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Clinical Study

MGMT inactivation and clinical response in newly diagnosed GBM patients treated with Gliadel ☆

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ABSTRACT

We examined the relationship between the O^6-methylguanine-methyltransferase (MGMT) methylation status and clinical outcomes in newly diagnosed glioblastoma multiforme (GBM) patients who were treated with Gliadel wafers (Eisai, Tokyo, Japan). MGMT promoter methylation has been associated with increased survival among patients with GBM who are treated with various alkylating agents. MGMT promoter methylation, in DNA from 122 of 160 newly diagnosed GBM patients treated with Gliadel, was determined by a quantitative methylation-specific polymerase chain reaction, and was correlated with overall survival (OS) and recurrence-free survival (RFS). The MGMT promoter was methylated in 40 (32.7%) of 122 patients. The median OS was 13.5 months (95% confidence interval [CI] 11.0–14.5) and RFS was 9.4 months (95% CI 7.8–10.2). After adjusting for age, Karnofsky performance score, extent of resection, temozolomide (TMZ) and radiation therapy (RT), the newly diagnosed GBM patients with MGMT methylation had a 15% reduced mortality risk, compared to patients with unmethylated MGMT (hazard ratio 0.85; 95% CI 0.56–1.31; $p = 0.46$). The patients aged over 70 years with MGMT methylation had a significantly longer median OS of 13.5 months, compared to 7.6 months in patients with unmethylated MGMT ($p = 0.027$). A significant difference was also found in older patients, with a median RFS of 13.1 *versus* 7.6 months for methylated and unmethylated MGMT groups, respectively ($p = 0.01$). Methylation of the MGMT promoter in newly diagnosed GBM patients treated with Gliadel, RT and TMZ, was associated with significantly improved OS compared to the unmethylated population. In elderly patients, methylation of the MGMT promoter was associated with significantly better OS and RFS.

1. Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor, with a median survival of less than 2 years [1]. To date, only two alkylating agents have been shown to be consistently associated with prolonged survival: temozolomide (TMZ) and locally delivered Gliadel wafers (Eisai, Tokyo, Japan) [1–3].

Gliadel wafers are intracranially implanted and locally deliver carmustine (1,3-bis[2-chloroethyl]-1; nitrosourea [BCNU]) at the site of tumor resection, allowing for a higher concentration of local chemotherapeutic dose while minimizing systemic adverse effects [2–4]. These wafers provide a controlled release form of local chemotherapy over approximately 3 weeks [4,5].

Methylation of the O^6-methylguanine-methyltransferase (MGMT) promoter in gliomas has been found to be an important predictor of tumor responsiveness after several cytotoxic regimens [1], including BCNU treatment [6]. Expression of the DNA repair protein, MGMT, results in GBM resistance to alkylating agents. Alkylating agents cause cell death by binding to DNA, most commonly to the O^6 position of guanine, and forming cross links

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between adjacent DNA strands. This cross linking of double stranded DNA is inhibited by the cellular DNA repair protein MGMT.

In this study, through a unique analysis of 122 patients with newly diagnosed GBM who had enough tumor tissue for MGMT analysis and were treated with Gliadel, we retrospectively examined the association between the MGMT promoter methylation status and overall survival (OS) and recurrence-free survival (RFS).

2. Methods

2.1. Patients and tumor specimens

We retrospectively reviewed 160 patients with newly diagnosed GBM, who received Gliadel after their tumor resections at Johns Hopkins Hospital in Baltimore, Maryland, USA, between July 1997 and December 2006. Of these patients, only 122 had stored tumor samples that were available for MGMT analysis; 38 (24%) did not have enough tumor tissue. The clinical, radiological and hospital courses of these patients were retrospectively reviewed. Age, sex, Karnofsky performance score (KPS) at time of diagnosis, tumor location, time to recurrence and dates of death were recorded. GBM was histologically confirmed in all patients. The extent of surgical resection was determined based on a postoperative MRI performed <48 hours after surgery. Gross total resection was defined as no residual tumor enhancement on MRI, while subtotal resection was defined as residual nodular enhancement on MRI. The study was approved by the Johns Hopkins Institutional Review Board.

2.2. Treatment algorithm

Gliadel wafers were not implanted in patients after tumor resection when the tumor significantly extended into the ventricles or was multifocal.

