Chemotherapy in glioma

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Practice points

- Chemoradiotherapy with temozolomide is currently the standard of care for newly diagnosed glioblastoma.
- Temozolomide chemotherapy is more effective than radiotherapy alone in elderly glioblastoma patients with a methylated MGMT promoter.
- Treatment with lomustine or retreatment with temozolomide can be considered in recurrent glioblastoma.
- Bevacizumab combined with lomustine may play a role in recurrent glioblastoma.
- Other 'targeted' therapies have shown poor results in diffuse glioma until now.
- Adjuvant PCV (procarbazine, CCNU [lomustine] and vincristine) chemotherapy is currently the standard of care in newly diagnosed anaplastic oligodendroglioma with combined loss of 1p/19q.
- Adjuvant PCV clearly improves survival in newly diagnosed high-risk low-grade glioma.
- Chemotherapy is the standard for recurrent low-grade WHO grade II and high-grade gliomas grade III.
- Temozolomide is commonly used instead of nitrosoureas containing regimen (e.g., PCV) due to better tolerance.
- It is unlikely that outcome in glioma can be further enhanced with the currently available cytotoxic drugs and new (targeted) drugs are needed.

SUMMARY The treatment of glial brain tumors begins with surgery, and standard adjuvant treatment at the end of the past millennium for high-grade glioma and high-risk low-grade glioma was radiotherapy and chemotherapy was given at recurrence. However, over the past 10 years much has changed regarding the role of chemotherapy in gliomas and it is now clear that chemotherapy has a role in the treatment of almost all newly diagnosed diffuse gliomas (WHO grade II–IV). This is the result of several prospective studies that showed survival benefit after combined chemoradiotherapy with temozolomide in glioblastoma (WHO grade IV) or after procarbazine, CCNU (lomustine) and vincristine chemotherapy in diffuse low-grade (WHO grade II) and anaplastic (WHO grade III) glioma. The current standard of treatment for diffuse gliomas is described in this overview and in addition some attention is given to targeted therapies.

The primary brain tumors include astrocytomas, oligodendrogliomas and mixed oligoastrocytomas (**Figure 1**). These tumors arise from the supporting tissues of the brain, the glia, and are called

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KEYWORDS

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- glioblastoma • glioma
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- oligodendroglioma

glioma. Besides this classification into different types, these tumors are graded according to the presence of anaplastic features in low-grade (or WHO grade II) and high-grade tumors (either WHO grade III or anaplastic and WHO grade IV or glioblastoma). Grade I gliomas are well-demarcated gliomas, and if completely resectable can be cured. The grade II–IV gliomas are among the so-called diffuse gliomas for which a curative treatment is not available. Over the past 10 years, much has changed regarding the role of chemotherapy in glial brain tumors. The purpose of this overview is to describe the current standard of treatment for gliomas, and in addition some attention is given to targeted therapies, for example, angiogenesis inhibitors. Currently, there is a lot of attention for this category, but the role for this class of agents in diffuse gliomas has by no means been defined.

Pathophysiology

Most brain tumors occur sporadically and no obvious environmental causes of brain tumors are known. Occasionally, gliomas arise as a part of rare genetic syndromes (e.g., neurofibromatosis type 1, Li-Fraumeni syndrome and Turcot syndrome type I) [1]. The various types of diffuse glioma are characterized by different molecular abnormalities, such as loss of chromosome 10, *EGF* receptor (*EGFR*) amplification, *PTEN* mutations and *TERT* mutations in glioblastoma, combined 1p/19q loss and *ATRX* mutations in oligodendroglial tumors, *TP53* mutations and *TERT* mutations in grade II and

III astrocytic tumors and mutations in isocitrate dehydrogenase (*IDH*) genes in grade II and III diffuse glioma and secondary glioblastoma (see Figure 2) [2–4]. Epigenetic factors such as methylation of the *MGMT* gene and other genes are involved in the pathogenesis, but their role is far from understood.

Diagnosis & prognosis of primary brain tumors

The MRI scan is sensitive for the diagnosis of a brain tumor, but it is nonspecific for the type of tumor. The diagnosis of glial tumors is therefore based on the histology of the tumor after biopsy or resection. The histological distinction between different types of glial tumors, such as astrocytomas, oligodendroglial tumors and mixed oligoastrocytoma (with characteristics of both oligodendrogliomas and astrocytomas) is difficult and is subject to ‘interobserver variation’ [5]. Specific markers for these tumors do not exist. The hallmark of glioblastoma is the presence of endothelial proliferation and/or necrosis in an astrocytic tumor. The prognosis of brain tumors depends on tumor grade and type: high-grade tumors and astrocytic tumors have a worse prognosis than low-grade tumors and oligodendrogliomas. Also, genetic defects have an important influence on the prognosis. For example, the presence of combined 1p/19q loss and/or *IDH1* mutations are correlated with a favorable prognosis. Other non-treatment-related prognostic factors are the age of the patient and the clinical condition: the prognosis is worse in older

Type \ Grade	WHO grade I	WHO grade II	WHO grade III	WHO grade IV
	Circumscribed		Diffuse	
		Low grade	High grade	
Astrocytoma	Pilocytic astrocytoma	Low-grade astrocytoma	Anaplastic astrocytoma	Glioblastoma
Oligodendroglioma		Low-grade oligodendroglioma	Anaplastic oligodendroglioma	
Oligoastrocytoma		Low-grade oligoastrocytoma	Anaplastic oligoastrocytoma	

Figure 1. Classification of glioma in types and WHO grades.

Figure 2. Possible genetic pathways in glioma. Glioblastomas (WHO grade IV) can be differentiated into *de novo* glioblastomas (primary) or secondary glioblastomas, which originate from low-grade astrocytomas (WHO grade II) directly or via malignant transformation from anaplastic astrocytomas (WHO grade III). It has been demonstrated that the two glioblastoma pathways show different genetic alterations. Mutations of *IDH1/2* are almost exclusively present in WHO grade II and III gliomas and are early events occurring before codeletion of chromosomes 1p and 19q in oligodendrogliomas or *TP53* mutation in astrocytomas. CIMP: CpG island hypermethylated phenotype.

patients in a poor condition (usually expressed as performance status). The median survival of the most aggressive glioma, the glioblastoma, in patients treated with combined chemoradiotherapy, is approximately 15 months [6]. For grade III tumors without 1p/19q loss, the median survival is 2–3 years, and for anaplastic oligodendrogliomas with combined 1p/19q loss, the median survival is more than 12–14 years. The prognosis of patients with low-grade glioma varies in most series between 5 and 15 years.

Outcome assessment in glioma studies

Response rate, progression-free survival (PFS) and survival are used for the assessment of outcome in studies on chemotherapy in gliomas. The objective response or response rate (ORR) is the percentage of patients with a complete or partial response, usually measured using MRI of the brain in conjunction with clinical assessment and corticosteroid dose. In the USA, conditional

approval may be granted based on response rate only, since this is expected to reflect the activity of the investigational agent. In such studies as a rule a confirmatory scan made 4 weeks after the first scan documenting the response is required.

