Preliminary Individualized Chemotherapy for Malignant Astrocytomas Based on O⁶-Methylguanine-Deoxyribonucleic Acid Methyltransferase Methylation Analysis

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Abstract

*O*⁶-methylguanine-deoxyribonucleic acid methyltransferase gene (MGMT) methylation is apparently correlated with responsiveness to nitrosourea chemotherapy, suggesting this alkylating agent should be effective against MGMT-methylated tumors. MGMT appears not to be linked to platinum resistance, so platinum chemotherapy should be used for MGMT-unmethylated tumors. This study was a preliminary trial of individualized chemotherapy based on MGMT methylation status in a total of 20 patients with newly diagnosed malignant astrocytomas (9 anaplastic astrocytomas and 11 glioblastomas multiforme). The procarbazine, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-2(2-chloroethyl)-3-nitrosourea, and vincristine (PAV) regimen was administered to seven patients with MGMT-methylated tumors, and the carboplatin and etoposide (CE) regimen was administered to 13 patients with MGMT-unmethylated tumors. Objective response to the PAV therapy was noted in all three patients with measurable residual tumor (2 complete responses and 1 partial response). Five of the seven patients continued to be disease-free after initiation of the PAV therapy. Objective response to the CE therapy was seen in only one of seven patients with measurable residual tumor (1 partial response). Three of the 13 patients were free from progression, whereas the remaining 10 patients showed early progression. The PAV regimen is effective against MGMT-methylated malignant astrocytomas, but the CE regimen is not useful at the given dose and schedule in MGMT-unmethylated tumors.

Key words: anaplastic astrocytoma, chemotherapy, glioblastoma multiforme, *O*⁶-methylguanine-deoxyribonucleic acid methyltransferase methylation

Introduction

Alkylating nitrosoureas are the main chemotherapeutic agents for the treatment of malignant astrocytomas (anaplastic astrocytoma [AA] and glioblastoma multiforme [GBM]) and have been shown to improve survival modestly.²⁰⁾ The effectiveness of these agents is frequently reduced by inherent or acquired drug resistance. *O*⁶-methylguanine-deoxyribonucleic acid (DNA) methyltransferase (MGMT) is a major factor in increasing the resistance of glioma cells to alkylating chemotherapeutic agents, since the transfer of cytotoxic alkyl adducts from the O⁶ position of guanine to an internal MGMT cytosine prevents the cross-linking of double-stranded

DNA by alkylating agents.⁷⁾

The presence of MGMT methylation is associated with increased sensitivity to nitrosourea chemotherapy and/or a better survival in patients with malignant astrocytomas.^{2,5)} MGMT activity is controlled by a promoter, and methylation of the CpG islands in the MGMT promoter region prevents transcription of the gene.⁶⁾ Our previous investigation of a homogeneous cohort of patients with malignant astrocytomas treated with 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-2(2-chloroethyl)-3-nitrosourea (ACNU) also revealed differences in clinical response and survival according to MGMT methylation status.²⁴⁾ Such observations suggest a rationale for tailored chemotherapeutic strategies: patients with MGMT-methylated tumors undergo a more intensive nitrosourea-based chemotherapy containing other alkylating agents, whereas patients with

Table 1 Summary of 20 patients treated with individualized chemotherapy

Case No.	Age (yrs)/ Sex	Histology	Location	Chemotherapy	Cycles	Response	PFS (mos)	OS (mos)
MGMT-unmethylated tumors:								
1	52/F	GBM	lt parietal	CE	3	SD	6	16
2	54/M	GBM	rt frontal	CE	2	PD	0	7
3	58/F	GBM	lt parietal	CE	3	SD	6	12
4	65/M	GBM	lt frontal	CE	2	PD	0	13
5	44/M	AA	rt frontal	CE	8	NE	20	24 +
10	66/F	GBM	rt temporal	CE	1	PD	0	10
11	64/M	GBM	rt temporal	CE	3	SD	7	13
12	39/M	AA	lt frontal	CE	8	NE	22 +	22 +
15	29/F	AA	lt parietal	CE	8	PR	16 +	16 +
16	53/F	GBM	lt temporal	CE	6	NE	12	15 +
17	32/M	AA	rt frontal	CE	6	NE	11	14 +
18	60/F	GBM	lt temporal	CE	2	NE	5	9
19	24/F	GBM	rt occipital	CE	6 +	NE	13 +	13 +
MGMT-methylated tumors:								
6	67/M	GBM	rt thalamus	PAV	4	PR	10	16
7	38/F	AA	lt frontal	PAV	8	CR	28 +	28 +
8	58/M	AA	rt temporal	PAV	8	CR	24 +	24 +
9	61/F	GBM	rt occipital	PAV	6	NE	16	24 +
13	49/F	AA	rt parietal	PAV	8	NE	21 +	21 +
14	60/M	AA	lt frontal	PAV	7 +	NE	19 +	19 +
20	66/F	AA	lt frontal	PAV	5 +	NE	12 +	12 +