2.3. DNA extraction

After the initial patient de-identification, all original histologic slides from the GBM specimens were reviewed by a senior neuropathologist (PB) to reconfirm the diagnosis of GBM. A representative block with tumor was retrieved for DNA extraction. Histologic slides from the formalin fixed, paraffin embedded tissue were obtained, one representative slide was stained with hematoxylin and eosin (H&E) and the tumor was marked by the senior neuropathologist (PB). An additional five correlating unstained 10 micron slides were also obtained. The tumor cells in the unstained slides were microdissected according to the marked H&E stained reference slide. DNA was extracted from the paraffin embedded tissue after xylene deparafinization. The microdissected tissue was digested with 1% sodium dodecyl sulfate and 200 μg/mL proteinase K (Hoffmann-La Roche, Basel, Switzerland) at 48°C for 48 hours, followed by phenol/chloroform extraction and ethanol precipitation of the DNA. The extracted DNA was dissolved in either LoTE (2.5 mM ethylenediaminetetraacetic acid, 10 mM tris-hydrochloric acid [pH 8]) or distilled water.

2.4. Bisulfite treatment

Extracted DNA was subjected to bisulfite treatment to convert the unmethylated cytosine residues to uracil residues. Briefly, 2 μg of genomic DNA from each sample was treated with bisulfite using the EpiTect Bisulfite kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The converted DNA was stored at −80°C.

2.5. Methylation analyses

The methylation analyses were performed using quantitative methylation-specific PCR. The bisulfite modified DNA was used as a template for fluorescence-based real time polymerase chain reactions (PCR). The amplification reactions were carried out in triplicate in a final volume of 20 μL that contained 3 μL bisulfite-modified DNA, 600 nmol/L concentrations of forward and reverse primers, 200 nmol/L of probe, 0.6 units of platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA), 200 μmol/L concentrations each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate, and 6.7 mmol/L of magnesium chloride. The primers and probes were designed to specifically amplify the promoter of MGMT and the promoter of a reference gene, ACTIN B. The primer and probe sequences and annealing temperatures are provided in Table 1. The amplifications were carried out using the following profile: 95°C for 3 min, 50 cycles at 95°C for 15 s, 60°C for 1 min. The amplification reactions were carried out in 384-well plates in a 7900 sequence detector (PerkinElmer, Waltham, MA, USA) and analyzed by a sequence detector system (Applied Biosystems, Foster City, CA, USA). Each plate included the patient DNA samples, positive controls (Bisulfite-converted universal methylated human DNA standards [Zymo Research, Irvine, CA, USA] in serial dilutions of 20 ng to 2 pg), and molecular grade water was used as a non-template control. The β-actin gene was used to normalize and act as an internal loading control. The methylation ratio was the ratio of values for the gene-specific PCR products to those of the ACTIN B, and then multiplied by 1000 for more efficient tabulation.

2.6. Statistical analyses

The baseline patient and disease characteristics were summarised using descriptive statistics for all 160 patients. The OS time was defined from the date of initial diagnosis of the disease (surgery) to the time of death, or it was censored at the time the patient was last known to be alive. The RFS was counted from the date of the initial diagnosis to the time of disease recurrence, or it was censored at the time the patient was last known to be alive and recurrence-free. The probabilities of OS and RFS were estimated using the Kaplan–Meier method [7] and compared using the log-rank test. Confidence intervals (CI) were calculated using the method of Brookmeyer and Crowley. The Cox proportional hazards model was used to estimate the association between OS or RFS and MGMT methylation status, treatments and well known prognostic factors among the 122 patients who had enough tissue

Table 1
Primer and probe sequences

Gene	Forward primer	Probe	Reverse primer
MGMT	CGA ATA TAC TAA AAC AAC CCG CG (1029–1051)	AAT CCT CGC GAT ACG CAC CGT TTA CG (1084–1109)	GTA TTT TTT CGG GAG CGA GGC (1130–1150)
ACTIN B	TGG TGA TGG AGG AGG TTT AGT AAG T (390–414)	ACC ACC ACC CAA CAC ACA ATA ACA AAC ACA (432–461)	AAC CAA TAA AAC CTA CTC CTC CCT TAA (496–522)

MGMT = O^6-methylguanine-methyltransferase.

for MGMT analysis. Schoenfeld residuals were used to test the proportionality of factors in the Cox proportional hazards models. The radiation status was treated as a stratification factor in the Cox regression model. TMZ has USA Federal Drug Administration approval for newly diagnosed GBM patients aged 18–70 years. Subgroup analyses were also performed for patients who were aged over 70. All p values were two-sided, and all analyses were performed using the Statistical Analysis System software (version 9.2; SAS Institute, Cary, NC, USA). MGMT was considered to be promoter methylated if the methylation ratio was higher than 8, and unmethylated if below 8.