PFS is the time between the start of treatment (or randomization) until progression or death. PFS is particularly used in Phase II studies on recurrent glioblastoma as the primary end point in which it is usually expressed as the percentage of patients that are alive and do not have progression after 6 or 12 months (6mo-PFS or 12 moPFS, respectively). However, PFS is not accepted by US regulators as an end point in uncontrolled trials, as PFS may be subject to prognostic factors and scanning intervals and does not only reflect treatment activity. The use of PFS as the primary end point in randomized Phase III trials on newly diagnosed glioblastomas has become controversial since the Phase III trials on bevacizumab and an increase in PFS

should at least be supported by a clear survival signal [7,8].

Both the ORR and PFS are largely dependent on the MRI findings, but changes in the enhancing lesion on an MRI or CT scan do not necessarily reflect changes in tumor activity. The phenomenon of pseudoprogression after chemoradiotherapy in glioblastoma patients is now well established [9,10]. Similarly, pseudoresponses occur after treatment with VEGF receptor signaling pathway inhibitors [11]. Both these phenomena confuse the assessment of ORR and PFS in clinical trials [12].

Because of the above, alternative end points and response criteria have been developed by an international working group (Response Assessment in Neuro-Oncology or RANO group) [13,14].

Survival or overall survival (OS) is defined by the time between the start of treatment until death, in trials usually measured from the time of randomization. OS is not influenced by imaging. In oncology, OS is usually seen as the golden end point, although salvage treatments given at progression may have an influence on this end point.

Treatment

The treatment begins with surgery, to resect as much of the tumor as safely possible. The surgery of brain tumors performs three functions: obtaining a histological diagnosis, improving the condition of the patient by a rapid reduction in tumor volume and finally improving the prognosis. If no meaningful resection is possible, a biopsy must be performed to obtain tissue for diagnosis. Although no randomized studies exist showing a survival benefit from extensive resection, uncontrolled studies have suggested that more extensive resection contributes to survival [15]. Safe extensive resections are more often

possible with advanced surgical and imaging techniques.

Radiotherapy was the first treatment proven to be effective for patients with high-grade gliomas in randomized trials. Postoperative radiotherapy of 60 Gy in 30–33 fractions of 1.8–2.0 Gy improved the median OS from 3–5 months to 8–10 months for glioblastoma (see Table 1) [16,17]. A higher radiotherapy dose, either with conventional techniques or high-dose techniques (i.e., interstitial brachytherapy or stereotactic radiotherapy), has not been proved to be more successful. Many centers give a short radiotherapy schedule (e.g., 14 fractions of 3 Gy; category 1C) to older patients with a poor prognosis (aged over 65–70 years, moderate clinical condition, only a biopsy) [18]. In very poor prognosis patients (e.g., older patients with a poor performance status), when it is assumed that the burden of treatment does not outweigh the very limited survival benefit, a palliative supportive strategy is often followed.

Early studies investigating the role of adjuvant chemotherapy (mostly nitrosoureas) in newly diagnosed high-grade gliomas (WHO grade III and IV) showed at best a small and statistically insignificant increase in survival from adjuvant chemotherapy (see Table 1) [16,17,19,21–24].

Consequently, in most areas in Europe adjuvant chemotherapy was not considered to be part of the standard treatment of newly diagnosed high-grade glioma. In Europe, until quite recently, chemotherapy was mainly used for recurrent high-grade (WHO grade III and IV) tumors after radiotherapy in which case temozolomide or a combination of procarbazine, CCNU (lomustine) and vincristine (PCV) chemotherapy was used. Recent results of several trials are discussed below, showing an early role for classical cytotoxic chemotherapy in glioma.

Table 1. Early studies in WHO grade III and IV gliomas on various postoperative adjuvant treatments.				
Therapy	Median survival (months)	1-year survival (%)	2-year survival (%)	Ref.
Supportive care	3	3	0	[16]
BCNU	4	12	0	
RT	8	24	1	
RT and BCNU	7.5	32	5	[19]
RT	–	40	15	
RT and chemotherapy	–	46	20	
RT	11.6	49.6	–	[20]
RT + BCNU wafer	13.9	59.2	–	
BCNU: Carmustine; RT: Radiotherapy.				

In recent years, inhibitors of blood vessel formation (angiogenesis inhibitors) have been extensively evaluated in glioblastoma, in particular, inhibitors of the VEGF signaling pathway. VEGF is expressed by hypoxic tumor cells and released into the bloodstream, leading to angiogenesis in the tumor. Especially, bevacizumab (a humanized monoclonal antibody against circulating VEGF) and cediranib (receptor tyrosine kinase inhibitor of VEGF receptor) have been extensively investigated.

Temozolomide

Temozolomide is an alkylating cytostatic, which has the same active metabolite as dacarbazine (DTIC). It is a relatively small molecule (194 Da) and it penetrates the blood–brain barrier easily due to its lipophilic character. Unlike dacarbazine, temozolomide does not require hepatic metabolism: a spontaneous conversion to the active ingredient MTIC finds place at a physiological pH. After oral administration, the bioavailability is almost 100%, minimally (10%) affected by taking food [25]. Elimination occurs in the liver and excretion is renal. Temozolomide is registered for use in gliomas. The standard schedule of temozolomide when used as monotherapy is 150–200 mg/m² on days 1–5 every 4 weeks. There are many alternative ‘dose dense’ regimens in use, resulting in a twofold greater dose intensity.

Temozolomide is generally well tolerated. Side effects are mainly nausea and vomiting (well avoidable with 5HT₃ antagonists), bone marrow depression (in particular leukopenia and thrombocytopenia; nadir after 21–28 days), lymphopenia (with a decrease in the CD4⁺ population) and hepatotoxicity. Sporadically allergic skin reactions are seen with the use of temozolomide. Pregnancy and lactation are contraindications. In the continuous dosing schedules a relative lymphopenia occurs, with a risk of opportunistic infections, especially *Pneumocystis carinii* (PCP) infections. PCP prophylaxis, for example cotrimoxazole, is indicated in these dose dense schedules.

Lomustine

Due to their good penetration across the blood–brain barrier, the nitrosoureas (especially CCNU [lomustine], BCNU [carmustine], ACNU [nimustine] and fotemustine) have been investigated early on for their efficacy in brain tumors [17]. Lomustine is also used as part of

the PCV combination regimen. PCV was widely used in the 1990s, especially in oligodendroglial tumors. The PCV schedule is currently used less frequently due to the more favorable side effect profile and easier dosing schedule of temozolomide.

Recently, a number of studies in recurrent glioblastoma used single-agent lomustine as a control arm and proved that lomustine is at least as effective as temozolomide in that setting. This has led to widespread use in recurrent glioblastoma patients. For single-agent use, the recommended dose is 110–130 mg/m² with a maximum of 200 mg, after combined chemoradiotherapy with temozolomide the maximum recommended dose of lomustine is 110 mg/m². One of the main side effects of lomustine is cumulative bone marrow suppression, with a relatively late nadir occurring at 4–6 weeks. Therefore, nitrosourea are given in 6–8-week cycles. Other side effects are nausea, vomiting and hepatotoxicity. The use of prophylactic 5HT₃ antagonist is recommended with lomustine. The 6-weekly PCV combination regimen consists of lomustine 110 mg/m² on day 1, procarbazine (60 mg/m² on days 8–21) and intravenous vincristine (1.4 mg/m², maximum 2 mg on days 8 and 29). The added value of vincristine in this schedule is questionable, and other combination schedules have been used. Other side effects of the PCV chemotherapy schedule are the vincristine-associated peripheral neurotoxicity, loss of appetite, weight loss and malaise symptoms, including fatigue. These side effects seem largely due to procarbazine. Furthermore, the myelosuppression of the PCV schedule is more prominent compared with that of lomustine alone. Pregnancy and lactation are contraindications.

