Biochemical Pharmacology, Vol. 46, No. 2, pp. 285–290, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

EFFECT OF O^6-BENZYLGUANINE ON THE SENSITIVITY OF HUMAN COLON TUMOR XENOGRAFTS TO 1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA (BCNU)

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(*Received* 28 *January* 1993; *accepted* 16 *March* 1993)

Abstract—A number of trials were conducted to determine the effect of O^6-benzylguanine pretreatment on the sensitivity of human colon tumor xenografts to the antitumor effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). O^6-Benzylguanine has been shown to inactivate the DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT), which is primarily responsible for resistance to alkylnitrosoureas including BCNU. Colon tumor xenografts carried in nude mice were analyzed for their AGT content, and tumors with low, intermediate and high levels were chosen for further study. The AGT activity of HC-1, GC-3, VRC-5 and CX-1 human colon tumor xenografts was 16, 113, 180 and 367 fmol/mg protein, respectively. Treatment of mice consisted of vehicle alone, 6.25 to 50 mg/kg BCNU administered alone or BCNU (6.25 to 25 mg/kg) 1 hr after 120 mg/kg O^6-benzylguanine on days 7 and 14 post-inoculation. Toxicity studies revealed that pretreatment with O^6-benzylguanine increased the toxicity of BCNU, requiring administration of about 4-fold less drug. The growth of the VRC-5 tumor at day 42 post-inoculation was inhibited by 39% following treatment with 12.5 mg/kg BCNU alone and 92% when BCNU was combined with O^6-benzylguanine pretreatment. The combination of O^6-benzylguanine and BCNU (12.5 mg/kg) at day 42 resulted in an inhibition of HC-1 and CX-1 tumor growth by 84 and 72%, whereas BCNU alone inhibited growth by 54 and 14%, respectively. Therefore, the degree to which the antitumor effect of BCNU was increased by O^6-benzylguanine pretreatment was dependent on the AGT activity, with a greater effect in tumors of intermediate or high activity. These data suggest that there is a role for O^6-benzylguanine combined with BCNU in the treatment of human colon tumors.

Human colon cancer is resistant to most chemotherapeutic agents, including chloroethylnitrosoureas (BCNU, CCNU and meCCNU) [1, 2]. The failure of chloroethylnitrosoureas to destroy tumor cells is often the result of cellular drug resistance. Although several mechanisms are involved in alkylating agent resistance, repair mechanisms that recognize and remove alkylation damage to DNA are important contributors [3, 4]. The mechanism by which chloroethylnitrosoureas are thought to exert their antitumor effect involves reaction with the O^6-position of guanine in DNA to form O^6-chloroethylguanine. Subsequent rearrangement produces an N1, O^6-ethanoguanine intermediate that can react with the opposite strand of DNA to produce a cytotoxic cross-link with cytosine [5–8]. Adducts formed at the O^6-position of guanine are removed by a DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT¶) [4]. Formation of cross-links by chloroethylating agents is prevented by AGT through transfer of the alkyl group to the cysteine residue in the active site of the protein [9] or by attack at the carbon adjacent to the oxygen position of the N1, O^6-ethanoguanine intermediate producing a protein-DNA cross-link [10]. The repair protein is inactivated in either process and new synthesis is required for restoration of activity. Xenograft tumors and cell lines with low AGT activity are sensitive to the killing effects of chloroethylnitrosoureas [4, 11, 12]. There is a high degree of correlation between alkyltransferase activity in human cancer cell lines and their resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in cell culture [13].

There have been several attempts to increase the chemotherapeutic effectiveness of chloroethylnitrosoureas by depletion of the AGT protein. One method of depletion has utilized methylating agents such as streptozotocin or dacarbazine (DTIC), which results in inactivation of the protein by repair of

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¶ Abbreviations: AGT, O^6-alkylguanine-DNA alkyltransferase; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BG, O^6-benzylguanine; PBS, phosphate-buffered saline; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; meCCNU, 1-(2-chloroethyl)-3-*p*-methylcyclohexyl-1-nitrosourea.

methylated DNA [14–16]. A limitation to the use of methylating agents is their introduction of toxic, mutagenic and/or carcinogenic DNA lesions [17] in addition to those produced by a chloroethylnitrosourea. A less deleterious method for AGT depletion involves the use of low molecular weight alternative substrates for the alkyltransferase such as O^6-benzylguanine [18–21]. O^6-Benzylguanine produces a dramatic and rapid depletion of cellular AGT activity [18]. The depletion of AGT in human colon and brain tumor cells in culture by O^6-benzylguanine produces an increase in the sensitivity of these cells to the cytotoxic effects of BCNU, CCNU and methylnitrosoureas [20]. In addition, we recently reported an increase in the therapeutic effect of BCNU in animals bearing SF767 brain tumor xenografts after treatment with O^6-benzylguanine [21].

