Clinical response of O⁶-methylguanine-DNA methyltransferase levels to 1,3-(2-chloroethyl)-1-nitrosourea chemotherapy in glioma patients

Zhong-Ping Chen, M.D., Ph.D., Daniel Yarosh, Ph.D., Yesenia Garcia, B.A., Donatella Tampieri, M.D., Gérard Mohr, M.D., Adrian Langleben, M.D., and Lawrence C. Panasci, M.D.

Divisions of Medical Oncology and Neurosurgery, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, Canada; Montreal Neurological Institute, Montreal, Quebec, Canada; Division of Medical Oncology, Royal Victoria Hospital, Montreal, Quebec, Canada; and Applied Genetics Inc., Freeport, New York

Adjuvant nitrosourea chemotherapy fails to prolong patient survival significantly as many tumors demonstrate resistance to these drugs. It has been documented in cell lines that O⁶-methylguanine-DNA methyltransferase (MGMT) plays an important role in chloroethylnitrosourea (CENU) drug resistance.

The authors evaluated MGMT expression in 22 glioma specimens by using an immunofluorescence assay and compared the results with clinical response of the patients to CENU-based chemotherapy.

The patients were treated with CENU after evidence of progressive disease following surgery and radiotherapy. Eight tumor samples had no detectable MGMT, whereas other samples had from 9989 to 982,401 molecules/nucleus. In one group (12 patients), the tumor decreased in size or was stable (effective group), whereas in the other group (10 patients), the tumor demonstrated continuous growth during chemotherapy (progressive group). The median time to progression (TTP) was 6.7 months with a median survival of 13 months. The Mer⁻ patients (MGMT < 60,000 molecules/nucleus) appeared to have more chance of stable disease or response to CENU therapy than the Mer⁺ patients (MGMT > 60,000 molecules/nucleus) (chi-square = 4.791, p = 0.0286). In patients with glioblastomas multiforme (GBMs), the TTP of Mer⁺ patients was shorter than that of Mer⁻ patients (t = 2.04, p = 0.049). As a corollary, the MGMT levels were significantly higher in GBM tumors from the progressive group than those from the effective group (t = -2.26, p = 0.029). The TTP and survival time in the effective GBM group were also longer than those in the progressive GBM group. However, there was no significant correlation between MGMT levels and either the survival time (r = 0.04, p = 0.8595) or TTP (r = 0.107, p = 0.6444).

Results from this study suggested that MGMT positivity is indicative of more aggressive disease that progresses more rapidly when exposed to CENU therapy. However, MGMT-negative tumors are not always sensitive to CENU agents, suggesting that other factors may also be important.

Key Words * O⁶-methylguanine-DNA methyltransferase * brain tumor * drug resistance

In treatment for malignant brain tumors, adjuvant radiotherapy plus chemotherapy with chloroethylnitrosoureas (CENUs) alone or in combination has produced modest increase in survival as compared to radiotherapy alone.[31] Chlorethylnitrosoureas have long been used as front-line drugs for malignant gliomas. However, adjuvant nitrosourea chemotherapy only has a minimal effect on survival time because many tumors are resistant to these drugs.

Chlorethylnitrosoureas act by releasing a chloroethyldiazonium ion that alkylates several sites in DNA, the most important of which is at the O⁶-position of deoxyguanosine. This chloroethyl adduct undergoes an intramolecular circularization and then crosslinks the DNA, producing a lethal lesion.[19,34]

O⁶-methylguanine-DNA methyltransferase (MGMT) can transfer the alkyladduct from O⁶-guanine to a cysteine residue at position 145 of the alkyltransferase peptide chain, thereby inactivating the alkyladduct.[26,35] By repairing O⁶-alkylguanine DNA adducts formed by CENUs, MGMT prevents further formation of DNA crosslinks that decrease the cytotoxicity of CENUs.[7] This stoichiometric "suicide" method of repair is limited by the number of alkyltransferase molecules available. It has been documented in tumor cell lines and xenografts that MGMT plays an important role in nitrosourea drug resistance.[7,13-15,29,30] Free base inhibitors such as O⁶-benzylguanine inactivate the alkyltransferase molecules in the cells and therefore reverse CENU drug resistance.[6,28,29] Clinical trials are currently underway to determine its efficacy;[18] however, there have been only a few clinical investigations of MGMT expression in brain tumor samples in relation to patient survival and/or clinical response to chemotherapy.[5,23,25]