Carmustine wafers

BCNU-containing wafers are registered in Europe for use in newly diagnosed high-grade gliomas and in recurrent glioblastoma [20,26]. However, the possible survival benefit is limited and not statistically significant in newly diagnosed glioblastoma.

Mechanism of action

The nitrosoureas cause in particular chloroethyl-adducts at the O⁶ position of guanine. This results in N¹-guanine, N³-cytosine DNA interstrand cross-links which are cytotoxic. Temozolomide causes single- and double-stranded DNA breaks

by adding methyl groups to N⁷ guanine (70% of total number of adducts), N³ adenine (9%), and O⁶ guanine (5%). The cytotoxic effects of temozolomide are mainly attributed to the O⁶-methylguanine adducts. The DNA repair protein O⁶-methylguanine-DNA methyltransferase (MGMT) removes both methyl and 2-chloroethyl adducts from the O⁶ position of guanine, and is an important mechanism of resistance against these agents [27]. Removing the MGMT protein, for example, from O⁶-benzylguanine leads to a higher cytotoxicity of nitrosourea and temozolomide. Expression of the *MGMT* gene is affected by epigenetic changes such as DNA methylation of the promoter gene, thus inhibiting the expression of MGMT. The presence of *MGMT* promoter methylation results in an increased effectiveness of temozolomide chemotherapy in glioma and perhaps also of PCV chemotherapy [28,29]. Once O⁶-methylguanine adducts are present, an intact mismatch repair (MMR) system is necessary for the induction of apoptosis. Temozolomide is not effective in cells with MMR deficiency [30]. A defect in the MMR system results in microsatellite instability and tolerance to O⁶-methylguanine DNA adduct mismatch. More than 80% of the lesions induced by temozolomide are N-methylated bases, recognized by DNA glycosylases and not by MGMT. Therefore, the resistance to temozolomide is also determined by the base excision repair (BER) system. The enzyme PARP, a nuclear enzyme that recognizes both double- and single-stranded DNA breaks and plays a central role in the activity of the BER system and the removal of methylated N³ and N⁷ adducts. Possibly, PARP inhibitors may disrupt temozolomide resistance by blocking BER through which N³- and N⁷-methyl adducts are still becoming cytotoxic. The combination of temozolomide and PARP inhibitors is therefore potentially interesting.

Chemotherapy in diffuse gliomas

• Newly diagnosed glioblastoma

The European Organization for Research and Treatment of Cancer (EORTC) 26981/NCIC

CE3 randomized Phase III study in 563 glioblastoma patients showed that radiotherapy combined with temozolomide chemotherapy (60 Gy radiotherapy in 30 daily fractions of 2 Gy in combination with daily 75 mg/m² temozolomide followed by adjuvant six cycles of temozolomide at a dose of 150–200 mg/m² on days 1–5 every 4 weeks) significantly improves survival compared with treatment with radiotherapy alone (see Table 2) [6]. A small randomized Phase II study showed a similar result [31]. The long-term follow-up of the EORTC study confirmed the results of the initial publication (hazard ratio [HR] for death with chemotherapy: 0.6; 95% CI: 0.5–0.7]; see Table 2) [32]. Combined chemoradiotherapy increases 2-year survival to 27 versus 11% after radiotherapy alone. The 5-year survival was 10% in the combination arm versus 2% in the radiotherapy only arm. With these results chemoradiation with temozolomide became the world-wide accepted standard treatment for glioblastoma patients.

The methylation status of the *MGMT* gene promoter is of prognostic significance for survival in the EORTC 26981 study and there is a suggestion of predictive significance but the study was not powered to show this. In tumors without methylation of the *MGMT* gene promoter the 2-year survival was 14.8% whereas it was 49% in the *MGMT*-methylated tumors (and 24% after radiotherapy alone) [33]. Predictive value of *MGMT* promoter methylation status was found in two studies on elderly glioblastoma patients (see below). The survival benefit from the combined chemoradiotherapy was less in patients with a poorer prognosis (only biopsy, older patients, poorer performance status) [34].

• Newly diagnosed glioblastoma in elderly patients

Elderly patients in a poor clinical condition are often treated with a short radiotherapy schedule [18,35]. The main consideration for this choice is the decreased survival in this group compared with younger patients. Subgroup analyses of EORTC 26981 show that even in

Table 2. Survival in months measured from the date of randomization in EORTC study 26981 of the combined chemoradiotherapy and temozolomide in glioblastoma					
Therapy	n	Survival			
		Median, months (95% CI)	2-year, % (95% CI)	3-year, % (95% CI)	5-year, % (95% CI)
Radiotherapy	286	12.1 (11.2–13.0)	10.9 (7.6–14.8)	4.4 (2.4–7.2)	1.9 (0.6–4.4)
Chemoradiotherapy	287	14.6 (13.2–16.8)	27.2 (22.2–32.5)	16.0 (12.0–20.6)	9.8 (6.4–14.0)

elderly patients (>70 years) with favorable prognostic factors the median survival is more than 12 months. Therefore, a longer radiotherapy schedule, combined with chemotherapy, can be considered in patients over 60 with favorable prognostic factors (macroscopic total resection and with a good performance status [KPS >70, MMSE >27]). Two trials have investigated treatment with temozolomide alone in an elderly population. The Nordic trial showed that a long-schedule radiotherapy leads to shorter survival compared with a short radiotherapy schedule or temozolomide alone [36]. In both the Nordic and the NOA-08 trials a better survival was found after temozolomide monotherapy compared with a short-schedule radiotherapy in patients with a methylated *MGMT* gene promoter, whereas *MGMT* promoter methylation status did not impact outcome to radiotherapy [36,37]. Therefore, temozolomide can be considered in elderly patients with *MGMT* promoter methylation instead of a short radiotherapy schedule. However, neither the Nordic nor the NOA-08 trial investigated the combination of a short-schedule radiotherapy and temozolomide chemotherapy. Such a trial was conducted by the EORTC/NCI (ClinicalTrials.gov [38] identifier: NCT00482677). In this trial on elderly glioblastoma patients hypofractionated radiotherapy is compared with hypofractionated radiotherapy with temozolomide. Results are expected by the end of 2015.

• Recurrent glioblastoma

In Europe, a randomized Phase II study in recurrent glioblastoma led to the registration of temozolomide. In this study, a significant improvement in 6mo-PFS was found after treatment with temozolomide compared with the procarbazine-treated control group (see Table 3; HR: 1.47; 95% CI: 1.11–1.95) [39]. Although the median survival in the temozolomide arm was 1.5 months longer, this difference was not significant. A single-arm study with temozolomide showed similar results [40]. The 6mo-PFS of 19–21% also shows that temozolomide chemotherapy in this situation is only marginally effective, with an ORR in glioblastoma of 5–10%. Although most relapsed glioblastoma patients will have received prior treatment with temozolomide, a Canadian study has shown that temozolomide may still be effective for recurrent glioblastoma with a temozolomide therapy-free interval of >3 months [41].