In the present studies, we have compared the effects of BCNU alone and BCNU in combination with O^6-benzylguanine on the growth of human colon tumor xenografts carried in nude mice. The objective was to determine whether O^6-benzylguanine as an adjuvant inhibited the growth of colon tumors to a greater extent than treatment with BCNU alone and to determine which types of xenografts were most responsive. Human colon tumors exhibiting a range of alkyltransferase activity were evaluated for this purpose.

Table 1. AGT activity of colon tumor xenografts and toxicity associated with BCNU and/or BG treatment

Tumor	Initial AGT activity* (fmol/mg protein)	BG† (mg/kg)	BCNU† (mg/kg)	Deaths‡
HC-1	16	0	25	0/10
		120	6.25	0/10
		120	12.5	1/10
GC-3	113	0	12.5	1/20
		0	25	0/20
		120	6.25	2/20
		120	12.5	18/20
VRC-5	180	0	25	0/10
		120	12.5	1/10
CX-1	367	0	25	1/9
		0	50	4/9
		120	6.25	0/9
		120	12.5	2/9

* AGT activity was assayed in duplicate as described in Materials and Methods on at least two separate tumors with the exception of the HC-1 tumor in which only one tumor was assayed in duplicate.

† Animals were treated on days 7 and 14 post-inoculation with BCNU alone or BG 1 hr prior to BCNU at the doses indicated.

‡ The ratio of animal deaths at 28 days post-inoculation to total animals in the group.

MATERIALS AND METHODS

Drug treatment. BCNU (NSC 409962) was provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. O^6-Benzylguanine was synthesized as described [18]. [^{3}H]Methylnitrosourea (3 or 16 Ci/mmol) was purchased from Moravek (Brea, CA) or Amersham (Arlington Heights, IL). All other biochemicals were purchased through Sigma (St. Louis, MO).

Animal treatment. CD-1 female nude mice obtained from Charles River were housed in an isolation facility with water and food provided *ad lib.* Therapy was initiated 1 week (CX-1, GC-3, VRC-5) or 2 weeks (HC-1) following inoculation with human colon tumors and animals were grouped accordingly (9–20 animals/group). Tumors at the time of treatment were approximately 75 mm^3. Treatment consisted of an i.p. injection of vehicle alone [emulphor or phosphate-buffered saline (PBS)], O^6-benzylguanine alone (120 mg/kg), BCNU alone (6.25 to 25 mg/kg) or the combination of O^6-benzylguanine followed 1 hr later by BCNU on days 7 and 14 post-inoculation. O^6-Benzylguanine was dissolved in emulphor and administered i.p. in a total volume of 0.5 mL. Immediately prior to injection, BCNU was prepared in PBS and administered i.p. Tumors were measured every 7 days beginning at day 21 post-inoculation. Toxicity was determined by animal survival. Mean tumor size and standard deviation were determined for each group as described previously [22].

Alkyltransferase activity. Animals bearing xenografts were anesthetized and killed by cervical dislocation. Tumors were excised and frozen in liquid nitrogen prior to storage at −70°. Alkyltransferase activity was measured as removal of [^{3}H]methyl groups from the O^6-position of guanine in a methylated DNA substrate. Methylated DNA was prepared from the reaction of [^{3}H]-methylnitrosourea and purified calf thymus DNA [23]. Protein was determined by the method of Bradford [24].

RESULTS

Alkyltransferase activity. Alkyltransferase activity as determined by the loss of O^6-methylguanine from a methylated DNA substrate varied from 16 to 367 fmol/mg protein for the four colon tumors studied (Table 1).