3. Results

3.1. Patient population

At the Johns Hopkins Hospital between 1997 and 2006, 600 patients with newly diagnosed GBM underwent craniotomies. Of these patients, 185 received Gliadel (30.8%) after their tumor resections. Methylation-specific PCR was performed for 122 of the 160 patients (76%), because 38 did not have sufficient paraffin embedded tumor tissue for MGMT analysis. The patient characteristics and treatment details are shown in Table 2. The clinical course of 40 patients who had MGMT promoter methylation was compared to 82 patients without methylation. The similarity of distributions of patient characteristics and treatments between the two groups is shown in Table 2. There was a slight male predominance in both groups. The median age of the MGMT methylated group was 65.5 years compared to 60.5 years in the unmethylated MGMT group ($p = 0.59$). Most of the patients had a KPS score of $\leqslant$80 ($p = 0.67$). Most patients from both groups underwent a gross total resection (85% methylated *versus* 74% unmethylated MGMT; $p = 0.19$).

Most of the patients in the methylated and unmethylated groups received postoperative radiation therapy (RT; 80% and 72%, respectively). However, there were 31 patients (25%) without any RT recorded in their medical charts. Only 33% and 29% of the MGMT methylated and unmethylated patients, respectively, were treated with TMZ.

Table 2
Patient demographics and clinical characteristics

Characteristics	Methylated MGMT, n (%)	Unmethylated MGMT, n (%)	Total, n (%)
Patients	40	82	122
Age, years			
Median (range)	65.5 (29–85)	60.5 (37–81)	63 (29–85)
>70	10 (25)	20 (25)	30 (25)
50–70	24 (60)	47 (57)	71 (58)
$\leqslant$50	6 (15)	15 (18)	21 (17)
Sex			
Male	24 (60)	47 (57)	71 (58)
Female	16 (40)	35 (43)	51 (42)
KPS			
90–100	15 (37)	34 (41)	49 (40)
$\leqslant$80	25 (63)	48 (59)	73 (60)
Treatment			
GTR			
Yes	34 (85)	61 (74)	95 (78)
No	6 (15)	21 (26)	27 (22)
Gliadel	40 (100)	82 (100)	122 (100)
RT			
Yes	32 (80)	59 (72)	91 (75)
Unknown	8 (20)	23 (28)	31 (25)
TMZ			
Yes	13 (33)	24 (29)	37 (30)
No	27 (67)	58 (71)	85 (70)

GTR = gross total resection, KPS = Karnofsky performance score, MGMT = O^6-methylguanine-methyltransferase, RT = radiotherapy, TMZ = temozolomide.

3.2. Overall survival

The median OS, from Kaplan–Meier analysis, for the total 160 patients with newly diagnosed GBM was 13.7 months (95% CI 11.5–15.3). In a subset analysis of the patients with enough tumor tissue for MGMT analysis, the median OS for those with MGMT methylation was 13.9 months (95% CI 9.5–17.1) compared to 12.9 months (95% CI 10.9–14.5; $p = 0.86$) for patients with unmethylated MGMT. The univariate and multivariate association of OS with treatment factors, baseline prognostic factors, and MGMT methylation status are shown in Table 3. There was a 15% reduction in the hazard of death (hazard ratio 0.85; 95% CI 0.56–1.31; $p = 0.46$) for patients with methylated MGMT compared to those with unmethylated MGMT after adjusting for age, KPS, extent of resection, TMZ and RT. A subgroup analysis was performed among 35 patients who were aged 18–70 years and treated with Gliadel, RT and TMZ (Gliadel plus Stupp's regimen) [1]. The median OS was 19.8 months (95% CI 14.5–22.2) in this subset of patients. There was no statistically significant difference in OS in this age group when comparing MGMT promoter methylation (median OS 20 months; 95% CI 9.2–37.0) with unmethylated MGMT promoter patients (median OS 18.9 months; 95% CI 11.9–22.2; Table 4). There were 38 patients with an unknown MGMT methylation status, who had a median OS of 16 months (95% CI 10.0–22.0) and median RFS of 10.4 months (95% CI 6.9–22.0)

Only two out of 30 elderly patients aged over 70 years were treated with TMZ, one of whom had methylated MGMT and the other unmethylated. The elderly patient with MGMT promoter methylation showed a significantly longer median survival of 13.5 months (95% CI 0.49–17.1) compared to 7.6 months (95% CI 2.9–9.4; $p = 0.027$) in the other. A similar significant difference was found for the median RFS, with 13.1 *versus* 7.6 months ($p = 0.01$) for methylated and unmethylated MGMT, respectively. The median OS and RFS were 9.8 and 5.1 months, respectively, among the eight patents with an unknown MGMT status and age above 70 years.