Recent Phase III studies using lomustine as a control arm showed that nitrosoureas may be a useful treatment alternative in recurrent glioblastoma, with 20–30% 6mo-PFS (see Table 3) [42,43]. A large British randomized Phase III study in recurrent glioblastoma patients after radiotherapy alone showed no difference between temozolomide and the PCV regimen (HR: 0.89; 95% CI: 0.73–1.08) [44].

• Dose-intensified temozolomide schedules

Continuous or ‘dose-dense’ temozolomide dosing regimens were based on theoretical considerations to overcome *MGMT*-dependent resistance to temozolomide [47]. Some uncontrolled studies suggested relatively favorable results with these intensified regimens for recurrent glioblastoma [48]. Many studies, however, failed to show such promising results [49–51]. In line with the latter studies was a British randomized Phase III study in chemotherapy naive patients with a recurrent glioblastoma. This study showed a better survival of the standard 1–5 days every 4 weeks temozolomide schedule compared with temozolomide given in a 3-weeks-on/1-week-off dose intensified regimen (HR: 1.32; 95% CI: 0.99–1.75) [44]. Furthermore, a large international RTOG lead study showed no survival benefit of a dose-intensified adjuvant schedule in newly diagnosed glioblastoma after radiotherapy, regardless of the *MGMT* promoter status [52]. With the currently available data it can be concluded that there is no proven efficacy for dose-intensive temozolomide regimens, and that retreatment with temozolomide can be considered in carefully selected patients.

• Newly diagnosed grade II & III gliomas

Anaplastic tumors including oligodendrogliomas

In the late eighties of the past century the first reports emerged that recurrent anaplastic oligodendrogliomas were sensitive to PCV chemotherapy with response rates in the 55–65% range and a median duration of response of 12–18 months [53–55]. In particular, tumors with combined 1p/19q loss were very chemotherapy responsive, with 90–100% of these patients showing a response to the PCV regimen or temozolomide [56,57]. These studies in recurrent tumors were the reason to investigate whether outcome would improve if the chemotherapy was given early in the treatment of these patients. Two open randomized controlled

Table 3. Studies with chemotherapy in patients with recurrent glioblastoma

Chemotherapy	n	6mo-PFS	Median PFS (months)	Median OS (months)	Ref.
Temozolomide	112	21%	3	–	[39]
Procarbazine	113	8%	2	–	
Temozolomide	138	19%	2.1	–	[40]
Lomustine	84	19%	1.6	7.1	[42]
Lomustine	65	25%	2.7	9.8	[43]
Lomustine/cediranib	129	35%	4	9.4	
Cediranib	131	16%	3	8.0	
PCV	224	–	3.6	6.7	[44]
Temozolomide	223	–	4.7	7.2	
PCV	63	29%	3	7.5	[45]
Bevacizumab	85	43%	4.2	9.2	[46]
Bevacizumab/irinotecan	82	50%	5.6	–	

6mo-PFS: Percentage of patients free of progression 6 months after the start of the chemotherapy; OS: Overall survival; PCV: Procarbazine, CCNU (lomustine) and vincristine; PFS: Progression-free survival.

Phase III trials (EORTC: 368 patients, RTOG: 289 patients) investigated the value of adjuvant PCV immediately before or after radiotherapy in newly diagnosed anaplastic oligodendroglial tumors compared with radiotherapy only and chemotherapy for progression [58,59]. Both had survival measured from the time of randomization as their primary end point. At the time of their first report both studies did not show an overall survival benefit from early adjuvant PCV chemotherapy although both studies showed a significant improvement in PFS after adjuvant chemotherapy [58,59]. However, the long-term results (median follow-up >11 years) of both studies showed survival benefit in anaplastic oligodendroglial tumors with combined 1p/19q loss after adjuvant PCV chemotherapy (see Table 4) despite crossover treatment with chemotherapy at the time of progression in 70% of patients randomized to radiotherapy only [60,61]. Both studies have shown that the presence of combined 1p/19q loss in oligodendrogliomas is of important prognostic significance and identifies patients with more benefit from adjuvant PCV chemotherapy. The median survival is over 10–14 years in patients with tumors with combined 1p/19q loss as opposed to 2–3 years for tumors without combined 1p/19q loss. Correlative side studies conducted within the context of these clinical trials have suggest that the CpG island hypermethylated phenotype (CIMP), a methylated *MGMT* gene promoter and mutations of *IDH* in anaplastic oligodendroglioma can also be used to identify patients that may benefit from adjuvant PCV chemotherapy [29,62].

A randomized German study in 318 patients with grade III gliomas compared initial treatment

with radiotherapy with initial treatment with chemotherapy, and randomized patients in the chemotherapy arm between PCV and temozolomide [63]. While previous studies consistently showed a better survival when high-grade tumors were treated with radiotherapy compared with chemotherapy, this first report of the study with still rather immature survival data showed no difference in PFS between patients treated with one line of chemotherapy followed at progression by radiotherapy and patients treated with radiotherapy followed at progression by one line of chemotherapy. This shows that in grade III tumors there is no difference whether the treatment is started with chemotherapy or radiotherapy, as long as patients are being surveyed carefully and further treatment is given at the time of progression. This study and the EORTC study 26951 in anaplastic oligodendrogliomas revealed that grade III tumors have a hypermethylation of the *MGMT* gene promoter in 70–80% of the cases, and that this is also a favorable prognostic factor after radiotherapy treatment. This was subsequently found to be related to hypermethylation of CpG islands (CIMP) in *IDH*-mutated tumors. In these tumors, methylation is induced by metabolic alterations that are the consequence of the altered substrate affinity of the *IDH1* mutation product resulting in an increased 2HG glutarate production [64–67]. This shows that the clinical significance of *MGMT* promoter methylation is determined by the molecular background of the tumor. An ongoing randomized trial (CATNON) investigates the value of combined chemoradiotherapy with temozolomide in grade III tumors without combined 1p/19q loss (anaplastic astrocytomas).

Low-grade gliomas

The role of chemotherapy in newly diagnosed low-grade gliomas is slowly being clarified. Uncontrolled studies with up-front temozolomide and PCV showed favorable results, especially in tumors with combined 1p/19q loss. In a study of 149 patients treated with up-front temozolomide for a low-grade glioma, the median time to progression was 28 months and significantly longer in the group with combined 1p/19q loss [68]. Similar observations have been made with PCV [69]. A first and still early analysis of the EORTC study 22033 presented at the ASCO meeting in 2013 suggests that PFS does not differ between patients with 1p loss receiving upfront temozolomide versus patients receiving radiotherapy, but radiotherapy may provide a superior PFS in patients without 1p loss ($p = 0.06$) [70]. The median OS was not yet reached in that study.

An updated report from an American randomized study (RTOG 9802) in 251 patients with newly diagnosed low-grade gliomas with a relatively unfavorable prognostic profile (less than gross total resection and/or over 40 years of age) showed that adjuvant PCV chemotherapy increased both PFS and OS [71]. With 55% of the patients having died, the median survival was 7.8 years after radiotherapy alone and 13.3 years after radiotherapy followed by PCV (HR: 0.59; $p = 0.002$) [72]. This implies that radiotherapy followed by adjuvant chemotherapy should now perhaps be standard therapy in high-risk low-grade glioma. Unfortunately, molecular data from RTOG 9802 are not available yet and it is not clear whether all molecular subgroups benefit equally from combined treatment. Another unsolved question is whether temozolomide will provide the same OS advantage as PCV.