Traditionally, MGMT levels have been determined by bioenzymatic assay in which fresh tissue is used.[7] The characterization of the **MGMT** gene has made it possible to determine MGMT expression by RNA and DNA blot analyses and the reverse transcription-polymerase chain reaction techniques.[9,23,32] However, determining MGMT expression with cellular extracts may be misleading because they may contain normal tissues that influence the results. Immunofluorescence assays have the advantage of allowing quantitation of MGMT activity in only the malignant cells. Furthermore, one can use paraffin-embedded specimens.[4,11] In the present study, we evaluated MGMT expression in human glioma specimens by an immunofluorescence assay and compared these results with the responses of patients to CENU-based chemotherapy.

CLINICAL MATERIAL AND METHODS

Clinical Data

There were 22 patients with gliomas (13 men and nine women) involved in this study ranging in age from 32 to 71 years (mean 50 years). Diagnosis was confirmed by routine pathological examination. Paraffin-embedded slides were used for MGMT detection (details following). All patients underwent radiotherapy after resective surgery. Following radiotherapy, chemotherapy was begun when there was evidence of progression defined on computerized tomography (CT) or magnetic resonance (MR) imaging. Either BCNU (200 mg/m²) or PCV (CCNU 110 mg/m² at Day 1; procarbazine 60 mg/m² Days 8-21; and vincristine 1.4 mg/m² Days 8 and 29) was given every 6 to 8 weeks (Table 1). The CT and/or MR images were obtained before and after chemotherapy to measure changes in tumor size. A reduction in the tumor mass greater than 25% but less than 50% was considered an improvement, whereas a reduction greater than or equal to 50% was considered a partial response. An increase in tumor size of more than 25% was considered progression. The tumor was considered stable if it neither increased nor decreased by 25% or more. Patients received one to five cycles of treatment and were followed for 2 to

43 months (Table 1). Patient response to chemotherapy was also evaluated by the time to progression (TTP), which was the time in months from the date of first treatment with CENU until the date that the patient developed progressive disease. Survival time was determined from the date of initial chemotherapy.

TABLE 1 CLINICAL DATA OF 22 PATIENTS WITH GLIOMAS*									
Case No.	Age (yrs), Sex	His-tology	MGMT		Size Response of Tumor	No. of Cycles	TTP (m os)	Survival Time (m os)	
			Level	Phenotype					
1	71, M	GBM	0	Mer ⁻	im proved (25%)	4	6	12	
2	57, F	GBM	0	Mer ⁻	partial	3	9	11	
3	64, M	GBM	98,136	Mer ⁺	partial	2	2	6	
4	63, M	GBM	0	Mer ⁻	stable	2	4	4	
5 [∞]	43, M	GBM	9989	Mer ⁻	stable	5	9	11	
6	60, M	GBM	92,685	Mer ⁺	progressive	3	2	5	
7	61, F	GBM	0	Mer ⁻	progressive	2	2	3	
8 [∞]	42, M	GBM	63,430	Mer ⁺	progressive	1	1.5	6	
9 [∞]	49, M	GBM	261,940	Mer ⁺	progressive	2	1	7	
10	55, F	GBM	519,000	Mer ⁺	progressive	2	3	5	
11	52, F	GBM	496,000	Mer ⁺	progressive	3	2	8	
12	65, F	GBM	0	Mer ⁻	progressive	1	1	3	
13†	62, M	GBM	127,000	Mer ⁺	progressive	2	4	5	
14	39, M	AA	387,066	Mer ⁺	progressive	4	5	>34	
15§	44, F	AA	83,234	Mer ⁺	progressive	4	5	>42	
16†	32, F	AA	0	Mer ⁻	partial	5	>43	>43	
17	32, M	AA	0	Mer ⁻	stable	4	6	10	
18 [∞]	36, F	AA	0	Mer ⁻	stable	4	13	19	
19 [∞]	41, M	AA	826,073	Mer ⁺	partial	5	9	12	
20 [∞]	44, M	AA	326,845	Mer ⁺	im proved (40%)	4	10	12	
21	51, F	AA	982,401	Mer ⁺	stable	2	9	12	
22‡ [∞]	43, M	AA	53,112	Mer ⁻	im proved (31%)	1	NE	14	
* NE = could not be evaluated. † Only these two patients had received BCNU with radiotherapy as initial treatment and on relapse were treated with BCNU alone. ‡ Refused further chemotherapy after one dose. § Received two cycles of PCV, then two cycles of BCNU. ∞ Received concomitant high-dose tamoxifen therapy but disease had progressed while patient received tamoxifen therapy before starting BCNU therapy									

