patients can re-establish BBB integrity. Third, high-grade gliomas seem to be refractory to most of cytotoxic agents, and occasional responses are often short-lived with rapid development of resistance: this phenomenon is partly due to genetic transformation and instability, leading to a high heterogeneous tumor cell population. In addition, the concurrent use of antiepileptic drugs, especially older agents, may dramatically influence the pharmacokinetics of several antineoplastic agents by the induction of hepatic cytochrome P450 enzymes. Finally, recent findings show that cancer stem cells in gliomas may play a critical role in tumor behavior and that these

may explain many aspects of growth modality and therapy resistance, which add to the complexity of these tumors.

## 2. From alkylant agents to the standardization of first-line treatment

Historically chemotherapy of brain gliomas was based on alkylating agents such as nitrosoureas, owing to their liposolubility and their ability to cross the BBB and reach effective concentrations. Carmustine (BCNU) and lomustine (CCNU) in the PCV regimen (procarbazine-CCNU-vincristine) were the most frequently employed agents [2,3]. Studies examining BCNU originally reported a 10 – 15% survival benefit at 18 months, but no differences in median overall survival or in survival at 1 and 2 years [3] could be observed and no particular benefit appeared from polichemotherapy over monochemotherapy [4,5]. For many years the efficacy of such adjuvant chemotherapy, administered after completion of radiotherapy, was questioned, but the meta-analyses of many randomized trials conducted by Fine *et al.* [3] and Stewart [6] showed the modest but meaningful advantage in overall survival that can be obtained by the use of adjuvant nitrosoureas for both grade 3 and 4 gliomas, with the 1-year survival rate increased from 40% to 46% and the 2-year survival rate increased from 15% to 20%. No differences were observed with age, sex, performance status and extent of resection for the effectiveness of adjuvant chemotherapy.

Early in the 1980s, the new alkylating agent temozolomide (TMZ) was developed with the intent to treat brain metastases from melanoma [7]. Pharmacokinetic properties of TMZ are rapid oral absorption, spontaneous conversion into the active metabolite MTIC, plasma peak of concentration in 30 – 90' with a t/2 of 2 h, tropism for basic ambient and good biodisponibility with a safe therapeutic-toxic profile [8]. The key-methylation of TMZ is that over the O6 of guanine, which induces citotoxicity and apoptosis [9]. TMZ was initially administered in a 5-day repeated scheme and demonstrated modest activity as a single agent against recurrent GBM after standard surgical and radiotherapy treatment, with response rates of 5% and progression-free survival at 6 months (PFS6) of 21% [10-11].

Given the good tolerability and the oral formulation of TMZ, physicians began to evaluate continuous administration schedules: Newlands *et al.* and Brock *et al.* developed a continuous TMZ administration for 49 days at a dosage between 75 and 85 mg/m$^2$ of body surface [7,12]. The rationale for this study was the possibility to use TMZ concomitantly to adjuvant fractionated radiotherapy (RT) administration, given the established radiosensitizing properties of TMZ [13,14]. On this basis, a Phase II study by Stupp *et al.* was published in 2002 [15], which treated 64 cases of primary GBM with TMZ, concomitant to the 30 fractions of RT (5 fractions per week 20 cGy each, for a total of 6 weeks). The dosage was 75 mg/m$^2$ of body surface for 7 days per week, followed by TMZ 200 mg/m$^2$ of body surface administered for 5 days every 28 per cycle for a total of six cycles as adjuvant treatment. The results of this study were so promising that they led to a Phase III multicenter randomized study with the same treatment protocol, involving the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) Trials Group [16]. A total of 573 patients were enrolled into this study, divided in two groups, one receiving RT alone followed by six cycles of standard TMZ as adjuvant treatment, the other receiving the protocol known as 'Stupp protocol', with TMZ given concomitantly and subsequently to RT. Results confirmed the observations of the pilot study: median survival was 14.6 months for the RT + TMZ group compared with 12.1 months for the RT-alone group ($p < 0{,}001$), with a 37% reduction of the risk of death, but the main results were lasting over time. In fact, the 2-year survival was 26.5% for the RT + TMZ group versus 10.4% for the RT-alone group. These data do not refer to patients who had a poor preoperative performance status and only biopsy as entity of tumor removal. Another remarkable finding of this study was the good tolerability profile of the adjuvant treatment, with a grade 3 or 4 observation of hematologic toxicity in only 7% of patients during the concomitant phase, and 14% during adjuvant TMZ: at 28 months of median follow-up, no increase in treatment-related toxicity could be observed. Moreover, a correlative study on health-related quality of life (QoL) showed no negative impact for the combined treatment group in comparison with the RT-alone group [17]. This study suggested for the first time that chemotherapy may result in a more effective treatment when employed early in the course of the disease, instead of later, at recurrence.