Although the tolerability of temozolomide is better than that of PCV, no comparable trials with temozolomide have been performed. This suggests that some fundamental questions on how to best select patients for adjuvant PCV chemotherapy in this population may remain unanswered.

Another important and unanswered question is whether it is safe to use only up-front chemotherapy in low-grade glioma patients with a large chemotherapy sensitive tumor (e.g., 1p/19q loss), in which a large radiation field is required. Such a policy is used by some to delay radiotherapy as this has been associated with cognitive deficits [73,74].

• Recurrent grade II & III gliomas

Recurrent grade II and III tumors and in particular the anaplastic oligodendroglioma with 1p/19q loss are significantly more sensitive to chemotherapy than the glioblastoma (see Table 5). In oligodendrogliomas with combined loss of 1p/19q a response is seen in 90–100% of the tumors, with a median response duration of 1–2 years. However, even in these tumors the response to a second line of chemotherapy is limited, with an ORR in 20–25% of cases and a 6-month PFS of approximately 50% [75,76]. Both temozolomide and nitrosoureas or PCV apply as the standard treatment in this situation.

Angiogenesis inhibitors, integrin inhibitors & other targeted agents

In recent years there is much interest in treatment of glioblastoma with substances inhibiting the signal transduction by the VEGF system, either by catching circulating VEGF (such as bevacizumab, a humanized monoclonal antibody against circulating VEGF) or by blocking

Table 4. Median overall survival (months) and median progression-free survival (months) in relation to the 1p/19q status.

Chromosomal status	Median OS, months (95% CI)		Median PFS, months (95% CI)	
	RT/PCV	RT	RT/PCV	RT
Combined 1p/19q loss				
EORTC	NR	111.8 (75.7–134.3)	156.8 (68.1–NR)	49.9 (27.8–101.8)
RTOG	176.4	87.6	100.8	34.8
No 1p/19q loss				
EORTC	25 (18–37)	21 (18–29)	14.8 (9.9–21.1)	8.7 (7.1–11.7)
RTOG	31.2	32.4	14.4	12.0

EORTC study 26951 and RTOG 9402 study with (neo)adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors [60,61]. EORTC: European Organization of Research and Treatment of Cancer; NR: Not reached; OS: Overall survival; PCV: Procarbazine, CCNU (lomustine) and vincristine; PFS: Progression-free survival; RT: Radiotherapy; RTOG: Radiotherapy and Oncology Group.

the receptor (such as cediranib, an orally tyrosine kinase inhibitor of VEGF receptors 2 and 3). Uncontrolled Phase II studies showed high response rates and 6mo-PFS with bevacizumab (see Table 3) [46,81]. Based on the randomized Phase II BRAIN trial (bevacizumab vs bevacizumab with irinotecan), the US FDA registered bevacizumab for use in recurrent glioblastoma ('conditional approval') in the USA. The lack of a control arm without bevacizumab was the reason for the European Medicines Agency to reject the registration of bevacizumab. Nonetheless, bevacizumab is currently used in many European countries for this indication.

An unsolved issue of the BRAIN trial is the use of imaging end points: the percentage of patients showing an objective response and/or 6mo-PFS [82]. Inhibition of the VEGF signaling system results also in a normalization of the abnormal permeability of the tumor blood vessels, reducing contrast enhancement on MRI scans even in the absence of a true tumor response [83]. The occurrence of these 'pseudoresponses' makes the interpretation of the MRI scans troublesome: it is questionable whether any reduction of contrast uptake on MRI scans in anti-VEGF agents treated glioblastoma patients indeed reflects a real tumor reduction or only a decrease in contrast uptake and edema. Indeed, a large placebo-controlled Phase III study (REGAL trial) in 325 patients showed no survival benefit of cediranib alone or in combination with lomustine compared with only lomustine single agent in recurrent glioblastoma, despite a >20% response rate in an uncontrolled Phase II study [43,84]. While the PFS in this trial was slightly better in patients treated with cediranib, this did not translate into a survival benefit. Though initial reports suggested that inhibition of tumor angiogenesis

might induce an infiltrative growth pattern by co-opting existing blood vessels, some recent studies failed to find evidence for this [85].

Two large Phase III trials (AVAglio trial and RTOG 0825) on bevacizumab in newly diagnosed glioblastoma have failed to show an improvement of OS despite a major improvement of PFS [7,8]. Still, a longer PFS could translate into an improved quality of survival, as progression is often accompanied by neurological deterioration. Therefore, in both studies a quality of life assessment was included as a mandatory secondary end point. Surprisingly, the AVAglio trial showed maintenance of the quality of life in the bevacizumab-treated patients prior to progression, whereas the RTOG 0825 trial showed a decreased quality of life despite using similar questionnaires. The present results do not justify the use of bevacizumab in newly diagnosed glioblastoma, and the role of bevacizumab in recurrent glioblastoma is still unclear. The Dutch-randomized Phase II BELOB trial is the only study with a control arm without bevacizumab in recurrent glioblastoma [86]. This trial also failed to show a survival benefit of bevacizumab single agent, although the results within the combination of lomustine and bevacizumab with an OS at 9 months of 59% met the prespecified criterion for further Phase III studies. The ongoing Phase III EORTC trial 26101 in recurrent glioblastoma is designed to demonstrate whether there is indeed survival benefit of the combination bevacizumab/lomustine compared with lomustine alone (ClinicalTrials.gov [38] identifier: NCT01290939).

A near endless list of compounds aiming at established molecular aberrations (e.g., PI3 kinase inhibitors, mTOR antagonists, CDK4 and 6 antagonists, etc.) in particular glioblastoma but also in other diffuse glioma has been investigated. Unfortunately, despite all these

Table 5. Studies with first-line temozolomide or procarbazine, lomustine and vincristine chemotherapy in patients with recurrent grade II and III gliomas.

Histology	n	Drugs	Response (%)	6mo-PFS (%)	12mo-PFS (%)	mOS (months)	Ref.
All	70	TMZ	47	63	–	13	[77]
AA/AOA	65	TMZ	43	50	–	11.5	[78]
AOD/AOA	67	TMZ	46	–	50	31	[79]
AOD/AOA	38	TMZ	54	71	40	NR	[55]
AO/AOA	52	PCV	63	–	50	20	[54]
AOD	37	PCV	59	72	52	30.7	[80]

6mo-PFS: Progression-free survival at 6 months; 12mo-PFS: Progression-free survival at 12 months; AA: Anaplastic (grade III) astrocytoma; All: Low-grade (grade II) astrocytoma; AOA: Anaplastic oligoastrocytoma; AOD: Anaplastic oligodendroglioma; mOS: Median overall survival; PCV: Procarbazine, CCNU (lomustine) and vincristine; Response: Percentage of patients with an objective response (partial and complete response); TMZ: Temozolomide.

efforts, none of these have improved outcome (e.g., CENTRIC, Erlotinib) [87,88].

Conclusion & future perspective

In the last decade, a shift has occurred from chemotherapy for recurrent disease toward the use of adjuvant treatment for newly diagnosed tumors. In addition, there is a better understanding of molecular factors that predict responsiveness to adjuvant chemotherapy.