AA: anaplastic astrocytoma; CE: carboplatin and etoposide; CR: complete response; GBM: glioblastoma multiforme; MGMT: *O*⁶-methylguanine-deoxyribonucleic acid methyltransferase gene; NE: not able to be evaluated; OS: overall survival; PAV: procarbazine, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-2(2-chloroethyl)-3-nitrosourea (ACNU), and vincristine; PD: progressive disease; PFS: progression-free survival; PR: partial response; SD: stable disease.

MGMT-unmethylated tumors receive chemotherapeutic regimens not affected by MGMT-related resistance mechanisms.

Platinum compounds such as cis-diamminedichloroplatinum (cisplatin; CDDP) and cis-diammine-1,1-cyclobutane-dicarboxylatoplatinum II (carboplatin; CBDCA) are some of the most potent antitumor agents against a wide variety of solid tumors. Platinum compounds given as a single drug or in combination with epipodophyllotoxin etoposide (VP-16) failed to improve survival in patients with malignant astrocytoma compared to nitrosourea-based chemotherapy when employed as the first adjuvant modality.^{8,11,16)} However, combination chemotherapy with CDDP or CBDCA and VP-16 as the second line chemotherapy following nitrosourea failure has shown encouraging results.^{1,10,23)} Furthermore, the antitumor activity of platinum compounds is not related to MGMT activity,^{3,17)} so platinum compounds are good candidates for the treatment of MGMT-unmethylated tumors.

The present study instituted a preliminary trial of individualized chemotherapy for malignant astrocytoma according to MGMT methylation status.

Patients with MGMT-methylated tumors were treated with procarbazine, ACNU, and vincristine (PAV) chemotherapy, whereas patients with MGMT-unmethylated tumors were treated with CBDCA and VP-16 (CE) chemotherapy. The feasibility and efficacy of such individualized chemotherapy was assessed as the first adjuvant modality combined with conventional radiation and interferon therapy.

Patients and Methods

I. Patient population

Twenty patients with malignant astrocytomas, nine men and 11 women aged 24 to 67 years (median 56 years), were enrolled between April 2002 and December 2003 in this prospective study designed to evaluate the efficacy of individualized adjuvant chemotherapy according to MGMT methylation status. The histological diagnosis was AA in nine patients and GBM in 11 patients (Table 1). The eligibility requirements included the following criteria: new histological diagnosis of supratentorial AA or GBM classified according to the World Health Organization (WHO),¹²⁾ age of 19 years or

older, Karnofsky performance status score of at least 40%, presence of contrast enhancement on preoperative T₁-weighted magnetic resonance (MR) imaging, adequate major organ function (leukocyte count of greater than 3500/ μ l, platelet count of greater than 130,000/ μ l, serum creatinine level of less than 1.5 mg/dl, and serum bilirubin and aminotransferase levels of less than two times the upper normal limit), and written informed consent. The study protocol was approved by the Clinical Research Ethics Committee.

II. Identification of MGMT methylation status

Genomic DNA was extracted from at least two frozen samples from different tumor sites using a QIAamp[®] DNA Mini Kit (QIAGEN Inc., Chatsworth, Calif., U.S.A.). Promoter hypermethylation of the MGMT gene was determined by the methylation-specific polymerase chain reaction (PCR).⁹⁾ Sodium bisulfite modification was performed with a CpGenome[™] DNA Modification Kit (Intergen, Oxford, U.K.) as described previously.^{26,27)} The primer sequences of MGMT for the methylated and unmethylated reactions were reported previously.⁶⁾ PCR was carried out as described previously.^{13,24)} Amplified products were electrophoresed on 3% agarose gels, and were visualized with ethidium bromide. CpGenome Universal Methylated DNA (Intergen) and normal blood DNA were included in each PCR experiment as methylated and unmethylated controls, respectively.