Detection of MGMT in Tumor Specimens

A quantitative immunofluorescence assay for MGMT was performed as previously described.[4,5] Briefly, two slides from each tumor sample were stained in parallel: one (test sample) with antitransferase monoclonal antibody 3B8, which was raised against recombinant human MGMT[3] and one without this antibody (background control). Quantum Simple Cellular Microbeads (Flow Cytometry Standard, Hato Rey, Puerto Rico), used in calibrating the digitized fluorescence intensity, were spotted on slides and fixed in parallel. The tissue samples and beads were treated with 0.1% Triton X-100 in phosphate-buffered saline, blocked with 5% nonfat milk/phosphate-buffered saline, and stained with the first antibody at 37 µg/ml and the second goat anti-mouse immunoglobulin G antibody linked to fluorescein isothiocyanate at 15 µg/ml (Sigma Chemical Co., St. Louis, MO). Nuclei were stained with 0.2 µg/ml 4'6-diamidino-2-phenylindole-2HCl for 3 minutes, and the slides were rinsed and mounted with 50% glycerol and Slowfade antifade reagent (Molecular Probes Inc., Eugene, OR).

Images from the slides of the tissues and beads were captured by epifluorescence microscopy for which a

Nikon Diaphot microscope equipped with Fluorite lenses and with green and blue filter sets was used (for fluorescein isothiocyanate and 4'6-diamidino-2-phenylindole-2HCl fluorescence, respectively). The images were digitized using a Star I CCD Camera (Photometrics Ltd., Tucson, AZ). The images were analyzed with the Optimas image analysis system (Bioscan Inc., Edmonds, WA), by using a macroinstruction that automates data collection and performs all calculations. A value for MGMT (in molecules/nucleus) for each nucleus was derived from measurements of nuclear areas and densitometric fluorescent intensities. Intensity was quantified following comparison to a calibration curve derived from positive control standards. For each tumor specimen, a distribution was determined from densitometric intensity measurements of approximately 100 nuclei in the test and background control sections. The MGMT level was scored as zero if the mean value on the test section did not differ significantly from the mean value on the background control section. The value in molecules/nucleus for each tumor specimen was defined as the difference between the means of the two distributions for the test and background control slide for each given tumor sample. Calculations were performed in duplicate for each sample and results were averaged.

Description of Groups

The MGMT-rich (Mer⁺) group had an MGMT level higher than 60,000 molecules/nucleus, whereas a tumor with an MGMT level below 60,000 molecules/nucleus was considered MGMT-poor (Mer⁻). The patients were placed in the progression group if the tumor mass increased in size during chemotherapy, whereas if the tumor remained stable or decreased in volume during treatment, the patients were assigned to the effective group. The patients were also separated into groups by histological tumor type: a glioblastoma multiforme (GBM) tumor group and an anaplastic astrocytoma (AA) tumor group.

Statistical Analysis

The relationships between MGMT expression and survival time or TTP of the patients were evaluated using linear regression analysis. In addition, the patients were divided into several groups for comparisons using the Students t-test and contingency analysis.

RESULTS

Twenty-two patients were entered in this study. Thirteen patients had GBMs and nine patients had AAs. The expression of O⁶-methylguanine-DNA methyltransferase in tumor samples varied widely. Eight samples had no detectable MGMT; the remaining tumors had MGMT expression ranging from 9989 to 982,401 molecules/nucleus. The response to chemotherapy was modest; only four patients had a partial response (tumor volume decrease > 50%). Three patients showed improvement (tumor volume decrease >= 25% but < 50%), and five patients remained stable during BCNU-based chemotherapy. With almost half of the patients (10 of 22), the tumor continuously grew during chemotherapy. The average TTP in patients was 6.7 months and the average survival was 13 months (range 2-43 months) (Table 1). By linear regression analysis, there was no significant correlation between MGMT levels in tumor cells and either the patient survival time ($r = 0.04$, $p = 0.8595$) or TTP ($r = 0.107$, $p = 0.6444$). However, the age of the patients, as expected, was negatively correlated with TTP ($r = 0.507$, $p = 0.019$) and survival time ($r = 0.573$, $p = 0.0053$). Patients who were Mer⁻ were less likely to be in the progression group (two of 10) than Mer⁺ patients (eight of 12), (chi-square = 4.791, $p = 0.0286$). However, there was no significant difference between the Mer⁺ and Mer⁻ groups for survival time ($t = -0.094$, $p = 0.4633$) and TTP ($t = 1.156$, $p = 0.1428$). Furthermore, there was no significant difference in MGMT levels between the

