Role of \(O^6\)-methylguanine-DNA methyltransferase in protecting from alkylating agent-induced toxicity and mutations in mice

Ryan J. Hansen, Ramamoorthy Nagasubramanian, Shannon M. Delaney, Leona D. Samson and M. Eileen Dolan

Committee on Cancer Biology, Department of Pediatrics, Department of Medicine and Committee on Clinical Pharmacology and Pharmacogenomics, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637, USA and Biological Engineering Division and Center for Environmental Health Sciences, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Abstract

As expected, tumors overexpressing MGMT are resistant to BCNU and TMZ (3,10) and there has been considerable attention to sensitizing tumors to these agents. Potent MGMT inactivators, such as 6-benzylguanine (BG), have been developed exploiting the fact that MGMT is inactivated after transfer of alkyl groups to the MGMT protein (11). BG was found to rapidly deplete MGMT activity to undetectable levels at micromolar concentrations and increase the sensitivity of human tumor cells and xenografts to the cytotoxic effects of \(O^6\)-alkylating agents (11–13). BG is currently in clinical trials in combination with TMZ, BCNU and Gliadel (14–17).

In contrast to TMZ and BCNU, the role of MGMT in protecting from the oxazaphosphorine, cyclophosphamide is less clear. Cyclophosphamide is metabolized into phosphoramid mustard and acrolein, both of which form adducts on DNA (18). Although phosphoramid mustard is thought to produce an antitumor effect by causing interstrand cross-links, acrolein is thought to contribute to bladder toxicity and carcinogenesis. Through a minor pathway (<10%), cyclophosphamide can also undergo N-dechloroethylation to produce chloroacetalddehyde (CAA) (18,19), a neuro- and nephrotoxic by-product. Recent evidence demonstrates that cells overexpressing MGMT are less sensitive to the toxic and mutagenic effects of acrolein (20,21) that suggests that acrolein may be producing lesions that are recognized and repaired by MGMT.

Several studies have shown that mice overexpressing human MGMT in the thymus are protected against \(N\)-methyl-\(N\)-nitrosourea-induced thymic lymphomas that correlated with lower \(O^6\)-methylguanine adduct levels in the thymus in MGMT transgenic mice compared with non-transgenic mice (22–24). Overexpression of MGMT also protects against aberrant crypt formation and mutations in \(K\)-ras following treatment with the methylating agent, azoxymethane (25) and against skin tumors induced by the chlorohydrating agent 3-(4-amino-2-methyl-5-pyrimidinyl)ethyl-1-(2-chloroethyl)-1-nitrosourea, an agent similar to BCNU (26). In this study, we evaluated MGMT’s ability to protect from mutations induced by TMZ, BCNU, cyclophosphamide, CAA and acrolein using \(O^6\)-methylguanine-DNA methyltransferase (MGMT) in BCNU-induced mutations.

Introduction

Clinically used alkylating agents (e.g. methylating agents, chloroethylnitrosoureas and oxazaphosphorines) produce their toxic effect by transferring alkyl groups to cellular molecules, such as proteins and DNA (1). These agents react with the nucleophilic oxygen and nitrogen of nucleic acids and the oxygen of the phosphodiester group in the backbone of DNA. The DNA repair protein MGMT repairs alkylation at the \(O^6\)-position of guanine without the aid of any other cofactors or proteins (2,3). In a single step, adducts at the \(O^6\)-guanine position are removed, leaving the DNA intact. This irreversible transfer results in ‘suicide’ inactivation of MGMT by changing the conformation of its DNA-binding domain. MGMT then becomes detached from the DNA and is targeted for degradation by ubiquitination (4,5).

The methylating agent temozolomide (TMZ) produces \(O^6\)-methylguanine, as well as other methylated bases. Unrepaired \(O^6\)-methylguanine lesions are mutagenic and procarcinogenic and can lead to GC→AT transition mutations, sister chromatid exchanges, chromosome aberrations and recombination (6). Chloroethylnitrosoureas [i.e. 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] produce G-C interstrand cross-links. The cross-link is formed by a rearrangement of the initial \(O^6\)-chloroethylnitrosourea adduct to 1, \(O^6\)-ethanoguanine, which then reacts with cytosine in the opposite DNA strand (7). Both \(O^6\)-chloroethylnitrosourea and 1, \(O^6\)-ethanoguanine are substrates for MGMT (8,9). Thus, repair of \(O^6\)-guanine lesions by MGMT protects from toxicity and mutagenicity induced by TMZ and BCNU.