III. Treatment plan

The chemotherapy regimen was initiated within 2 weeks after surgery. Patients with MGMT-methylated tumors received 120 mg/m² ACNU intravenously on day 1, 60 mg/m² procarbazine orally on days 8 to 21, and 1.5 mg/m² vincristine (max. 2 mg) intravenously on days 8 and 29. Patients with MGMT-unmethylated tumors received 300 mg/m² CBDCA intravenously on day 1 and 60 mg/m² VP-16 orally on days 1 to 5. The chemotherapy regimen was repeated every 6 weeks for a total of eight cycles unless the disease progressed.

Beginning on day 1, adjuvant radiation therapy was given by delivering using 2.0 Gy per fraction five times a week to total doses of 50 to 60 Gy. The radiation field covered the high-intensity area on T₂-weighted MR images including the enhanced tumor mass on T₁-weighted images. Human fibroblast interferon natural beta type (IFN- β) at a dose of 2×10^6 IU/m² was administered intravenously three to five times weekly during the radiation therapy course, as reported previously.²⁵⁾ Following completion of the radiation therapy, intravenous adminis-

tration of IFN- β was repeated at 2-week intervals.

Postoperative MR imaging with contrast medium was performed within 72 hours of the operation. The extent of surgery was assessed as follows: absence of contrast enhancement on postoperative T₁-weighted MR imaging was defined as radical surgery; and presence of remnant enhancement was defined as palliative surgery. The treatment responses were assessed by comparing the immediate postoperative MR images with follow-up images obtained using the same scanner, with standard cross-sectional diameter measurements of the enhanced tumor. Complete response (CR) was defined as total disappearance of all enhanced tumor on consecutive MR images at least 1 month apart. Partial response (PR) was defined as 50% or more reduction in the area of the enhanced tumor on consecutive MR images at least 1 month apart. Stable disease (SD) was defined as no change or less than 50% reduction or less than 25% increase in the area of the enhanced tumor. Progressive disease (PD) was defined as 25% or more increase in the area of the enhanced tumor or the appearance of new lesions. In situations where there had been no measurable residual tumor following total resection, patients were considered as being not able to be evaluated. MR images with contrast medium were obtained every 2 weeks for the duration of the radiation therapy and every 2–3 months thereafter.

IV. Statistical analysis

Progression-free survival (PFS) as the time period from the start of chemotherapy to the point when radiographic evidence of PD was noted. If PD was evident at the time of the first evaluation, the PFS was set at zero. The overall survival (OS) was defined as the interval between the start of chemotherapy and the date of death or the most recent evaluation. The Kaplan-Meier method was employed to calculate the PFS and OS. Analyses used a personal computer running Stat View J-5.0 software (Abacus Concepts, Berkeley, Calif., U.S.A.).

Results

I. Patient outcomes

Cytoreductive surgery was performed in all patients, except for stereotactic biopsy in one patient (Case 6). All patients completed the planned radiation therapy. Total IFN- β administered was $28\text{--}142 \times 10^6$ IU/m² (mean 79.6×10^6 IU/m²). Corticosteroids were given to seven patients (Cases 2, 6, 10, 11, 13, 16, and 18) with intracranial hypertension. Methylation-specific PCR for MGMT promoter

was evaluated in all 20 patients. Thirteen tumors were MGMT-unmethylated, and seven tumors were MGMT-methylated.

The response to adjuvant therapy could be assessed in 10 of the 20 patients. Two patients exhibited CR, two had PR, three had SD, and three exhibited PD, giving an overall objective response rate of 40%. The objective response rate was 100% (3/3) for AA and 14% (1/7) for GBM.

At the end point of observation (January 10, 2005), eight patients were still alive without PD, four were alive with PD, and eight had died of PD. Among the 12 survivors, the duration of follow-up evaluations (from the onset of chemotherapy) ranged from 12 to 28 months (median 18 months). Overall, the estimated median PFS was 12 months with a 1-year PFS rate of 55%, and the 1-year OS rate was 85%. For patients with AA, the 1-year PFS rate was 89%, and the 1-year OS rate was 100%. For patients with GBM, the median PFS was 6 months with a 1-year PFS rate of 27%, and the median OS was 13 months with a 1-year OS rate of 73%.