Moreover, this effectiveness seems to last over time: a very recently published study [18] analyzes the long-term follow-up results at 5 years of the mentioned EORTC study [16]. Results of this further analysis are clear: the improvement on overall survival (OS) is maintained at 2, 3, 4 and 5 years, respectively for the concomitant TMZ and RT arm versus the RT-alone arm, as follows: 27.2% vs 10.9% are still alive at 2 years, 16.0% vs 4.4% at 3 years, 12.1% vs 3% at 4 years and 9.8% vs 1.9% at 5 years. The difference is statistically significative and is maintained over analyzed subgroups.

This randomized Phase III study by EORTC had a profound worldwide impact on GBM management, to the extent of changing management paradigms and view of approaching all high-grade gliomas at an experimental and clinical level [19]. In fact, as this treatment has become a standard, more European and North American clinical research protocols were modified incorporating concomitant adjuvant TMZ plus radiation rather than radiation alone as their standard treatment arm. The worldwide acceptation of the 'Stupp protocol' as a standard leads to many clinical and research problems, some of which may be explained as follows.

- There is a lack of clinical confirmation studies allowing the standardization of treatment.
- 'Stupp protocol' is based on radiosensitizing properties of TMZ [13,14], but this hypothesis is not yet confirmed. There is a clear association between concomitant RT and TMZ administration and OS improvement, but the true mechanism is not yet known, and the relative contribution of the subsequent adjuvant TMZ cycles is not well understood.
- In the EORTC/NCIC study the number of postradiotherapic adjuvant TMZ cycles is six, with a progression-free survival (PFS) rate of 54%. However, because chemotherapy was discontinued before disease progression in a substantial proportion of patients, one could hypothesize that the 2-year survival rate of 26% could have been higher if all patients had received TMZ until disease progression [20]. Even though this long-term treatment appears to be safe and increasingly employed both in USA and Europe, data regarding a superior efficacy above the standard regimen are lacking.
- Patients aged over 70 years represent approximately 20% of the entire GBM population [1], but they are often excluded from clinical trials.
- It appears difficult to extrapolate Stupp protocol results for GBM from other anaplastic (grade 3 WHO) gliomas. For this reason, the question whether concomitant and adjuvant TMZ and radiation should be the standard care for grade 3 gliomas arises. An argument in favor of this hypothesis is that a meta-analysis study did not show any difference between GBM and anaplastic grade 3 gliomas with respect to adjuvant chemotherapy [6], and high response rates were observed in recurrent grade 3 gliomas after TMZ treatment [21]. On the other hand, there is a potential risk of neurotoxicity in long-term survivors. Moreover, there is a lack of benefit in terms of overall survival after adjuvant chemotherapy with PCV regimen in newly diagnosed anaplastic oligodendroglial tumors, and we must consider that TMZ, even though different from PCV, is equally an alkylating agent [22,23]. On this topic, there is general agreement on the need for randomized trials of confirmation.