Materials and methods

Animal care

All animal procedures were approved by the University of Chicago’s Institutional Animal Care and Use Committee. C57BL/6 \(O^6\)-methylguanine-DNA methyltransferase (MGMT)−/− mice were generated as described previously (27). C57BL/6 and F1 (C57BL/6 × 129/Sv) wild-type mice were either bred in house or purchased from Jackson Laboratories (Bar Harbor, ME). F1 (C57BL/6 × 129/Sv) wild-type mice were used for BG × BCNU experiments to complement previous work from our laboratory (28). All other experiments used C57BL/6 mice. All mice were housed in an isolation facility with water and food provided ad libitum. Euthanasia was
performed at experiment end point or on morbid animals using CO₂ followed by necropsy or cervical dislocation to confirm death.

Chemicals and drugs
BG (NSC 637037) was generously provided by Dr Robert C. Moschel (National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD). TMZ (NSC 362856) and BCNU (NSC 409962) were kindly provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Cyclophosphamide monohydrate, acrolein (90%) and CAA (≈50% weight, percentage of solution in water) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO). All other chemicals were purchased from Sigma-Aldrich Chemical Company or Fisher Scientific (Fair Lawn, NJ), unless otherwise noted.

Animal treatment
Male mice were treated between 8 and 12 weeks with a single i.p. injection of alkylating agent or vehicle. All alkylating agents were prepared fresh prior to injection. Injection volumes for all drugs were 200 μl/20 g of body weight. TMZ in 50% dimethyl sulfoxide was dissolved in 100% dimethyl sulfoxide and diluted with filtered saline (0.9% NaCl) to a final concentration of either 0.7 or 1.0 mg/ml (final dosage: 7 and 10 mg/kg). BCNU in 15% ethanol was prepared fresh by dissolving in 100% ethanol and adding filtered saline such that the final concentration was 0.75, 1.5 or 5 mg/ml (final dosage: 7.5, 15 or 50 mg/kg, respectively). Cyclophosphamide monohydrate was dissolved in filtered saline so that final concentration was 25 mg/ml (final dosage: 250 mg/kg). Acrolein and CAA were diluted in filtered saline to a final concentration of 0.45 and 0.9 mg/ml for acrolein and 1.75 mg/ml for CAA (final dosage: 4.5 and 9.0 mg/kg for acrolein and 17.5 mg/kg for CAA). To evaluate the mutation frequencies in Mgmt+/+ mice following BG ± BCNU, mice were treated via intraperitoneal injections with two cycles 3 weeks apart, beginning at 8–12 weeks of age. BG was administered to mice at 30 mg/kg. BG (3 mg/ml) was made fresh in 40% (vol/vol) polyethylene glycol 400/60% saline by initially dissolving BG in polyethylene glycol 400 followed by the addition of 37°C saline. This solution was filter sterilized prior to injection. Mice were randomly assigned to one of five treatment groups consisting of: (i) vehicle (40% polyethylene glycol 400/60% saline 1 h prior to 15% ethanol in saline); (ii) BG (30 mg/kg) given 1 h prior to 15% ethanol in saline; (iii) 40% polyethylene glycol 400/60% saline followed 1 h later with 15 mg/kg BCNU; (iv) BG (30 mg/kg) given 1 h prior to BCNU (15 mg/kg) and (v) 40% polyethylene glycol 400/60% saline followed 1 h later with 50 mg/kg BCNU.

Toxicity studies
Animals were weighed prior to injection (baseline) and on days 3, 5 and/or 7 after injection and at time of sacrifice. Weight gain/loss was determined as a percentage of baseline weight.