Chemoradiotherapy with temozolomide is currently the standard of care for newly diagnosed glioblastoma. Two studies have showed that chemotherapy is more effective than radiotherapy alone in elderly glioblastoma patients with a methylated *MGMT* promoter. Furthermore, it has been shown that bevacizumab added to chemoradiotherapy in newly diagnosed glioblastoma does not improve survival. Treatment with lomustine or retreatment with temozolomide (in patients with a longer temozolomide-free interval) can be considered in recurrent glioblastoma, but with modest effectivity. Additionally, bevacizumab combined with lomustine may play a role in the recurrent glioblastoma setting and is currently being investigated in an adequately powered Phase III trial (EORTC 26101). Until now other 'targeted' therapies have shown poor results.

Adjuvant PCV chemotherapy is now part of standard of care in newly diagnosed WHO grade III oligodendroglial tumors at least in the tumors with combined loss of 1p/19q. Adjuvant PCV also clearly improves survival in newly diagnosed high-risk low-grade glioma.

For recurrent WHO grade II and grade III tumors, chemotherapy is the standard and commonly temozolomide is used instead of lomustine or PCV due to better tolerance. Although about half of the patients show an objective response to this treatment, this response however is usually of limited duration, with the exception of oligodendroglial tumors with combined 1p/19q loss.

With the current data available, early chemotherapy is now part of the management of nearly all diffuse glioma.

Ongoing studies investigate whether chemoradiotherapy with concomitant and adjuvant temozolomide also improves outcome in elderly patients with glioblastoma treated with a short schedule of radiotherapy. Furthermore, the value of combined chemoradiotherapy with temozolomide for grade III astrocytoma without 1p/19q loss is currently being investigated, but it will take however many years before the results of this study become available. Clearly, low-grade glioma patients benefit from adjuvant chemotherapy, but the optimal way of identifying these patients (1p/19q loss? *IDH* mutated? CIMP or *MGMT* promoter methylated tumors?) remains to be established. Also, whether in large low-grade gliomas, initial treatment with chemotherapy alone can be considered is at present unclear, but the prevailing data suggest that this approach may decrease survival.

Current treatment results in the recurrent glioma are of rather modest effectivity, and it is recommended that these patients are enrolled in clinical trials aiming at the improvement of outcome. Although patient selection can still be improved, it is unlikely that with the currently available cytotoxic drugs outcome can be further enhanced. This implies that for further improvement of outcome new drugs are needed. This should be a high priority.

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References

Papers of special note have been highlighted as:
•• of considerable interest

- Ohgaki H, Kim YH, Steinbach JP. Nervous system tumors associated with familial tumor syndromes. *Curr. Opin. Neurol.* 23(6), 583–591 (2010).
- Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. *Clin. Cancer Res.* 19(4), 764–772 (2013).
- Labussiere M, Di Stefano AL, Gleize V *et al.* TERT promoter mutations in gliomas, genetic associations and clinico-pathological correlations. *Br. J. Cancer* (2014).
- Jiao Y, Killela PJ, Reitman ZJ *et al.* Frequent *ATRX*, *CIC*, *FUBP1* and *IDH1* mutations refine the classification of malignant gliomas. *Oncotarget* 3(7), 709–722 (2012).
- Van Den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. *Acta Neuropathol.* 120(3), 297–304 (2010).
- Stupp R, Mason WP, Van Den Bent MJ *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* 352(10), 987–996 (2005).
- First trial showing survival benefit in glioblastoma with early chemotherapy
- Gilbert MR, Dignam JJ, Armstrong TS *et al.* A randomized trial of bevacizumab for newly

- diagnosed glioblastoma. *N. Engl. J. Med.* 370(8), 699–708 (2014).
- 8 Chinot OL, Wick W, Mason W *et al.* Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N. Engl. J. Med.* 370(8), 709–722 (2014).
- 9 Taal W, Brandsma D, De Bruin HG *et al.* Incidence of early pseudo-progression in a cohort of malignant glioma patients treated with chemoirradiation with temozolomide. *Cancer* 113(2), 405–410 (2008).
- **First research showing a high incidence of pseudoprogression in glioblastoma after chemoradiotherapy.**
- 10 Brandsma D, Stalpers L, Taal W, Sminia P, Van Den Bent M. Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. *Lancet Oncol.* 9(5), 453–461 (2008).
- 11 Brandsma D, Van Den Bent MJ. Pseudoprogression and pseudoreponse in the treatment of gliomas. *Curr. Opin. Neurol.* 22(6), 633–638 (2009).
- 12 Reardon DA, Galanis E, Degroot JF *et al.* Clinical trial end points for high-grade glioma: the evolving landscape. *Neuro Oncol.* 13(3), 353–361 (2011).
- 13 Van Den Bent MJ, Wefel JS, Schiff D *et al.* Response assessment in neuro-oncology (a report of the RANO group): assessment of outcome in trials of diffuse low-grade gliomas. *Lancet Oncol.* 12(6), 583–593 (2011).
- **Much-needed update of response criteria in low-grade gliomas.**
- 14 Wen PY, Macdonald DR, Reardon DA *et al.* Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J. Clin. Oncol.* 28(11), 1963–1972 (2010).
- **Much-needed update of response criteria in high-grade gliomas.**
- 15 Lacroix M, Abi-Said D, Fourney DR *et al.* A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J. Neurosurg.* 95(2), 190–198 (2001).
- 16 Walker MD, Alexander E Jr, Hunt WE *et al.* Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J. Neurosurg.* 49(3), 333–343 (1978).
- 17 Walker MD, Green SB, Byar DP *et al.* Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N. Engl. J. Med.* 303(23), 1323–1329 (1980).
- 18 Roa W, Brasher PM, Bauman G *et al.* Abbreviated course of radiation therapy in older patients with glioblastoma multiforme: a prospective randomized clinical trial. *J. Clin. Oncol.* 22(9), 1583–1588 (2004).
- 19 Stewart LA. Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials. *Lancet* 359(9311), 1011–1018 (2002).
- 20 Westphal M, Hilt DC, Bortey E *et al.* A Phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro Oncol.* 5(2), 79–88 (2003).
- 21 Chang CH, Horton J, Schoenfeld D *et al.* Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. A joint Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group study. *Cancer* 52(6), 997–1007 (1983).
- 22 Green SB, Byar DP, Walker MD *et al.* Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. *Cancer Treat. Rep.* 67(2), 121–132 (1983).
- 23 Hildebrand J, Sahmoud T, Mignolet F, Brucher JM, Afra D. Adjuvant therapy with dibromodulcitol and BCNU increases survival of adults with malignant gliomas. EORTC Brain Tumor Group. *Neurology* 44(8), 1479–1483 (1994).
- 24 Medical Research Council Brain Tumor Working P Randomized trial of procarbazine, lomustine, and vincristine in the adjuvant treatment of high-grade astrocytoma: a Medical Research Council trial. *J. Clin. Oncol.* 19(2), 509–518 (2001).
- 25 Brada M, Judson I, Beale P *et al.* Phase I dose-escalation and pharmacokinetic study of temozolomide (SCH 52365) for refractory or relapsing malignancies. *Br. J. Cancer* 81(6), 1022–1030 (1999).
- 26 Brem H, Piantadosi S, Burger PC *et al.* Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. *Lancet* 345(8956), 1008–1012 (1995).
- 27 Pegg AE. Repair of O(6)-alkylguanine by alkyltransferases. *Mutat. Res.* 462(2–3), 83–100 (2000).
- 28 Esteller M, Garcia-Foncillas J, Andion E *et al.* Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *N. Engl. J. Med.* 343(19), 1350–1354 (2000).
- 29 Van Den Bent MJ, Erdem-Eraslan L, Idhah A *et al.* *MGMT*-STP27 methylation status as predictive marker for response to PCV in anaplastic oligodendrogliomas and oligoastrocytomas. A report from EORTC study 26951. *Clin. Cancer Res.* 19(19), 5513–5522 (2013).
- 30 D’atri S, Tentori L, Lacal PM *et al.* Involvement of the mismatch repair system in temozolomide-induced apoptosis. *Mol. Pharmacol.* 54(2), 334–341 (1998).
- 31 Athanassiou H, Synodinou M, Maragoudakis E *et al.* Randomized Phase II study of temozolomide and radiotherapy compared with radiotherapy alone in newly diagnosed glioblastoma multiforme. *J. Clin. Oncol.* 23(10), 2372–2377 (2005).
- 32 Stupp R, Hegi ME, Mason WP *et al.* Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised Phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 10(5), 459–466 (2009).
- 33 Hegi ME, Diserens AC, Gorlia T *et al.* *MGMT* gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* 352(10), 997–1003 (2005).
- 34 Mirimanoff RO, Gorlia T, Mason W *et al.* Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 Phase III randomized trial. *J. Clin. Oncol.* 24(16), 2563–2569 (2006).
- 35 Keime-Guibert F, Chinot O, Taillandier L *et al.* Radiotherapy for glioblastoma in the elderly. *N. Engl. J. Med.* 356(15), 1527–1535 (2007).
- 36 Malmstrom A, Gronberg BH, Marosi C *et al.* Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, Phase 3 trial. *Lancet Oncol.* 13(9), 916–926 (2012).
- **This trial shows that elderly glioblastoma patients with a methylated *MGMT* gene promoter have a better survival with temozolomide monotherapy compared with a short-schedule radiotherapy in patients.**
- 37 Wick W, Platten M, Meisner C *et al.* Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised,