The median RFS was 9.7 months (95% CI 8.1–11.5) for the whole cohort (n = 160). There was no difference in RFS between the 18–70-year-old patients with and without MGMT methylation.

Table 3
Univariate and multivariate analyses of patient characteristics and survival

Characteristic	Hazard ratio (95% CI)	p value
Univariate		
Age >70 *versus* <50 years	3.54 (1.90–6.59)	0.0001
Age 50–70 *versus* <50 years	1.56 (0.91–2.670	0.11
Biopsy *versus* craniotomy	1.05 (0.64–1.72)	0.84
MGMT unmethylated *versus* methylated	1.04 (0.69–1.55)	0.86
KPS 50–80 *versus* 90–100	1.02 (0.69–1.51)	0.93
RT unknown *versus* known	1.84 (1.18–2.88)	0.008
No TMZ *versus* TMZ	1.90 (1.24–2.91)	0.003
Multivariate		
Age >70 *versus* $\leqslant$70 years	2.13 (1.30–3.48)	0.003
KPS $\leqslant$80 *versus* 90–100	1.03 (0.69–1.53)	0.8
No GTR *versus* GTR	1.04 (0.63–1.70)	0.9
MGMT methylated *versus* unmethylated	0.85 (0.56–1.31)	0.46
No TMZ *versus* TMZ	1.65 (1.03–2.64)	0.037

CI = confidence interval, GTR = gross total resection, KPS = Karnofsky performance score, MGMT = O^6-methylguanine-methyltransferase, RT = radiotherapy, TMZ = temozolomide.

Table 4
Median OS comparison

Age, years	Study	Treatment	Methylated MGMT	Patients, n	Median OS (95% CI), months
⩽70	EORTC	RT + TMZ		287	14.6 (13.2–16.8)
			Y	46	21.7 (17.4–30.4)
			N	60	12.7 (11.6–14.4)
	Hopkins	Gliadel[a] + RT + TMZ		35	19.8 (14.5–22.2)
			Y	12	20.0 (9.2–37.0)
			N	23	18.9 (11.9–22.2)
>70	Hopkins	Gliadel[a] + RT		30	9.0 (4.9–10.9)
			Y	9	13.5 (0.49–17.1)
			N	19	7.6 (2.9–9.4)

[a] Gliadel; Eisai, Tokyo, Japan.
CI = confidence interval, EORTC = European Organization for Research and Treatment of Cancer Phase III trial, MGMT = O^6-methylguanine-methyltransferase, N = no, OS = overall survival, RT = radiotherapy, TMZ = temozolomide, Y = yes.

4. Discussion

We investigated the significance of MGMT methylation status in a series of 122 patients with newly diagnosed GBM, who underwent surgical resection and implantation of Gliadel wafers. The results of our series show a reduction in the hazard of death for patients who had MGMT methylation compared to those with unmethylated MGMT. Interestingly, this effect was much more profound in the patients who were aged >70 years at GBM diagnosis. The elderly patients with methylated MGMT had significantly longer OS compared to those with unmethylated MGMT (13.5 *versus* 7.6 months; $p = 0.027$).

Methylation of the MGMT promoter region leads to a reduced ability to repair the DNA damage that is induced by alkylating chemotherapeutic agents [6]. Methylation of the MGMT promoter was found to be associated with responsiveness to alkylating chemotherapeutic agents such as TMZ [1] and BCNU [6], and led to an increase in OS and PFS. The median OS of patients in our cohort who received Gliadel, TMZ and RT ranged from 18.9–20 months, which is 6 months greater than that of an historical RT and TMZ-treated cohort (Fig. 1) [1]. For patients younger than 70 years, the median OS of the methylated MGMT subgroup was slightly greater than that of the unmethylated MGMT group.