Hprt mutation analysis
Mice were killed 8 weeks following injection and their spleens were isolated and weighed. General procedures for isolating splenic lymphocytes and selecting for hypoxanthine–guanine phosphoribosyltransferase (Hprt) mutant T-cell colonies were followed as described elsewhere (29) with slight modifications. Briefly, splenic lymphocytes were harvested and stimulated overnight in Hprt media freshly supplemented with mouse IL-2 (10 U/ml of media; Roche, Indianapolis, IN) and concanavalin A (4 μg/ml, Sigma-Aldrich Chemical Company). The following day, viable cells (as determined by trypan blue exclusion) were seeded in 96-well round-bottomed plates at 8 or 16 cells per well in media without selection or at 4 × 10⁵ cells per well in media containing 2 μg/ml 6-thioguanine (Sigma-Aldrich Chemical Company). All plates received 5 × 10⁴ feeder cells per well. Feeder cells were lethally gamma irradiated with 9000 rads prior to plating. After 10–14 days in culture, positive (6-thioguanine resistant) colonies were detected with visual and microscopic confirmation and the mutant frequency (ratio of the cloning efficiency with and without 6-thioguanine) was compared using Poisson statistics. All experiments were done a minimum of three times with at least two mice per group for each experiment.

Statistical analysis
Mutation frequencies were initially analyzed pairwise for equality of variance using Levene’s test followed by pairwise t-test, performed with either equal or unequal variance depending on Levene results.

Results
In vivo Hprt mutation frequency following TMZ treatment
Mgmt−/− mice were more sensitive to the toxic effects of TMZ than Mgmt+/+ mice (Table I). Mgmt−/− mice lost 6.6 and 8.6% baseline weight after TMZ treatment (7 and 10 mg/kg, respectively) compared with an increase of 1.6 and 5.6% in Mgmt+/+ mice. In addition, there were six deaths out of 14 mice following 10 mg/kg TMZ in Mgmt−/− with no deaths in Mgmt+/+ mice. Eight weeks after treatment, the mutation frequency was determined in splenic T-lymphocytes. As shown in Figure 1, the mutation frequency in Mgmt−/− mice, as measured by the number of 6-thioguanine-resistant colonies, significantly increased in a dose-dependent manner following treatment with TMZ (1.0, 5.5 and 9.8 × 10⁻⁶ for vehicle, 7 and 10 mg/kg, respectively, P ≤ 0.05). This increase was not observed in Mgmt+/+ mice following the same treatment (1.8, 2.4, 1.2 × 10⁻⁶ for vehicle, 7 or 10 mg/kg, respectively). The mutation frequency following 7 mg/kg TMZ observed in Mgmt−/− mice was significantly greater than in Mgmt+/+ mice (P ≤ 0.0001).

In vivo Hprt mutation frequency following BCNU treatment
Following BCNU treatment, Mgmt+/+ mice gained weight (3.8 and 1.1% for 7.5 and 15 mg/kg, respectively) compared with 4.5 and 23.0% weight loss in Mgmt−/− mice (Table I). All Mgmt−/− mice died following 15 mg/kg BCNU, similar to reports from Glassner et al. (27) that 14 mg/kg BCNU was the LD₅₀ in Mgmt−/− mice. Therefore, we determined the Hprt mutation frequency in mice treated with 7.5 mg/kg BCNU. In contrast to TMZ, we did not observe an increase in mutation frequency in Mgmt−/− and Mgmt+/+ mice following treatment with BCNU (Figure 2A).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mgmt+/+</th>
<th>Mgmt−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% baseline (SD)</td>
<td>Day</td>
<td>% baseline (SD)</td>
</tr>
<tr>
<td>TMZ</td>
<td>10</td>
<td>5.6 (5.9)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.6 (1.9)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.0 (3.1)</td>
<td>7</td>
</tr>
<tr>
<td>BCNU</td>
<td>15</td>
<td>1.1 (1.9)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>3.8 (6.0)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3.7 (2.8)</td>
<td>7</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>250</td>
<td>–3.0 (2.1)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.5 (2.2)</td>
<td>7</td>
</tr>
<tr>
<td>Acrolein</td>
<td>9</td>
<td>–10.6 (4.0)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>–3.0 (4.8)</td>
<td>3</td>
</tr>
<tr>
<td>CAA</td>
<td>17.5</td>
<td>–6.3 (5.1)</td>
<td>3</td>
</tr>
</tbody>
</table>

*Weight change represents mean of 4–20 mice.