## 3. Methylguanine methyltransferase and modulation strategies

The cytotoxic activity of TMZ is mainly due to DNA methylation at the O6-position of guanine [7]. Even though the methylation of the O6 of the guanine corresponds to a small number of TMZ alkylations (about 7%), with the N7-site of guanine and N3-site of adenine representing the major sites [7], the resulting O6-methylguanine is mutagenic and able to trigger cytotoxicity and apoptosis [24]. These methyl adducts are removed by the ubiquitous DNA repair enzyme methylguanine methyltransferase (MGMT) [25,26]. MGMT acts as a suicide acceptor protein for the methyl group [27], and cytotoxic stress induced by chemotherapy or radiotherapy may induce upregulation of MGMT in cell lines, thus leading to an increased resistance to treatments [28]. Moreover, in GBM patients the MGMT gene promoter is frequently methylated, leading to silencing of the gene and lack of MGMT protein [29]. A correlation between tumor MGMT levels, evaluated by immunohistochemistry, and outcome of malignant glioma patients has been reported with both BCNU [30] and TMZ [31]. More accurately, using small quantities of DNA extracted from fresh-frozen or paraffin-embedded tumor tissue, the methylation status of the CpG islands of the gene promoter can be analyzed. Promoter methylation represents an epigenetic regulatory system that leads to silencing of the gene. Thus, evaluation of gene function on DNA level by methylation-specific polymerase (MSP) chain reaction allows one to predict the ability of the tumor to repair DNA damage induced by alkylating agents, and, even more important, correlates well with prognosis [32]. On these assumptions, Hegi *et al.* assessed, first in a Phase II study [33] and successively in a companion study of the previously described EORTC-NCIC [16], the methylation status of the MGMT gene promoter for 206 patients. These authors found a methylated gene in 45% of cases [34], and this study confirmed MGMT promoter status as an independent prognostic factor: the median OS for the entire population irrespective of treatments was 18.2 months for patients with methylated MGMT gene promoter compared with 12.2 months for patients with unmethylated MGMT gene promoter ($p < 0.001$). When treatment modalities were considered, for patients with methylated MGMT gene promoter median survival was 21.7 months for those receiving TMZ plus radiotherapy versus 15.3 months for those receiving radiotherapy alone ($p < 0.007$), with 2-year survival rates of 46% and 22.7% respectively. On the contrary, for patients with unmethylated MGMT gene promoter, the difference in survival of the group treated with TMZ plus radiotherapy was less significant, with a median survival of 12.7 months versus 11.8 months for the radiotherapy- only group, and 2-year survival rates of 13.8% and < 2% respectively. The predictive value of MGMT gene promoter status was confirmed by the analysis of PFS according to the received treatment. Among patients with methylated MGMT gene promoter, those who received combined treatment had a PFS of 10.3 months compared with 5.9 months for those patients who received radiotherapy alone; for patients with unmethylated MGMT gene promoter, those who received the combined treatment had a PFS of 5.3 months versus 4.4 months for those who received radiotherapy alone.

This correlative study had a profound impact on the research and clinic community, leaving the following important practical questions open:

- Additional information confirming the correlation of MGMT methylation status and outcome after TMZ are needed, and it is not yet clear whether MGMT promoter methylation status represents an intrinsic prognostic factor instead of a marker of clinical response to alkylating agents.
- It is not clear if MGMT gene promoter methylation status must be considered as an essential stratification test for new clinical trials.

- The methylation status of the MGMT gene promoter may change from newly diagnosed to recurrent tumors [35], thus rendering the analysis of biomarkers in the original tumor as unreliable to predict response to treatment or outcome at relapse [36].
- Moreover, changes in the methylation status of MGMT gene promoter may be induced by corticosteroids, ionizing radiations and genotoxic agents [28], thus leading to a more complex genetic pathway alteration that is not always easy to interpret.
- A more complex question arises for patients with unmethylated gene. These patients may be considered for trials that aim at modulating the chemoresistance or testing new chemotherapeutic agents.

An important issue regarding the need for strategies overcoming MGMT protein expression originate from the observation that continuous exposure to an alkylating agent will exhaust the endogenous reservoir of MGMT [22,37].

The depletion of MGMT in peripheral mononuclear blood cells after continuous daily administration of TMZ has been shown and quantified [37,38]: it seemed to be a time- and dose-dependent phenomenon. Under this light a variety of schedule extensions of TMZ administration were developed, the so-called dose-dense regimens, allowing the TMZ dose intensity to be increased just over twice the conventional regimens [37,39,40], and have been employed especially in the recurrence setting of GBM. An increase in PFS6 of up to 48% has been reported with the 1 week on–1 week off schedule at 150 mg/m$^2$/day [41]. The 21 days' therapy over 28 days with TMZ at 75 – 100 mg/m$^2$/day was among the first 'dose-dense' schedules to be developed [37], and a Phase II study showed a PFS6 of 30.3% [42]. Moreover, a protracted dose-intense TMZ administered on a continuous schedule ('28 over 28 days') at a dosage of 50 mg/m$^2$/day was recently employed [43]. Results on PFS at 6 months depended largely on the time to tumor progression and showed a 35% rate for those patients who recurred after stopping adjuvant treatment. This study also showed a PFS6 of 53% for anaplastic glioma patients. In general, the major problem when employing protracted low-dose schedules is lymphopenia, particularly with CD4+ T-cell depletion. Clinical experience showed that these regimens were relatively safe and easy to manage in the major part of cases, but the question of prophylaxis for opportunistic infections during treatment did arise [44,45]. It is still to be proven whether resuming TMZ at disease progression, either as a standard or a dose-dense regimen, is safe and effective [46]. This strategy may be indicated for previous TMZ responders (patient candidates for a 'rechallenge' treatment with TMZ) [47], and more clinical data are expected.