Animal toxicity. Toxicity studies indicated that O^6-benzylguanine combined with BCNU was more toxic to these nude mice than BCNU alone. This required lowering the dose of BCNU in the combination treatment by 2- to 4-fold to administer similarly non-toxic dosages (Table 1). Similar treatments with 10% toxic deaths were observed with animals bearing HC-1 and VRC-5 tumors when O^6-benzylguanine was combined with 12.5 mg/kg BCNU treatment. No obvious toxicity was observed at twice this dose of BCNU alone. Animals carrying the GC-3 tumor were unusually sensitive to the combination of O^6-benzylguanine and BCNU with 90% deaths at day 28 when BCNU (12.5 mg/kg) was administered with O^6-benzylguanine. Although one of twenty animals died after treatment with 12.5 mg/kg BCNU alone, there were no deaths at 25 mg/kg BCNU alone. Tumor effects on the host may be a factor in this toxicity since the same strains of mice were used in all studies. O^6-Benzylguanine alone

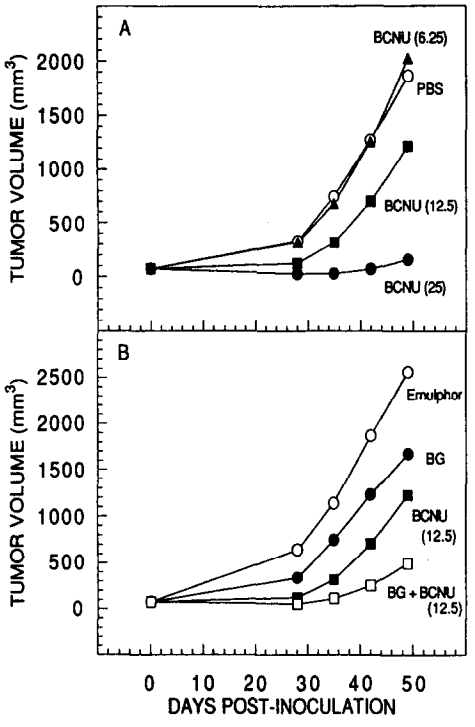

Fig. 1. Tumor growth rate of HC-1 xenografts treated with BG and/or BCNU. Nude mice carrying HC-1 xenograft tumors were administered i.p. injections of (A) PBS, BCNU at 6.25 mg/kg, 12.5 mg/kg, or 25 mg/kg, or (B) emulphor control, 120 mg/kg BG alone, BCNU at 12.5 mg/kg alone or with BG (120 mg/kg) prior to BCNU (12.5 mg/kg) on days 7 and 14 post-inoculation. Data points represent the mean tumor volume for 10 animals per group.

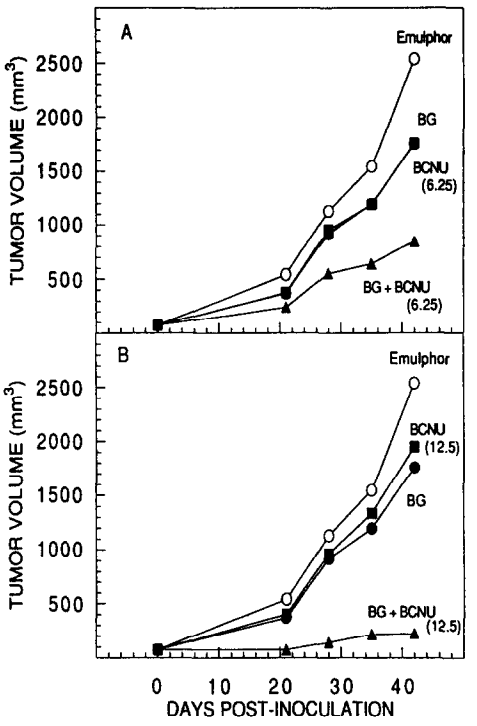

Fig. 2. Tumor growth rate of GC-3 xenografts treated with BG and/or BCNU. Mice carrying GC-3 xenograft tumors were administered i.p. injections of (A) emulphor control, 120 mg/kg BG alone, BCNU at 6.25 mg/kg alone, or with BG (120 mg/kg) administered 1 hr prior to BCNU at 6.25 mg/kg on days 7 and 14 post-inoculation. (B) Same as above except that the dose of BCNU was 12.5 mg/kg for either treatment. Data points represent the mean tumor volume for 10 animals per group.

was non-toxic at 120 mg/kg administered on days 7 and 14 post-inoculation; however, 3/10 toxic deaths resulted from administration of 200 mg/kg on the same schedule (data not shown).

