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Chapter V

Alkylating Agents and Treatment of Gliomas

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Abstract

Nitrosoureas have been commonly used for the treatment of malignant gliomas since the 1970s. However, the prognosis of patients with malignant gliomas remains extremely poor despite the fact that multidisciplinary approaches involving surgery, chemotherapy, and radiotherapy have been used for several decades. Recently, the novel alkylating agent temozolomide (TMZ) was shown to improve the survival of patients with malignant gliomas (including glioblastomas) in many clinical studies and has become one of the standard modalities for treatment of newly diagnosed and recurrent malignant gliomas. Temozolomide is a prodrug that can be orally administered and is hydrolytically processed in the blood to yield the methyldiazonium

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cation, which has DNA methylating activity. Patients can receive ambulatory treatment with TMZ because of its oral administration route and minimal side effects. The expression level of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) is the most important factor for a favorable outcome in patients treated with TMZ as well as those treated with nitrosoureas, and the epigenetic silencing of *MGMT* is the strongest predictive marker. The treatment course for patients with tumors lacking *MGMT* promoter methylation is unknown, and recurrence is unavoidable even in patients with TMZ-sensitive glioblastoma. While TMZ has provided a significant survival benefit, innovative modalities that can be combined with TMZ and radiotherapy are still required. In this chapter, we review the history and chemistry of alkylating agents for the treatment of malignant gliomas. In particular, we focus on TMZ and its effects on tumor cells. Furthermore, chemoresistance to TMZ and current chemotherapies used for the treatment of malignant gliomas are discussed.

Introduction

Gliomas represent the most common primary tumors of the adult central nervous system (CNS). Despite innovations in neurosurgical devices and techniques, the development of antineoplastic drugs and molecular target drugs, and advances in radiotherapy over the past decades, malignant gliomas—especially glioblastoma (glioblastoma multiforme, GBM)—are still fatal diseases. The World Health Organization (WHO) grading system is widely accepted as a tool for predicting the biological behavior of CNS neoplasms including gliomas. Anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic oligoastrocytoma are classified as grade III, and GBM is classified as grade IV. Alkylating agents, especially nitrosoureas such as lomustine (CCNU), have been used to treat high-grade gliomas or malignant gliomas (Grades III and IV). Optionally, subsets of unresectable grade II gliomas could be adaptation diseases. The standard of treatment for malignant gliomas is surgery followed by chemotherapy with radiotherapy. Optionally, boost radiotherapy or chemotherapy is added, and stereotactic radiotherapy or chemotherapy is performed as a salvage therapy upon recurrence/regrowth [1, 2, 3]. Gross total resection is directly associated with longer survival [4], and a randomized trial showed that fluorescence-guided maximum surgical resection improves 6-month progression-free survival [5]. However, patients with malignant gliomas cannot be cured by surgery alone regardless of the surgical technique employed. In the late 1970s, it was

reported that the addition of radiotherapy to surgery was more beneficial than surgery alone for patients with malignant gliomas [6, 7]. Although chemotherapy was mainly combined with alkylating agents during this era, there was controversy regarding the benefit of chemotherapy for patients with malignant gliomas. PCV-3, which is a combination of procarbazine, CCNU, and vincristine, is the most common chemotherapy regimen and conferred improved survival and increased time to progression in patients with anaplastic astrocytoma in comparison with the outcomes obtained using a single agent and radiotherapy alone [8]. However, no regimen for the treatment of GBM showed a significant benefit relative to radiotherapy alone until 2003. The Neuro-Oncology Working Group of the German Cancer Society (NOA) reported that a nimustine (ACNU)-based regimen caused a significant improvement in median survival relative to radiotherapy alone [9]. Furthermore, Stupp et al. reported that a novel alkylating agent, temozolomide (TMZ), improved survival in GBM when administered with concomitant radiotherapy in phase II and III studies [10, 11]. TMZ has been regarded as a well-tolerated oral alkylating agent, and the favorable results of clinical studies led to the widespread use of TMZ [12, 13]. The mammalian DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) has been implicated in the resistance of tumor cells to alkylating agents [14]. To date, many clinical trials have aimed to reduce TMZ resistance. In this chapter, we review the use of alkylating agents, including TMZ, in the treatment of malignant gliomas, their mechanism of antiglioma action, and the findings of clinical trials. Current concepts of chemotherapy in the context of a multidisciplinary approach to the treatment of malignant gliomas are also discussed.

Alkylating Agents for the Treatment of Brain Tumors

Alkylating agents contribute alkyl groups to DNA causing apoptosis of tumor cells. These agents include nitrogen mustards, ethyleneimines and methylmelamines, methylhydrazine derivatives, alkyl sulfonates, nitrosoureas, and triazenes [15]. Nitrosoureas such as carmustine (BCNU), ACNU, CCNU, semustine (methyl-CCNU), and ranimustine(MCNU) are lipophilic and can pass through the blood-brain barrier, which is one reason why they are the most widely used agents for the treatment of brain tumors. The nitrosoureas

exert cytotoxicity via spontaneous breakdown to an alkylating intermediate, the 2-chloroethyl diazoniumion, which alkylates guanine residues in DNA. One of the triazenes, TMZ, also targets guanine and induces methylation. Moreover, the methylhydrazine derivative procarbabazine also induces guanine methylation and is a component of the PCV-3 regimen. The structural formulas of these agents are shown in Figure 1. The other component of PCV-3, vincristine, is not an alkylating agent but rather a vinca alkaloid, which is a natural cell cycle-specific compound.

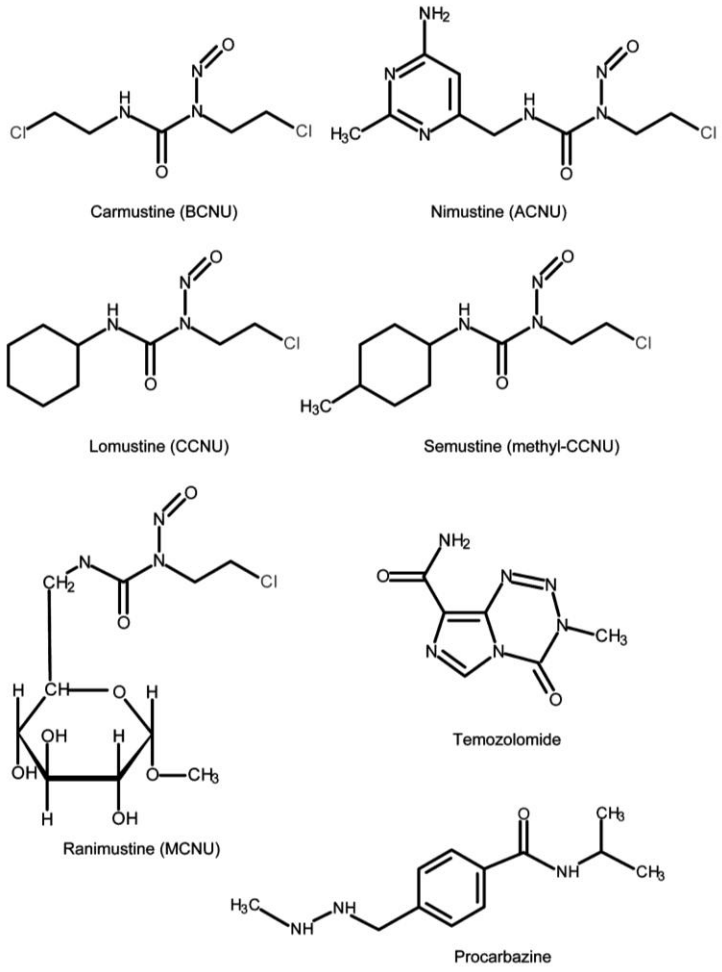

Figure 1. Structural formulae of alkylating agents.