II. MGMT-methylated tumors

All seven patients with MGMT-methylated tumors underwent PAV chemotherapy. All three patients with measurable residual tumor on immediately postoperative MR images responded to adjuvant therapy. The two patients with AA achieved CR (Fig. 1) and remained alive without disease at the most recent follow-up evaluation at 24 and 28 months after initiation of PAV chemotherapy. The patient with GBM achieved PR, but displayed evidence of progression at 10 months following commencement of the PAV administration and eventually died at 6 months after recurrence. Four patients had no measurable residual tumor. The patient with GBM showed recurrence at 16 months, whereas the three patients with AA showed no disease progression as of 12, 19, and 21 months since commencing the therapy. The 1-year PFS rate was 86%, and the 1-year OS rate was 100% in these seven patients.

A total of 46 cycles of PAV chemotherapy were administered. Three patients completed the full eight cycles of chemotherapy without progression. The protocol was discontinued in two patients because of progressive tumor. The protocol was continuing at the last follow-up examination in two patients. No patient suffered severe toxicity requiring the therapy to be discontinued. Treatment-related toxicity was assessed in accordance with the WHO criteria. Grade 3 or 4 neutropenia occurred in three patients, and grade 3 thrombocytopenia in one patient. Nausea and vomiting were controlled in all

Fig. 1 Case 7. A 38-year-old woman with O⁶-methylguanine-deoxyribonucleic acid methyltransferase gene (MGMT)-methylated anaplastic astrocytoma. Preoperative T₁-weighted magnetic resonance (MR) image with contrast medium demonstrating a huge heterogeneous enhanced tumor in the left frontal lobe (upper left). Immediately postoperative T₁-weighted MR image with contrast medium revealing residual tumor at the anterior horn of the lateral ventricle (upper right). T₁-weighted MR image with contrast medium obtained after three cycles of procarbazine, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-2(2-chloroethyl)-3-nitrosourea, and vincristine chemotherapy showing the residual enhanced lesion had completely disappeared (lower right). This patient was classified as showing complete response. The methylation-specific polymerase chain reaction (PCR) for MGMT promoter revealed hypermethylation in the tumor tissues (lower left). M: PCR product amplified by methylated-specific primers, NC: normal control, PC: positive control, S: molecular size marker, T: tumor tissue, U: PCR product amplified by unmethylated-specific primers.

patients by anti-emetic medication. No episodes of hepatic toxicity or renal toxicity were encountered.

III. MGMT-unmethylated tumors

All 13 patients with MGMT-unmethylated tumors received CE therapy. Six patients had measurable residual tumor, but no patient had CR. Only one patient with AA partially responded to the therapy and continued to respond for 16 months. Three

Fig. 2 Case 4. A 65-year-old man with *O*⁶-methylguanine-deoxyribonucleic acid methyltransferase gene (*MGMT*)-unmethylated glioblastoma multiforme manifesting as focal seizure originating from the right side of the face. Preoperative T₁-weighted magnetic resonance (MR) image with contrast medium revealing a small enhanced lesion in the left motor cortex (upper left). T₁-weighted MR image with contrast medium taken immediately after surgery demonstrating complete removal of the tumor (upper right). T₁-weighted MR image with contrast medium obtained after one cycle of carboplatin and etoposide chemotherapy showing the tumor had progressed (lower right). The methylation-specific polymerase chain reaction (PCR) for *MGMT* promoter showed no methylation in the tumor tissues (lower left). M: PCR product amplified by methylated-specific primers, NC: normal control, PC: positive control, S: molecular size marker, T: tumor tissue, U: PCR product amplified by unmethylated-specific primers.

patients with GBM exhibited SD for 6, 6, and 7 months before the tumors progressed. Three other patients with GBM including one without measurable residual tumor on the immediately postoperative MR image demonstrated progression at the first evaluation after initiation of the CE chemotherapy (Fig. 2). An objective response was identified in only one of the seven patients with measurable residual tumor (1/1 for AA and 0/6 for GBM). The patients with GBM eventually died at 7, 10, 12, 13, 13, and 16 months. Six other patients without measurable residual tumor, two with GBM and two with AA

displayed evidence of recurrence at 5, 12, 11, and 20 months, respectively, and one with GBM and one with AA were disease-free at the most recent follow-up evaluation (at 13 and 22 months, respectively). For all 13 patients, the estimated median PFS was 7 months with a 1-year PFS rate of 39%, and the estimated median OS was 16 months with a 1-year OS rate of 77%.