Table I. Weight change and deaths in Mgmt+/+ and −/− mice following treatment with alkylating agents

Downloaded from https://academic.oup.com/carcin/article-abstract/28/5/1111/2476254 by guest on 02 March 2019
In vivo Hprt mutation frequency following BG ± BCNU
We have reported previously the maximal tolerated dose of BCNU in Mgmt+/- mice to be 50 mg/kg and the combination of BG (30 mg/kg) plus BCNU (15 mg/kg) to result in similar weight loss and toxicities (28). While Mgmt+/- mice treated with vehicle, BG alone or BCNU (15 mg/kg) were at or above baseline weight 7 days following first treatment, BG plus BCNU and BCNU (50 mg/kg) treatment resulted in 14.8 and 22.9% weight loss (Table II). There were also four deaths out of 17 mice following 50 mg/kg of BCNU. The mutation frequencies following treatments of 15 mg/kg BCNU (10.5 × 10^{-6}) or BG plus BCNU (16.6 × 10^{-6}) were significantly higher than vehicle and BG alone (1.5 and 2.8 × 10^{-6}, respectively, P < 0.05, Figure 2B). Importantly, the mutation frequency following BG plus BCNU was not significantly greater than BCNU (15 mg/kg) alone. The mutation frequency following 15 mg/kg BCNU was also significantly greater than the mutation frequency in mice treated with 50 mg/kg BCNU (1.7 × 10^{-6}, P < 0.02).

In vivo Hprt mutation frequency following cyclophosphamide, acrolein or CAA
Weight loss was similar following cyclophosphamide, acrolein and CAA regardless of Mgmt expression (Table I). Cyclophosphamide-induced mutations were significantly higher compared with vehicle treatment in Mgmt+/- and Mgmt-/-- mice (P < 0.005); however, the difference between the two genotypes was not significant (Figure 3A). While acrolein and CAA resulted in weight loss, neither was mutagenic in mice (Figure 3A and B).

Discussion
In vitro studies have implicated MGMT as a means of resistance to the toxicity and mutagenicity of methylation and chloroethylating agents including TMZ and BCNU (2,3). The development of MGMT-deficient mice has provided an in vivo model to evaluate these biological effects of alkylating agents (27,30,31). Our results have confirmed MGMT’s role in protecting from in vivo toxicities of O’-alkylating agents. In this study, we observed greater toxicity, as evidenced by 20- and 7-fold reduction of the maximal tolerated doses of
TMZ and BCNU, respectively, in Mgmt−/− mice compared with Mgmt+/+ mice. In contrast, cyclophosphamide, CAA and acrolein were equally toxic in Mgmt+/+ and Mgmt−/− mice. In MGMT-deficient mice, TMZ-induced mutation frequencies rose 6- to 10-fold above vehicle-treated mice. Equivalent doses of TMZ were not mutagenic in wild-type mice demonstrating MGMT’s importance in protecting against in vivo TMZ-induced mutations. On the other hand, the maximal tolerated dose of BCNU in Mgmt−/− mice (7.5 mg/kg) failed to induce mutations in either Mgmt+/+ or Mgmt−/− mice. Additionally, BG administered prior to BCNU in wild-type mice did not result in a significant increase in mutations compared with wild-type mice receiving BCNU alone. While in vitro evidence suggests a role of MGMT in protecting against mutations induced by both TMZ and BCNU (2,32,33), these results do not support a role for MGMT in vivo in protecting against BCNU mutagenicity. In addition, cyclophosphamide was found to be mutagenic in both Mgmt+/+ and Mgmt−/− mice; however, MGMT deficiency did not result in a significant enhancement of cyclophosphamide-induced mutations. Neither acrolein nor CAA was found to be mutagenic in vivo at the determined maximal tolerated dose and the absence of MGMT did not alter this.