TMZ is orally administered, while the other components are systemically administered. The Brain Tumor Study Group revealed that intra-arterial administration of BCNU did not yield more favorable results than intravenous administration [16]. Recently, intraoperative local treatment with Gliadel (BCNU) wafers was approved, and malignant glioma patients treated with BCNU wafers at the initial surgery in combination with radiation therapy demonstrated a survival advantage compared with those treated with placebo [17].

Prognosis of GBM and Therapeutic Effects of Chemoradiotherapy

The median survival durations of anaplastic astrocytoma patients treated with PCV-3 and BCNU were 157 weeks and 82.1 weeks, respectively. The time to progression was also doubled. However, there was no statistically significant difference in the survival duration of GBM patients regardless of treatment (median, 50.4 weeks with PCV and 57.4 weeks with BCNU) [8, 18]. Despite these results, PCV-3 has been the most extensively used treatment for malignant glioma for a long time. Recent studies have demonstrated that loss of heterozygosity (LOH) on chromosomes 1p and 19q in patients with anaplastic oligodendroglioma predicts sensitivity to chemotherapy and better overall survival [19, 20]. PCV-3 is preferably used in patients who have malignant gliomas with oligodendroglial components. The NOA reported the beneficial effects of ACNU-based chemotherapy in the NOA-1 trial. In phase III of this trial, ACNU plus teniposide (VM26) resulted in a median survival of 17.3 months in 154 GBM patients, and ACNU plus cytosine arabinoside (ara-C) in addition to radiotherapy resulted in a median survival of 15.7 months in 147 GBM patients. These survival benefits are more favorable than those described in any other phase III trial, including later TMZ studies [9, 21]. In the phase III trial reported by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG), a combined initial treatment for glioblastoma, including TMZ and radiotherapy, improved survival in comparison with radiotherapy alone over a 5-year follow-up period [11]. The overall survival rate at 5 years was 9.8% (of 287 GBM patients) with TMZ and radiotherapy and 1.9% (of 286 GBM patients) with radiotherapy alone. The median survival duration was 14.6 months with combined therapy and

12.1 months with radiotherapy alone. It is noteworthy that patients with a methylated MGMT promoter who were treated with TMZ and radiotherapy had a longer progression-free survival (23.4 months) [11].

Mechanism of Anticancer Action of TMZ

Similar to dacarbazine (DTIC), TMZ is a triazene. Stevens et al. synthesized TMZ as an analogue of mitozolomide, one of the antitumor imidazotetrazines, in the 1980s [22]. Although mitozolomide showed severe myelosuppression in a phase I study [23], TMZ (a 3-methyl derivative of mitozolomide) was less toxic than mitozolomide and exhibited broad-spectrum activity in mouse tumors [24]. TMZ was also well tolerated and effective in a phase I study [25].

Orally administered TMZ is converted to 5-(3-methyltriazen-l-yl) imidazole-4-carboximide (MTIC) in water/blood with little or no enzymatic component [26, 27]. MTIC is also an intermediate of DTIC degradation and is broken down to the methyldiazonium cation and 5-aminoimidazole-4-carboxamide (AIC) [27]. Although AIC is excreted via the kidneys, the methyldiazonium cation delivers a methyl group to DNA [27]. This methyl group is transferred to the oxygen atom at the 6th position of guanine to generate O6-methylguanine. O6-methylguanine mispairs with thymine instead of cytosine during DNA replication. The O6-methylguanine: thymine mispair can be recognized by the post-replication mismatch repair system, which removes the daughter strand along with the thymine, leaving the O6-methylguanine to again pair with thymine during gap filling. If replication of the gapped DNA occurs, double strand breaks can be formed that result in cell death unless they are repaired by recombination repair pathways. The generation of a methylating intermediate from TMZ is shown in Figure 2. Because this cytotoxicity is replication-dependent, methylating agents including TMZ are more effective in tumor cells than in quiescent cells [26, 27].

MGMT Expression and Resistance to TMZ

DNA repair and apoptosis are important chemoresistance mechanisms, especially when alkylating agents such as TMZ are involved.

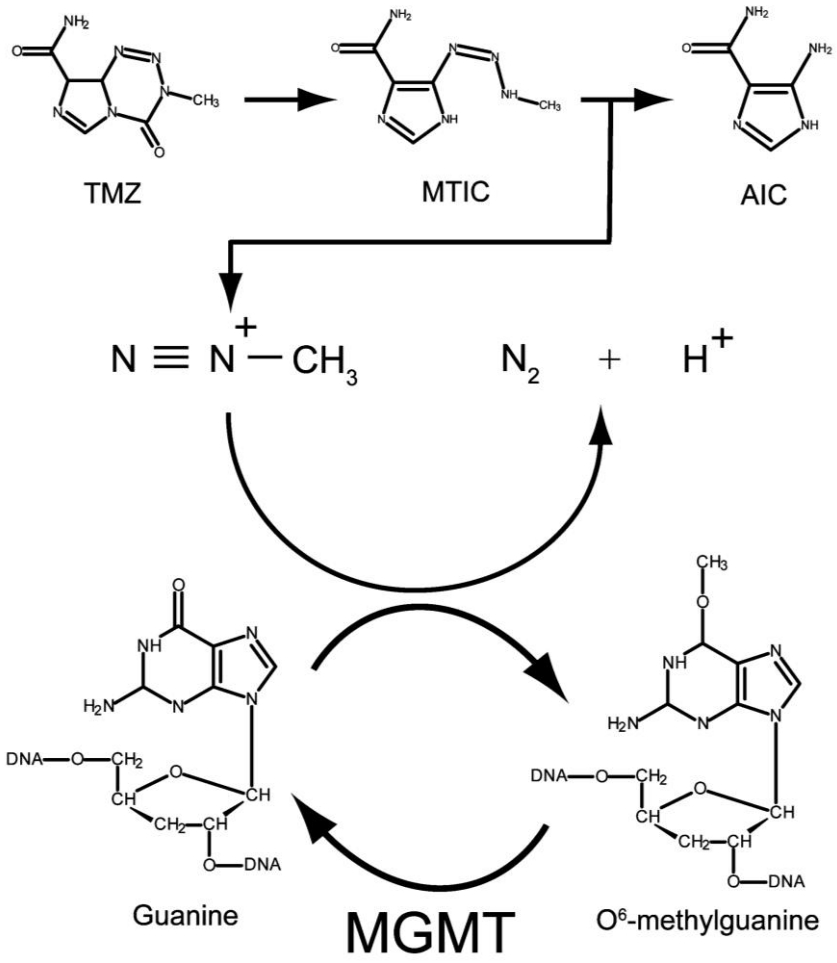

Figure 2. Mechanism of action of TMZ. TMZ is converted to 5-(3-methyltriazen-l-yl)imidazole-4-carboximide (MTIC) in water/blood with little or no enzymatic component. MTIC is broken down to methyldiazonium cation and 5-aminoimidazole-4-carboxamide (AIC). AIC is excreted via kidneys and methyldiazonium cations deliver methyl groups to DNA. Methyl groups are transferred to the 6th position oxygen atoms of guanines and O6-methylguanines are formed. O6-methylguanine mispairs with thymine instead of cytosine during DNA replication. The O6-methylguanine causes DNA break and apoptosis. MGMT removes methyl groups from O6-methylguanines to repair the genome. The expression of MGMT is epigenetically controlled. If the promoter region is methylated, the expression of MGMT is kept in low level.