In related work, strategies have been designed to deplete tumor alkylguanine DNA alkyltransferase (AGT) levels before therapy with carmustine. O^6-benzyl guanine (O^6-BG) is an AGT substrate that inactivates AGT and enhances alkylnitrosourea activity *in vitro* and *in vivo* [8]. Phase I studies have been completed with intravenous O^6-BG and either systemically delivered BCNU or BCNU delivered via Gliadel, to establish the maximal tolerated dose in patients with recurrent or progressive malignant glioma [9]. A Phase II clinical study in patients with recurrent or progressive malignant gliomas included treatment with concomitant infusion of O^6-BG and systemically delivered carmustine [10,11]. The results indicated that there was dose limiting toxicity of BCNU, and that tumor regression was not significant. In a Phase II trial of Gliadel with O^6-BG in patients with recurrently malignant glioma, the efficacy of Gliadel was improved, with a 6 month OS of 82% and 1 and 2 year OS rates of 47 and 10%, respectively [12]. Future directions could include the investigation of a sequential combination of O^6-BG, Gliadel and effective systemic chemotherapies.

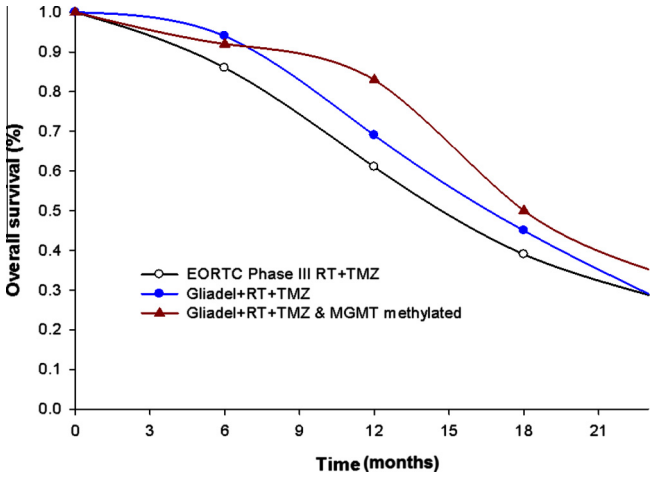

Fig. 1. Kaplan–Meier survival curve for overall survival of patients who had promoter methylation of O^6-methylguanine-methyltransferase (MGMT) and were treated with Gliadel (Eisai, Tokyo, Japan), radiation therapy (RT) and temozolomide (TMZ) (red line) *versus* patients who were treated with Gliadel, RT and TMZ (blue line) *versus* GBM patients in the European Organization for Research and Treatment of Cancer (EORTC) Phase III trial (black line).

KPS in a known prognostic factor for patients with brain tumors [13]. Most of the patients in our study cohort had a poor KPS of <80. Regardless, our results were in line with the report by Lechapt-Zalcman et al. [14], which assessed the prognostic impact of MGMT promoter methylation in patients with newly diagnosed GBM who received Gliadel in addition to RT and TMZ. The OS of their study cohort was 17.5 months. The patients with MGMT methylation had a significantly longer OS of 21.7 months compared with patients without MGMT methylation, who had an OS of 15.1 months.

Two recent Phase III clinical trials in elderly patients with malignant astrocytoma, the NOA-08 [15] and Nordic trials [16], have demonstrated that TMZ therapy alone is not inferior to RT alone, and methylation of the MGMT gene promoter was associated with a benefit from TMZ. However, there is a concern that the combination of RT and TMZ may be less active and less well tolerated in the elderly population [17]. The European Organization for Research and Treatment of Cancer-26981/National Cancer Institute of Canada CE3 trial has suggested that with increasing age, the relative benefit of the addition of TMZ to RT decreases, and patients suffer from increased chemotherapy-associated side effects, including neutropenia, lymphocytopenia, thrombocytopenia, raised liver enzyme concentrations, infections and thromboembolic events. As opposed to the limitations of systemic chemotherapy, the local delivery of Gliadel wafers may improve the side effects in this subset of patients. Chaichana et al. compared 45 elderly patients who were treated with Gliadel to 88 who did not [18]. The survival of the Gliadel-treated patients was significantly longer than for patients who did not receive Gliadel (8.7 *versus* 5.5 months; $p = 0.007$). In our cohort, the median survival of elderly patients with methylated MGMT was doubled. These results provide support for the use of Gliadel in this subpopulation.