## 4. Emerging antiangiogenic treatments

Endotelial proliferation is among the diagnostic hallmarks of GBM, and angiogenesis plays a critical role in the progression and clinical behavior of these tumors. In fact, the amount of surrounding brain tumor edema and the need for steroids is a direct consequence of the pathological vasculature. As tumor growth is critically dependent on the formation of new blood vessels, inhibition of this process has offered an attractive strategy to complement standard therapies [48,49]. Many extracellular, cell surface and intracellular molecules that modulate angiogenesis have been identified and characterized, involving numerous growth factor, tyrosine-kinase receptors and signalling cascades. Of these, VEGF (with its variants A, B, C, D) and its receptors (with the 1, 2 and 3-subtypes) have emerged as the most important [48,50,51]. Moreover, cancer stem cells in gliomas, which may play critical roles in tumor initiation and therapy resistance, have been proven to promote tumor angiogenesis through VEGF pathway stimulation [52]. Owing to its dominant role in tumor angiogenesis, targeting VEGF signaling has evolved into a promising therapeutic strategy.

Among the first antiangiogenetic drugs was bevacizumab, a humanized monoclonal antibody against VEGF-A. This drug was first approved in colorectal and lung cancer treatment and initially employed against malignant gliomas using a regimen borrowed from colorectal cancer, which combined bevacizumab and the cytotoxic agent irinotecan (CPT-11), a topoisomerase-I inhibitor [53]. In this never definitively published study, 29 patients with recurrent malignant glioma were treated with the combination of bevacizumab (5 mg/kg every 2 weeks) and CPT-11 (125 mg/m$^2$ weekly for 4 weeks, followed by 1 – 2-week breaks), and the results showed a dramatic overall radiographic response rate of 66%. In this first study, the reported side effects were bowel perforation in one patient and intracranial hemorrhage in another, though resulting as well tolerated. A subsequent study of 14 patients with high-grade recurrent glioma treated with bevacizumab combined with various cytotoxic drugs showed a radiographic response rate of 50% [54]; and a third retrospective study of 44 patients with recurrent malignant glioma treated with a similar protocol documented a response rate of 34%, with mild collateral effects like asymptomatic intracranial hemorrhages [55]. More interestingly, in the last study the requirement for concurrent steroid treatment seemed significantly reduced in approximately 50% of patients [55]. The first Phase II studies of bevacizumab plus irinotecan in recurrent high-grade glioma management were those of Vredenburgh *et al.* [56,57], in which 35 patients with GBM and 33 with grade 3 glioma were treated. The 6-week cycles used dosages of 10 and 15 mg/kg of bevacizumab and 125 mg/m$^2$ of irinotecan, raised to 340 mg/m$^2$ for those patients who were concomitantly assuming cytochrome P450-enzyme inducing antiepileptic drugs. According to revised Macdonald criteria [58], the overall response rate was of 59% (65% in grade 3 gliomas and 53% in GBM patients). Moreover, a recent survival update [59] showed a PFS6 of 43% for GBM patients and 59% for grade 3 glioma patients, with a 2-year OS of 15% for GBM and

33% for anaplastic glioma patients. In this series, adverse effects included thromboembolic events (12%) and intracranial hemorrhage in 2% of cases with an acceptable overall toxicity. Successively, a larger Phase II trial compared a total of 167 recurrent GBM patients at first or second relapse treated with bevacizumab, given every 2 weeks at a dosage of 10 mg/kg, with or without irinotecan [60]. Preliminary results reported a PFS6 of 43% with a radiological response rate of 28% in patients treated with bevacizumab alone, compared with a PFS6 of 50% and radiographic response rate of 38% in patients treated with the combined regimen. Median OS was 9.2 months for the group receiving bevacizumab only and 8.7 months for the combination group. The possibility of reducing steroids occurred in the majority of patients and toxicity was relatively modest [49,61]. Moreover, a Phase II trial was recently published addressing the efficacy of bevacizumab alone in the recurrent GBM setting with the adjunct of irinotecan at further tumor progression [61]. Forty-eight heavily pretreated patients were enrolled into this study. Results showed a PFS6 of 29%, with a median OS of 31 weeks for the single-agent bevacizumab-treated patients, and early radiographic response was predictive of long-term PFS. The adjunct of irinotecan at further tumor progression seemed ineffective in this setting. Overall, patients tolerated treatment well. Particularly, a diminished fluorodeoxy-glucose (FDG) uptake by PET scan was demonstrated in 49% of patients after 4 weeks of therapy.