Removal of alkyl groups, mismatch repair, base excision repair, and strand break repair are implicated in the resistance of gliomas to alkylating agents [28]. Above all, the removal of alkyl groups is suggested to have profound effects because a relationship between treatment and MGMT status has been observed [11, 29-31]. MGMT is a DNA repair protein that reverses alkylation at the O6 position of guanine to compensate for the effects of alkylating agents [32]. Human *MGMT* cDNA was isolated from a cDNA library based on its ability to rescue a methyltransferase-deficient *Escherichia coli* host [33]. The expression level of *MGMT* differs depending on species, organ, type of tumor, and cell line. In the early 1990s, subsets of cell lines that expressed low levels of *MGMT*, termed Mer- strains, were investigated to clarify the mechanism of decreased *MGMT* expression, and a correlation between DNA methylation and *MGMT* expression was revealed [34]. TMZ causes cell death by alkylation of the O6 position of guanine and subsequent disturbance of DNA replication; therefore, *MGMT* expression was implicated in resistance to TMZ chemotherapy. Indeed, *MGMT* expression and its contribution to resistance against TMZ have been reported in gliomas [35, 36]. Deletion, mutation, rearrangement, and mRNA instability of the *MGMT* gene are rare [37-41], and hypermethylation of the CpG island has been reported as the essential mechanism for *MGMT* silencing [29-31, 34, 37, 42-46]. Esteller et al. indicated that 40% of GBM cell lines, 50% of anaplastic astrocytoma cases, and 41% of GBM cases showed *MGMT* promoter methylation [37]. The incidence of *MGMT* promoter methylation in patients with GBM was 45% in the EORTC trial and the National Cancer Institute of Canada (NCSC) trial [11]. The MGMT protein level in tumor tissues can be evaluated by immunohistochemistry [47], and the activity of MGMT is measurable by an enzyme assay [48]. *MGMT* mRNA can be evaluated with reverse transcription-PCR (RT-PCR) [49] and real-time RT-PCR [50]. The methylation status of the *MGMT* gene has been assessed with methylation-specific PCR using bisulfite-modified DNA samples [45]. For diagnostic purposes, methylation-specific PCR is more advantageous than measurement of MGMT protein activity or mRNA levels because tissue contamination with non-neoplastic cells does not interfere with the detection of genomic methylation in tumor cells [30, 51]. To date, methylation-specific PCR is widely used for the evaluation of MGMT, although a standardized and validated method for the evaluation of MGMT status is required for the diagnosis and prognostication of gliomas. On the other hand, the clinical significance of *MGMT* promoter methylation status remains controversial [52, 53].

Chemoresistance Mechanisms Independent of MGMT

The enzymatic activity of MGMT is the most important mechanism underlying resistance to TMZ. However, other DNA repair and apoptosis processes exist, and other unknown mechanisms may also be present. The mismatch repair system is thought to be one mechanism of TMZ resistance [54-56]. Yip et al. reported mutations in the mismatch repair gene *MSH6* and microsatellite instability in patients with GBM after exposure to TMZ. These authors suggested that MSH6 inactivation could be involved in therapeutic resistance along with other unknown mechanisms [56]. Felsberg et al. reported reduced expression of MSH2, MSH6, and PMS2 protein in recurrent glioblastomas and suggested the existence of a novel chemoresistance mechanism facilitated by these proteins and independent of MGMT methylation [57]. The nucleotide excision repair system may also be involved in the TMZ resistance [58-62]. More than 80% of the DNA lesions methylated by TMZ are N-methylated bases that are recognized not by MGMT but rather by DNA glycosylases. Thus, resistance to TMZ may be due in part to robust base excision repair (BER) [58]. Knockdown of the BER enzyme DNA polymerase beta increased TMZ-induced cytotoxicity [59]. Some human tumor cells treated with TMZ showed increased expression of the chromatin-associated gene poly(ADP-ribose) polymerase-1 (*PARP-1*), which is involved in nucleotide excision repair [60]. PARP inhibitors have been shown to enhance sensitivity to TMZ [60-63].TMZ chemotherapy is expected not only to be a cytotoxic modality but also to sensitize tumor cells to radiation effects. TMZ actually enhances the radiosensitivity of tumor cells [64], and the strand break repair system is thought to interrupt this effect. Although TMZ can disrupt Rad51-induced repair [64], knockdown of the Rad54-related repair gene *DNA ligase IV* resulted in enhanced TMZ sensitivity in a human glioblastoma cell line [65]. The tumor suppressor p53 is a pleiotropic protein that plays an important role in DNA repair and apoptosis, and it functions via a mechanism distinct from that of MGMT. Wild-type p53 can reduce the cellular expression level of MGMT *in vitro* [66]. Conversely, in another report, p53 silencing reduced *MGMT* expression in murine astrocytic glioma cells [67]. Moreover, a p53 inhibitor enhanced the effects of TMZ in a mouse intracranial tumor implantation model, suggesting that p53 may induce the expression of *MGMT*, which in turn negatively regulates TMZ [68]. During treatment with chloroethylating agents, p53 protects against cell death;

however, this is not the case during treatment with methylating agents although both agents alkylate DNA [69]. The role of p53 during TMZ treatments has been described as favorable [66, 71, 72, 74], unfavorable [67, 68, 70], and variable depending on the type of cell [69, 73]. Collectively, the role of p53 is complicated and varies depending on cell type, *p53* allele (wild type or mutant), and type of agent.

Chemoresistance and Glioma Stem Cells

The concept of cancer stem cells is attracting increasing interest, and cancer stem cells may function as mediators of chemoresistance. Resistance to chemoradiotherapy may be due to the expansion of cancer stem cells, which can escape therapy-induced cell death. Cancer stem cells exhibit a multidrug-resistant phenotype by overexpressing drug transporters such as members of the adenosine triphosphate (ATP) binding cassette (ABC) superfamily and anti-apoptotic proteins such as B cell lymphoma/leukemia-2 (BCL-2) [75, 76]. CD133, CD15/SSEA-1, L1CAM, A2B5, and integrin α6 are some of the known surface markers that are used to enrich for glioma stem cells [77]. Additionally, glioma stem cells frequently express *MGMT* [78] and exhibit a multidrug resistant phenotype; thus, they may be more important therapeutic targets than the more highly differentiated tumor cells in the heterogeneous GBM population [77-79]. It has been reported that one of the ABC superfamily members, multidrug resistance 1 (MDR1), plays an important role in the chemoresistance of GBM independent of *MGMT* status [79]. Additionally, a single nucleotide polymorphism in the *MDR1* gene determines TMZ sensitivity [80]. A representative stem cell marker, CD133, was reported to be a candidate predictor of poor survival in patients treated with concomitant TMZ chemoradiotherapy [81, 82]. On the other hand, TMZ administration was also reported to decrease the number of glioma stem cells [83]. Conversely, treatment with BCNU increased the proportion of glioma stem cells in some cell lines [84].

