ONCOLOGY REPORTS 16: 1117-1121, 2006

A pilot study of metronomic temozolomide treatment in patients with recurrent temozolomide-refractory glioblastoma

DOO-SIK KONG[1*], JUNG-IL LEE[1*], WON SEOG KIM[2], MYUNG JIN SON[1], DO HOON LIM[3], SUNG TAE KIM[4], KWAN PARK[1], JONG HYUN KIM[1], WHAN EOH[1] and DO-HYUN NAM[1]

[1]Department of Neurosurgery, [2]Division of Hematology-Oncology, Department of Medicine, [3]Department of Radiation Oncology, [4]Department of Radiology Center for Imaging Science, Samsung Medical Center and Samsung Biomedical Research Institute Sungkyunkwan University School of Medicine, Seoul, Korea

Received June 15, 2006; Accepted July 27, 2006

Abstract. Frequent regular administration of chemotherapeutic agents at low doses, known as 'metronomic chemotherapy', can increase the anti-angiogenic activity of the drugs, as has been confirmed by several other experimental tumor models. The aim of this pilot study was to evaluate the efficacy and safety of metronomic temozolomide (TMZ) treatment in twelve consecutive patients with recurrent TMZ-refractory glioblastoma. The patients were administered by metronomic treatment schedule (continuous low-dose chemotherapy) with TMZ at a daily dose of 40 mg/m^2. The median overall survival (OS) and progression-free survival (PFS) from the start of metronomic treatment were 11.0 months (95% CI, 5.2-10.5 months) and 6.0 months (95% CI, 0-12.3 months), respectively. During the follow-up period, complete response (CR) was not achieved in any patient, partial response (PR) in 2, and stable disease (SD) in 5 patients. Estimated PFS (CR+PR+SD) was 58.3% at 3 months. Grade III/IV toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) was not found. These results suggest that the change of chemotherapeutic schedule from conventional to metronomic treatment overcomes the chemo-resistance in patients with recurrent TMZ-refractory glio-blastoma without any major toxicity.

Correspondence to: Dr Do-Hyun Nam, Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Il-Won Dong, Kang-Nam Ku, Seoul 135-710, Korea
E-mail: nsnam@smc.samsung.co.kr

*Contributed equally

Abbreviations: TMZ, temozolomide; PFS, progression free survival; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease

Key words: temozolomide, metronomic treatment, recurrent glioblastoma

Introduction

Glioblastoma is a very aggressive neoplasm and poorly responsive to chemotherapy. Despite optimal treatment for glioblastoma, the prognosis of patients with recurrent glioblastoma remains extremely poor. Furthermore, there is little evidence of benefit of chemotherapy after tumor recurrence or progression. Since the majority of them are no longer candidates for further irradiation or surgical intervention, considerable efforts have been recently focused in the investigation of new chemotherapeutic agents and treatment schedules for recurrent glioblastoma (1-6).

Angiogenesis is one of the critical hallmarks of malignant glioma pathology. High grade glioma and glioblastoma are the most prominent tumors associated with endothelial cell proliferation (7). Therefore, vascular targeted therapy has been considered to be useful for glioblastoma treatment. Conventional administration of chemotherapeutic agents typically requires a treatment-free period for the recovery of normal host cells. However, endothelial cells may have a chance to repair the damage inflicted by the chemotherapy and resume tumor re-growth during this treatment-free interval (2). Recently, it has been suggested that this repair process could be compromised by frequent administration of lower doses of a chemotherapeutic drug without interruption (2,8,9). This frequent regular administration of chemotherapeutic agents at low doses, known as 'metronomic chemotherapy', increases the anti-angiogenic activity of the drugs (3,10). This hypothesis has been confirmed in several experimental tumor models which have led us to the concept of 'anti-angiogenic scheduling'. The metronomic scheduling for chemotherapy targets both proliferating tumor cells and endothelial cells, and minimizes toxicity (2,3,8-11).