These studies on bevacizumab plus irinotecan in recurrent glioblastoma patients management warrant some observations.

- A first question arises on whether the observed important clinical and radiological responses were secondary to decreased vascular permeability, as measured by MRI contrast enhancement changes or to a real antitumor effect. In fact, an accompanying lack of effect on tumor cell biology is likely to explain the rapid subsequent progression observed in nearly half of initial responders. Nevertheless, a bevacizumab-mediated antitumor effect probably occurs in a subpopulation of patients, because nearly half of the initial radiographic responders were progression-free for more than 6 months [61].
- A second question regards the role of irinotecan, and whether it represents the right cytotoxic agent to be combined to bevacizumab [61], since it had a limited or no role in gliomas as a single agent [62], with a clear addition of toxicity.
- Another concern has come from studies showing that blockade of VEGF-mediated angiogenesis may ultimately promote tumor infiltration represented by the continuous 'gliomatosis-like' spread [63-67]. This fact may be due to co-option of existing cerebral blood vessels in a VEGF-independent fashion [64,65]. The possibility that bevacizumab can accelerate this process cannot be excluded entirely and may explain why once disease progression is detected by standard radiographic criteria, most patients die shortly afterwards. In fact, current data seem to indicate that such short survival after drug failure seems to represent an end-stage disease in heavily treated patients [68].
- Another question regards the fact that it seems challenging to discontinue bevacizumab after tumor progression, because of rapid clinical deterioration and increasing edema: new neuro-imaging tools detecting treatment failure earlier than conventional MRI are expected.
- Finally, we must consider that results on PFS6 and OS of these studies are still superior to historical controls, so that compelling data from available studies in the recurrence setting of high-grade glioma patients justify all the enthusiasm related to this new management strategy.

Preliminary data indicate the role of VEGF expression as a marker: it may predict overall prognosis [69] and radiographic response to antiangiogenetic agents [70], but this marker seems to be unable to predict overall survival in recurrent patients treated with anti-VEGF bevacizumab [70].

There is increasing evidence that inhibition of angiogenesis may potentially enhance the effects of radiation therapy [71]: this fact leads to the possibility of a combination with TMZ during the concomitant phase of radiation therapy [72], and results are expected.

Another strategy targeting the VEGF pathway regards VEGF-trapping with a soluble decoy VEGF receptor that is fused to the constant region of IG1. Such a molecule, acting as a 'VEGF-trap', is aflibercept, which showed several hundred times greater VEGF-binding affinity than bevacizumab. Even though promising, a Phase II trial with aflibercept [73] was stopped because of 25% toxicity leading to interruption of treatment, and other studies with a safer dosage are expected.

Antiangiogenetics other than ligand sequestors represent a large number of competitive inhibitors for VEGF receptors and other angiogenic Tyr-kinase receptors (such PDGF receptors and stem-cell factor c-kit) inhibitors: among these, encouraging results have come from a recent Phase II trial of cediranib (AZD2171), a potent pan-VEGF inhibitor [74]. The oral daily administration of 45 mg of cediranib to 31 recurrent GBM patients gave the following results: the PFS6 was 26%, and the radiographic response rate was about 56%. In addition, a significant steroid-sparing effect was observed. Adverse effects required temporary drug suspension in 69% of the initial 16 patients and comprehended gastrointestinal toxicity, fatigue and hypertension. Advanced MRI studies and a subsequent histopathological confirmation study [75] from a subset of these patients showed decreased contrast enhancement followed by reduction in blood vessel size, permeability, blood flow and blood volume; but these effects were transient, and blood vessel size began to rebound after 8 weeks of treatment and after cessation of drug administration [76], mimicking bevacizumab treatment findings. The concept of vascular normalization of abnormal tumor blood vessel is of fundamental importance for antiangiogenetic drugs. It has been shown that the vascular normalization effect of antiangiogenetic drugs may facilitate delivery of concurrently administered cytotoxic drugs and potentially improve the efficacy of radiation therapy [77]. The observation that

vascular normalization is a transient phenomenon suggests that a specific therapeutic window exists, during which chemotherapy and radiation may be most effective, so that, for the single patient, adjuvant chemotherapeutic options must be individualized and also well-timed.