Clinical Trials for GBM

Various phase II and III studies of malignant gliomas including GBM were performed and several studies are currently underway. Historically

important and recent phase II and III clinical studies of newly diagnosed GBM [8, 9, 11, 17, 85-106] are shown in Table 1. TMZ and other alkylating agents are elementary modalities involved in most of these trials. Importantly, TMZ has become the standard of care for patients with GBM. On the other hand, an overall survival longer than 2 years has not been shown in any clinical trials except for a study of selected cases in which the patients had better performance statuses [105]. Many clinical trials exploring treatments for recurrent GBM, designated as salvage therapies, have also been performed. Most of these therapies involve a combination of existing modalities and/or alternative dose-dense schedules. Although efforts to utilize existing modalities are important, breakthrough and innovative modalities are eagerly anticipated for a complete cure of GBM.

Table 1. Important and recent phase II and III clinical trials for newly-diagnosed glioblastoma

Reference	Year of publication	Remarks	Regimen	Patients (n)	Overall survival (months)
Levin et al. [8]	1990	NCOG	PCV + radiation	31	11.8
Weller et al. [9]	2003	NOA-01	ACNU + teniposide + radiation	154	17.3
Buckner et al. [85]	2006		cisplatin + BCNU + radiation	451	10.5
Colman et al. [86]	2006	RTOG9710	interferon beta + radiation	109	13.6
Westphal et al. [17]	2006		BCNU polymer wafers (Gliadel) + radiation	59	13.8
Brown et al. [87]	2008		erlotinib + TMZ + radiation	97	15.3
Stupp et al. [11]	2009	EORTC-NCIC	TMZ + radiation	287	14.6
Prados et al. [88]	2009		erlotinib + TMZ + radiation	65	19.3
Grossman et al. [89]	2009		Talampanel + TMZ + radiation	72	18.3
Beier et al. [90]	2009		pegylated liposomal doxorubicin + TMZ + radiation	63	17.6
Balducci et al. [91]	2010		TMZ + radiation	25	18
Mizumoto et al. [92]	2010		hyperfructionated proton radiation	20	21.6

Table 1. (Continued)

Reference	Year of publication	Remarks	Regimen	Patients (n)	Overall survival (months)
Jaeckle et al. [93]	2010	NCCTG	irinotecan + radiation	24	10.8
Jenkinson et al. [94]	2010		BCNU (intratumoral injection)	8	11.8
Peereboom et al. [95]	2010		erlotinib + TMZ + radiation	27	8.6
Li et al. [96]	2010		(125)I-mAb + TMZ + radiation	60	20.2
Hainsworth et al. [97]	2010		sorafenib + TMZ + radiation	47	12
Lai et al. [98]	2011		bevacizumab + TMZ + radiotherapy	70	19.6
Balducci et al. [99]	2011		TMZ + radiation (stereotactic)	36	28
Stummer et al. [100]	2011		fluorescence-guided resection + TMZ + radiation	122	16.3
Ogawa et al. [101]	2011		modified PCV + radiation + hyperbaric oxygenation	39	17.2
Butowski et al. [102]	2011		enzastaurin + TMZ + radiation	66	17.3
Ananda et al. [103]	2011		TMZ + pegylsted liposomal doxorubicin + radiation	40	13.4
Gállego Pérez-Larraya et al. [104]	2011	ANOCEF phase II	TMZ	70	5.8
Cho et al. [105]	2011		immunotherapy + TMZ + radiation	18	31.9
Ardon et al. [106]	2011	HGG-2006 phase I/II	immunotherapy + TMZ + radiation	77	18.3

Abbreviation; NCOG, the Northern California Oncology Group; NOA, Neuro-Oncology Working Group (of the German Cancer Society); RTOG, Radiation Therapy Oncology Group; EORTC, European Organization for the Research and Treatment of Cancer; NCIC, the National Cancer Institute of Canada Clinical Trials Group; NCCTG, the North Central Cancer Treatment Group; ANOCEF, Association des Neuro-Oncologuesd' Expression Française; HGG-2006, Immunotherapy for High Grade Glioma 2006 trial; PCV, procarbazine, CCNU, and vincristine.

Conclusion

Chemotherapy is an essential component in the multidisciplinary treatment of malignant gliomas. Alkylating agents such as TMZ and BCNU play major roles in current chemotherapy regimens. However, it remains controversial whether TMZ represents an improvement over conventional nitrosoureas [21, 107]. Nonetheless, regimens such as PCV-3 [8] and Stupp's regimen [10] will be important in postoperative adjuvant chemotherapy for the foreseeable future. Although the new alkylating agent TMZ has improved the prognosis of GBM and had an impact on the treatment of malignant gliomas, GBM remains an incurable disease. In order to attain a complete cure, new breakthroughs will be required. Treatment selection based on the molecular mechanisms of glioma pathogenesis and drug effects will become an integral part of personalized medicine; the role of *MGMT* methylation status as a predictive indicator of TMZ efficacy represents an illustrative example. In addition to translational approaches and improvements in drug delivery applications, an understanding of the molecular and cellular biology of gliomas, especially regarding chemoresistance and stem cell phenotype, will be required. The detailed molecular mechanisms of chemoresistance and the roles of related molecules including MGMT, mismatch repair enzymes, DNA excision repair enzymes, the ABC superfamily, and apoptosis-related factors will aid further innovation in the treatment of malignant gliomas.

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References

[1] Burton, EC; Prados, MD. Malignant gliomas. *Curr. Treat Options Oncol.*, 2000 1(5), 459-468.

[2] Chamberlain, MC; Kormanik, PA. Practical guidelines for the treatment of malignant gliomas. *West J. Med.*, 1998 168(2), 114-120.

[3] Lefranc, F; Sadeghi, N; Camby, I; Metens, T; Dewitte, O; Kiss, R. Present and potential future issues in glioblastoma treatment. *Expert. Rev. Anticancer Ther.*, 2006 6(5), 719-732.

[4] Ammirati, M; Vick, N; Liao, YL; Ciric, I; Mikhael, M. Effect of the extent of surgical resection on survival and quality of life in patients with supratentorial glioblastomas and anaplastic astrocytomas. *Neurosurgery*, 1987 21(2), 201-206.

[5] Stummer, W; Pichlmeier, U; Meinel, T; Wiestler, OD; Zanella, F; Reulen, HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.*, 2006 7(5), 392-401.

[6] Walker, MD; Alexander, E Jr; Hunt, WE; MacCarty, CS; Mahaley, MS Jr; Mealey, J Jr; et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J. Neuro surg.*, 1978 49(3), 333-343.