Cyclophosphamide and BCNU have been used for several decades and evidence links these agents to therapy-induced acute myeloid leukemia (34,35). Interestingly, TMZ has only been approved for chemotherapeutic use since 1999 and already two cases of therapy-induced acute myeloid leukemia/myelodysplastic syndrome have been reported following TMZ treatment (36,37). Therapy-induced acute myeloid leukemia and myelodysplastic syndrome are observed in roughly 10–20% of patients treated with regimens containing BCNU and cyclophosphamide (1,34). However, because most regimens contain multiple drugs, assigning responsibility to a specific alkylating agent is difficult. Additionally, dissecting out the metabolite from alkylating agents with multiple metabolites, such as cyclophosphamide, requires investigation of each metabolite. By determining the mutagenicity of these agents in Mgmt−/− mice, we not only examined MGMT’s role but also shed light as to whether the mutagenic, and possibly tumorigenic, lesion of these agents is an O6-alkylguanine lesion. At vastly reduced doses than the maximal tolerated dose of TMZ in Mgmt+/+ mice, TMZ is mutagenic in Mgmt−/− mice. Additionally, Geiger et al. (38) have recently shown TMZ to be mutagenic in the bone marrow of wild-type mice harboring the lacZ reporter gene, with the vast majority (>90%) of the mutations being point mutations. Together, these data imply that in vivo TMZ-induced mutations are a result of O6-guanine adducts and that MGMT is important in repairing them.

TMZ was first approved for use in the chemotherapy of a malignant brain tumor, anaplastic astrocytoma (39). MGMT expression has been associated with reduced response to TMZ and therefore clinical trials have employed agents, such as BG, to inactivate MGMT (15). While this has resulted in increased efficacy of TMZ, depletion of MGMT would be expected to lead to increased TMZ-induced mutations and possibly therapy-induced acute myeloid leukemia. MGMT-deficient mice are more susceptible to tumor formation following N-methyl-N-nitrosourea and dacarbazine (30,40), stressing MGMT’s importance in protecting against methylation agent-induced tumorigenesis.

Fig. 3. Hprt mutation frequency in mice exposed to cyclophosphamide, acrolein or CAA. (A) Mgmt+/+ and −/− mice were treated with vehicle (Mgmt+/+, n = 5; Mgmt−/−, n = 6) or 250 mg/kg cyclophosphamide (Mgmt+/+, n = 5; Mgmt−/−, n = 6). (From R. Nagasubramanian, R. Hansen, S. Delaney, M. Cherian, L. Samson, S. Kogan and M. E. Dolan, in preparation.) (B) Mgmt+/+ and −/− mice were treated with vehicle (Mgmt+/+, n = 5; Mgmt−/−, n = 6), 4.5 mg/kg (Mgmt+/+, n = 3; Mgmt−/−, n = 3) or 9 mg/kg acrolein (Mgmt+/+, n = 3; Mgmt−/−, n = 3). (C) Mgmt+/+ and −/− mice were treated with vehicle (Mgmt+/+, n = 5; Mgmt−/−, n = 6) or 17.5 mg/kg CAA (Mgmt+/+, n = 4; Mgmt−/−, n = 4). Data represent mean of mutation plates ± SD. *** indicates P < 0.005.
Mgmt+/+ mice. With the increased mutagenicity of TMZ in the absence of MGMT, depletion of MGMT by BG is likely to result in an increase in the mutagenic potential of TMZ. Furthermore, our data suggest that if MGMT is absent when TMZ is administered, mutations that could lead to leukemia are a major risk that must be considered.