## 5. Noncytotoxic molecular targeted therapies

The oncogenetic process of GBM is driven by several biological events, including activation and amplification of several growth factor receptor signaling pathways [78]. In this way, several growth factor receptors, such as EGFR, platelet-derived growth factor receptor (PDGFR), C-kit and the abovementioned VEGFR may result overexpressed, amplified and mutated. This metabolic activation, which commonly results in an increased cellular tyrosine-kinase activity, is able to trigger downstream oncogenic signaling pathways.

To interfere with these pathological molecular signalling cascades, and given the common chemoresistence of malignant gliomas to conventional cytotoxic agents, much effort has been spent on the development of noncytotoxic targeted molecular therapies, which are able to act directly against the amplified pathways involved in the oncogenetic process.

Particular attention has been given to the EGFR pathway, owing to the strong prognostic correlation with prognosis in GBM patients. EGFR, a tyrosine kinase receptor, is frequently mutated in GBM leading to protein overexpression (60%) and gene amplification (40%) [79]. Truncated transcripts encoding for EGFRvIII produce a constitutively active and ligand-independent receptor which is present in 20% of GBM [80,81]. Another interesting finding is that the presence of EGFRvIII has been found to be an independent major prognostic factor of worst prognosis among GBM patients [82]. EGFR activation determines induction of several signaling tyrosine kinase mediated pathways that are involved in cellular biological processes promoting oncogenesis. Agents such as gefitinib and erlotinib, small molecules with tyrosine kinase inhibition properties, were used as single therapeutic agents and in combination with TMZ mainly in recurrent glioblastomas [83,84]. Erlotinib achieved more relevant results in terms of PFS and OS as it determines inhibition of EGFRvIII. Recent studies [85,86] have demonstrated that the response to erlotinib may be correlated to a subgroup of patients with coexpression of EGFRvIII and PTEN. Considering these different molecular subsets, it has been suggested that patients who may benefit from this targeted therapy be selected. Toxicity profiles demonstrated that these agents are well tolerated at low dose; a higher drug exposure determined diarrhea and rash [87]. Patients with these complications survived significantly longer than those without complications [88], showing that there was a major benefit in patients with maximal doses. However, not many clinical trials have been performed and the results obtained by these studies showed a modest activity of these agents. The 6-month PFS rate in recurrent gliomas varied between 9% and 14.3% [88,89] in Phase II trials using gefitinib alone, whereas it was up to 23.5% in Phase I studies in which gefitinib was combined with sirolimus. Sirolimus represents an inhibitor of the mammalian target of rapamycin (mTOR), which represents a distal target of the EGFR cascade: the combination of agents that act at different levels of a same-cascade pathway represents a promising strategy, and preliminary results are encouraging, with PFS6 between 23% and 26% in two recent studies [90,91]. Recently, the combination of erlotinib with TMZ has been investigated [87,90] with uncertain preliminary results, but they require validation in larger prospective studies. Particularly, a combination of erlotinib and TMZ was used in 65 newly diagnosed GBM patients during and after radiation therapy, with a median OS of 19.3 months, a median PFS of 8.2 months and a strong correlation with MGMT promoter methylation status [92]. Another study, by Brown *et al.* [87] with 97 patients treated with erlotinib before, during and after the Stupp protocol showed contrasting results: even though primary end point median survival were improved, recursive partitional analysis (RPA) classes showed no significant benefit compared with the concomitant arm of the EORTC study. The definition of the peculiar genetic and molecular profile for each patient seems mandatory for this kind of treatment. Other strategies targeting the EGFR signaling pathway comprise the murine humanized monoclonal anti-EGFR antibody cetuximab and more recent irreversible EGFR inhibitors. The results are expected in the near future.

## 6. Locoregional treatment with biodegradable polymers

Locoregional treatment with biodegradable polymers consists in the implantation into the resection cavity of wafers loaded with the abovementioned alkylant agent carmustine (BCNU) at the concentration of 3.85% after surgical tumor removal. These wafers (Gliadel® wafers) are designed to release BCNU slowly over a 2 – 3-week period. A prospective Phase III randomized control trial conducted on 240 patients with newly diagnosed high grade gliomas showed a good safety profile and efficacy, with a median survival for the GBM subset of 13.5 months compared with 11.4 months for the placebo-controlled patients ($p = 0.10$) and a 24% reduction of risk of death [93]. This survival advantage persists through longer follow-up in the entire patient population, with a 2-year survival of 15.8% compared with 8.3% for the placebo group, and a 3-year survival of 9.2% compared with 1.7% for the placebo group [94].