A total of 58 cycles of CE therapy were administered and well tolerated by all patients. Three patients completed all eight cycles of chemotherapy. The protocol was discontinued in nine patients because of progressive clinical deterioration associated with radiographic progression, and seven of these patients stopped chemotherapy early (3 courses or less). The remaining one patient continued chemotherapy without progression. None of the patients discontinued therapy because of severe toxicity. Grade 3 neutropenia occurred in two patients and grade 3 thrombocytopenia in one. Mild hepatic toxicity (grade 2) occurred in one patient. No patient developed renal or auditory toxicity. Grade 1 or 2 nausea was common but was generally well controlled with anti-emetics, but one patient experienced grade 3 nausea and vomiting.

Discussion

In the present study, PAV chemotherapy yielded favorable responses in *MGMT*-methylated malignant astrocytomas. The PAV protocol employed in the current trial is a substitute for procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine; CCNU), and vincristine (PCV) chemotherapy, as CCNU is not available in Japan. The PCV regimen is one of the most commonly used combination chemotherapies and has yielded promising results in the treatment of oligodendroglial tumors, although no clear clinical benefit has been conclusively demonstrated for patients with malignant astrocytomas in comparison with those treated by only nitrosourea.¹⁴⁾ Our data suggest that such combination chemotherapy may be effective in the treatment of patients with *MGMT*-methylated malignant astrocytomas. In contrast, the CE regimen at the dose schedule employed in the present study exhibited only modest activity against *MGMT*-unmethylated tumors.

A similar chemotherapeutic approach for diffuse gliomas was based on *MGMT* messenger ribonucleic acid expression: patients with *MGMT*-negative tumors were treated with ACNU, whereas patients with *MGMT*-positive tumors were treated with CBDCA or CDDP.²¹⁾ A high rate (over 50%) of responses were observed to adjuvant chemotherapy

in combination with interferon and radiation therapy irrespective of the chemotherapeutic regimens. However, levels of MGMT expression can be affected by contamination from normal cells such as endothelial cells, reactive astrocytes, and infiltrating lymphocytes, which express considerable amounts of MGMT. In addition, individual tumors appear to secrete varying amounts of MGMT.⁷⁾ Since the MGMT promoter is unmethylated in normal brain tissue adjacent to tumors,⁶⁾ the methylation-specific PCR assay has advantages for accurate assessment of MGMT and could be employed routinely in the clinical setting.

The most likely explanation for the failure to achieve tumor regression in MGMT-unmethylated tumors is an inadequate dose and schedule for the CE regimen. The present study adopted a dose schedule similar to that proposed for treating recurrent malignant astrocytomas.²³⁾ Despite using a less-intensive dosage as compared to other clinical studies,^{1,10)} PR was achieved in seven of 28 malignant astrocytomas. However, initial growth arrest was observed following five or six courses, and achieving tumor regression required eight courses on average. Considering that most of our patients with GBM who received the CE regimen showed early progression, as well as the relatively well-tolerated safety profile, a much more intensive dose and a tighter schedule seem to be applicable.

The chemotherapy of MGMT-unmethylated tumors could also be improved by innovative drug regimens, such as the novel alkylating agent, temozolomide, which has recently been shown to be effective against malignant astrocytomas. In vitro, CDDP can enhance the activity of temozolomide through the inhibition of MGMT activity,⁴⁾ which would suggest a combination of temozolomide with CDDP as a novel chemotherapeutic approach. The efficacy of CDDP plus temozolomide was recently demonstrated in patients with malignant gliomas who had developed recurrence after nitrosourea treatment.¹⁹⁾ Depletion of MGMT by O⁶-benzylguanine before receiving alkylating agents is also an attractive prospect for the treatment of MGMT-unmethylated tumors, although a recent phase II trial of carmustine plus O⁶-benzylguanine failed to achieve tumor regression in nitrosourea-resistant malignant gliomas.¹⁸⁾

A recent experimental study has demonstrated that the induction of MGMT by dexamethasone was associated with increased resistance to ACNU in glioma cells.²²⁾ Conversely, IFN- β down-regulates the expression of MGMT, enhancing chemotoxicity against glioma cells.¹⁵⁾ Therefore, the real responsiveness to adjuvant chemotherapy might be masked

by these therapeutic agents. In addition, radiation therapy was concomitantly administered, which makes the therapeutic contribution of chemotherapy even more difficult to elucidate. Most importantly, the number of our patients was too small to provide conclusive evidence. A larger cohort of patients treated with well-designed chemotherapeutic strategies is needed to assess the exact contribution of individualized adjuvant chemotherapy.