Antitumor effect. Growth of tumor xenografts was determined for animals treated with vehicle (PBS or emulphor), BCNU (6.25 to 25 mg/kg) or O^6-benzylguanine (120 mg/kg) prior to BCNU (6.25 to 25 mg/kg). Animals were treated on days 7 and 14 post-inoculation. Tumor measurements and animal survival were assessed weekly.

Figure 1 illustrates the results of HC-1 tumor growth rate in animals treated with increasing dosages of BCNU alone or the combination of O^6-benzylguanine and 12.5 mg/kg BCNU compared with the same dose of BCNU alone. The HC-1 tumor, which has low levels of alkyltransferase activity, responded to BCNU in a dose-dependent manner (Fig. 1A). Growth inhibition was greater in animals treated with O^6-benzylguanine prior to BCNU as compared with an equal dose of BCNU alone (Fig. 1B); however, a higher, non-toxic dose of BCNU alone (25 mg/kg) gave similar tumor growth inhibition (Fig. 1A).

Human colon tumors with intermediate levels of alkyltransferase activity (GC-3 and VRC-5) were also evaluated for their response to BCNU following O^6-benzylguanine pretreatment (Figs. 2 and 3). Results of GC-3 tumor-bearing animals illustrate a dramatic inhibition of tumor growth for animals treated with O^6-benzylguanine and BCNU compared with BCNU alone at a dose of 6.25 mg/kg BCNU (Fig. 2A) and 12.5 mg/kg BCNU (Fig. 2B). Figure 3 shows a dose-dependent decrease in tumor growth with BCNU alone; however, this was not as dramatic as that observed with the HC-1 tumor, presumably due to the higher AGT activity in these tumors (Fig. 3A). When animals were treated with the combination of O^6-benzylguanine and BCNU, there was a much greater inhibition of tumor growth (Fig. 3B).

When CX-1 tumor-bearing animals were treated with BCNU and/or O^6-benzylguanine, there was no growth delay observed for BCNU treatment alone at 6.25 and 12.5 mg/kg (Fig. 4). However, when either dose was combined with O^6-benzylguanine, there was a marked significant inhibition of tumor growth (Fig. 4).

A comparison of the mean tumor size for the four types of xenografts at day 42 post-inoculation for animals treated with vehicle, BCNU, or O^6-benzylguanine in combination with BCNU is listed in Table 2. The size of HC-1, GC-3, VRC-5 and CX-1 tumors at 42 days was 54, 6, 39 and 14% that of control animals when animals were treated with

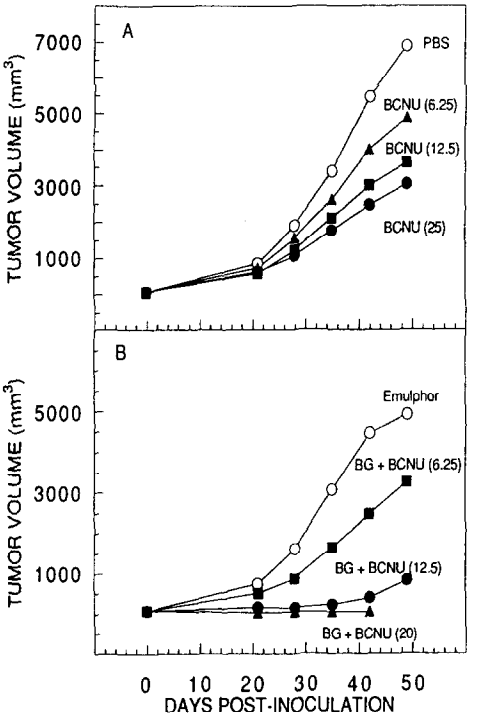

Fig. 3. Tumor growth rate of VRC-5 xenografts treated with BG and/or BCNU. Mice carrying VRC-5 xenograft tumors were administered i.p. injections of (A) PBS, BCNU at 6.25 mg/kg, 12.5 mg/kg, or 25 mg/kg, or (B) emulphor control, 120 mg/kg BG 1 hr prior to BCNU at 6.25 mg/kg, 12.5 mg/kg or 20 mg/kg on days 7 and 14 post-inoculation. Data points represent the mean tumor volume for 10 animals per group.