- Phase 3 trial. *Lancet Oncol.* 13(7), 707–715 (2012).
- This trial shows that elderly glioblastoma patients with a methylated MGMT gene promoter have a better survival with temozolomide monotherapy compared with a short-schedule radiotherapy in patients.
- 38 ClinicalTrials.gov. <https://clinicaltrials.gov/>
 - 39 Yung WK, Albright RE, Olson J *et al.* A Phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br. J. Cancer* 83(5), 588–593 (2000).
 - 40 Brada M, Hoang-Xuan K, Rampling R *et al.* Multicenter Phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. *Ann. Oncol.* 12(2), 259–266 (2001).
 - 41 Perry JR, Rizek P, Cashman R, Morrison M, Morrison T. Temozolomide rechallenge in recurrent malignant glioma by using a continuous temozolomide schedule: the “rescue” approach. *Cancer* 113(8), 2152–2157 (2008).
 - 42 Wick W, Puduvalli VK, Chamberlain MC *et al.* Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. *J. Clin. Oncol.* 28(7), 1168–1174 (2010).
 - 43 Batchelor T, Mulholland P, Neyns B *et al.* Phase III randomized study comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, with lomustine alone in recurrent glioblastoma patients. *J. Clin. Oncol.* 31(26), 3212–3218 (2013).
 - 44 Brada M, Stenning S, Gabe R *et al.* Temozolomide versus procarbazine, lomustine, and vincristine in recurrent high-grade glioma. *J. Clin. Oncol.* 28(30), 4601–4608 (2010).
 - 45 Kappelle AC, Postma TJ, Taphoorn MJ *et al.* PCV chemotherapy for recurrent glioblastoma multiforme. *Neurology* 56(1), 118–120 (2001).
 - 46 Friedman HS, Prados MD, Wen PY *et al.* Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J. Clin. Oncol.* 27(28), 4733–4740 (2009).
 - 47 Tolcher AW, Gerson SL, Denis L *et al.* Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br. J. Cancer* 88(7), 1004–1011 (2003).
 - 48 Wick A, Felsberg J, Steinbach JP *et al.* Efficacy and tolerability of temozolomide in an alternating weekly regimen in patients with recurrent glioma. *J. Clin. Oncol.* 25(22), 3357–3361 (2007).
 - 49 Han SJ, Rolston JD, Molinaro AM *et al.* Phase II trial of 7 days on/7 days off temozolomide for recurrent high-grade glioma. *Neuro Oncol.* 16(9), 1255–1262 (2014).
 - 50 Tabatabai G, Wick W, Wick A *et al.* MGMT promoter methylation as a prognostic biomarker for benefit from dose-intensified temozolomide rechallenge in progressive glioblastoma: first results from the randomized phase II DIRECTOR trial. *J. Clin. Oncol.* 32(Suppl. 5s), Abstract 2015 (2014).
 - 51 Perry JR, Belanger K, Mason WP *et al.* Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study. *J. Clin. Oncol.* 28(12), 2051–2057 (2010).
 - 52 Gilbert MR, Wang MH, Aldape KD *et al.* RTOG 0525: A randomized phase iii trial comparing standard adjuvant Temozolomide (TMZ) with a dose-dense (DD) schedule in newly diagnosed glioblastoma (GBM). *Neuro Oncol.* 13, 51–51 (2011).
 - 53 Cairncross G, Macdonald D, Ludwin S *et al.* Chemotherapy for anaplastic oligodendroglioma. National Cancer Institute of Canada Clinical Trials Group. *J. Clin. Oncol.* 12(10), 2013–2021 (1994).
 - 54 Van Den Bent MJ, Kros JM, Heimans JJ *et al.* Response rate and prognostic factors of recurrent oligodendroglioma treated with procarbazine, CCNU, and vincristine chemotherapy. Dutch Neuro-oncology Group. *Neurology* 51(4), 1140–1145 (1998).
 - 55 Van Den Bent MJ, Taphoorn MJ, Brandes AA *et al.* Phase II study of first-line chemotherapy with temozolomide in recurrent oligodendroglioma tumors: the European Organization for Research and Treatment of Cancer Brain Tumor Group Study 26971. *J. Clin. Oncol.* 21(13), 2525–2528 (2003).
 - 56 Cairncross JG, Ueki K, Zlatescu MC *et al.* Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J. Natl Cancer Inst.* 90(19), 1473–1479 (1998).
 - 57 Kouwenhoven MC, Kros JM, French PJ *et al.* 1p/19q loss within oligodendroglioma is predictive for response to first line temozolomide but not to salvage treatment. *Eur. J. Cancer* 42(15), 2499–2503 (2006).
 - 58 Van Den Bent MJ, Carpentier AF, Brandes AA *et al.* Adjuvant procarbazine, lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: a randomized European Organisation for Research and Treatment of Cancer Phase III trial. *J. Clin. Oncol.* 24(18), 2715–2722 (2006).
 - 59 Intergroup Radiation Therapy Oncology Group T, Cairncross G, Berkey B *et al.* Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. *J. Clin. Oncol.* 24(18), 2707–2714 (2006).
 - 60 Cairncross G, Wang M, Shaw E *et al.* Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. *J. Clin. Oncol.* 31(3), 337–343 (2013).
 - This trial showed survival benefit in anaplastic oligodendroglioma tumors with combined 1p/19q loss after adjuvant procarbazine, CCNU (lomustine) and vincristine chemotherapy.
 - 61 Van Den Bent MJ, Brandes AA, Taphoorn MJ *et al.* Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *J. Clin. Oncol.* 31(3), 344–350 (2013).
 - This trial showed survival benefit in anaplastic oligodendroglioma tumors with combined 1p/19q loss after adjuvant procarbazine, CCNU (lomustine) and vincristine chemotherapy.
 - 62 Cairncross JG, Wang M, Jenkins RB *et al.* Benefit from procarbazine, lomustine, and vincristine in oligodendroglioma tumors is associated with mutation of IDH. *J. Clin. Oncol.* 32(8), 783–790 (2014).
 - 63 Wick W, Hartmann C, Engel C *et al.* NOA-04 randomized Phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J. Clin. Oncol.* 27(35), 5874–5880 (2009).
 - 64 Van Den Bent MJ, Gravendeel LA, Gorlia T *et al.* A hypermethylated phenotype is a better predictor of survival than MGMT methylation in anaplastic oligodendroglioma brain tumors: a report from EORTC study 26951. *Clin. Cancer Res.* 17(22), 7148–7155 (2011).
 - 65 Noshmehr H, Weisenberger DJ, Diefes K *et al.* Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* 17(5), 510–522 (2010).
 - 66 Wiestler B, Capper D, Hovestadt V *et al.* Assessing CpG island methylator phenotype,