4.1. Limitations

There are several limitations to this study. Its retrospective nature carries a potential bias. Moreover, the time period of this study ended in 2006, only 1 year after TMZ became a common treatment for GBM. Therefore, most of the patients were not treated with the combination therapy of TMZ and RT. Furthermore, because it was conducted at a tertiary referral center, there are a large number of patients who were operated in this center but received further neuro-oncology treatments elsewhere. Therefore, their complementary oncology treatment information was not available.

Quantitative methylation-specific PCR is a robust quantitative method, but like all assays it has some limitations. The assay has an absolute sensitivity of about 7–8 methylated molecules, therefore, it cannot detect very rare methylated alleles. In addition, the assay was performed on only one tumor section. The methylation status of different regions due to tumor heterogeneity was not assessed. Additional techniques such as digital PCR could detect very rare alleles and quantify them throughout the tumor specimen. However, the value of rare allele detection and heterogeneity for clinical validation has not been shown. Regardless, this large and unique cohort of patients with newly diagnosed GBM, who were operated on in a single tertiary center, provides novel data that may assist in optimizing and personalizing treatments for GBM.

5. Conclusion

Our results show a reduction in the hazard of death for patients with methylated MGMT compared to unmethylated. This effect was more profound in the elderly (>70 years old). MGMT methylation showed a significantly better OS and RFS compared to unmethylated MGMT. These results may support Gliadel use in this subpopulation, since accumulating data indicate that with increasing age, the benefit of adding an alkylating agent such as TMZ to RT decreases, and may be less well tolerated. Gliadel, as opposed to systemic chemotherapy, may be a promising therapeutic in this subset of patients, and has the potential to not only prolong survival but also to improve the quality of life.

Conflicts of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

References

[1] Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997–1003.
[2] 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 1995;345:1008–12.
[3] 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 2003;5:79–88.
[4] Fleming AB, Saltzman WM. Pharmacokinetics of the carmustine implant. Clin Pharmacokinet 2002;41:403–19.
[5] Fung LK, Ewend MG, Sills A, et al. Pharmacokinetics of interstitial delivery of carmustine, 4-hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer implant in the monkey brain. Cancer Res 1998;58:672–84.
[6] 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 2000;343:1350–4.
[7] Dinse GE, Lagakos SW. Nonparametric estimation of lifetime and disease onset distributions from incomplete observations. Biometrics 1982;38:921–32.
[8] Rhines LD, Sampath P, Dolan ME, et al. O_6-benzylguanine potentiates the antitumor effect of locally delivered carmustine against an intracranial rat glioma. Cancer Res 2000;60:6307–10.
[9] Friedman HS, Pluda J, Quinn JA, et al. Phase I trial of carmustine plus O_6-benzylguanine for patients with recurrent or progressive malignant glioma. J Clin Oncol 2000;18:3522–8.
[10] Weingart J, Grossman SA, Carson KA, et al. Phase I trial of polifeprosan 20 with carmustine implant plus continuous infusion of intravenous O_6-benzylguanine in adults with recurrent malignant glioma: new approaches to brain tumor therapy CNS consortium trial. J Clin Oncol 2007;25:399–404.
[11] Quinn JA, Pluda J, Dolan ME, et al. Phase II trial of carmustine plus O(6)-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma. J Clin Oncol 2002;20:2277–83.
[12] Quinn JA, Jiang SX, Carter J, et al. Phase II trial of Gliadel plus O_6-benzylguanine in adults with recurrent glioblastoma multiforme. Clin Cancer Res 2009;15:1064–8.
[13] Soffietti R, Chio A, Giordana MT, et al. Prognostic factors in well-differentiated cerebral astrocytomas in the adult. Neurosurgery 1989;24:686–92.
[14] Lechapt-Zalcman E, Levallet G, Dugue AE, et al. O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation and low MGMT-encoded protein expression as prognostic markers in glioblastoma patients treated with biodegradable carmustine wafer implants after initial surgery followed by radiotherapy with concomitant and adjuvant temozolomide. Cancer 2012;118:4545–54.
[15] 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 2012;13:707–15.
[16] 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 2012;13:916–26.
[17] Tabatabai G, Stupp R, Wick W, et al. Malignant astrocytoma in elderly patients: where do we stand? Curr Opin Neurol 2013;26:693–700.
[18] Chaichana KL, Zaidi H, Pendleton C, et al. The efficacy of carmustine wafers for older patients with glioblastoma multiforme: prolonging survival. Neurol Res 2011;33:759–64.