progression and effective groups ($t = 0.098$, $p = 0.461$).

Because GBMs are much more aggressive than AAs, the survival time was significantly shorter for patients with these tumors (6.9 ± 0.8 months) than for those with AAs (22 ± 4.6 months). The TTP of the patients was also shorter in the GBM group than in the AA group. Patients in the GBM group were much older than those in the AA group (mean 57 compared with 40 years, respectively). However, the MGMT levels between these two groups were not significantly different ($t = -1.24$, $p = 0.122$). (Fig. 1, Table 2).

Fig. 1. Comparison between patients with GBM and AA for age, TTP, survival time, and MGMT levels in tumor cells.

Specific to the GBM group alone, the TTP of Mer⁺ patients (2.2 ± 0.4 months) was shorter than that of Mer⁻ patients (5.2 ± 1.4 months), but there was no significant difference in survival time between these two groups ($t = 0.889$, $p = 0.207$) (Table 2, Fig. 2).

Fig. 2. The difference of TTP and survival time between MGMT-rich (Mer⁺) and

MGMT-poor (Mer⁻) patients with GBMs.

When we compared the GBM patients in the effective and progressive groups, the MGMT levels were much higher ($195,007 \pm 74,223$ molecules/nucleus) in the progressive patients than in the effective patients ($21,625 \pm 19,225$ molecules/nucleus). The TTP and survival time in the effective group were also longer than those in the progressive group (Fig. 3, Table 2). However, there was no linear correlation between MGMT levels and TTP ($r = 0.338$, $p = 0.259$) or survival time ($r = 0.119$, $p = 0.698$) in these patients. Again in GBM patients, those who were Mer⁻ were less likely to have progressive disease (two of six) than those who were Mer⁺ (six of seven) (chi-square = 3.745, $p = 0.053$). There were only nine patients with AAs, and only two of these were in the progressive group. However, it is clear that progression in AA patients with high MGMT levels is not necessarily ominous because both of these patients were alive at 34 months or more.

Fig. 3. Comparison between progression (Prog) and effective (Eff) patients for age, TTP, survival time with GBM, and MGMT levels in GBMs.

DISCUSSION

It has been shown that O⁶-methylguanine-DNA methyltransferase can repair O⁶-alkylguanine adducts, such as the chloroethyl adduct formed by CENUs, preventing the formation of cytotoxic interstrand crosslinks.[7] Accumulating evidence indicates that MGMT-positive tumor cells are more resistant to CENUs than MGMT-negative tumor cells.[7,13-15,30] It has also been confirmed by many investigations that MGMT inhibitors such as O⁶-benzylguanine can sensitize tumor cells to CENUs via inhibition of MGMT.[6,13,28,29] However, MGMT expression does not always correlate linearly with drug resistance, and some MGMT-negative tumors also show resistance to these agents.[9] We previously investigated MGMT protein levels and MGMT activity in 14 human tumor cell lines and compared these results with BCNU cytotoxicity. Although there was no significant linear correlation between MGMT expression and CENU resistance, cell lines with high MGMT expression were more resistant to BCNU than MGMT-poor cell lines. In addition, BCNU-sensitive cell lines were all MGMT poor, indicating that MGMT plays a major role in BCNU drug resistance.[9]

In our present clinical observation, Mer⁺ patients appeared more likely to have progressive disease with

CENU-based chemotherapy than Mer⁻ patients. However, we did not find a statistically different TTP between Mer⁺ and Mer⁻ groups in the entire series of patients, possibly due to the small number of cases. However, the TTP of the Mer⁺ group was shorter than the Mer⁻ group (4.5 compared with 10.3 months, respectively). Because GBM and AA behave differently, we considered them separately. Even failing to respond to chemotherapy, patients with AA survive much longer than patients with GBM. In our series this is indicated by the two AA patients with high MGMT levels and no response to CENU therapy, who have survived more than 34 and 42 months, respectively, after initial CENU therapy, which was much longer than any GBM patient. The MGMT levels were similar between GBM and AA patients, whereas the survival times and TTP of AA patients were longer than GBM patients. The AA patients were much younger than the GBMs patients, which is also a factor affecting survival time.