[7] Walker, MD; Strike, TA; Sheline, GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int. J. Radiat. Oncol. Biol. Phys.*, 1979 5(10), 1725-1731.

[8] Levin, VA; Silver, P; Hannigan, J; Wara, WM; Gutin, PH; Davis, RL; et al. Superiority of post-radiotherapy adjuvant chemotherapy with CCNU, procarbazine, and vincristine (PCV) over BCNU for anaplastic gliomas: NCOG 6G61 final report. *J. Rad. Onc. Biol. Phys.*, 1990 18(2), 321-324.

[9] Weller, M; Muller, B; Koch, R; Bamberg, M; Krauseneck, P; Neuro-Oncology Working Group of the German Cancer Society. Neuro-Oncology Working Group 01 trial of nimustine plus teniposide versus nimustine plus cytarabine chemotherapy in addition to involved-field radiotherapy in the first-line treatment of malignant gliomas. *J. Clin. Oncol.*, 2003 21(17), 3276-3284.

[10] Stupp, R; Mason, WP; van den Bent, MJ; Weller, M; Fisher, B; Taphoorn, MJ; et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.*, 2005 352(10), 987-996.

[11] Stupp, R; Hegi, ME; Mason, WP; van den Bent, MJ; Taphoorn, MJ; Janzer, RC; 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.*, 2009 10(5), 459-466.

[12] Khasraw, M; Lassman, AB. Advances in the treatment of malignant gliomas. *Curr. Oncol. Rep.*, 2010 12(1), 26-33.

[13] Villano, JL; Seery, TE; Bressler, LR. Temozolomide in malignant gliomas: current use and future targets. *Cancer Chemother. Pharmacol.*, 2009 64(4), 647-655.

[14] Gonzaga, PE; Potter, PM; Niu, TQ; Yu, D; Ludlum, DB; Rafferty, JA; et al. Identification of the cross-link between human O6-methylguanine-DNA methyltransferase and chloroethylnitrosourea-treated DNA. *Cancer Res.*, 1992 52(21), 6052-6058.

[15] Chabner, BA; Amrein, PC; Druker, BJ; Michaelson, MD; Mitsidiades, CS; Goss, PE; et al. Antineoplastic agents. In Brunton, LL (eds.), Goodman and Gilman's the Pharmacological Basis of Therapeutics (11th ed., 1315-1335). New York, NY: McGraw-Hill: 2006.

[16] Shapiro, WR; Green, SB; Burger, PC; Selker, RG; VanGilder, JC; Robertson, JT; et al. A randomized comparison of intra-arterial versus intravenous BCNU, with or without intravenous 5-fluorouracil, for newly diagnosed patients with malignant glioma. *J. Neuro surg.*, 1992 76(5), 772-781.

[17] Westphal, M; Ram, Z; Riddle, V; Hilt, D; Bortey, E. (Executive Committee of the Gliadel Study Group). Gliadel wafer in initial surgery for malignant glioma: long-term follow-up of a multicenter controlled trial. *Acta Neurochir*. (Wien), 2006 148(3), 269-275.

[18] Levin, VA; Silver, P; Hannigan, J; Wara, WM; Gutin, PH; Davis, RL; et al. Superiority of post-radiotherapy adjuvant chemotherapy with CCNU, procarbazine, and vincristine (PCV) over BCNU for anaplastic gliomas: NCOG 6G61 final report. *Int. J. Radiat. Oncol. Biol. Phys.*, 1990 18(2), 321-324.

[19] Cairncross, JG; Ueki, K; Zlatescu, MC; Lisle, DK; Finkelstein, DM; Hammond, RR; et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J. Natl. Cancer Inst.*, 1998 90(19), 1473-1479.

[20] Smith, JS; Perry, A; Borell, TJ; Lee, HK; O'Fallon, J; Hosek, SM; et al. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. *J. Clin. Oncol.*, 2000 18(3), 636-645.

[21] Linz, U. Chemotherapy for glioblastoma: is costly better? *Cancer*, 2008 113(10), 2617-2622.

[22] Stevens, MF; Hickman, JA; Stone, R; Gibson, NW; Baig, GU; Lunt, E; et al. Antitumor imidazotetrazines. 1. Synthesis and chemistry of 8-carbamoyl-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3 H)-one,

a novel broad-spectrum antitumor agent. *J. Med. Chem.*, 1984 27(2), 196-201.

[23] Newlands, ES; Blackledge, G; Slack, JA; Goddard, C; Brindley, CJ; Holden, L; et al. Phase I clinical trial of mitozolomide. *Cancer Treat Rep.*, 1985 69(7-8), 801-805.

[24] Stevens, MF; Hickman, JA; Langdon, SP; Chubb, D; Vickers, L; Stone, R; et al. Antitumor activity and pharmacokinetics in mice of 8-carbamoyl-3-methyl-imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (CCRG 81045; MandB 39831), a novel drug with potential as an alternative to dacarbazine. *Cancer Res.*, 1987 47(22), 5846-5852.

[25] Newlands, ES; Blackledge, GR; Slack, JA; Rustin, GJ; Smith, DB; Stuart, NS; et al. Phase I trial of temozolomide (CCRG 81045: MandB 39831: NSC 362856). *Br. J. Cancer*, 1992 65(2), 287-291.

[26] Clark, AS; Deans, B; Stevens, MF; Tisdale, MJ; Wheelhouse, RT; Denny, BJ; et al. Antitumor imidazotetrazines. 32. Synthesis of novel imidazotetrazinones and related bicyclic heterocycles to probe the mode of action of the antitumor drug temozolomide. *J. Med. Chem.*, 1995 38(9), 1493-1504.

[27] Denny, BJ; Wheelhouse, RT; Stevens, MF; Tsang, LL; Slack, JA. NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. *Biochemistry*, 1994 33(31), 9045-9051.

[28] Frosina, G. DNA repair and resistance of gliomas to chemotherapy and radiotherapy. *Mol. Cancer Res.*, 2009 7(7), 989-999.

[29] Estellar, M; Garcia-Foncillas, J; Andion, E; Goodman, SN; Hidalgo, OF; Vanaclocha, V; et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N. Engl. J. Med.*, 2000 343(19), 1350-1354.

[30] Hegi, ME; Liu, L; Herman, JG; Stupp, R; Wick, W; Weller, M; et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. *J. Clin. Oncol.*, 2008 26(25), 4189-4199.

[31] Hegi, ME; Diserens, AC; Gorlia, T; Hamou, M. F; de Tribolet, N; Weller, M; et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.*, 2005 352(10), 997-1003.

[32] Dolan, ME; Moschel, RC; Pegg, AE. Depletion of mammalian O6-alkylguanine-DNA alkyltransferase activity by O6-benzylguanine provides a means to evaluate the role of this protein in protection against

carcinogenic and therapeutic alkylating agents. *Proc. Natl. Acad. Sci. USA*, 1990 87(14), 5368-5372.

[33] Tano, K; Shiota, S; Collier J; Foote R. S; Mitra, S. (1990). Isolation and structural characterization of a cDNA clone encoding the human DNA repair protein for O6-alkylguanine. *Proc. Natl. Acad. Sci. USA*, 87, 686-690.