This preliminary trial of individualized chemotherapy suggests that MGMT-methylated malignant astrocytomas are responsive to the PAV regimen. CE chemotherapy at the given dose and schedule seem unlikely to offer a promising regimen for MGMT-unmethylated tumors. Strategies for MGMT-unmethylated tumors will need to examine various dose schedules and combinations with other agents, as well as incorporate improved understanding of the molecular basis underlying the mechanisms of resistance to platinum compounds.

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Commentary

Most malignant astrocytomas are resistant to chemotherapeutic agents because of the presence of several mechanisms or substances such as the blood-brain barrier, genes, and proteins. Recently, many studies have been started to overcome the chemoresistance. Especially recent advances in the field of molecular biology have contributed to the examination of the chemosensitivities of tumor cells. Trial for the individualization of the treatment, so-called tailor-made therapy, is one of these challenges.

O⁶-methylguanine-DNA methyltransferase (MGMT)

is a DNA repair enzyme which reduces the cytotoxic effect of nitrosourea. In order to overcome chemoresistance, drugs except nitrosourea or some drugs which reduce the MGMT activity are used for tumors expressing MGMT. In this paper, the author found that the PAV regimen is effective against MGMT-methylated malignant astrocytomas, but the CE regimen is not useful at the given dose and schedule in MGMT-unmethylated tumors based on MGMT. Individualized chemotherapy for malignant astrocytomas by its specific biological character can improve the efficiency of agents which are valuable in clinical use. This study can be a model of clinical trials for malignant brain tumors. We expect larger clinical trials in the future.

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This article presents interesting initial data on the individualized treatment of patients with malignant gliomas (9 anaplastic astrocytomas and 11 glioblastoma multiforme). The authors suggest that PAV therapy is effective in MGMT-methylated astrocytomas. This work is provocative but, unfortunately, is difficult to make definitive conclusions in that the 20 patients are subdivided into anaplastic astrocytoma and glioblastoma, each of which have different expected responses and outcomes. These are further subdivided into MGMT-methylated and MGMT-unmethylated. This leaves very small numbers in each group. Moreover, of the MGMT-methylated tumors, there are only two glioblastomas, and one of these had only a biopsy (Case 6) whereas the other had a resection but was listed as NE, or not able to be evaluated (Case 9). Indeed, of the seven MGMT-methylated tumors, more (4) are listed as "not able to be evaluated" than those that were evaluable (3). Thus it is difficult with such small numbers to come to a definitive conclusion.

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The study by Watanabe et al. presents the preliminary results of a clinical trial of 20 patients with newly diagnosed malignant astrocytoma, in which MGMT methylation status was used to personalize drug selection. Administration of PAV to patients with MGMT-methylated tumors yielded favorable results in five of seven patients.

Compared to the previous reports, the current study

uses MGMT methylation status, which has more supporting evidence as a predictive factor than mRNA expression or protein levels. Although the number of patients is small and response to adjuvant therapy could only be assessed in half of the patients, it is only a preliminary study as the authors point out. The data is still valuable since this is one of the first attempts at individually tailored therapy based on a validated independent prognostic factor.

Use of interferon and corticosteroids could, as pertinently discussed, mask the real responsiveness to adjuvant therapy. However, since steroids are usually a part of glioma management, accumulating data and analyzing it seems more realistic than complete exclusion of steroids.

The unmethylated tumor group had poor response to CE. The authors trace the failure to achieve tumor regression to an inadequate dosing and scheduling of the regimen. It may be due to the fact that the majority of patients in the group had GBM (vs. AA in methylated group).

The authors' discussion of alternatives is comprehensive. A more aggressive CE regimen, the use of O⁶-benzylguanine (BG) to deplete MGMT or of a platinum-alkylating agent combination could be the answer for the unmethylated tumor group. Another combination in trial is topoisomerase I inhibitor + alkylating agent, the expected mechanism of action being a change in kinetics of topoisomerase I-DNA covalent complexes induced by O⁶-MG adducts. A phase II study of irinotecan + BCNU for second-line chemotherapy in GBM patients reported a 21.4% partial response rate and 50% stable disease rate.¹⁾ O⁶-BG + irinotecan + temozolomide has shown dramatic results for MGMT-positive glioma cells²⁾ and is now in phase I trial.

References

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