General limitations to systemic chemotherapies comprehend systemic toxicities, short half-life and limitations in traversing the blood–brain barrier, and in this context both temozolomide and the new approach with Gliadel wafer implantation present many advantages: as seen above, TMZ has a good bioavailability and cerebral tumor tropism, with good clinical effectiveness and low toxicity that allows

**Table 1. Major alternative chemotherapy strategies under study.**

| Strategy | Rationale |
|---|---|
| Addition of chemotherapy agents to TMZ during concomitant phase<br>Bevacizumab<br>CCNU | To increase radiosensitivity |
| Dose-dense TMZ<br>One week on – one week off<br>21 over 28<br>Daily administration<br>Others | Depletion of MGMT and other cellular chemoresistance systems |
| Addition of antiangiogenetics to citotoxic regimens<br>Fotemustine + bevacizumab<br>Dose-dense TMZ + bevacizumab<br>TMZ + cediranib (AZD2171)<br>TMZ + celecoxib<br>Others | Combining cytotoxic activity with strategies that lower tumor feeding |
| Molecular targeted therapies<br>EGFR inhibitors or Ab<br>VEGFR inhibitors or Ab<br>Integrine inhibitors<br>mTOR inhibitors<br>Antihormons (tamoxifen etc.)<br>Others | Individualize chemotherapy to peculiar tumor pattern of amplificated pathways |
| Rechallenge with precedently used regimens | Often good response |
| Delivery strategies<br>CED<br>Biodegradable polymers<br>Bacterial toxins, viral vectors<br>Immunological strategies<br>Dendritic cell vaccines<br>Others | Lower systemic side effects, allowing increased dosages |

CCNU: lomustine; CED: Convection enhanced delivery; MGMT: Methylguanine methyltransferase; mTOR: Mammalian target of rapamycin; TMZ: Temozolomide.

temozolomide to be used in combination with radiotherapy [16]. On the other hand, Gliadel wafer is a locoregional treatment designed to act in the period in which the patient is not being treated while waiting for the beginning of radiotherapy.

The absence of systemic side effects, namely the lack of significant hematologic toxicity from Gliadel wafers [93] make it an ideal agent to be used in combination with systemic agents in an attempt to achieve synergistic treatment for patients with malignant glioma [95]. This kind of integrated therapy represents an interesting and promising improvement to the current standard of therapy for high-grade gliomas, and more cases and follow-up will clarify the safety and true effectiveness of this treatment strategy in a near future.

## 7. Other strategies

Many different cytostatic chemotherapeutic approaches have been investigated using single or combined protocols with cytotoxic agents or radiotherapy (main strategies currently under investigation are summarized in **Table 1**). Highly malignant tumor cells are able to escape cell death by expressing various prosurvival factors, such as anti-apoptotic proteins, repair enzymes or protein kinases that are involved in intracellular signal transduction, like protein kinase C (PKC) or mitogen activated protein kinases.

Tamoxifen, primarily used as an estrogen antagonist in the treatment of breast cancer, produces estrogen-independent functions such as a potent inhibition of PKC activity. By reducing or inhibiting PKC's ability to stimulate the synthesis of pro-apoptotic protein bax and to decrease the anti-apoptotic protein Bcl-2, the loss of survival advantage of tumor cells can be achieved [96]. High-dose tamoxifen administration (80 mg/m$^2$ daily) alone used in recurrent malignant gliomas throughout various studies showed a response rate of 20 – 40% [97]. More interestingly, PKC inhibitors have been found to enhance the cytotoxic effect of other chemotherapeutic agents. Tamoxifen was combined with irinotecan [98] and TMZ [99,100], but unfortunately no real improvement was reported in terms of OS. In conclusion, the use of tamoxifen in the treatment of high-grade malignant gliomas still remains confined to highly selected patients who were nonresponsive to precedent second-line chemotherapy regimens [96,101,102].

All malignant gliomas are treated with radiotherapy, but recent studies have outlined that sublethal doses of irradiation at the outlines of the target might promote the migration and invasiveness of glioma cells by upregulation of angiogenesis involving enhanced $\alpha\upsilon\beta3$ integrin expression [103]. To interfere with these mechanisms, the use of integrin antagonists such as cilengitide were investigated in combination with TMZ [103,104]. As the latter agent is supposed to have certain anti-angiogenetic properties, it could be synergistic in inhibiting radio-induced tumor spread. Clinical trials have yet to be done.