Several studies suggest that MGMT is the principal mechanism protecting cells from toxic and mutagenic effects associated with BCNU (3,33). To overcome tumor resistance to BCNU, approaches to inactivate MGMT with BG or through the use of Mgmt−/− mice, does not significantly enhance mutations induced by BCNU in vivo. In the absence of MGMT, BCNU interstrand cross-links are formed to a much greater extent (42), therefore, BCNU results in cell death not allowing for cell survival required for mutations. In agreement with this explanation, a xenograft study showed that although BG plus BCNU resulted in increased tumor growth delay as compared with BCNU alone, no increase in genomic instability was observed in recurring tumors (43). This suggests that BG increased BCNU antitumor effects without increasing genomic instability. Other possibilities to explain why BG does not enhance the frequency of BCNU-induced mutations in our studies is that (i) other repair pathways such as base excision (44) and/or nucleotide excision repair (45) may be able to repair BCNU-induced mutagenic lesions and (ii) the mutagenic lesion induced by BCNU, or products of attempted repair of the lesion, may be a lesion(s) other than O6-chloroethylguanine.

In mice, cyclophosphamide has been shown to be mutagenic (46). We determined whether the lack of MGMT (using Mgmt−/−/C0 mice) results in increased somatic mutations following cyclophosphamide, acrolein and CAA when compared with Mgmt+/+ mice. Clinical studies, as well as xenograft studies, are inconsistent on MGMT’s role in protecting from mutations induced by alkylating agents. In vitro studies, investigating cyclophosphamide’s metabolites individually, suggest that MGMT protects against acrolein-induced toxicity and mutagenicity (20,21). Our results do not support a role for MGMT in protecting against in vivo toxicities and mutations following cyclophosphamide. Consistent with the observation of others (30), our results confirm that Mgmt−/− mice are as sensitive to acute cyclophosphamide-induced toxicity as wild-type mice and demonstrate cyclophosphamide-, acrolein- and CAA-induced acute toxicities or mutagenesis in equivalent Mgmt+/+ and Mgmt−/−/C0 mice. However, mutation frequencies were determined in the spleen and may not represent mutagenic events, as well as tumorigenesis, in other tissues or organs. In order to address this issue, additional studies conducted in our laboratory determined that MGMT deficiency does not significantly alter long-term cyclophosphamide-induced toxicity or tumorigenesis (R.Nagasubramaniam, R.Hansen, S.Delaney, M.Cherian, L.Samson, S.Kogan and M.E.Dolan, in preparation). Our current studies demonstrate that MGMT deficiency does not result in increased in vivo toxicities or mutations following cyclophosphamide, acrolein or CAA as compared with wild-type mice. Previous studies from our laboratory suggest that chemotherapeutic use of BG prior to oxazaphosphorines, such as cyclophosphamide or ifosfamide, enhances their toxicity without increasing mutations (48,49).

In conclusion, clinical use of TMZ and other mutagenic chemotherapeutic agents will continue to be a double-edged sword, weighing risks with antitumor response. If the clinical use of TMZ is increased consequent to observed increases in patient survival after TMZ, the incidence of therapy-induced acute myeloid leukemia and secondary malignancy will probably rise. Therefore, it is important to determine what repair mechanisms are involved in both the toxic and the mutagenic effects of this agent. From our work, it is clear that MGMT is important in protecting against the toxic and mutagenic effects of TMZ. On the other hand, mutations following BCNU or cyclophosphamide are not increased in the absence of MGMT. The result that BG did not enhance BCNU mutagenicity implies that clinically, pretreatment with BG will not increase BCNU therapy-induced acute myeloid leukemia, however, direct studies in an appropriate animal model have not been performed. BG does enhance weight loss and lung toxicity (28) associated with BCNU treatment, implying MGMT is important in protecting from BCNU toxicity that results in eventual death.

Acknowledgements

The authors would like to thank Mathew Cherian for his technical assistance and Dr Lisiane Meira for her comments on the manuscript. This work was supported in part by Public Health Service Grants CA81483 (M.E.D.), the University of Chicago’s Environmental Biology Training Grant, National Institutes of Health National Cancer Institute 5-T32 CA09273 (R.J.H.) and Graduate Training Program in Cancer Biology, NIH NCI ST2 CA09594 (R.J.H.).

References


27. Glassner, B.J. et al. (1999) DNA repair methyltransferase (Mgmt) knockout mice are sensitive to the lethal effects of chemotherapeutic alkylating agents. Mutagenesis, 14, 339–347.