We have studied the effect of metronomic treatment of temozolomide (TMZ) in the preclinical study using orthotopic model of TMZ resistant C6 rat glioma (12). Conventional TMZ treatment against C6 glioma model did not reveal significant effect on the tumor growth. However, frequent administration of TMZ at a low dose markedly inhibited angiogenesis as well as tumor growth (total amount of TMZ was similar to conventional treatment). Based on this result, we hypothesized that metronomic treatment would be effective

Table I. Patient characteristics.

No.	Age (years)	Sex	Histology	Biopsy or surgery	Previous treatments			KPS score
					Radiation therapy (cGy)	GKS	Conventional chemotherapy	
1	32	F	GBM	GTR	5400	Done	TMZ #14	70
2	61	M	GBM	Biopsy	5800	ND	TMZ #6 +ACNU	60
3	64	M	GBM	GTR	5400	Done	TMZ #10	70
4	50	M	GBM	STR	5000	ND	TMZ #2 + PCV	60
5	53	M	GBM	GTR	6000	Done	TMZ#2	70
6	69	F	GBM	GTR	5400	ND	TMZ #2	60
7	55	M	GBM	STR	6000	ND	TMZ #8	60
8	36	M	GBM	GTR	6000	ND	TMZ #2 + PCV	60
9	24	M	GBM	STR	5400	Done	TMZ #3	60
10	50	M	GBM	GTR	5800	Done	TMZ #6	70
11	51	F	GBM	GTR	6000	Done	TMZ #7	60
12	35	F	GBM	Biopsy	6000	Done	TMZ #5	80

GKS, gamma knife surgery; KPS, Karnofsky performance status; TMZ, temozolomide; ND, not done; GBM, glioblastoma; GTR, gross total removal; STR, subtotal removal; TMZ #, temozolomide 200 mg/m^2/5 consecutive days every 4 weeks.

for recurrent glioblastomas with drug resistance. The objective of this pilot study was to evaluate the efficacy and safety of metronomic treatment of TMZ for recurrent glioblastoma which was refractory to conventional cyclic administration.

Patients and methods

Patients' eligibility. This study was conducted at a single institute. Written informed consents were obtained from all patients according to the declaration of Helsinki. To be eligible for the study, patients had to fulfill the following criteria: i) adult patients with recurrent glioblastoma previously treated with irradiation, conventional TMZ after surgery, and/or gamma knife surgery; ii) whose age was 18-70 years; iii) confirmed histologic diagnosis of glioblastoma (WHO grade IV astrocytomas); iv) adequate pretreatment laboratory studies: granulocyte count >1500/Fl, hemoglobin level >10.0 g/dl, platelet count >100000/Fl, serum creatinine ≤1.5 mg/dl, total and direct bilirubin ≤1.5 times institutional normal, and aspartate aminotransferase ≤3 times the institutional upper limit of normal. Subjects presenting with any of following were excluded in the study; any other malignancies within the past 5 years; serious comorbid diseases; pregnancy or breast feeding; patients with allergy to TMZ.

Study design. Patients with recurrent glioblastoma received TMZ (Schering Plough Pharmaceuticals) at a dose of 40 mg/m^2/day everyday. Dosage of TMZ was adjusted according to neutrophil and platelet counts in the hematological study. During treatment period, a complete blood count was obtained at day 28 after the first dose or within 48 h of that day, and weekly until the ANC (absolute neutrophil count) was above 1.5x10^9/l (1500/μl) and the platelet count exceeded 100x10^9/l (75000/μl), if abnormalities were found in the hematological study.

Evaluation of disease. The criteria of MacDonald *et al* were used to determine treatment response (13); complete response (CR) was defined as the disappearance of all enhancing tumor, partial response (PR) as more than a 50% reduction in tumor size, progressive disease (PD) as an increase of enhanced tumor portion, appearance of new lesions, and neurologic decline not explained radiographically that cannot be attributed to another cause, and stable disease (SD) as all other radiographic situations. To differentiate from the radiation necrosis on the enhanced MRI, tumor progression was defined beyond the limit out of the gamma knife field. For an accurate measurement of tumor size, an outline of the tumor in each slice was downloaded to a personal computer with an image scanner (Adobe Photoshop), and the area of the tumor was measured with commercial available image software (SCION Corp.). The tumor volume was calculated by multiplying the total tumor areas in all slices with the slice interval. All these radiological findings were blindly reviewed by radiologist.