[34] Wang, Y; Kato, T; Ayaki, H; Ishizaki, K; Tano, K; Mitra, S; et al. Correlation between DNA methylation and expression of O6-methylguanine-DNA methyltransferase gene in cultured human tumor cells. *Mutat. Res.*, 1992 273(2), 221-230.

[35] Preuss, I; Eberhagen, I; Haas, S; Eibl, RH; Kaufmann, M; von Minckwitz, G; Kaina B. O6-methylguanine-DNA methyltransferase activity in breast and brain tumors. *Int. J. Cancer*, 1995 61(3), 321-326.

[36] Bobola, MS; Tseng SH; Blank A; Berger MS; Silber JR. Role of O6-methylguanine-DNA methyltransferase in resistance of human brain tumor cell lines to the clinically relevant methylating agents temozolomide and streptozotocin. *Clin. Cancer Res.*, 1996 2(4), 735-741.

[37] Esteller, M; Hamilton, SR; Burger, PC; Baylin, SB; Herman, JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.*, 1999 59(4), 793-797.

[38] Day, RS 3rd; Ziolkowski, CH; Scudiero, DA; Meyer, SA; Lubiniecki, AS; Girardi, AJ; et al. Defective repair of alkylated DNA by human tumor and SV40-transformed human cell strains. *Nature*, 1980 288(5792), 724-727.

[39] Fornace, AJ Jr; Papathanasiou, MA; Hollander, MC; Yarosh, DB. Expression of the O6-methylguanine DNA methyltransferase gene MGMT in MER+ and MER- human tumor cells. *Cancer Res.*, 1990 50(24), 7908-7911.

[40] Pieper, RO; Futscher, BW; Dong, Q; Ellis, TM; Erickson, LC. Comparison of O6-methylguanine-DNA methyltransferase gene (MGMT) mRNA levels in MER+ and MER- human tumor cell lines containing the MGMT gene by the polymerase chain reaction technique. *Cancer Commun.*, 1990 2(1), 13-20.

[41] Kroes, RA; Erickson, LC. The role of mRNA stability and transcription in O6-methylguanine-DNA methyltransferase (MGMT) expression in Mer- human tumor cell lines. *Carcinogenesis*, 1995 16(9), 2255-2257.

[42] Costello, JF; Futscher, BW; Tano, K; Graunke, DM; Pieper, RO. Graded methylation in the promoter and in the body of the O6-methylguanine-DNA methyltransferase gene correlates with MGMT expression in human glioma cells. *Cancer Res.*, 1994 269(25), 17228-17237.

[43] Qian, XC; Brent, TP. Methylation hot spots in the 5'-flanking region denote silencing of the O6-Methylguanine-DNA methyltransferase gene. *Cancer Res*., 1997 57(17), 3672-3677.

[44] Watts, GS; Pieper, RO; Costello, JF; Peng, Y-M; Dalton, WS; Futscher, BW. Methylation of discrete regions of the O6-Methylguanine DNA methyltransferase (MGMT) CpG island is associated with heterochromatinization of the MGMT transcription start site and silencing of the gene. *Mol. Cell Biol.*, 1997 17(9), 5612-5619.

[45] Paz, MF; Yaya-Tur, R; Rojas-Marcos, I; Reynes, G; Pollan, M; Aguirre-Cruz, L; et al. CpG island hypermethylation of the DNA repair enzyme methyltransferase predicts response to temozolomide in primary gliomas. *Clin. Cancer Res.*, 2004 10(15), 4933-4938.

[46] Hegi, ME; Diserens, AC; Godard, S; Dietrich, PY; Regli, L; Ostermann, S; et al. Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. *Clin. Cancer Res.*, 2004 10(6), 1871-1874.

[47] Sasai, K; Nodagashira, M; Nishihara, H; Aoyanagi, E; Wang, L; Katoh, M; et al. Careful Exclusion of Non-neoplastic Brain Components is Required for an Appropriate Evaluation of O6-methylguanine-DNA methyltransferase status in glioma: relationship between immunohistochemistry and methylation analysis. *Am. J. Surg. Pathol.*, 2008 32(8), 1220-1227.

[48] Spiro, TP; Gerson, SL; Liu, L; Majka, S; Haaga, J; Hoppel, CL; et al. O6-benzylguanine: a clinical trial establishing the biochemical modulatory dose in tumor tissue for alkyltransferase-directed DNA repair. *Cancer Res.*, 1999 59(10), 2402-2410.

[49] Mineura, K; Yanagisawa, T; Watanabe, K; Kowada, M; Yasui, N. Human brain tumor O(6)-methylguanine-DNA methyltransferase mRNA and its significance as an indicator of selective chloroethyl nitrosourea chemotherapy. *Int. J. Cancer*, 1996 69(5), 420-425.

[50] Tanaka, S; Kobayashi, I; Utsuki, S; Oka, H; Fujii, K; Watanabe, T; et al. O6-methylguanine-DNA methyltranspherase gene expression in gliomas by means of real-time quantitative RT-PCR and clinical response to nitrosoureas. *Int. J. Cancer*, 2003 103(1), 67-72.

[51] Rodriguez, FJ; Thibodeau, SN; Jenkins, RB; Schowalter, KV; Caron, BL; O'neill, BP; et al. MGMT immunohistochemical expression and promoter methylation in human glioblastoma. *Appl. Immunohistochem. Mol. Morphol.*, 2008 16(1), 59-65.

[52] Weller, M. Novel diagnostic and therapeutic approaches to malignant glioma. Swiss Med Wkly, 2011 141, w13210 (doi:10.4414/ smw.2011.13210).

[53] Jha, P; Suri, V; Jain, A; Sharma, MC; Pathak, P; Jha, P; et al. O6-methylguanine DNA methyltransferase gene promoter methylation status in gliomas and its correlation with other molecular alterations: first Indian report with review of challenges for use in customized treatment. *Neurosurgery*, 2010 67(6), 1681-1691.

[54] Friedman, HS; Johnson, SP; Dong, Q; Schold, SC; Rasheed, BK; Bigner, SH; et al. Methylator resistance mediated by mismatch repair deficiency in a glioblastoma multiforme xenograft. *Cancer Res.*, 1997 57(14), 2933-2936.

[55] Liu, L; Markowitz, S; Gerson, SL. Mismatch repair mutations override alkyltransferase in conferring resistance to temozolomide but not to 1,3-bis(2-chloroethyl)nitrosourea. *Cancer Res.*, 1996 56(23), 5375-5379.

[56] Yip, S; Miao, J; Cahill, DP; Iafrate, AJ; Aldape, K; Nutt, CL; et al. MSH6 mutations arise in glioblastomas during temozolomide therapy and mediate temozolomide resistance. *Clin. Cancer Res.*, 2009 15(14), 4622-4629.

[57] Felsberg, J; Thon, N; Eigenbrod, S; Hentschel, B; Sabel, MC; Westphal, M; et al. Promoter methylation and expression of MGMT and the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in paired primary and recurrent glioblastomas. *Int. J. Cancer*, 2011 129(3), 659-670.

[58] Trivedi, RN; Almeida, KH; Fornsaglio, JL; Schamus, S; Sobol, RW. The role of base excision repair in the sensitivity and resistance to temozolomide-mediated cell death. *Cancer Res.*, 2005 65(14), 6394-6400.