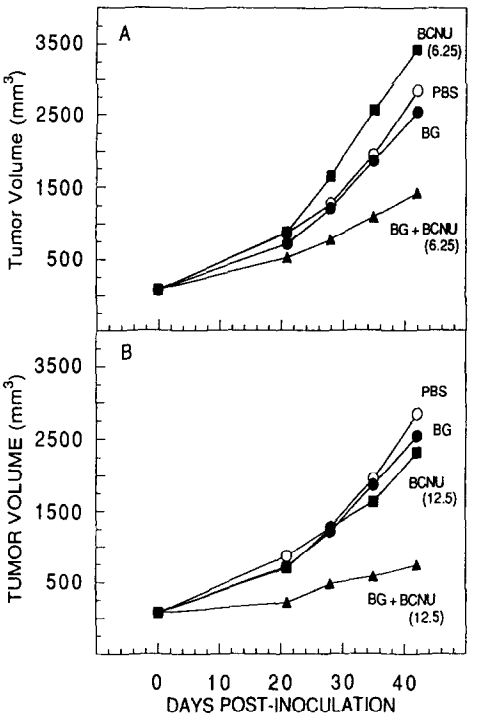

Fig. 4. Tumor growth rate of CX-1 xenografts treated with BG and/or BCNU. Mice carrying CX-1 xenograft tumors were administered i.p. injections of (A) PBS control, 120 mg/kg BG alone, BCNU at 6.25 mg/kg alone or BG administered 1 hr prior to BCNU on days 7 and 14 post-inoculation. (B) Same as above except that the dose of BCNU was 12.5 mg/kg for either treatment. Data points represent the mean tumor volume for 10 animals per group.

BCNU alone (12.5 mg/kg). With the exception of the GC-3 tumor, inhibition of tumor growth was inversely related to the AGT activity. However, tumor inhibition for all tumors was similar when O^6-benzylguanine was added to the regimen (84, 89, 92 and 72% for HC-1, GC-3, VRC-5 and CX-1 tumor, respectively).

DISCUSSION

These results demonstrate that O^6-benzylguanine pretreatment can be used to enhance the antitumor effect of BCNU against human colon tumor xenografts in nude mice. The sensitivity of colon tumors to BCNU was greatest for the tumor with the lowest AGT activity (HC-1) compared with colon tumors with intermediate or high AGT activity (GC-3, VRC-5, CX-1). In contrast, the sensitivity to the combination of O^6-benzylguanine and BCNU was similar for all four tumors. Thus, the enhancement of tumor inhibition was greater for tumors with higher levels of AGT activity. These results are in agreement with cell culture data which indicated that the enhancement of BCNU cytotoxicity by O^6-benzylguanine was greatest in cell lines with high alkyltransferase activity relative to cells with low AGT activity [20]. The vast majority of primary

Table 2. Effect of BG on the mean tumor volume of human colon tumor xenografts treated with BCNU

Tumor	BG* (mg/kg)	BCNU* (mg/kg)	Tumor size (mm³) on day 42† (% inhibition)
HC-1	0	0	1542 ± 781
	0	12.5	703 ± 430 (54)
	120	12.5	253 ± 225 (84)
GC-3	0	0	2078 ± 860
	0	6.25	1768 ± 988 (15)
	0	12.5	1950 ± 942 (6)
	120	6.25	846 ± 333 (59)
	120	12.5	225 ± 63 (89)
VRC-5	0	0	4964 ± 1630
	0	12.5	3017 ± 791 (39)
	120	12.5	400 ± 215 (92)
CX-1	0	0	2687 ± 926
	0	12.5	2313 ± 744 (14)
	120	12.5	747 ± 191 (72)

* Animals were treated on days 7 and 14 post-inoculation with BCNU alone or BG (120 mg/kg) 1 hr prior to BCNU (12.5 mg/kg).