Safety assessments. Patients were monitored for TMZ-related toxicity each month. Toxicity was graded using the NCI CTC. Dose reductions and/or administration delays were planned in case of severe hematological and/or non-hematological toxicities. If grade III or IV toxicity occurred despite a dose reduction, the patient was taken off protocol treatment.

Statistical analysis. The Kaplan-Meier method was used to estimate overall survival (OS) and progression-free survival (PFS) in the population. OS and PFS were calculated from the time of study inclusion.

Results

Patient characteristics. Twelve consecutive patients were included in this study. Their clinical characteristics are presented in Table I. All patients had recurrent or progressive

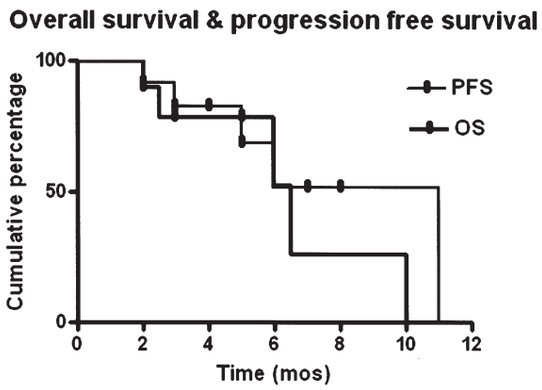

Figure 1. Overall survival and progression free survival from the study inclusion.

glioblastoma refractory to the conventional administration of TMZ (150-200 mg/m^2/day for 5 consecutive days every 4 weeks). The median age of patients was 48.3 years (range, 24-69 years). Ten out of twelve patients had undergone surgical removal of tumors and two patients received stereotactic biopsy for lesions located in eloquent area. For the previous treatments, all patients had then received local radiation therapy at a dose of 5000-6000 cGy and seven out of twelve patients had undergone gamma knife surgery for remnant or recurrent lesions. All patients administered conventional chemotherapy with TMZ. It is notable that seven patients had poor neurological status (KPS <70), while five other patients had relatively good neurological status (KPS ≥70). None of the patients were a candidate for reoperation.

Efficacy of metronomic treatment with low-dose TMZ. Twelve patients were assessed for response to metronomic treatment with TMZ. The median OS and PFS from the time of study inclusion were 11 months (95% CI, 5.2-10.5 months) and 6.0 months (95% CI, 0-12.3 months), respectively (Fig. 1). During the follow-up period after metronomic treatment, CR was not achieved in any patient, PR in 2, and SD in 5 patients (Fig. 2). Estimated PFS rate (CR+PR+SD) was 58.3% at 3 months (Table II).

Safety. No grades III/IV toxicity were observed according to the CTC classification. All patients received 40 mg/m^2/day dose of TMZ, which was well tolerated.

Discussion

TMZ is an oral alkylating agent and acts as an inhibitor of DNA mismatch repair and can induce apoptosis (14,15). Recent clinical studies have demonstrated that TMZ is effective for malignant glioma and accepted as first-line chemotherapy (1,6,16-18). It should be administered at a regular interval in current protocol, giving treatment-free period to avoid the dose-limiting toxicity like non-cumulative myelosuppression (17). However, tumor cell targeted, conventional treatment having treatment-free interval gives recovery time to tumor associated endothelial cells, allowing tumor cells to resume growth (19). To overcome this disadvantage of conventional schedules of cytotoxic agents, a metronomic treatment schedule was introduced. The frequent administration of certain cytotoxic agents at low doses, known as 'metronomic chemotherapy', was more effective in targeting tumor endothelium than large single bolus doses followed by treatment-free period (2,3,9,20). The current studies demonstrated efficacy as salvage therapy in the treatment of patients with breast cancer and hormone-refractory prostate cancer (4,18-20).