[59] Liu, L; Taverna, P; Whitacre, CM; Chatterjee, S; Gerson, SL. Pharmacologic disruption of base excision repair sensitizes mismatch repair-deficient and -proficient colon cancer cells to methylating agents. *Clin. Cancer Res.*, 1999 5(10), 2908-2917.

[60] Tentori, L; Portarena, I; Torino, F; Scerrati, M; Navarra, P; Graziani, G. Poly(ADP-ribose) polymerase inhibitor increases growth inhibition and

reduces G(2)/M cell accumulation induced by temozolomide in malignant glioma cells. *Glia*, 2002 40(1), 44-54.

[61] Curtin, NJ; Wang, LZ; Yiakouvaki, A; Kyle, S; Arris, CA; Canan-Koch, S; et al. Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. *Clin. Cancer Res.*, 2004 10(3), 881-889.

[62] Dungey, FA; Löser, DA; Chalmers, AJ. Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-Ribose) polymerase: mechanisms and therapeutic potential. *Int. J. Radiat. Oncol. Biol. Phys.*, 2008 72(4), 1188-1197.

[63] Tentori, L; Leonetti, C; Scarsella, M; d'Amati, G; Portarena, I; Zupi, G; et al. Combined treatment with temozolomide and poly(ADP-ribose) polymerase inhibitor enhances survival of mice bearing hematologic malignancy at the central nervous system site. *Blood*, 2002 99(6), 2241-2224.

[64] Kil, WJ; Cerna, D; Burgan, WE; Beam, K; Carter, D; Steeg, PS; et al. In vitro and in vivo radiosensitization induced by the DNA methylating agent temozolomide. *Clin. Cancer Res.*, 2008 14(3), 931-938.

[65] Kondo, N; Takahashi, A; Mori, E; Ohnishi, K; McKinnon, PJ; Sakaki, T. DNA ligase IV as a new molecular target for temozolomide. *Biochem. Biophys. Res. Commun.*, 2009 387(4), 656-660.

[66] Harris, L. C; Remack, JS; Houghton, PJ; Brent, TP. Wild-type p53 suppresses transcription of the human O6-methylguanine-DNA methyltransferase gene. *Cancer Res*., 1996 56(9), 2029-2032.

[67] Blough, MD; Zlatescu, MC; Cairncross, JG. O6-methylguanine-DNA methyltransferase regulation by p53 in astrocytic cells. *Cancer Res.*, 2007 67(2), 580-584.

[68] Dinca, EB; Lu, KV; Sarkaria, JN; Pieper, RO; Prados, MD; Haas-Kogan, DA; et al. p53 Small-molecule inhibitor enhances temozolomide cytotoxic activity against intracranial glioblastoma xenografts. *Cancer Res.*, 2008 68(24), 10034-10039.

[69] Batista, LF; Roos, WP; Christmann, M; Menck, CF; Kaina, B. Differential sensitivity of malignant glioma cells to methylating and chloroethylating anticancer drugs: p53 determines the switch by regulating xpc, ddb2, and DNA double-strand breaks. *Cancer Res*., 2007 67(24), 11886-11895.

[70] Li, S; Zhang, W; Chen, B; Jiang, T; Wang, Z. Prognostic and predictive value of p53 in low MGMT expressing glioblastoma treated with

surgery, radiation and adjuvant temozolomide chemotherapy. *Neurol. Res.*, 2010 32(7), 690-694.

[71] Bocangel, D; Sengupta, S; Mitra, S; Bhakat, KK. p53-Mediated down-regulation of the human DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) via interaction with Sp1 transcription factor. *Anticancer Res.*, 2009 29(10), 3741-3750.

[72] Bobustuc, GC; Baker, CH; Limaye, A; Jenkins, WD; Pearl, G; Avgeropoulos, NG; et al. Levetiracetam enhances p53-mediated MGMT inhibition and sensitizes glioblastoma cells to temozolomide. *Neuro Oncol.*, 2010 12(9), 917-927.

[73] Blough, MD; Beauchamp, DC; Westgate, MR; Kelly, JJ; Cairncross, JG. Effect of aberrant p53 function on temozolomide sensitivity of glioma cell lines and brain tumor initiating cells from glioblastoma. *J. Neuro oncol.*, 2011 102(1), 1-7.

[74] Sato, A; Sunayama, J; Matsuda, K; Seino, S; Suzuki, K; Watanabe, E; et al. MEK-ERK signaling dictates DNA-repair gene MGMT expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-p53 axis. *Stem. Cells*, 2011 29 (12), 1942-1951.

[75] Singh, SK; Clarke, ID; Hide, T; Dirks, PB. Cancer stem cells in nervous system tumors. *Oncogene*, 2004 23(43), 7267-7273.

[76] Lu, C; Shervington, A. Chemoresistance in gliomas. Mol Cell Biochem, 2008 312(1-2), 71-80.

[77] Venere, M; Fine, HA; Dirks, PB; Rich, JN. Cancer stem cells in gliomas: identifying and understanding the apex cell in cancer's hierarchy. *Glia*, 2011 59(8), 1148-1154.

[78] Beier, D; Schulz, JB; Beier, CP. Chemoresistance of glioblastoma cancer stem cells--much more complex than expected. *Mol. Cancer*, 2011 10, 128 (doi:10.1186/1476-4598-10-128)

[79] Nakai, E; Park, K; Yawata, T; Chihara, T; Kumazawa, A; Nakabayashi, H; et al. Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. *Cancer Invest.*, 2009 27(9), 901-908.

[80] Schaich, M; Kestel, L; Pfirrmann, M; Robel, K; Illmer, T; Kramer, M; et al. A MDR1 (ABCB1) gene single nucleotide polymorphism predicts outcome of temozolomide treatment in glioblastoma patients. *Ann. Oncol.*, 2009 20(1), 175-181.

[81] Murat, A; Migliavacca, E; Gorlia, T; Lambiv, WL; Shay, T; Hamou, MF; et al. Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to

concomitant chemoradiotherapy in glioblastoma. *J. Clin. Oncol.*, 2008 26(18), 3015-3024.

[82] Pallini, R; Ricci-Vitiani, L; Banna, GL; Signore, M; Lombardi, D; Todaro, M; et al. Cancer stem cell analysis and clinical outcome in patients with glioblastoma multiforme. *Clin. Cancer Res.*, 2008 14(24), 8205-8212.

[83] Beier, D; Röhrl, S; Pillai, DR; Schwarz, S; Kunz-Schughart, LA; Leukel, P; et al. Temozolomide preferentially depletes cancer stem cells in glioblastoma. *Cancer Res*., 2008 68(14), 5706-5715.

[84] Kang, MK; Kang, SK. Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. *Stem. Cells Dev.*, 2007 16(5), 837-847.

[85] Buckner, JC; Ballman, KV; Michalak, JC; Burton; GV, Cascino; TL, Schomberg, PJ; et al. North Central Cancer Treatment Group 93-72-52; Southwest Oncology Group 9503 Trials. Phase III trial of carmustine and cisplatin compared with carmustine alone and standard radiation therapy or accelerated radiation therapy in patients with glioblastoma multiforme: North Central Cancer Treatment Group 93-72-52 and Southwest Oncology Group 9503 Trials. *J. Clin. Oncol.*, 2006 24(24), 3871-3879.