An alternative anti-angiogenetic strategy is represented by cyclooxygenase-2 (COX-2) inhibitors. In malignant gliomas this enzyme is often upregulated. Clinical trials used celecoxib or rofecoxib in combination with TMZ [105] or irinotecan [106]. The results showed a tolerable toxicity of these agents, grade 3 toxicity occurring in 8% of cases. The OS rates showed encouraging activity of these agents, but their use is still limited.

## 8. The heterogeneous grade 3 glioma group

Grade 3 oligodendroglial tumors (i.e., anaplastic oligodendrogliomas and oligoastrocytomas) are treated and studied apart from pure anaplastic astrocytomas. The differences are based on histopathological characterization and recently on the more reliable molecular analysis confirmation. The standard care for newly diagnosed anaplastic astrocytoma is

radiotherapy alone (in Europe) or in combination with BCNU (USA) [107]. TMZ is used at recurrence, since a multicenter Phase II study that showed a response rate of 35% and a PFS6 of 46% [108].

In grade 3 glioma tumors with an oligodendroglial component, molecular genetic analysis of chromosome alleles 1p and 19q status (loss of heterozygosity (LOH) on 1p and 19q, or LOH 1p 19q) seems to have not only a diagnostic and prognostic significance but also a strong prediction of response to therapy [1,19,109]. Cairncross *et al*. were the first to demonstrate a strong correlation of patients with anaplastic oligodendroglioma response to therapy with PCV and the presence of LOH 1p 19q codeletion [109]. Given the best tolerability and the same efficacy demonstrated by other studies [110,111], TMZ has replaced PCV as standard of care for recurrent anaplastic oligodendrogliomas, reserving PCV chemotherapy as an option for nonresponder patients, but the response rate to second-line chemotherapy seems to be less important, about 17 – 26% of patients [111,112]. Particularly, the association of PCV chemotherapy to radiotherapy does not seem to improve OS in this kind of patient, showing only a modest benefit for PFS [113,114]. Studies on the presence of LOH 1p 19q mutation are now commonly performed worldwide, but treatment recommendations for anaplastic oligodendroglial tumors vary widely among physicians and are often independent of the molecular data [115].

## 9. Expert opinion

In the past ten years, great progress has been made in the field of chemotherapy of high-grade gliomas and particularly in the understanding of molecular pathways and tumor biology. Unfortunately, clinical results and efficacy of treatments are still disappointing and lie under our expectancies. The most important progress and improvements in clinical results were obtained mainly with the application of the Stupp protocol in the newly diagnosed GBM population and in the individuation and treatment of more chemosensitive grade 3 glioma subtypes. Given that there is still lack of agreement and validation of guidelines for the treatment of high-grade gliomas embracing the entire course of disease, the management of the single patient strongly depends on the experience and sensibility of the physician. From our experience, we can point out some topics that are still under debate among the neuro-oncologist's community.

- For patients aged over 70 years ('elderly patients') with malignant glioma, which represents a consistent percentage of the high-grade glioma population, the optimal treatment remains controversial: it ranges from palliative care to aggressive treatment overcoming surgery, radiation- and chemotherapy. We believe that elderly patients could benefit from aggressive treatment as do younger people, depending primarily on their neurological and functional status and on the general health condition instead of on age itself.
- Intensified TMZ regimens are often a feasible and advantageous opportunity for recurrent patients that have already been treated with the Stupp protocol ('rechallenge'), and in many cases this strategy obtains immediate results.
- Combination of Gliadel therapy and subsequent Stupp protocol seems to be a safe strategy, and our preliminary results on progression-free survival and disease control are encouraging.
- Molecular markers are not easy to manage in the single patient treatment, as prognostic significance is not yet sufficiently reliable for many of them. The importance of molecular markers rises more often at recurrence, and this happens for two principal reasons. First, There is still a lack of chemotherapeutic options clearly superior to the Stupp protocol as first-line therapy: this fact implies that molecular data will not change the first therapeutic approach in the majority of cases, but successively the best molecular characterization of the tumor can guide second-line chemotherapy choices. Second, there is always a delay in the availability of molecular analyses results, and this practical inconvenience is often due to the real time required for performing molecular analyses for all patients by the majority of laboratories.
- Given the possibility of individualizing treatment and orienting chemotherapy options on the basis of peculiar molecular alterations in each patient, the characterization of molecular markers becomes particularly important in the stratification of grade 3 glioma patients. In fact, thanks to these biomolecular studies, the complexity of this heterogeneous class of glial tumors had been raised, augmenting the difficulties in the establishment of the best adjuvant treatment.

## Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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