The main target of metronomic treatment is tumor associated endothelial cells (EC), which are known to be genetically stable and have significantly higher proliferating rates than tumor cells (3,21). This has been attributed to an interference not only with angiogenic signals, but also with EC survival signals resulting in EC apoptosis, regression of existing tumor blood vessels, and subsequent tumor cell apoptosis (11,20). It has less possibility of developing drug

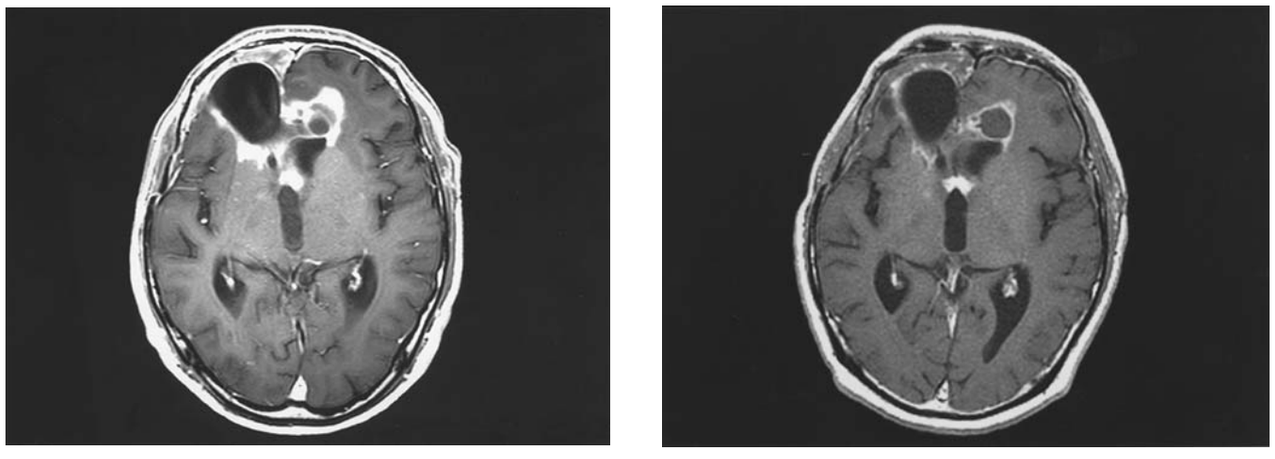

Figure 2. Follow-up of MR images before and after metronomic temozolomide (TMZ) treatment in a 36-year male patient with glioblastoma (case 8). The left MR image shows tumor recurrence in spite of conventional TMZ chemotherapy with PCV administration. The right demonstrates decrease of size in enhanced tumor portion at 2 months after initiation of metronomic treatment (dose 40 mg/m^2/day).

Table II. Results of metronomic treatment.

No.	Age (years)	Sex	Metronomic dosage of TMZ (mg/m^2/d)	Response at 3 mos	PFS (mos)	OS (mos)	Survival
1	32	F	40	SD	9	9	Alive
2	61	M	40	PD	2	2	Death
3	64	M	40	PD	0	4	Alive
4	50	M	40	PD	2.5	5	Death
5	53	M	40	SD	3	6	Death
6	69	F	40	SD	3	6	Alive
7	55	M	40	SD	5	7	Alive
8	36	M	40	PR	6.5	8	Death
9	24	M	40	PD	0	3	Death
10	50	M	40	SD	3	3	Alive
11	51	F	40	PD	2	3	Alive
12	35	F	40	PR	5	5	Alive

PFS, progression free survival; OS, overall survival from the inclusion of study; mos, months.

resistance, no difficulty in reaching the concentration of drug needed for the cellular target, fewer systemic side-effects, and feasibility of long-term administration (3). In addition, continuous administration of an alkylating agent depletes DNA repair gene, MGMT (O^6-methylguanine-DNA-methyltransferase), which may be an important determinant of treatment failure, by MGMT promoter methylation (22-24). Consequently, change of dosage and schedule of chemotherapeutic agents can overcome drug resistance.