[86] Colman, H; Berkey, BA; Maor, MH; Groves, MD; Schultz, CJ; Vermeulen, S; et al. Radiation Therapy Oncology Group. Phase II Radiation Therapy Oncology Group trial of conventional radiation therapy followed by treatment with recombinant interferon-beta for supratentorial glioblastoma: results of RTOG 9710. *Int. J. Radiat. Oncol. Biol. Phys.*, 2006 66(3), 818-824.

[87] Brown, PD, Krishnan, S, Sarkaria, JN, Wu, W, Jaeckle, KA, Uhm, JH, et al. North Central Cancer Treatment Group Study N0177. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J. Clin. Oncol.*, 2008 26(34), 5603-5609.

[88] Prados, MD; Chang, SM; Butowski, N; DeBoer, R; Parvataneni R; Carliner, H; et al. Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. *J. Clin. Oncol.*, 2009 27(4), 579-584.

[89] Grossman, SA; Ye, X; Chamberlain, M; Mikkelsen, T; Batchelor, T; Desideri, S; et al. Talampanel with standard radiation and temozolomide

in patients with newly diagnosed glioblastoma: a multicenter phase II trial. *J. Clin. Oncol.*, 2009 27(25), 4155-4161.

[90] Beier, CP; Schmid, C; Gorlia, T; Kleinletzenberger, C; Beier, D; Grauer, O; et al. RNOP-09: pegylated liposomal doxorubicine and prolonged temozolomide in addition to radiotherapy in newly diagnosed glioblastoma--a phase II study. *BMC Cancer*, 2009 9, 308 (doi:10.1186/1471-2407-9-308).

[91] Balducci, M; D'Agostino, GR; Manfrida, S; De Renzi, F; Colicchio, G; Apicella, G; et al. Radiotherapy and concomitant temozolomide during the first and last weeks in high grade gliomas: long-term analysis of a phase II study. *J. Neuro oncol.*, 2010 97(1), 95-100.

[92] Mizumoto, M; Tsuboi, K; Igaki, H; Yamamoto, T; Takano, S; Oshiro, Y; et al. Phase I/II trial of hyperfractionated concomitant boost proton radiotherapy for supratentorial glioblastoma multiforme. *Int. J. Radiat. Oncol. Biol. Phys.*, 2010 77(1), 98-105.

[93] Jaeckle, KA; Ballman, KV; Giannini, C; Schomberg, PJ; Ames, MM; Reid, JM; et al. Phase II NCCTG trial of RT + irinotecan and adjuvant BCNU plus irinotecan for newly diagnosed GBM. *J. Neuro oncol.*, 2010 99(1), 73-80.

[94] Jenkinson, MD; Smith, TS; Haylock, B; Husband, D; Shenoy, A; Vinjamuri, S; et al. Phase II trial of intratumoral BCNU injection and radiotherapy on untreated adult malignant glioma. *J. Neuro oncol.*, 2010 99(1), 103-113.

[95] Peereboom, DM; Shepard, DR; Ahluwalia, MS; Brewer, CJ; Agarwal, N; Stevens, GH; et al. Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme. *J. Neuro oncol.*, 2010 98(1), 93-99.

[96] Li, L; Quang, TS; Gracely, EJ; Kim, JH; Emrich, JG; Yaeger, TE; et al. A Phase II study of anti-epidermal growth factor receptor radioimmunotherapy in the treatment of glioblastoma multiforme. *J. Neuro surg.*, 2010 113(2), 192-198.

[97] Hainsworth, JD; Ervin, T; Friedman, E; Priego, V; Murphy, PB; Clark, BL; et al. Concurrent radiotherapy and temozolomide followed by temozolomide and sorafenib in the first-line treatment of patients with glioblastoma multiforme. *Cancer*, 2010 116(15), 3663-3669.

[98] Lai, A, Tran, A; Nghiemphu, PL; Pope, WB; Solis, OE; Selch, M; Filka, E; et al. Phase II study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. *J. Clin. Oncol.*, 2011 29(2), 142-148.

[99] Balducci, M; Apicella, G; Manfrida, S; Mangiola, A; Fiorentino, A; Azario, L; et al. Single-arm phase II study of conformal radiation therapy and temozolomide plus fractionated stereotactic conformal boost in high-grade gliomas: final report. *Strahlenther Onkol.*, 2010 186(10), 558-564.

[100] Stummer, W; Nestler, U; Stockhammer, F; Krex, D; Kern, BC; Mehdorn, HM; et al. Favorable outcome in the elderly cohort treated by concomitant temozolomide radiochemotherapy in a multicentric phase II safety study of 5-ALA. *J. Neuro oncol.*, 2011 103(2), 361-370.

[101] Ogawa, K; Ishiuchi, S; Inoue, O; Yoshii, Y; Saito, A; Watanabe, T; et al. Phase II trial of radiotherapy after hyperbaric oxygenation with multiagent chemotherapy (procarbazine, nimustine, and vincristine) for high-grade gliomas: long-term results. *Int. J. Radiat. Oncol. Biol. Phys.*, 2012 82(2), 732-738.

[102] Butowski, N; Chang, SM; Lamborn, KR; Polley, MY; Pieper, R; Costello, JF; et al. Phase II and pharmacogenomics study of enzastaurin plus temozolomide during and following radiation therapy in patients with newly diagnosed glioblastoma multiforme and gliosarcoma. *Neuro Oncol.*, 2011 13(12), 1331-1338.

[103] Ananda, S; Nowak, AK; Cher, L; Dowling, A; Brown, C; Simes, J; et al. Cooperative Trials Group for Neuro-Oncology (COGNO). Phase 2 trial of temozolomide and pegylated liposomal doxorubicin in the treatment of patients with glioblastoma multiforme following concurrent radiotherapy and chemotherapy. *J. Clin. Neuro sci.*, 2011 18(11), 1444-1448.

[104] Gállego Pérez-Larraya, J; Ducray, F; Chinot, O; Catry-Thomas, I; Taillandier, L; Guillamo, JS; et al. Temozolomide in elderly patients with newly diagnosed glioblastoma and poor performance status: an ANOCEF phase II trial. *J. Clin. Oncol.*, 2011 29(22), 3050-3055.

[105] Cho, DY; Yang, WK; Lee, HC; Hsu, DM; Lin, HL; Lin, SZ; et al. Adjuvant immunotherapy with whole-cell lysate dendritic cells vaccine for glioblastoma multiforme: a phase II clinical trial. *World Neuro surg.*, 2012 77(5-6), 736-744.

[106] Ardon, H; Van Gool, SW; Verschuere, T; Maes, W; Fieuws, S; Sciot, R; et al. Integration of autologous dendritic cell-based immunotherapy in the standard of care treatment for patients with newly diagnosed glioblastoma: results of the HGG-2006 phase I/II trial. *Cancer Immunol. Immunother.*, 2012 61(11), 2033-2044.

[107] Wick, W; Hartmann, C; Engel, C; Stoffels, M; Felsberg, J; Stockhammer, F; et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J. Clin. Oncol.*, 2009 27(35), 5874-5880.