In the GBM patients, those who demonstrated progressive disease with CENU therapy had higher MGMT expression than the patients in the effective group. Thus, the TTP in patients in the effective group was longer compared to patients in the progressive group, suggesting that MGMT contributes to CENU resistance in GBM. In the previous reported large multicenter series,[5] patients whose gliomas showed high MGMT levels had a shorter time to treatment failure and shorter survival time, with a mortality rate 1.7 times greater than patients whose gliomas showed low MGMT levels. In this series, although we did not find a significant difference in survival time between patients harboring MGMT-rich and MGMT-poor GBMs, possibly due to the small sample size, patients with MGMT-positive tumors were more likely to have progressive disease with CENU therapy. Nonetheless, the results are complicated by the fact that some patients with low levels of MGMT in their gliomas failed to respond to CENU-based chemotherapy. This is in agreement with our previous finding with human tumor cell lines demonstrating that some MGMT-poor cell lines are also resistant to CENU.[9]

The mechanism of drug resistance in brain tumors is multifactorial.[27] There are at least three possible sites of drug resistance concerning the tumor cell: drug inactivation, such as glutathione S-transferase-mediated metabolism of nitrosoureas;[16,33] transportation of the drug out of the tumor cells, such as mediated by multidrug resistance gene;[22,24] and repair of the damaged DNA.[2,7-10,14,36] One of the best recognized mechanisms of nitrosourea resistance in human tumors relating to DNA repair is O⁶-methylguanine-DNA methyltransferase. Nucleotide excision repair (NER), a multienzyme complex, is responsible for repairing a wide variety of DNA lesions,[20] including lesions induced by ultraviolet (UV) light or by some chemotherapeutic agents.[1,2,36] Authors of several studies have suggested that NER may also be implicated in anticancer drug resistance.[2,8-10,12,36] Recently, we examined **ERCC2** gene expression in 10 human brain tumor cell lines by reverse transcriptase-polymerase chain reaction and found a significant correlation between **ERCC2** expression and the cytotoxicity of BCNU or SarCNU, a novel CENU analog with enhanced antitumor activity against human gliomas.[10,21] We also examined four samples of this series, and ERCC2-positive tumors were found in two patients with progressive disease, one of which was MGMT negative, whereas two patients whose treatment was considered effective had ERCC2-negative tumors (data not shown). Previously we determined that ERCC2 protein levels were significantly correlated with UV sensitivity and BCNU resistance. Moreover, UV sensitivity was correlated with BCNU cytotoxicity, suggesting that NER, specifically ERCC2, is involved in nitrosourea resistance in human tumor cell lines.[8] Currently, DNA-dependent protein kinase (DNA-PK), a recently identified nuclear protein serine/threonine kinase, has been unequivocally identified as an important mammalian DNA repair complex involved in DNA double-strand break repair and in the joining step of recombination processes.[17] We recently investigated DNA-PK activity in 34 patients with chronic lymphocytic leukemia and found that the

lymphocytes of the patients resistant to chlorambucil had a higher DNA-PK activity than those of sensitive patients. Furthermore, DNA-PK activity is linearly correlated with the sensitivity of chronic lymphocytic leukemia lymphocytes to chlorambucil in vitro, suggesting that DNA-PK might also be involved in drug resistance in specific human tumors (unpublished data).

CONCLUSIONS

Our present clinical observations confirm that MGMT plays an important role in CENU drug resistance in human gliomas. Patients with MGMT-negative gliomas are less likely to progress on CENU chemotherapy than patients with MGMT-positive gliomas. This notwithstanding, MGMT-positive tumors are usually, but not always, resistant to CENUs, whereas MGMT-negative tumors are not always sensitive to CENUs, suggesting that other factors may also affect a patient's response to chemotherapy.