† Data represent the mean tumor size (±SD) for 9–10 animals/group. Values for control animals represent the mean tumor size of two groups of animals treated with vehicle alone (PBS or emulphor).

tumor samples contain AGT activity [25]. From our results and those of others it seems reasonable that tumors with low AGT activity might be expected to respond to BCNU alone and biochemical modulation of AGT would not be necessary. For tumors with intermediate or high levels of AGT, however, inactivation of the protein by O^6-benzylguanine pretreatment may be a very effective means for enhancing the therapeutic effectiveness of BCNU or other chloroethylating antitumor drugs.

Both BCNU alone and the combination of O^6-benzylguanine and BCNU were quite toxic to mice carrying the GC-3 tumor. Treatment of animals with 25 mg/kg BCNU alone resulted in no animal death at 28 days post-inoculation; however, the LD_{90} of BCNU when combined with O^6-benzylguanine was 12.5 mg/kg. This phenomenon can only be explained by tumor effects on host since the same strains of mice were used in all studies. Toxicity studies are necessary to fully evaluate this phenomenon.

The data presented here extend other reports demonstrating the usefulness of O^6-benzylguanine therapy for human tumor xenografts in nude mice [21, 26, 27]. Previously, we found no effect of BCNU alone at 20 mg/kg for HT29 tumor-bearing mice that have AGT activity comparable to the CX-1 tumor; however, combining BCNU with O^6-benzylguanine resulted in inhibition of tumor growth [21]. Furthermore, the combination of O^6-benzylguanine and BCNU inhibited the growth of human brain tumor xenografts to a greater extent than the maximal tolerated dose of BCNU alone [21]. We now illustrate the effect of O^6-benzylguanine on the antitumor response of a number of human colon tumor xenografts containing different levels of AGT activity to BCNU. Our data suggest that to overcome resistance to the chloroethylnitrosoureas due to AGT activity, O^6-benzylguanine should be added to the chemotherapeutic regimen. Enhanced toxicity may be overcome by designing analogs of O^6-benzylguanine that more effectively target tumor tissue. We have now designed, synthesized and tested a number of agents including water-soluble analogs for their ability to deplete AGT activity in cells [28]. Further animal testing is required to assess their usefulness *in vivo*.

Acknowledgements—This work was supported by National Institutes of Health Grants CA47228 (M.E.D.), CA18137 (A.E.P.) and Contract NO1-CO-74101 with ABL (R.C.M.).