For optimal metronomic treatment, TMZ could be considered as the first choice, because it can easily be taken everyday by the patient, whereas many other drugs need to be administered via an intravenous route. Therefore, it can provide high patient compliance without requiring further hospitalization. Tuttenberg *et al* demonstrated that metronomic treatment of TMZ combined with COX2 inhibitor was effective in controling tumors as an initial chemotherapy (11). This schedule also has some similarities with the recent study showing combination treatment of radiation therapy and concomitant continuous TMZ administration for glioblastomas (22). One of the reasons for better outcome in that study was the daily administration of low doses during the radiation therapy, which had potent anti-angiogenic effect (22,25). In our preclinical study, we elucidated that frequent administration of TMZ at a low dose significantly inhibits the growth of tumor in relatively TMZ resistant rat glioma. Based on these preclinical findings, we have performed a pilot study regarding metronomic treatment with low-dose TMZ in cases of recurrent glioblastoma refractory to the conventional administration of TMZ. No clinical studies with similar agents as first-line chemotherapy administered by metronomic treatment have been published. A notable finding in our study is that even in the patients with poor general condition (KPS score <70), it could be a safe and effective treatment for glioblastoma without developing major side effects.

In conclusion, metronomic treatment using TMZ proved to be feasible and safe chemotherapeutic modality for patients with recurrent glioblastoma. This novel schedule of anticancer therapy can be an alternative for conventional schedule of chemotherapy in the recurrent glioblastoma with drug resistance. Nevertheless, much more challenges to improve clinical outcome are required. In addition, phase II clinical trial of larger series and controlled randomized study are required.

Acknowledgements

This study was supported by the Samsung Medical Center Clinical Research Development Program grant (CRS106-03-1). The authors thank Dr Jin Hye Seo for critically reviewing the manuscript.

References

1. Brada M, Hoang-Xuan K, Rampling R, Dietrich PY, Dirix LY, Macdonald D, Heimans JJ, Zonnenberg BA, Bravo-Marques JM, Henriksson R, Stupp R, Yue N, Bruner J, Dugan M, Rao S and Zaknoen S: Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. Ann Oncol 12: 259-266, 2001.
2. Browder T, Butterfield CE, Kraling BM, Shi B, Marshall B, O'Reilly MS and Folkman J: Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 60: 1878-1886, 2000.
3. Gasparini G: Metronomic scheduling: the future of chemotherapy? Lancet Oncol 2: 733-740, 2001.
4. Jaeckle KA, Hess KR, Yung WK, Greenberg H, Fine H, Schiff D, Pollack IF, Kuhn J, Fink K, Mehta M, Cloughesy T, Nicholas MK, Chang S and Prados M: Phase II evaluation of temozolomide and 13-cis-retinoic acid for the treatment of recurrent and progressive malignant glioma: a North American Brain Tumor Consortium study. J Clin Oncol 21: 2305-2311, 2003.
5. Prados MD, Yung WK, Fine HA, Greenberg HS, Junck L, Chang SM, Nicholas MK, Robins HI, Mehta MP, Fink KL, Jaeckle KA, Kuhn J, Hess KR and Schold SC Jr: Phase 2 study of BCNU and temozolomide for recurrent glioblastoma multiforme: North American Brain Tumor Consortium Study. Neuro-oncol 6: 33-37, 2004.