References

1. Abooussekhra A, Wood RD: Repair of UV-damaged DNA by mammalian cells and *Saccharomyces cerevisiae*. **Curr Opin Genet Dev** 4:212-220, 1994
2. Andersson BS, Sadeghi T, Siciliano MJ, et al: Nucleotide excision repair genes as determinants of cellular sensitivity to cyclophosphamide analogs. **Cancer Chemother Pharmacol** 38:406-416, 1996
3. Ayi TC, Loh KC, Ali RB, et al: Intracellular localization of human DNA repair enzyme methylguanine-DNA methyltransferase by antibodies and its importance. **Cancer Res** 52:6423-6430, 1992
4. Belanich M, Ayi TC, Li BF, et al: Analysis of O⁶-methylguanine-DNA methyltransferase in individual human cells by quantitative immunofluorescence microscopy. **Oncol Res** 6:129-137, 1994
5. Belanich M, Pastor M, Randall T, et al: Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. **Cancer Res** 56:783-778, 1996
6. Bobola MS, Berger MS, Silber JR: Contribution of O⁶-methylguanine-DNA methyltransferase to resistance to 1,3-(2-chloroethyl)-1-nitrosourea in human brain tumor-derived cell lines. **Mol Carcinogen** 13:81-88, 1995
7. Brent TP, Houghton PJ, Houghton JA: O⁶-Alkylguanine-DNA alkyltransferase activity correlates with the therapeutic response of human rhabdomyosarcoma xenografts to 1-(2-chloroethyl)-3(trans-4-methylcyclohexyl)-1-nitrosourea. **Proc Natl Acad Sci USA** 82:2985-2989, 1985
8. Chen ZP, Malapetsa A, Marcantonio D, et al: Correlation of chloroethylnitrosourea resistance with ERCC-2 expression in human tumor cell lines as determined by quantitative competitive polymerase chain reaction. **Cancer Res** 56:2475-2478, 1996
9. Chen ZP, Malapetsa A, McQuillan A, et al: Evidence for nucleotide excision repair as a modifying factor of O⁶-methylguanine-DNA methyltransferase-mediated innate chloroethylnitrosourea resistance in human tumor cell lines. **Mol Pharmacol** 52:815-820, 1997

10. Chen ZP, McQuillan A, Mohr G, et al: Excision repair cross-complementing rodent repair deficiency gene 2 expression and chloroethylnitrosourea resistance in human glioma cell lines. **Neurosurgery** **42**:1112-1119, 1998
11. Citron M, White A, Decker R, et al: O⁶-methylguanine-DNA methyltransferase in human brain tumors detected by activity assay and monoclonal antibodies. **Oncol Res** **7**:49-55, 1995
12. Dabholkar M, Bostick-Bruton F, Weber C, et al: ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients. **J Natl Cancer Inst** **84**:1512-1517, 1992
13. Dolan ME, Mitchell RB, Mummert C, et al: Effect of O⁶-benzylguanine analogues on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. **Cancer Res** **51**:3367-3372, 1991
14. Erickson LC, Laurent G, Sharkey NA, et al: DNA cross-linking and monoadduct repair in nitrosourea-treated human tumour cells. **Nature** **288**:727-729, 1980
15. Gonzaga PE, Potter PM, Niu TQ, et al: Identification of the cross-link between human O⁶-methylguanine-DNA methyltransferase and chloroethylnitrosourea-treated DNA. **Cancer Res** **52**:6052-6058, 1992
16. Hayes JD, Pulford DJ: The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. **Crit Rev Biochem Mol Biol** **30**:445-600, 1995
17. Jin S, Inoue S, Weaver DT: Function of the DNA dependent protein kinase. **Cancer Surveys** **29**:221-261, 1997
18. Koc ON, Phillips WP Jr, Lee K, et al: Role of DNA repair in resistance to drugs that alkylate O⁶ of guanine. **Cancer Treat Res** **87**:123-146, 1996
19. Kohn KW, Erickson LC, Laurent G, et al: DNA crosslinking and the origin of sensitivity to chloroethylnitrosoureas, in Prestayko AW, Crooke ST, Baker LH, et al (eds): **Nitrosoureas. Current Status and New Developments**. New York: Academic Press, 1981, pp 69-83
20. Ma L, Hoeijmakers JHJ, van der Eb AJ: Mammalian nucleotide excision repair. **Biochim Biophys Acta** **1242**:137-164, 1995
21. Marcantonio D, Panasci LC, Hollingshead MG, et al: 2-Chloroethyl-3-sarcosinamide-1-nitrosourea, a novel chloroethylnitrosourea analogue with enhanced antitumor activity against human glioma xenografts. **Cancer Res** **57**:3895-3898, 1997
22. Matsumoto T, Tani E, Yamaura I, et al: Effects of protein kinase C modulators on multidrug resistance in human glioma cells. **Neurosurgery** **36**:565-572, 1995
23. Mineura K, Watanabe K, Yanagisawa T, et al: Quantification of O⁶-methylguanine-DNA methyltransferase mRNA in human brain tumors. **Biochim Biophys Acta** **1289**:105-109, 1996
24. Nabors MW, Griffin CA, Zehnbauser BA, et al: Multidrug resistance gene (MDR1) expression in human brain tumors. **J Neurosurg** **75**:941-946, 1991
25. Nutt CL, Costello JF, Bambrick LL, et al: O⁶-methylguanine-DNA methyltransferase in tumors and