- 1p/19q codeletion, and *MGMT* promoter methylation from epigenome-wide data in the biomarker cohort of the NOA-04 trial. *Neuro Oncol.* 16(12), 1630–1638 (2014).
- 67 Wick W, Meisner C, Hentschel B *et al.* Prognostic or predictive value of *MGMT* promoter methylation in gliomas depends on *IDH1* mutation. *Neurology* 81(17), 1515–1522 (2013).
- 68 Kaloshi G, Benouaich-Amiel A, Diakite F *et al.* Temozolomide for low-grade gliomas: predictive impact of 1p/19q loss on response and outcome. *Neurology* 68(21), 1831–1836 (2007).
- 69 Stege EM, Kros JM, De Bruin HG *et al.* Successful treatment of low-grade oligodendroglial tumors with a chemotherapy regimen of procarbazine, lomustine, and vincristine. *Cancer* 103(4), 802–809 (2005).
- 70 Baumert BG, Mason WP, Ryan G *et al.* Temozolomide chemotherapy versus radiotherapy in molecularly characterized (1p loss) low-grade glioma: a randomized Phase III intergroup study by the EORTC/NCIC-CTG/TROG/MRC-CTU (EORTC 22033–26033). *J. Clin. Oncol.* 31(Suppl.), Abstract 2007 (2013).
- 71 Shaw E, Wang MH, Coons SW *et al.* Radiation therapy (RT) versus RT plus procarbazine, CCNU, and vincristine (PCV) chemotherapy for adult low-grade glioma (LGG): Radiation Therapy Oncology Group (RTOG) protocol 9802. *Neuro Oncol.* 10(5), 884–885 (2008).
- 72 (Nci) NCI. Adding chemotherapy following radiation treatment improves survival for adults with a slow-growing type of brain tumor. Press release (2014).
- 73 Habets EJ, Taphoorn MJ, Nederend S *et al.* Health-related quality of life and cognitive functioning in long-term anaplastic oligodendroglioma and oligoastrocytoma survivors. *J. Neurooncol.* 116(1), 161–168 (2014).
- 74 Douw L, Klein M, Fagel SS *et al.* Cognitive and radiological effects of radiotherapy in patients with low-grade glioma: long-term follow-up. *Lancet Neurol.* 8(9), 810–818 (2009).
- 75 Triebels VH, Taphoorn MJ, Brandes AA *et al.* Salvage PCV chemotherapy for temozolomide-resistant oligodendrogliomas. *Neurology* 63(5), 904–906 (2004).
- 76 Van Den Bent MJ, Chinot O, Boogerd W *et al.* Second-line chemotherapy with temozolomide in recurrent oligodendroglioma after PCV (procarbazine, lomustine and vincristine) chemotherapy: EORTC Brain Tumor Group Phase II study 26972. *Ann. Oncol.* 14(4), 599–602 (2003).
- 77 Taal W, Dubbink HJ, Zonnenberg CB *et al.* First-line temozolomide chemotherapy in progressive low-grade astrocytomas after radiotherapy: molecular characteristics in relation to response. *Neuro Oncol.* 13(2), 235–241 (2011).
- 78 Yung WK, Prados MD, Yaya-Tur R *et al.* Multicenter Phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group. *J. Clin. Oncol.* 17(9), 2762–2771 (1999).
- 79 Brandes AA, Tosoni A, Cavallo G *et al.* Correlations between O6-methylguanine DNA methyltransferase promoter methylation status, 1p and 19q deletions, and response to temozolomide in anaplastic and recurrent oligodendroglioma: a prospective GICNO study. *J. Clin. Oncol.* 24(29), 4746–4753 (2006).
- 80 Brandes AA, Tosoni A, Vastola F *et al.* Efficacy and feasibility of standard procarbazine, lomustine, and vincristine chemotherapy in anaplastic oligodendroglioma and oligoastrocytoma recurrent after radiotherapy. A Phase II study. *Cancer* 101(9), 2079–2085 (2004).
- 81 Batchelor TT, Duda DG, Di Tomaso E *et al.* Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. *J. Clin. Oncol.* 28(17), 2817–2823 (2010).
- 82 Van Den Bent MJ, Vogelbaum MA, Wen PY, Macdonald DR, Chang SM. End point assessment in gliomas: novel treatments limit usefulness of classical Macdonald's Criteria. *J. Clin. Oncol.* 27(18), 2905–2908 (2009).
- 83 Batchelor TT, Sorensen AG, Di Tomaso E *et al.* AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 11(1), 83–95 (2007).
- 84 Batchelor TT, Mulholland P, Neyns B *et al.* Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J. Clin. Oncol.* 31(26), 3212–3218 (2013).
- 85 Wick A, Dörner N, Schafer N *et al.* Bevacizumab does not increase the risk of remote relapse in malignant glioma. *Ann. Neurol.* 69(3), 586–592 (2011).
- 86 Taal W, Oosterkamp H, Walenkamp A *et al.* A randomized Phase II study on bevacizumab versus bevacizumab plus lomustine versus lomustine single agent in recurrent glioblastoma: the Dutch Belob Study. *Neuro Oncol.* 15, 83–84 (2013).
- 87 Stupp R, Hegi ME, Gorlia T *et al.* Standard chemoradiotherapy +/- cilengitide in newly diagnosed glioblastoma (GBM): updated results and subgroup analyses of the international randomized Phase III CENTRIC trial (EORTC trial #26071–22072/Canadian Brain Tumor Consortium). *Eur. J. Cancer* 49, S775–S775 (2013).
- 88 Van Den Bent MJ, Brandes AA, Rampling R *et al.* Randomized Phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J. Clin. Oncol.* 27(8), 1268–1274 (2009).