6. Silvani A, Eoli M, Salmaggi A, Lamperti E, Maccagnano E, Broggi G and Boiardi A: Phase II trial of cisplatin plus temozolomide, in recurrent and progressive malignant glioma patients. J Neurooncol 66: 203-208, 2004.
7. Plate KH and Risau W: Angiogenesis in malignant gliomas. Glia 15: 339-347, 1995.
8. Kerbel RS, Klement G, Pritchard KI and Kamen B: Continuous low-dose anti-angiogenic/metronomic chemotherapy: from the research laboratory into the oncology clinic. Ann Oncol 13: 12-15, 2002.
9. Klement G, Huang P, Mayer B, Green SK, Man S, Bohlen P, Hicklin D and Kerbel RS: Differences in therapeutic indexes of combination metronomic chemotherapy and an anti-VEGFR-2 antibody in multidrug-resistant human breast cancer xenografts. Clin Cancer Res 8: 221-232, 2002.
10. Kerbel RS and Kamen BA: The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 4: 423-436, 2004.
11. Tuettenberg J, Grobholz R, Korn T, Wenz F, Erber R and Vajkoczy P: Continuous low-dose chemotherapy plus inhibition of cyclooxygenase-2 as an antiangiogenic therapy of glioblastoma multiforme. J Cancer Res Clin Oncol 131: 31-40, 2005.
12. Kim JT, Kim JS, Ko KW, Kong DS, Kang CM, Kim MH, Son MJ, Song HS, Shin HJ, Lee DS, Eoh W and Nam DH: Metronomic treatment of temozolomide inhibits tumor cell growth through reduction of angiogenesis and augmentation of apoptosis in orthotopic models of gliomas. Oncol Rep 16: 33-39, 2006.
13. MacDonald DR, Cascino TL, Schold SC Jr and Cairncross JG: Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 8: 1277-1280, 1990.
14. D'Atri S, Tentori L, Lacal PM, Graziani G, Pagani E, Benincasa E, Zambruno G, Bonmassar E and Jiricny J: Involvement of the mismatch repair system in temozolomide-induced apoptosis. Mol Pharmacol 54: 334-341, 1998.
15. Kokkinakis DM, Bocangel DB, Schold SC, Moschel RC and Pegg AE: Thresholds of O6-alkylguanine-DNA alkyltransferase which confer significant resistance of human glial tumor xenografts to treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea or temozolomide. Clin Cancer Res 7: 421-428, 2001.
16. Gruber ML and Buster WP: Temozolomide in combination with irinotecan for treatment of recurrent malignant glioma. Am J Clin Oncol 27: 33-38, 2004.
17. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, Brada M, Spence A, Hohl RJ, Shapiro W, Glantz M, Greenberg H, Selker RG, Vick NA, Rampling R, Friedman H, Phillips P, Bruner J, Yue N, Osoba D, Zaknoen S and Levin VA: A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. Br J Cancer 83: 588-593, 2000.
18. Yung WK, Prados MD, Yaya-Tur R, Rosenfeld SS, Brada M, Friedman HS, Albright R, Olson J, Chang SM, O'Neill AM, Friedman AH, Bruner J, Yue N, Dugan M, Zaknoen S and Levin VA: 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: 2762-2771, 1999.
19. Folkman J: Tumor angiogenesis: therapeutic implications. N Engl J Med 285: 1182-1186, 1971.
20. Bello L, Carrabba G, Giussani C, Lucini V, Cerutti F, Scaglione F, Landre J, Pluderi M, Tomei G, Villani R, Carroll RS, Black PM and Bikfalvi A: Low-dose chemotherapy combined with an anti-angiogenic drug reduces human glioma growth *in vivo*. Cancer Res 61: 7501-7506, 2001.
21. Folkman J: Angiogenesis and apoptosis. Semin Cancer Biol 13: 159-167, 2003.
22. Hegi ME, Diserens AC, Gorlia T, Hamou MF, De Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC and Stupp R: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352: 997-1003, 2005.
23. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E and Mirimanoff RO: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987-996, 2005.
24. Tolcher AW, Gerson SL, Denis L, Geyer C, Hammond LA, Patnaik A, Goetz AD, Schwartz G, Edwards T, Reyderman L, Statkevich P, Cutler DL and Rowinsky EK: Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. Br J Cancer 88: 1004-1011, 2003.
25. Stupp R, Dietrich PY, Ostermann Kraljevic S, Pica A, Maillard I, Maeder P, Meuli R, Janzer R, Pizzolato G, Miralbell R, Porchet F, Regli L, De Tribolet N, Mirimanoff RO and Leyvraz S: Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol 20: 1375-1382, 2002.