REFERENCES

1. Moertel GG, Therapy of advanced gastrointestinal cancer with the nitrosoureas. *Cancer Chemother Rep* **4**: 27–32, 1973.
2. Gastrointestinal Tumor Study Group, Adjuvant therapy of colon cancer: Results of a prospectively randomized trial. *N Engl J Med* **310**: 737–743, 1984.
3. Woolley PV and Tew KD, *Mechanisms of Drug Resistance in Neoplastic Cells*. Academic Press, San Diego, 1988.
4. Pegg AE, Mammalian O^6-alkylguanine-DNA alkyltransferase: Regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res* **50**: 6119–6129, 1990.
5. Tong WP, Kirk MC and Ludlum DB, Formation of the cross-link 1-[N^3-deoxycytidyl],2-[N^1-deoxyguanosinyl]-ethane in DNA treated with N,N'-bis(2-chloroethyl)-N-nitrosourea. *Cancer Res* **42**: 3102–3105, 1982.
6. Ludlum DB, DNA alkylation by the haloethylnitrosoureas: Nature of modifications produced and their enzymatic repair or removal. *Mutat Res* **233**: 117–126, 1990.
7. Brent TP, Isolation and purification of O^6-alkylguanine-DNA alkyltransferase from human leukemic cells. Prevention of chloroethylnitrosourea-induced cross-links by purified enzyme. *Pharmacol Ther* **31**: 121–140, 1985.
8. Kohn KW, Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. *Cancer Res* **37**: 1450–1454, 1977.
9. Robins P, Harris AL, Goldsmith I and Lindahl T, Cross-linking of DNA induced by chloroethylnitrosourea is prevented by O^6-methylguanine-DNA methyltransferase. *Nucleic Acids Res* **11**: 7743–7758, 1983.
10. Brent TP and Remack JS, Formation of covalent complexes between human O^6-alkylguanine-DNA alkyltransferase and BCNU-treated defined length synthetic oligodeoxynucleotides. *Nucleic Acids Res* **16**: 6779–6788, 1988.
11. Yarosh DB, The role of O^6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. *Mutat Res* **145**: 1–16, 1985.
12. Pegg AE and Dolan ME, Properties and assay of mammalian O^6-alkylguanine-DNA alkyltransferase. *Pharmacol Ther* **34**: 167–179, 1987.
13. Gerson SL, Berger NA, Arce C, Petzold S and Wilson JKV, Modulation of nitrosourea resistance in human colon cancer by O^6-methylguanine. *Biochem Pharmacol* **43**: 1101–1107, 1992.
14. Micetich KC, Futscher B, Koch D, Fisher RI and Erickson LC, Phase I study of streptozocin and carmustine-sequenced administration in patients with advanced cancer. *J Natl Cancer Inst* **84**: 256–260, 1992.
15. Futscher BW, Micetich KC, Barnes DM, Fisher RI and Erickson LC, Inhibition of a specific DNA repair system and nitrosourea cytotoxicity in resistant human cancer cells. *Cancer Commun* **1**: 65–73, 1989.
16. Gerson SL, Modulation of human lymphocyte O^6-alkylguanine-DNA alkyltransferase by streptozotocin *in vivo*. *Cancer Res* **49**: 3134–3138, 1989.
17. Saffhill R, Margison GP and O'Connor PJ, Mechanism of carcinogenesis induced by alkylating agents. *Biochim Biophys Acta* **823**: 111–145, 1985.
18. Dolan ME, Moschel RC and Pegg AE, Depletion of mammalian O^6-alkylguanine-DNA alkyltransferase activity by O^6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. *Proc Natl Acad Sci USA* **87**: 5368–5372, 1990.
19. Dolan ME, Stine L, Mitchell RB, Moschel RC and Pegg AE, Modulation of mammalian O^6-alkylguanine-DNA alkyltransferase *in vivo* by O^6-benzylguanine and its effect on the sensitivity of a human glioma tumor to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea. *Cancer Commun* **2**: 371–377, 1990.
20. Dolan ME, Mitchell RB, Mummert C, Moschel RC and Pegg AE, Effect of O^6-benzylguanine analogues on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. *Cancer Res* **51**: 3367–3372, 1991.
21. Mitchell RB, Moschel RC and Dolan ME, Effect of O^6-benzylguanine on the sensitivity of human tumor xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea and on DNA-interstrand cross-link formation. *Cancer Res* **52**: 1171–1175, 1992.

22. Worzalla JF, Bewley JR and Grindey GB, Automated measurement of transplantable solid tumors using digital electronic calipers interfaced to a microcomputer. *Invest New Drugs* **8**: 241–251, 1990.
23. Domoradzki J, Pegg AE, Dolan ME, Maher VM and McCormick JJ, Correlation between O^6-methylguanine-DNA-methyltransferase activity and resistance of human cells to the cytotoxic and mutagenic effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Carcinogenesis* **5**: 1641–1647, 1984.
24. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
25. Citron M, Decker R, Chen S, Schneider S, Graver M, Kleynerman L, Kahn LB, White A, Schoenhaus M and Yarosh D, O^6-Methylguanine-DNA methyltransferase in human normal and tumor tissue from brain, lung, and ovary. *Cancer Res* **51**: 4131–4134, 1991.
26. Friedman HS, Dolan ME, Moschel RC, Pegg AE, Felker M, Rich J, Bigner DD and Schold SC, Enhancement of nitrosourea activity in medulloblastoma and glioblastoma multiforme. *J Natl Cancer Inst* **84**: 1926–1931, 1992.
27. Gerson SL, Zborowska E, Norton K, Gordon NH and Willson JKV, Synergistic efficacy of O^6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in a human colon cancer xenograft completely resistant to BCNU alone. *Biochem Pharmacol* **45**: 483–491, 1993.
28. Moschel RC, McDougall MG, Dolan ME, Stine L and Pegg AE, Structural features of substituted purine derivatives compatible with depletion of human O^6-alkylguanine-DNA alkyltransferase. *J Med Chem* **35**: 4486–4491, 1992.