cells of the oligodendrocyte lineage. **Can J Neurol Sci** **22**:111-115, 1995

26. Pegg AE: Mammalian O⁶-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. **Cancer Res** **50**:6119-6129, 1990
27. Phillips PC: Antineoplastic drug resistance in brain tumors. **Neurol Clin** **9**:383-404, 1991
28. Phillips WP Jr, Willson JKV, Markowitz SD, et al: O⁶-methylguanine-DNA methyltransferase (MGMT) transfectants of a 1,3-(2-chloroethyl-1-nitrosourea (BCNU)-sensitive colon cancer cell line selectively repopulate heterogenous MGMT+/MGMT- xenografts after BCNU and O⁶-benzylguanine plus BCNU. **Cancer Res** **57**:4817-4823, 1997
29. Schold SC Jr, Kokkinakis DM, Rudy JL, et al: Treatment of human brain tumor xenografts with O⁶-benzyl-2'-deoxyguanosine and BCNU. **Cancer Res** **56**:2076-2081, 1996
30. Scudiero DA, Meyer SA, Clatterbuck BE, et al: Sensitivity of human cell strains having different abilities to repair O⁶-methylguanine in DNA to inactivation by alkylating agents including chloroethylnitrosoureas. **Cancer Res** **44**:2467-2474, 1984
31. Shapiro WR: Therapy of adult malignant brain tumors: what have the clinical trials taught us? **Semin Oncol** **13**:38-45, 1986
32. Tano K, Shiota S, Collier J, et al: 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, 1990
33. Tew KD, Monks A, Barone L, et al: Glutathione-associated enzymes in the human cell lines of the National Cancer Institute Drug Screening Program. **Mol Pharmacol** **50**:149-159, 1996
34. Tong WP, Kirk MC, Ludlum DB: Formation of the cross-link 1-[N3-deoxycytidyl],2-[N1-deoxyguanosinyl]ethane in DNA treated with N,N'-bis(2-chloroethyl)-N-nitrosourea. **Cancer Res** **42**:3102-3105, 1982
35. Yarosh DB: The role of O⁶-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. **Mutat Res** **145**:1-16, 1985
36. Zeng-Rong N, Paterson J, Alpert L, et al: Elevated DNA repair capacity is associated with intrinsic resistance of lung cancer to chemotherapy. **Cancer Res** **55**:4760-4764, 1995

Manuscript received April 7, 1998.

Accepted in final form May 20, 1998.

This work was supported by the National Institute of Neurological Disorders Stroke Grant No. NS22230, and a private donation from Helen and Nicki Lang. Dr. Zhong-Ping Chen is a recipient of the Francis McNamilton Memorial Prize from the Canadian Congress of Neurological Sciences. Dr. Lawrence Panasci is a recipient of the Gertrude and Stanley Vineberg clinical scientist award.

Address reprint requests to: Lawrence C. Panasci, M.D., Lady Davis Institute for Medical Research, Sir Mortimer B. Davis Jewish General Hospital, 3755 Côte Ste. Catherine, Montreal, Quebec, H3T 1E2 Canada. email: cxzc@musica.mcgill.ca.